

# Program and Abstract Book

## ERRS 2017

*43<sup>rd</sup> Annual Meeting of the European  
Radiation Research Society*

## GBS 2017

*20<sup>th</sup> Annual Meeting of the Society for  
Biological Radiation Research*

**17-21<sup>st</sup>.09.2017, Essen, Germany**



**Organizers**  
*George Iliakis and Verena Jendrossek*



**Gesellschaft für  
Biologische  
Strahlenforschung e.V.**

# Commercial Sponsors



# Sponsors



● ● ● **Stiftung Universitätsmedizin Essen**



# Table of Content

## Keynote Lectures

<i>Keynote Lecture 1</i> .....	27
<i>Keynote Lecture 2</i> .....	29
<i>Keynote Lecture 3</i> .....	87
<i>Keynote Lecture 4</i> .....	145
<i>Keynote Lecture 5</i> .....	203

## Plenary Sessions

<i>PS1 . "Radiation Exposure and Immune Response"</i> .....	31
<i>PS2 . "DNA Damage Response and DNA Repair"</i> .....	89
<i>PS3 . "Mechanisms of Radiation Effects"</i> .....	147
<i>PS4 . "Clinical Translation of Radiation Biology"</i> .....	205

## Sessions

<i>S01. "Immune Response"</i> .....	39
<i>S02 "DNA Damage Response 1"</i> .....	47
<i>S03. "Tumor Response"</i> .....	55
<i>S04 "DNA Repair in Chromatin Context"</i> .....	63
<i>S05. "Novel Radiosensitizers"</i> .....	71
<i>S06. "Radiation Protection"</i> .....	79
<i>S07. "DNA Damage Response 2"</i> .....	97
<i>S08. "Particle Radiation Modalities 1"</i> .....	105
<i>S09. "Intercellular Communication/Bystander Effects"</i> .....	113
<i>S10. "DNA Damage Response 3"</i> .....	121



# Table of Content

## Sessions

<i>S11. "Radiation Therapy 1" .....</i>	<i>129</i>
<i>S12. "Predictive Assays/Biomarkers 1" .....</i>	<i>137</i>
<i>S13. "Particle Radiation Modalities 2" .....</i>	<i>155</i>
<i>S14. "Radiation Toxicity and Carcinogenesis" .....</i>	<i>163</i>
<i>S15. "Normal Tissue Response 1" .....</i>	<i>171</i>
<i>S16. "Low Dose Radiation Effects" .....</i>	<i>179</i>
<i>S17. "Predictive Assays/Biomarkers 2" .....</i>	<i>187</i>
<i>S18. "Radiation Therapy/Stem Cells" .....</i>	<i>195</i>
<i>S19. "Normal Tissue/Stem Cells Response" .....</i>	<i>213</i>
<i>S20. "Low Dose Radiation Effects/Countermeasures" .....</i>	<i>221</i>
<i>S21. "Modelling of DNA Damage Responses" .....</i>	<i>229</i>

## Poster Sessions

<i>Poster Session 1 .....</i>	<i>237</i>
<i>Poster Session 2 .....</i>	<i>293</i>
<i>Poster Session 3 .....</i>	<i>345</i>





# Organizers

*George Iliakis and Verena Jendrossek*

## Committees

### Organization and Logistics

---

*George Iliakis  
Simon Magin  
Emil Mladenov  
Christian Möllers  
Christian Streffer  
Marcel Thissen*

### Scientific Committee

---

*Udo Gaipf  
George Iliakis  
Verena Jendrossek  
Markus Löbrich  
Gabriele  
Niedermann  
Franz Rödel  
Kai Rothkamm*

### Local Scientific Committee

---

*Diana Klein  
Fanghua Li  
Simon Magin  
Johann Matschke  
Veronika Mladenova  
Yasser Mohd  
Justine Rudner  
Aashish Soni  
Florian Wirsdörfer*

### Advisory Scientific Committees GBS

---

*Nils Cordes  
Claudia Fournier  
Michael Hausmann  
Julia Hess  
Verena Jendrossek  
Kirsten Lauber  
Peter Rodemann  
Claudia Rübe  
Beate Volkmer*

### ERRS

---

*Sarah Baatout  
Marc Benderitter  
Kerstin Borgmann  
Wolfgang Dörr  
Klaas Franken  
Siamak Haghdoust  
Michal Hofer  
Marek Janiak  
George Don Jones  
Katalin Lumniczky  
Fiona Lyng  
Lorenzo Manti  
Marjan Moreels  
Gabriel Pantelias*

### Local Organizing Committee

---

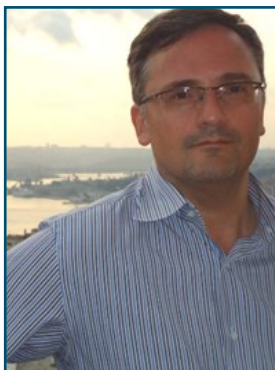
*Anna Broich  
Shipra Chaudhary  
Julia Ketteler  
Lisa Krieger  
Adam Kryzstofiak  
Pelin Kucuk  
Malihe Mesbah  
Tamara Mußfeldt  
Ivonne Schulte  
Mortoga Sharif  
Vasiliki Tasiou  
You Wang  
Marcus Wolf*





# ERRS Council

## President



**Prof. Lorenzo Manti**  
(Italy)

*University of Naples  
Federico II, Physics Dept.  
Radiation Biophysics  
Laboratory,  
Complesso Universitario  
di Monte S. Angelo,  
80126 Naples, Italy*

[lorenzo.manti@unina.it](mailto:lorenzo.manti@unina.it)

## Honorary President



**Prof. Adayapalam  
Natarajan**  
(Netherlands)

† 28.08.2017

*Leiden University  
Medical Centre (LUMC),  
Dept. Toxicogenetics/  
Human Genetics, Leiden,  
The Netherlands*

[natarajan@live.nl](mailto:natarajan@live.nl)

## Vice-President



**Prof. Fiona Lyng**  
(Ireland)

*Dublin Institute of  
Technology,  
Centre for Radiation and  
Environmental Studies,  
Kevin Street, Dublin 8,  
Ireland*

[fiona.lyng@dit.ie](mailto:fiona.lyng@dit.ie)

## Past President



**Prof. Dr. Wolfgang  
Dörr (Austria)**

*Dept. of Radiation Onco-  
logy & Christian Doppler  
Laboratory for Medical  
Radiation Research,  
Radiooncology, Medical  
University of Vienna,  
Währingergürtel 18-20,  
1090 Wien, Austria  
[wolfgang.doerr@  
meduniwien.ac.at](mailto:wolfgang.doerr@meduniwien.ac.at)*

## Secretary



**Prof. Sarah Baatout**  
(Belgium)

*Radiobiology Unit,  
Belgian Nuclear  
Research Centre  
SCK-CEN, Boeretang  
200, B-2400 MOL,  
Belgium*

[err\\_secretariat@  
sckcen.be](mailto:err_secretariat@sckcen.be)

## Deputy Secretary



**Dr. Marjan Moreels**  
(Belgium)

*Radiobiology Unit,  
Belgian Nuclear  
Research Centre  
SCK-CEN, Boeretang  
200, B-2400 MOL,  
Belgium*

[marjan.moreels@  
sckcen.be](mailto:marjan.moreels@sckcen.be)



# ERRS Council

## Deputy Secretary



**Dr. Michal Hofer**  
(Czech Republic)

*Institute of Biophysics,  
Academy of Sciences of  
the Czech Republic,  
Královopolská 135, 612  
65 Brno, Czech Republic*

**zhofer@ibp.cz**

## Deputy Secretary



**Prof. Marek K. Janiak**  
(Poland)

*Department of Radio-  
biology and Health  
Protection Military  
Institute of Hygiene and  
Epidemiology,  
Kozielska 4, 01-163  
Warsaw, Poland*

**mjanjak@wihe.waw.pl**

## Deputy Secretary



**Prof. Klaas Franken**  
(The Netherlands)

*Academic Medical Cen-  
ter, University of Ams-  
terdam, Laboratory of  
Experimental Oncology  
and Radiobiology,  
Meibergdreef 9, 1105 AZ  
Amsterdam, The  
Netherlands*

**n.a.franken@  
amc.uva.nl**

## Deputy Secretary

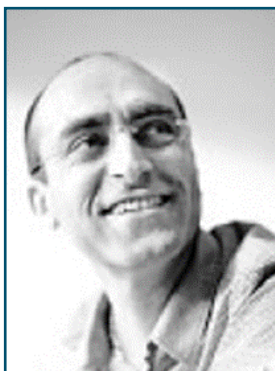


**Prof. Gabriel  
Pantelias (Greece)**

*Radiobiology and Bio-  
dosimetry Laboratory,  
I/R-RP, National Center  
for Scientific Research  
(NCSR) Demokritos,  
Neapoleos str., 153 41,  
Agia Paraskevi, Athens,  
Greece*

**gabriel@  
ipta.demokritos.gr**

## Deputy Secretary



**Dr. Marc Benderitter**  
(France)

*Institute de Radio-  
protection et de Surrete  
Nucleaire,  
BP N°17 92262,  
Fontenay-aux-Roses  
Cedex, France*

**marc.benderitter@  
irsn.fr**

## Deputy Secretary



**Dr. Kerstin Borgmann**  
(Germany)

*Lab. of Radiobiology &  
Experimental Radio-  
oncology, Univ. Med.Cen.  
Hamburg-Eppendorf,  
Martinistr. 52, 20246  
Hamburg, Germany*

**borgmann@  
uke.uni-hamburg.de**





# ERRS Council

## Deputy Secretary



**Prof. Geza Safrany  
(Hungary)**

*Frédéric Joliot-Curie  
National Research  
Institute for Radio-  
biology and Radio-  
hygiene, 1221 Budapest,  
H-1775 Budafok 1. P.O.  
Box 101, Hungary*

***safrany.geza@osski.hu***

## Deputy Secretary



**Prof. Siamak  
Haghdoost (Sweden)**

*Department of Mole-  
cular Biosciences, the  
Wenner-Gren Institute,  
Stockholm University,  
Svante Arrheniusväg  
20C, 10691 Stockholm,  
Sweden*

***siamak.haghdoost@  
su.se***

## Deputy Secretary



**Prof. Ester Hammond  
(United Kingdom)**

*CRUK/MRC Oxford  
Institute for Radiation  
Oncology, Department  
of Oncology, Oxford  
University, Roosevelt  
Drive Oxford, OX3 7DQ,  
UK*

***ester.hammond@  
oncology.ox.ac.uk***







# GBS Board

## Chairman



**Prof. Dr. Michael Hausmann**

*Kirchhoff-Institute for Physics, Faculty of Physics and Astronomy, University of Heidelberg, Im Neuenheimer Feld 227, 69120 Heidelberg, Germany*

## Vice Chairman



**Prof. Dr. Nils Cordes**

*OncoRay - National Center for Radiation Research in Oncology and Dept. of Radiation Oncology, Universitätsklinikum und Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Fetscherstrasse 74, Dresden, Germany*

## Observer Biology



**Prof. Dr. Kirsten Lauber**

*Molecular Oncology LMU Clinic for Radiotherapy and Radiation Oncology Campus Grosshadern Marchioninistr. 15 D-81377 Munich Germany*

## Observer Medicine



**Prof. Dr. med. Claudia E. Rübe**

*Labor für Molekulare Radioonkologie, Klinik für Strahlentherapie, Universitätsklinikum des Saarlandes, Kirrbergerstr. Geb. 6.5, 66421 Homburg/Saar, Germany*



# GBS Board

## Observer Physics



**Dr. Beate Volkmer**

*Dept. of Dermatology  
Dermatologie  
Elbeklinikum Buxtehude  
Am Krankenhaus 1  
21614 Buxtehude  
Germany*

## Observer Young Investigators



**Dr. Julia Hess**

*Research Unit Radiation  
Cytogenetics,  
Helmholtz Zentrum  
München  
Deutsches Forschungs-  
zentrum für Gesundheit  
und Umwelt (GmbH)  
Ingolstädter Landstr. 1  
85764 Neuherberg  
Germany*

## Secretary



**Prof. Dr. Verena  
Jendrossek (PhD)**

*Institute of Cell Biology  
(Cancer Research),  
Molecular Cell Biology,  
University of Duisburg-  
Essen Medical School,  
Virchowstrasse 173,  
45147 Essen,  
Germany*

## Treasurer



**Prof. Dr. Claudia  
Fournier**

*GSI Helmholtz Center for  
Heavy Ion Research  
Department of  
Biophysics  
Planckstr. 1  
64291 Darmstadt  
Germany*

## Past-Chairman

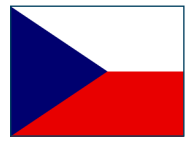


**Prof. Dr. H. Peter  
Rodemann**

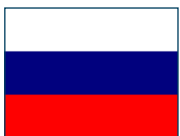
*Molecular Environmental  
Research  
Dept. of Radiation  
Oncology  
Univ. of Tuebingen  
Roentgenweg 11  
72076 Tuebingen  
Germany*



# Participating Countries (32)



Australia  
Austria  
Belgium  
Canada  
Colombia  
Czech Republic  
Denmark  
France  
Germany  
Greece



Hungary

India

Iran

Ireland

Italy

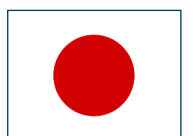
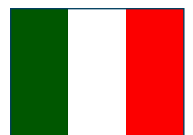
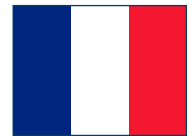
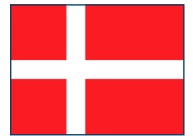
Japan

Lithuania

Netherlands

Nigeria

Norway



Poland

Romania

Russia

Singapore

South Africa

Spain

Sweden

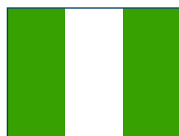
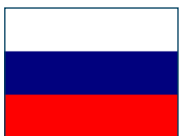
Switzerland

Syria

Turkey

United Kingdom

United States







# Travel Awards ERRS/GBS 2017

## ERRS Awards (20)

1. **Adrian, Gabriel** ([gabriel.adrian@med.lu.se](mailto:gabriel.adrian@med.lu.se))  
M, Lund University, Lund, Sweden
2. **Barbieri, Sofia** ([sofia.barbieri01@universitadipavia.it](mailto:sofia.barbieri01@universitadipavia.it))  
F, University of Pavia, Pavia, Italy
3. **Belmans, Niels** ([nbelmans@sckcen.be](mailto:nbelmans@sckcen.be))  
M, Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium
4. **Carante, Mario Pietro** ([mariopietro.carante01@ateneopv.it](mailto:mariopietro.carante01@ateneopv.it))  
M, University of Pavia and INFN, Pavia, Italy
5. **Casolaro, Pierluigi** ([casolaro@na.infn.it](mailto:casolaro@na.infn.it))  
M, University of Napoli "Federico II" and INFN-Napoli, Naples, Italy
6. **Cheng, Lei** ([lei.cheng@su.se](mailto:lei.cheng@su.se))  
F, Stockholm University, Stockholm, Sweden
7. **Coninx, Emma** ([emma.coninx@sckcen.be](mailto:emma.coninx@sckcen.be))  
F, Belgian Nuclear Research Centre (SCK•CEN), Mol, Belgium
8. **Cullen, Daniel** ([d13123391@mydit.ie](mailto:d13123391@mydit.ie))  
M, Dublin Institute of Technology, Dublin, Ireland
9. **Daems, Naomi** ([noami.daems@sckcen.be](mailto:noami.daems@sckcen.be))  
F, SCK•CEN, Belgian Nuclear Research Centre, Mol, Belgium
10. **Doix, Bastien** ([bastien.doix@uclouvain.be](mailto:bastien.doix@uclouvain.be))  
M, Université Catholique de Louvain, Brussels, Belgium
11. **Gasol Garcia, Ana** ([a.gasolgarcia@vumc.nl](mailto:a.gasolgarcia@vumc.nl))  
F, Cancer Center Amsterdam, Amsterdam, Netherlands
12. **Gorodetska, Ielizaveta** ([Liza.Gorodetska@uniklinikum-dresden.de](mailto:Liza.Gorodetska@uniklinikum-dresden.de))  
F, OncoRay-National Center for Radiation Research in Oncology, Dresden, Germany
13. **Koshanskaya, Maria** ([maria.koshanskaya@meduniwien.ac.at](mailto:maria.koshanskaya@meduniwien.ac.at))  
F, Medical University of Vienna, Vienna, Austria
14. **Kowaliuk, Jakob** ([jakob.kowaliuk@meduniwien.ac.at](mailto:jakob.kowaliuk@meduniwien.ac.at))  
M, Medical University of Vienna, Vienna, Austria
15. **Mboumboua Mfossa, Andre Claude** ([ammfossa@sckcen.be](mailto:ammfossa@sckcen.be))  
M, Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium
16. **Meneceur, Sarah** ([sarah.meneceur@uniklinikum-dresden.de](mailto:sarah.meneceur@uniklinikum-dresden.de))  
F, OncoRay – National Center for Radiation Research in Oncology, Dresden, Germany
17. **Persa, Eszter** ([persaeszter@yahoo.com](mailto:persaeszter@yahoo.com))  
F, National Public Health Institute, Budapest, Hungary
18. **Rassamegevanon, Treewut** ([Treewut.Rassamegevanon@uniklinikum-dresden.de](mailto:Treewut.Rassamegevanon@uniklinikum-dresden.de))  
M, OncoRay – National Center for Radiation Research in Oncology, Dresden, Germany
19. **Siragusa, Mattia** ([masir@dtu.dk](mailto:masir@dtu.dk))  
M, Technical University of Denmark, Roskilde, Denmark
20. **Souli, Maria** ([maria\\_souli@yahoo.gr](mailto:maria_souli@yahoo.gr))  
F, National Technical University of Athens, Athens, Greece

## GBS Awards (5)

1. **Deville, Sara Sofia** ([SaraSofia.Deville@uniklinikum-dresden.de](mailto:SaraSofia.Deville@uniklinikum-dresden.de))  
F, OncoRay – National Center for Radiation Research in Oncology, Dresden, Germany
2. **Mirsch, Johanna** ([mirsch@bio.tu-darmstadt.de](mailto:mirsch@bio.tu-darmstadt.de))  
F, TU Darmstadt, Darmstadt, Germany
3. **Papenfuß, Franziska** ([franziska.papenfuss@kip.uni-heidelberg.de](mailto:franziska.papenfuss@kip.uni-heidelberg.de))  
F, Kirchhoff-Institute for Physics, Heidelberg University, Heidelberg, Germany
4. **Rühle, Alexander** ([a.ruehle@stud.uni-heidelberg.de](mailto:a.ruehle@stud.uni-heidelberg.de))  
M, German Cancer Research Center, Heidelberg, Germany
5. **Timm, Sara** ([sara.timm@uks.eu](mailto:sara.timm@uks.eu))  
F, Saarland University, Homburg/Saar, Germany



## Organizers' Awards (25)

1. **Ahire, Vidula** ([vidhula4@gmail.com](mailto:vidhula4@gmail.com))  
F, Academic Medical Center, Amsterdam, Netherlands
2. **Al-Refae, Klaudia** ([klaudia.al-refae@uk-essen.de](mailto:klaudia.al-refae@uk-essen.de))  
F, Institute of Cell Biology, University Hospital Essen, Essen, Germany
3. **Bucher, Martin** ([mbucher@bfs.de](mailto:mbucher@bfs.de))  
M, Federal Office for Radiation Protection, Neuherberg, Germany
4. **Castelletti, Noemi** ([noemi.castelletti@helmholtz-muenchen.de](mailto:noemi.castelletti@helmholtz-muenchen.de))  
F, Helmholtz Center Munich, Institute of Radiation Protection (ISS), Munich, Germany
5. **Frister, Moritz** ([m.frister@dkfz-heidelberg.de](mailto:m.frister@dkfz-heidelberg.de))  
M, DKFZ, Heidelberg, Germany
6. **Giuranno, Lorena** ([l.giuranno@maastrichtuniversity.nl](mailto:l.giuranno@maastrichtuniversity.nl))  
F, Maastricht University, Maastricht, Netherlands
7. **Grasso, Debora** ([d.grasso@uclouvain.be](mailto:d.grasso@uclouvain.be))  
F, University of Louvain, Brussels, Belgium
8. **Gupta, Noopur** ([noopurgupta3105@gmail.com](mailto:noopurgupta3105@gmail.com))  
F, Institute of Medicine and allied Sciences, Delhi, India
9. **Haritwal, Teena** ([haritwalteena@gmail.com](mailto:haritwalteena@gmail.com))  
F, Institute of Medicine and allied Sciences, Delhi, India
10. **Ilicic, Katarina** ([katarina.ilicic@tum.de](mailto:katarina.ilicic@tum.de))  
F, Technical University Munich, Germany
11. **Kabacik, Sylwia** ([sylwia.kabacik@phe.gov.uk](mailto:sylwia.kabacik@phe.gov.uk))  
F, Public Health England, Chilton, UK
12. **Kainthola, Anup** ([anupmicrobio@gmail.com](mailto:anupmicrobio@gmail.com))  
M, Institute of Medicine and allied Sciences, Delhi, India
13. **Ketteler, Julia** ([julia.ketteler@uk-essen.de](mailto:julia.ketteler@uk-essen.de))  
F, Institute of Cell Biology, University Hospital Essen, Essen, Germany
14. **Kumari, Neeraj** ([kmneeraj05@yahoo.com](mailto:kmneeraj05@yahoo.com))  
F, Institute of Medicine and allied Sciences, Delhi, India
15. **Manning, Grainne** ([Grainne.Manning@phe.gov.uk](mailto:Grainne.Manning@phe.gov.uk))  
F, Public Health England, Centre for Radiation, Chemical and Environmental Hazards, Oxfordshire, U.K.
16. **Matsuya, Yusuke** ([y-matsuya1028@frontier.hokudai.ac.jp](mailto:y-matsuya1028@frontier.hokudai.ac.jp))  
M, Graduate School of Health Sciences, Hokkaido University, Japan
17. **Meyer, Felix** ([fe.meyer@uke.de](mailto:fe.meyer@uke.de))  
M, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
18. **Nguyen, Lily** ([lily.nguyen@helmholtz-muenchen.de](mailto:lily.nguyen@helmholtz-muenchen.de))  
F, Helmholtz Zentrum München, Neuherberg, Germany
19. **Nimker, Shwetanjali** ([shwetanjaly1@yahoo.com](mailto:shwetanjaly1@yahoo.com))  
F, Institute of Medicine and allied Sciences, Delhi, India
20. **Paithankar, Jagdish Gopal** ([jagdish.paithankar@gmail.com](mailto:jagdish.paithankar@gmail.com))  
M, Mangalore University, Mangalore, Karnataka state, India
21. **Vandevoorde, Charlot Rosa** ([cvandevoorde@tlabs.ac.za](mailto:cvandevoorde@tlabs.ac.za))  
F, NRF iThemba LABS, Somerset West, South Africa
22. **Verstraete, Bram** ([Bram.Verstraete@UGent.be](mailto:Bram.Verstraete@UGent.be))  
M, Ghent University, Belgium
23. **Vitale, Ermenegilda** ([erm.vitale@studenti.unina.it](mailto:erm.vitale@studenti.unina.it))  
F, University of Naples Federico II, Naples, Italy
24. **Walsh, Dietrich** ([dietrich.walsh@unibw.de](mailto:dietrich.walsh@unibw.de))  
M, Universität der Bundeswehr München, Neubiberg, Germany
25. **Yudhistiara, Brian** ([yudhistiara@stud.uni-heidelberg.de](mailto:yudhistiara@stud.uni-heidelberg.de))  
M, University of Heidelberg, Germany





# Detailed Program

**Registration: Monday to Wednesday, 8:00-16:00**  
**Sunday, 10:00-20:00**  
**Deichmann Auditorium**

13:30-14:30

## Lunch for Scholars in Training

## Special Primer for Scholars in Training

Deichmann Auditorium

### Session 1: "Career Development"

*Host and Chair: J. Hess*

14:30-15:15

**Bernd Pulverer**  
Heidelberg, Germany

*"Transparent publishing, preprints and open science"*

15:15-16:00

**Julia Verse**  
Berlin, Germany

*"Good Scientific Practice - Protecting Scientific Integrity"*

16:00-16:30

## Coffee Break

### Session 2: "Applied Science" and Round Table Discussion

*Chairs: K. Al-Refae and A. Krysztofiak*

16:30-17:15

**Nicholas McGranahan**  
London, United Kingdom

*"Tumor heterogeneity and therapy outcome"*

17:15-18:00

**M.H. Barcellos-Hoff, M. Foiani, N. McGranahan, G. McKenna, B. Pulverer  
J. Verse and E. Rogakou**

*"Round Table Discussion on career paths in science"*

## Inaugural Session

Audimax

18:30-19:00

### Opening Ceremony

**Prof. Dr. Jan Buer**

*Dean, Medical Faculty, University of Duisburg-Essen*

**Prof. Lorenzo Manti**

*President, ERRS*

**Prof. Michael Hausmann**

*President, GBS*

**Prof. Till Engel**

*Music for intellectual stimulation  
Beethoven Piano Sonata Nr. 1 in f-Minor op. 2/1*

**Prof. Dr. Verena Jendrossek**

*Institute of Cell Biology, University of Duisburg-Essen*

**Prof. George Iliakis**

*Inst. of Med. Rad. Biology, University of Duisburg-Essen*

19:00-20:00

### Keynote Lecture 1

*Chair: G. Iliakis*

**Silvia Formenti**

*"Immune effects of radiation therapy"*

New York, United States

20:00-22:00

## Welcome Reception

## Keynote Lecture 2, Deichmann Auditorium

*Chair: V. Jendrossek*

8:15-9:00

**Mary Helen Barcellos-Hoff** *"Looking for Achilles' heel: HPV, TGF- $\beta$ , and DDR"*  
San Francisco, United States

## Plenary Session 1, "Radiation Exposure and Immune Response"

*Chairs: G. Niedermann/B. Frey*

9:00-9:20

**Silvia Formenti** *"TREX1 regulates radiation immunogenicity: clinical implications"*  
New York, United States

9:20-9:40

**Udo Gaipi** *"Immune modulatory properties of radiotherapy - rationales for combination with immunotherapy"*  
Erlangen, Germany

9:40-10:00

**Gabriele Niedermann** *"Preclinical evaluation and imaging of immunoradiotherapy approaches"*  
Freiburg, Germany

10:00-10:20

**Conrad Rauber** *"Gut microbiota in radioimmunotherapy"*  
Villejuif, France

10:20-10:40

**Lisa Sevenich** *"Effects of ionizing radiation on brain metastasis-associated inflammation"*  
Frankfurt am Main, Germany

10:40-11:00

## Coffee Break

### S01, "Immune Response"

#### Deichmann Auditorium

*Chairs: G. Safrany/F. Wirsdörfer*

11:00-11:15

**Ruth Muschel**  
Oxford, United Kingdom  
*"FGF2 is a switch controlling the tumor response to radiation through macrophage polarization"*

11:15-11:30

**Benjamin Frey**  
Erlangen, Germany  
*"Pre-clinical and clinical hints for immune modulation by low doses of ionizing radiation"*

11:30-11:45

**Amir Abdollahi**  
Heidelberg, Germany  
*"Radiation induced lung fibrosis-a dynamic interplay between immune response, angiogenesis ..."*

11:45-12:00

**Ludwig Dubois**  
Maastricht, Netherlands  
*"Radiotherapy and immunocytokines: a perfect match for abscopal effects with long-lasting memory?"*

12:00-12:15

**Roman Hennel**  
Munich, Germany  
*"Immunostimulatory effects induced by radiotherapy of breast cancer cells"*

12:15-12:30

**Veronica Olivo Pimentel\***  
Maastricht, Netherlands  
*"Radiotherapy causes long-lasting antitumor immunological memory when combined with immunotherapy"*

### S02, "DNA Damage Response 1"

#### Audimax

*Chairs: C. Sorensen/M. Wolf*

**Beate Volkmer**  
Buxtehude, Germany  
*"Epigenetic alterations in skin cancer tissue and UV-exposed skin"*

**Stephanie Hehlhans**  
Frankfurt am Main, Germany  
*"Nek1 depletion modulates apoptosis, DNA repair and radiation survival of 3D-cultured colorectal and cervix ..."*

**Aashish Soni**  
Essen, Germany  
*"Increased DSB end resection causes enhanced formation of chromosomal translocations through Parp-1 ..."*

**Klaudia Al-Refae\***  
Essen, Germany  
*"Impact of Akt1 phospho-mutants on the cellular response to ionizing radiation"*

**Aadhya Tiwari\***  
Tübingen, Germany  
*"Y-box binding protein-1 stimulates repair of ionizing radiation-induced DNA double strand breaks ..."*

**Jagdish Paithankar\***  
Mangalore, India  
*"Variations in radiation tolerance in life stages of D. melanogaster provide clues to radiation tolerance ..."*

### S03, "Tumor Response"

#### IG1, Hörsaal 1

*Chairs: S. Haghdoust/A. Vehlou*

**Peter Huber**  
Heidelberg, Germany  
*"CTGF (connective tissue growth factor) in radiation tumor and normal tissue response"*

**Marc Vooijs**  
Maastricht, Netherlands  
*"Modeling tumour and normal tissue effects of combination treatments"*

**Heidi Lyng**  
Oslo, Norway  
*"Imaging hypoxia in prostate cancer"*

**Simone Moertl**  
Munich, Germany  
*"Exosomes from irradiated squamous head and neck cancer cells with altered protein cargo boost ..."*

**Sofia Ferreira\***  
Orsay Ville, France  
*"Dbait and radiation treatments in pediatric brain tumors"*

**Emmy Rogakou**  
Athens, Greece  
*"20 years after the discovery of the  $\gamma$ H2AX biomarker"*

12:30-14:30

## Poster Session 1, Audimax (Lunch will be provided)



# MONDAY, 18.09.2017

	S04, "DNA Repair in Chromatin Context"	S05, "Novel Radiosensitizers"	S06, "Radiation Protection"
	Deichmann Auditorium	Audimax	IG1, Hörsaal 1
14:30-14:45	<p><i>Chairs: A. Rapp/V. Mladenova</i></p> <p><b>Timothy Humphrey</b> Oxford, United Kingdom <i>"Histone H3K36 trimethylation, genome stability and cancer"</i></p>	<p><i>Chairs: Y. Saintigny/S. Ferreira</i></p> <p><b>Gillies McKenna</b> Oxford, United Kingdom <i>"High-throughput screens for drugs to modify hypoxia"</i></p>	<p><i>Chairs: C. Streffer/N. Castelletti</i></p> <p><b>Christian Streffer</b> Essen, Germany <i>"LNT-model for radiological protection: reasonable use and misuse"</i></p>
14:45-15:00	<p><b>Claudia Rübe</b> Homburg, Germany <i>"Hair follicle stem cell fate is dependent on chromatin remodeling capacity following low-dose radiation"</i></p>	<p><b>Martin Pruschy</b> Zurich, Switzerland <i>"Targeting ADAM17 for radiosensitization"</i></p>	<p><b>Wolfgang-Ulrich Müller</b> Essen, Germany <i>"Attributing health effects to ionizing radiation exposure and inferring risks"</i></p>
15:00-15:15	<p><b>Burkhard Jakob</b> Darmstadt, Germany <i>"Dynamics of DNA repair proteins in response to radiation induced DNA damage of different complexity"</i></p>	<p><b>Peter Sminia</b> Amsterdam, Netherlands <i>"The MAPK targeted agent MEK162 acts as radiosensitizer in glioblastoma therapy"</i></p>	<p><b>Ulrike Kulka</b> Oberschleißheim, Germany <i>"Research for radiation protection"</i></p>
15:15-15:30	<p><b>Alexander Rapp</b> Darmstadt, Germany <i>"Identification of the elementary structural chromatin units of the DNA damage response"</i></p>	<p><b>Simon Magin</b> Essen, Germany <i>"ATR dependent reactivation of suppressed alt-EJ in G0"</i></p>	<p><b>Herbert Braselmann</b> Neuherberg, Germany <i>"A 9-feature genomic copy number signature is associated with radiation exposure in post-Chernobyl breast ..."</i></p>
15:30-15:45	<p><b>Lovisa Lundholm</b> Stockholm, Sweden <i>"The role of chromatin in response to alpha and gamma radiation in breast cancer cells"</i></p>	<p><b>Mike Atkinson</b> Neuherberg, Germany <i>"Do non-coding RNAs play a role in the response to radiation?"</i></p>	<p><b>Niels Belmans*</b> Mol, Belgium <i>"Age-related biological effects of dental cone-beam CT exposure"</i></p>
15:45-16:00	<p><b>Nataša Anastasov</b> Neuherberg, Germany <i>"Non-coding RNAs as regulators of cellular epigenome and response to radiation"</i></p>	<p><b>Bodo Laube</b> Darmstadt, Germany <i>"Impact of glutamate receptor mediated signaling on the induction and repair of DNA DSBs ..."</i></p>	<p><b>Christine Hellweg</b> Cologne, Germany <i>"Radiation protection for human space flights"</i></p>
16:00-16:30	Coffee Break		
	GBS - Function		
16:30-17:10	<p><b>"Best Paper of the Year/Dieter Frankenberg Award Lecture", Deichmann Auditorium</b></p>		
17:15-18:30	<p><b>"GBS Mitgliederversammlung", Deichmann Auditorium</b></p>		
18:30-20:00	<p><b>Get Together with Beer and Snacks</b></p>		

43rd Annual Meeting of ERRS and 20th Annual Meeting of GBS, Essen, Germany, 2017

\*Denotes young investigators

## Keynote Lecture 3, Deichmann Auditorium

*Chair: M. Löbrich*

8:15-9:00

**Simon Boulton**

London, United Kingdom

*"Mechanics of homologous recombination and its exploitation in cancer therapy"*

## Plenary Session 2, "DNA Damage Response and DNA Repair"

*Chairs: D. van Gent/ E. Mladenov*

9:00-9:20

**Markus Löbrich**

Darmstadt, Germany

*"ATR promotes DNA repair synthesis and cross-over formation during homologous recombination"*

9:20-9:40

**Haico van Attikum**

Leiden, Netherlands

*"Regulation and dynamics of DNA repair in a chromatin context"*

9:40-10:00

**George Iliakis**

Essen, Germany

*"Logic and necessities in the repair of DNA double strand breaks in higher eukaryotes"*

10:00-10:20

**Dik van Gent**

Rotterdam, Netherlands

*"Modulation of DNA repair enhances the ionizing radiation effect in anti-tumor treatment"*

10:20-10:40

**Ester Hammond**

Oxford, United Kingdom

*"Understanding and exploiting the hypoxia-induced DNA damage response"*

10:40-11:00

## Coffee Break

### S07, "DNA Damage Response 2"

#### Deichmann Auditorium

*Chairs: B. Volkmer/S. Magin*

11:00-11:15

**Carsten Herskind**

Mannheim, Germany

*"A possible role of cell-cycle checkpoints in radioresistance and the shape of cell survival ..."*

11:15-11:30

**Wael Mansour**

Hamburg, Germany

*"Loss of PTEN-assisted G2/M checkpoint impedes homologous recombination repair ..."*

11:30-11:45

**Emil Mladenov**

Essen, Germany

*"CRISPR/Cas9 based model system to investigate the repair of DSB clusters with increased complexity"*

11:45-12:00

**Michael Ensminger\***

Darmstadt, Germany

*"Nek1 coordinates the repair of one-ended DSBs with cell-cycle progression"*

12:00-12:15

**Stefanie Mosel\***

Essen, Germany

*"A novel role for the anti-apoptotic protein Survivin in DNA repair and replication"*

12:15-12:30

**Ramon Lopez Perez\***

Heidelberg, Germany

*"Towards a better understanding of the ultrastructural organization of DNA double-strand break repair foci"*

### S08, "Particle Radiation Modalities 1"

#### Audimax

*Chairs: M. Moreels/P. Casolaro*

**Beate Timmermann**

Essen, Germany

*"The pediatric program at the West German Proton Therapy Centre Essen (WPE): aiming to reduce late ..."*

**Thomas Schmid**

Neuherberg, Germany

*"Proton minibeam radiation therapy is a novel approach to minimize normal tissue damage"*

**Sara Timm\***

Homburg, Germany

*"Clustering of DSB following high-LET irradiation perturbs DNA repair and is associated with long-lasting ..."*

**Katrien Konings\***

Mol, Belgium

*"How do different radiation qualities affect the Hedgehog signaling pathway?"*

**Ralf Kriehuber**

Jülich, Germany

*"Geno- and cytotoxicity of DNA-associated Auger electron emitters"*

**Elke Beyreuther**

Dresden, Germany

*"Analysing methods of animal irradiation experiments with deviations from prescribed dose"*

### S09, "Intercellular Comm./Bystander Effects"

#### IG1, Hörsaal 1

*Chairs: F. Lyng/L. Dubois*

**Carmel Mothersill**

Hamilton, Canada

*"Harnessing radiation induced bystander signalling mechanisms for radiotherapy"*

**Katalin Lumniczky**

Budapest, Hungary

*"Radiation-induced bystander effects in the haematopoietic system mediated by extracellular vesicles"*

**Géza Sáfrány**

Budapest, Hungary

*"Intracranial or local thorax irradiation-induced non-targeted effects in bone-marrow-derived ..."*

**Colin Seymour**

Hamilton, Canada

*"Low doses and non-targeted effects in environmental radiation protection; where are we now and where ..."*

**An Aerts**

Mol, Belgium

*"Effect of ionizing radiation on intercellular communication in vascular endothelial cells"*

**Werner Rühm**

Neuherberg, Germany

*"Relevance of low dose and low dose rate research for radiation protection"*

12:30-14:30

## Poster Session 2, Audimax (Lunch will be provided)

## TUESDAY, 19.09.2017

	S10, "DNA Damage Response 3"	S11, "Radiation Therapy 1"	S12, "Predictive Assays/Biomarkers 1"
	Deichmann Auditorium	Audimax	IG1, Hörsaal 1
14:30-14:45	<b>Chairs: M. Toulany/R. L. Perez</b> <b>Kerstin Borgmann</b> Hamburg, Germany <i>"CHK1-mediated replication fork stabilization confers radioresistance in homologous recombination ..."</i>	<b>Chairs: H. Lyng/A. Rühle</b> <b>Marie Dutreix</b> Orsay Ville, France <i>"DNA repair inhibitors and radiotherapy"</i>	<b>Chairs: K. Franken/C. von Neubeck</b> <b>George Don Jones</b> Leicester, United Kingdom <i>"Comet assay measures of cancer treatment cell sensitivity"</i>
14:45-15:00	<b>Claus Sørensen</b> Copenhagen, Denmark <i>"Novel DNA nuclease function governs cell fate decisions after ionising radiation"</i>	<b>Alexander Schramm</b> Essen, Germany <i>"Modulation of the DNA damage response by neurotrophin receptor signalling"</i>	<b>Nicolaas Franken</b> Amsterdam, Netherlands <i>"Reduced activity of DSB Repair Genes in prostate cancer patients with late normal tissue radiation-toxicity"</i>
15:00-15:15	<b>Aurelie Vaurijoux*</b> Fontenay-aux-Roses, France <i>"Persistent ionizing radiation-induced foci in human primary cells: transmission through cell division"</i>	<b>Heike Anders*</b> Munich, Germany <i>"HSP90 inhibition: sensitization of aggressive soft tissue sarcomas to radiotherapy by enhancing the ..."</i>	<b>Pavel Lobachevsky</b> Melbourne, Australia <i>"γ-H2AX assay as a basis for prediction of individual radiosensitivity"</i>
15:15-15:30	<b>Fanghua Li</b> Essen, Germany <i>"Suppression of CtIP-controlled DNA end-resection impairs alt-EJ in quiescent cells"</i>	<b>Justine Rudner</b> Essen, Germany <i>"USP9x-regulated Mcl-1 stability is important for prostate cancer survival in response to ionizing radiation"</i>	<b>Dietrich Walsh*</b> Neubiberg, Germany <i>"Real time imaging of ROS propagation after targeted mitochondrial irradiation"</i>
15:30-15:45	<b>Michael Hausmann</b> Heidelberg, Germany <i>"Nano-probes and localization microscopy: a pointilist view on mechanisms of radiation ..."</i>	<b>Hua Jing*</b> Heidelberg, Germany <i>"Combination of hypofractionated radiotherapy and IL-2/anti-IL-2 complexes and its theranostic ..."</i>	<b>Ulrike Schötz</b> Munich, Germany <i>"Biomarkers and targets for personalization of radiotherapy of HNSCC: CD44v6"</i>
15:45-16:00	<b>Moritz Frister*</b> Heidelberg, Germany <i>"Ultrastructural studies on radiation-induced DNA double strand break repair foci with super-resolution ..."</i>	<b>Erika Zernikel*</b> Essen, Germany <i>"A synthetic lethality-based strategy for individual sensitization of lung cancer cell lines with ..."</i>	<b>Michael Orth</b> Munich, Germany <i>"Mechanism and biomarkers predicting the outcome of taxane-based, concurrent ..."</i>



### 16:30-18:00 (optional guided tour)

**Art Museum Folkwang: Collections of painting and sculpture from the 19th century, Classical Modernism and the post-1945 period**



### 18:30-23:00

#### Conference Dinner, Philharmonie Essen

*Prof. Dr. Ulrich Radtke, President, University of Duisburg-Essen*

*Prof. Till Engel:*

*W.A. Mozart - Piano Sonata No 11 in A - Major, K.331*

*F. Liszt - Hungarian Rhapsody No. 13*

**Awards**

*Jörg Hegemann, Boogie Woogie and lots of dance and fun*

43rd Annual Meeting of ERRS and 20th Annual Meeting of GBS, Essen, Germany, 2017

## Keynote Lecture 4, Deichmann Auditorium

*Chair: T. Halazonetis*

8:15-9:00

**Marco Foiani**

Milan, Italy

*"An integrated ATR, ATM and mTOR-mechanical network controlling nuclear plasticity and cell migration"*

## Plenary Session 3, "Mechanisms of Radiation Effects"

*Chairs: M. Foiani/Z. Nikitaki*

9:00-9:20

**Thanos Halazonetis**

Geneva, Switzerland

*"A vertebrate DNA replication completion checkpoint shows similarities to the spindle checkpoint"*

9:20-9:40

**Christian Reinhardt**

Cologne, Germany

*"ATM restoration in vivo induces lymphoma regression through cell autonomous and non-cell autonomous mechanisms"*

9:40-10:00

**Björn Schumacher**

Cologne, Germany

*"Genome stability in aging and disease"*

10:00-10:20

**George Garinis**

Heraklion, Greece

*"Nucleotide excision repair: from persistent DNA damage to chronic inflammation"*

10:20-10:40

**Petra Boukamp**

Heidelberg, Germany

*"A role for telomeres in UV-induced skin cancer"*

10:40-11:00

## Coffee Break

### S13, "Particle Radiation Modalities 2"

#### Deichmann Auditorium

*Chairs: L. Manti/S. Timm*

11:00-11:15

**Lorenzo Manti**

Naples, Italy

*"Enhancement of clinical proton biological effectiveness by means of proton-boron fusion reaction"*

11:15-11:30

**Andrzej Wojcik**

Stockholm, Sweden

*"X-rays and  $\alpha$ -particles interact in inducing DNA damage in U2OS cells"*

11:30-11:45

**Alexandros Georgakilas**

Athens, Greece

*"Uniting ionizing radiation, DNA damage and immune response through an integrative ..."*

11:45-12:00

**Georgia Terzoudi**

Athens, Greece

*"Biological effectiveness of protons in comparison to  $\alpha$ -particles and accelerated C-ions as measured ..."*

12:00-12:15

**Charlot Vandevoorde\***

Cape Town, South Africa

*"Assessment of out-of-field DNA damage and the impact of neutron RBE on secondary cancer risk in ..."*

12:15-12:30

**Marco Durante**

Trento, Italy

*"Ground-based research for space radiation protection"*

### S14, "Radiation Toxicity and Carcinogenesis"

#### Audimax

*Chairs: M. Atkinson/J. Hess*

**Christophe Badie**

Didcot, United Kingdom

*"Tracking pre-leukemic live cells in murine radiation-induced leukaemia"*

**Neeraj Kumari\***

Delhi, India

*"IL-6 protects cells from radiation induced cell death by activating anti-oxidant defence and STAT-3 ..."*

**Mieke Verslegers\***

Mol, Belgium

*"Investigating congenital eye defects in prenatally-irradiated mice and the efficacy of folic acid as a ..."*

**André Claude M. Mfossa\***

Mol, Belgium

*"Mechanistic analysis of radiation-induced microcephaly in mice"*

**Emma Coninx\***

Mol, Belgium

*"The hippocampus: a target for premature aging after early-life irradiation"*

**Paul Schofield**

Cambridge, United Kingdom

*"The STORE database; a platform for sharing experimental and epidemiological data and resources in ..."*

### S15, "Normal Tissue Response 1"

#### IG1, Hörsaal 1

*Chairs: S. Moertl/L. Barazzuol*

**Florian Wirsdörfer**

Essen, Germany

*"Loss of CD73 prevents macrophage accumulation and phenotype changes in radiation-induced lung fibrosis"*

**Noëlle Mathieu**

Fontenay-aux-Roses, France

*"Bowel radiation injury: complexity of the pathophysiology and promises of cell and tissue engineering"*

**Shwetanjali Nimker\***

Delhi, India

*"Dose-dependent morphological and biochemical alterations in the peripheral blood erythrocytes of ..."*

**Arjan van Dijk**

Bilthoven, Netherlands

*"A mechanistic model for radiation induced atherosclerosis"*

**Sylwia Kabacik\***

Didcot, United Kingdom

*"Ionising radiation increases permeability of endothelium through ADAM 10-mediated cleavage of VE-cadherin"*

**Lorena Giuranno\***

Maastricht, Netherlands

*"Improving lung cancer outcome by reducing normal lung toxicity"*

12:30-14:30

## Poster Session 3, Audimax (Lunch will be provided)

# WEDNESDAY, 20.09.2017

	S16, "Low Dose Radiation Effects"	S17, "Predictive Assays/Biomarkers 2"	S18, "Radiation Therapy/Stem Cells"
	Deichmann Auditorium	Audimax	IG1, Hörsaal 1
14:30-14:45	<p><i>Chairs: M. Hausmann/A. Soni</i></p> <p><b>Horst Zitzelsberger</b> Munich, Germany <i>"Radiation markers in breast carcinogenesis"</i></p>	<p><i>Chairs: C. Rübe/J. Matschke</i></p> <p><b>Gabriel Pantelias</b> Athens, Greece <i>"The use of PCC for elucidating the mechanism underlying chromothripsis and for early triage biodosimetry ... "</i></p>	<p><i>Chairs: F. Rödel/R. Hennel</i></p> <p><b>Lisa Wiesmüller</b> Ulm, Germany <i>"The MLL breakpoint cluster region: a target for chemo- and radiotherapy"</i></p>
14:45-15:00	<p><b>Claudia Fournier</b> Darmstadt, Germany <i>"Immune-related, low dose effects in adipose and other joint cells and tissue"</i></p>	<p><b>Malte Kriegs</b> Hamburg, Germany <i>"Kinomic profiling in radiation oncology: strategies towards personalized molecular targeting"</i></p>	<p><b>Daniel Cullen*</b> Dublin, Ireland <i>"Identification of prostate cancer patients at risk of late radiotoxicity following radiation therapy ... "</i></p>
15:00-15:15	<p><b>Roel Quintens</b> Mol, Belgium <i>"Prenatal irradiation as a potential inducer of premature differentiation during corticogenesis"</i></p>	<p><b>Sarah Meneceur</b> Dresden, Germany <i>"Are residual <math>\gamma</math>-H2AX foci predictive for radiosensitivity? Evaluation of in vivo irradiated tumors ... "</i></p>	<p><b>Daniel Lopez*</b> Bogota, Colombia <i>"Effect of NIS expression in response to treatment with external beam radiotherapy in a colon carcinoma ... "</i></p>
15:15-15:30	<p><b>Maria Gomolka</b> Oberschleißheim, Germany <i>"Gene expression changes in former uranium workers of the sag/sdag wismut"</i></p>	<p><b>Cläre von Neubeck</b> Dresden, Germany <i>"Using <math>\gamma</math>-H2AX Foci as potential predictors for individual radiosensitivity"</i></p>	<p><b>Mozhgan Dehghan Harati*</b> Tübingen, Germany <i>"Akt isoforms differently regulate ALDH activity and the expression of stem cell markers in cancer cells"</i></p>
15:30-15:45	<p><b>Claudia Dalke</b> Neuherberg, Germany <i>"A lifetime study in mice – assessing several biological endpoints after exposure to low doses of IR"</i></p>	<p><b>Judith Reindl*</b> Neubiberg, Germany <i>"Using DNA DSB protein clusters as a potent marker for biological micro-dosimetry on high-LET particle ... "</i></p>	<p><b>Sylvia Ritter*</b> Darmstadt, Germany <i>"Ionizing radiation alters the differentiation potential of human embryonic stem cells"</i></p>
15:45-16:00	<p><b>Daniela Hladik*</b> Neuherberg, Germany <i>"Low-dose radiation exposure leads to irreversible changes in the proteome of the murine hippocampus"</i></p>	<p><b>Kristian Unger</b> Neuherberg, Germany <i>"Prediction models in radiation oncology"</i></p>	<p><b>Yannick Saintigny</b> Caen, France <i>"Multimodal treatments of radio-resistant glioblastoma stem cells: emerging effective tryptic of ... "</i></p>
16:00-16:30	Coffee Break		
	ERRS - Function		
16:30-17:30	<p><b>"Bacq and Alexander Award Lecture", Deichmann Auditorium</b></p> <p><b>Catharine West</b> <i>"Radiobiology biomarkers and how to minimise fake news"</i> Manchester, United Kingdom</p>		
17:30-18:30	<b>"ERRS General Assembly, Deichmann Auditorium"</b>		
18:30-20:00	Get Together with Beer and Snacks		

43rd Annual Meeting of ERRS and 20th Annual Meeting of GBS, Essen, Germany, 2017

\*Denotes young investigators



## Keynote Lecture 5, Deichmann Auditorium

*Chair: R. Coppes*

8:15-9:00

**Thomas Helleday**  
Stockholm, Sweden

*"Novel regulators of IR-induced DNA repair and dNTP generation at DNA damage sites, and implications for cancer treatments"*

## Plenary Session 4, "Clinical Translation of Radiation Biology"

*Chairs: M. Benderitter/F. Meyer*

9:00-9:20

**Penny Jeggo**  
Sussex, United Kingdom

*"Double-strand breaks repair disorders "*

9:20-9:40

**Wolfgang Dörr**  
Vienna, Austria

*"Stem cells for amelioration of radiation-induced oral mucositis: preclinical studies"*

9:40-10:00

**Verena Jendrossek**  
Essen, Germany

*"Adaptation to the adverse tumor environment - towards novel combined treatment strategies"*

10:00-10:20

**Franz Rödel**  
Frankfurt am Main, Germany

*"PD-1/PD-L1, CD8 and FOXP3 expression and human papilloma virus load in patients with anal squamous cell carcinoma treated ..."*

10:20-10:40

**Rob Coppes**  
Groningen, Netherlands

*"Use of organoid cultures in the study of radiation response and regeneration"*

10:40-11:00

## Coffee Break

### S19, "Normal Tissue/Stem Cells Response"

#### Deichmann Auditorium

*Chairs: G. Terzoudi/D. Klein*

11:00-11:15

**Diana Klein**  
Essen, Germany  
*"Radioprotection of normal lung tissue by mesenchymal stem cell therapy"*

11:15-11:30

**Lara Barazzuol**  
Groningen, Netherlands  
*"A coordinated DNA damage response activates adult quiescent neural stem cells in a ..."*

11:30-11:45

**Insa Sigrid Schröder**  
Darmstadt, Germany  
*"Ionizing radiation negatively affects cardiac differentiation of pluripotent stem cells"*

11:45-12:00

**Felix Meyer\***  
Hamburg, Germany  
*"Interplay of DNA repair and stem-like phenotype determines the sensitizing effect of CHK1, RAD51 and PARP1 ..."*

12:00-12:15

**Danuta Galetzka**  
Mainz, Germany  
*"Molecular karyotyping and DNA methylation pattern analysis in primary fibroblasts of patients ..."*

12:15-12:30

**Harry Scherthan**  
Munich, Germany  
*"DNA damage in leukocytes after internal exposure to beta and alpha emitters"*

### S20, "Low Dose Radiation Effects/Countermeasures"

#### Audimax

*Chairs: C. Rios/N. Daems*

**Carmen Rios**  
Rockville, United States  
*"Expediting the drug development process by repurposing licensed products – a NIAID/RNCP viewpoint"*

**Serge Candéas**  
Grenoble, France  
*"Low dose radiation accelerates ageing of the T cell receptor repertoire in mice"*

**Michael Abend**  
Munich, Germany  
*"Pre-exposure gene expression in baboons with and without pancytopenia after radiation exposure"*

**Johanna Mirsch\***  
Darmstadt, Germany  
*"Efficient DSB repair after low irradiation doses requires a critical cellular radical level"*

**Lisa Deloch\***  
Erlangen, Germany  
*"Low-dose irradiation has an impact on bone metabolism and reduces fibroblast-like synoviocytes ..."*

**Vijay Singh**  
Bethesda, United States  
*"Gamma-tocotrienol as a promising radiation countermeasure for acute radiation syndrome: efficacy ..."*

### S21, "Modelling of DNA Damage Responses"

#### IG1, Hörsaal 1

*Chairs: M. Scholz/K. Sasaki*

**Michael Scholz**  
Darmstadt, Germany  
*"Modelling the impact of cell cycle dependent repair mechanisms and repair deficiencies based on ..."*

**Werner Friedland**  
Neuherberg, Germany  
*"Modelling of DNA damage by light ions from radiotherapy-relevant energies down to stopping"*

**Andrea Ottolenghi**  
Pavia, Italy  
*"From track structure to systems biology: How many roads must a man walk down ..."*

**Mario Pietro Carante**  
Pavia, Italy  
*"Full predictions of cell death and chromosome damage along hadron-therapy dose profiles by the ..."*

**Kohei Sasaki\***  
Sapporo, Japan  
*"A simulation study for both of the targeted and untargeted effect on the uniformly irradiated cells"*

**Thomas Friedrich**  
Darmstadt, Germany  
*"Which spatial dimensions of radiation damage interaction are relevant for the high effectiveness ..."*

12:30-13:30

## Closing Ceremony and Farewell





# Keynote Lecture 1

**Sylvia Formenti**

New York, United States

*„Immune effects of radiation therapy“*

# Immune effects of radiation therapy

**Silvia C. Formenti**

*NYP/Weill Cornell Medicine, New York, United States*

Radiation therapy induces both immunogenic and immunosuppressive signals to the tumor and its microenvironment. Preclinical strategies to enhance the formers and/or mitigate the latter have proven the concrete possibility to shift the balance toward a therapeutic success (J Natl Cancer Inst. 2013;105(4):256-265). Preclinical experiments in multiple syngeneic mouse models that mimic the setting of advanced human cancers have demonstrated promise of combining radiotherapy with immunotherapy. The preclinical data has consistently reflected on clinical confirmation. Particularly, when combined with immune checkpoint blockade, radiotherapy has demonstrated to be a powerful adjuvant to immunotherapy (Clin Cancer Res. 2005;11:728-734). Clinical examples of synergy between radiation and immune checkpoint inhibitors have been reported (N Engl J Med. 2012;366(10):925-931; Transl Oncol. 2012;5(6):404-407; Int J Radiat Oncol Biol Phys. 2013;85(2):293-295; Cancer Immunol Res 2013;1(6):365-372) and interim results in our prospective clinical trial confirm this finding (ESTRO proceedings 2017, Abstract #E36-2149). Currently, multiple clinical trials are exploring optimal combinations and scheduling of radiotherapy and immunotherapy. Despite the fact that radiation can enhance responses to immune checkpoint inhibitors, in the majority of patients tumors remain unresponsive, warranting research to identify markers that predict response. A recent study testing radiation with ipilimumab in melanoma suggested that tumor expression of PDL-1 may predict lack of response to radiation and ipilimumab. Conversely, in lung cancer patients treated with radiation and ipilimumab we found high PDL-1 expression among patients achieving durable complete and partial responses, without addition of PD-1 pathway inhibitors (ASTRO Proceedings 2015, abstract #149). In fact, higher expression of immune checkpoints in tumors has been hypothesized as a marker for identifying more immunogenic cancers (Science, 2015, October 9: 207-211). Similarly, pre-treatment mutational load has been found to be associated with responses to immune checkpoint inhibitors (Science, 2015 Apr 3: 124-8). It will be important to determine if radiation can compensate tumors with a low mutational load, by inducing de novo T cell priming to multiple tumor antigens and could, therefore, achieve responses in the absence of pre-existing neoantigens (Science 2015;348(6230):69-74). The overall degree of immune impairment of the patients may also be a critical predictor of response to radiation + immunotherapy. For instance, we found the pretreatment neutrophil/lymphocyte ratio might enable a priori selection of individuals with a propensity to develop abscopal responses to the combination of radiation and GM-CSF (Lancet Oncol. 2015 Jul;16(7):795-803). Strategies at reducing radiation-induced lymphopenia are warranted to assure adequate availability of naïve T cells when radiotherapy is harnessed to convert the tumor into an individualized cancer vaccine. Finally, both dose and fractionation are key at optimizing the combination of radiotherapy with immune-check point blockade (Vanpouille-Box et al, Nature Communications, in press). Overall, while radiation has emerged as a promising partner for immunotherapy and current research is focusing at identifying tumor and patient characteristics to help predict which patients should receive upfront the combination of immunotherapy with radiotherapy instead of immunotherapy alone.

[formenti@med.cornell.edu](mailto:formenti@med.cornell.edu)

A decorative graphic on the left side of the slide, showing a cross-section of a virus particle. It features a spherical, textured outer shell and a smooth, segmented inner core. The graphic is rendered in a light blue, semi-transparent style.

# Keynote Lecture 2

**Mary Helen Barcellos-Hoff**

San Francisco, United States

*„Looking for Achilles’ heel: HPV, TGF- $\beta$ , and DDR“*



# Looking for Achilles' Heel: HPV, TGF- $\beta$ and DDR

**Mary Helen Barcellos-Hoff**

*University of California, San Francisco, USA*

Transforming growth factor beta (TGF $\beta$ ) is an extracellular cytokine that regulates aspects of proliferation, phenotype and differentiation in all cells, and plays an underappreciated role in regulation of the DNA damage response. Our prior studies demonstrate that TGF $\beta$  inhibition synergizes with radiation treatment to improve tumor control in preclinical models of breast, brain and lung cancer. The substantially better prognosis of human papilloma virus (HPV) positive head and neck squamous cell carcinoma in response to treatment suggests that HPV creates an intrinsic molecular vulnerability that is exploited by standard of care therapy. Here we determined that HPV positive cancers are unresponsive to TGF $\beta$  and that loss of TGF $\beta$  signaling compromises DNA damage recognition. These studies identify the mechanism by which impaired TGF $\beta$  signaling in HPV-positive head and neck cancer alter default settings of DNA damage repair pathways, resulting in increased sensitivity to radiation, cis-platinum and PARP inhibitors. Thus, HPV positive head and neck cancer is an 'experiment of nature' that supports the potential for significant benefit of pharmaceutical inhibition of TGF $\beta$  during radiotherapy.

# Plenary Session 1

## „Radiation Exposure and Immune Response“

**Silvia Formenti**

New York, United States

*„TREX1 regulates radiation immunogenicity: clinical implications“*

**Udo Gaipl**

Erlangen, Germany

*„Immune modulatory properties of radiotherapy—rationales for combination with immunotherapy“*

**Gabriele Niedermann**

Freiburg, Germany

*„Preclinical evaluation and imaging of immunoradiotherapy approaches“*

**Conrad Rauber**

Villejuif, France

*„Gut microbiota in radioimmunotherapy“*

**Lisa Sevenich**

Frankfurt am Main, Germany

*„Effects of ionizing radiation on brain metastasis-associated inflammation“*





# TREX1 regulates radiation immunogenicity: clinical implications

**Silvia Formenti**, C. Vanpouille-Box and S. Demaria

*Department of Radiation oncology Weill Cornell Medical College and NewYork  
Presbyterian Hospital, USA*

The issue of dose and fractionation seems to be particularly relevant to abscopal effects (responses at a distant, synchronous, un-irradiated established tumor or metastasis). During immune checkpoint blockade (ICB), inferior abscopal effects occurred after a single 20Gy dose of radiation compared to regimens of 8GyX3 or 6GyX5 fractions (Clin Cancer Res 2009;15:5379-88; Int J Radiat Oncol Biol Phys. 2012; Jul 15;83(4):1306-10). A mechanism underlying the dose dependence of abscopal response was recently elucidated (Nature Communications 2017; Jun 9;8: 15618 ). In mice bearing bilateral TSA murine breast carcinoma when combined with ICB a single dose of 20 or 30Gy achieved comparable in field control to that of a regimen of 8GyX3 fractions, but only the fractionated regimen induced abscopal responses. An interferon type I (IFN-I) gene signature was associated with the 8GyX3 fractions but not with a single dose of 20 or 30 Gy. RT-generated double strands (ds) DNA fragments reach the cytoplasm of irradiated cells where they are “sensed” by the cGAS/STING pathway (cGAS=cyclic GMP-AMP synthase and its adaptor protein STING= stimulator of interferon genes, aka transmembrane protein 173 – TMEM173). cGAS binds cytosolic dsDNA to initiate IFN-I responses upon STING stimulation, resulting in dendritic cell recruitment and cross-priming of effector T-cells, the key steps to convert the tumor into an in situ vaccine. When tested in multiple carcinoma murine and human carcinoma cells as the radiation dose increases, cytosolic dsDNA was found to accumulate to a threshold above which induction of three prime repair exonuclease 1 (Trex1) occurred, an enzyme that degrades cytoplasmic DNA. (J Exp Med 2011; 208:2005-16). When single doses in excess of 10-12 Gy were applied Trex1 induction rapidly degraded cytosolic dsDNA, the substrate for cGAS/STING. As a result, signaling to induce IFN was abrogated, impairing RT-induced abscopal effects. The clinical implications of this findings will be reviewed with focus on strategies to identify biomarkers predictive on radiation immunogenicity during ICB (Clin Can Res. 2017 Jul 27).

# Immune modulatory properties of radiotherapy - rationales for combination with immunotherapy

B. Frey, A. Derer, M. Rückert, M. Hecht, A. Harrer, D. Riegel, L. Deloch, R. Fietkau  
and Udo Gaipl

*Department of Radiation Oncology, Universitätsklinikum Erlangen, Friedrich-Alexander-  
Universität Erlangen-Nürnberg, Erlangen, Germany*

Radiotherapy (RT) is a common treatment for cancer and about 60% of all cancer patients will receive it during their course of illness. RT primarily aims to achieve local tumor control. The induction of DNA damage, tumor cell death and the modulation of the tumor microenvironment are the main effects of ionizing irradiation to reduce tumor masses, but also to modulate the immune system. RT might act as an in situ cancer vaccine under certain microenvironmental conditions. However, RT also fosters the upregulation of immune suppressive molecules such as the programmed cell death receptor ligand 1 (PD-L1, CD274). Ionizing radiation in general has been demonstrated to impact on the immune system and in dependence on the radiation dose particular immune modulations take place. The presentation will focus on how local irradiation changes the tumor cell phenotype and the tumor microenvironment and consecutively does impact on local and systemic changes in immune cell compositions. The dynamics of immune changes, the radiosensitivity of distinct immune cells as well as biological basis for reasonable combination of RT with immune stimulation will be discussed in detail, as well as how radiation-induced immune suppression can be overcome. Regarding the latter, the impact of radiotherapy and chemotherapy on PD-L1 and PD-L2 expression of melanoma and glioblastoma cells will be outlined and possible mechanisms that contribute to increased expression of these immune checkpoint molecules on tumor cell surfaces will be discussed. Based on the pre-clinical knowledge, innovative clinical study concepts of radio-immune treatments will be presented. We conclude that knowledge on immune modulations induced by ionizing radiation is important to optimize multimodal cancer therapies aiming to achieve local and systemic tumor control and to define immune-related biomarkers of radiation exposure for prognosis and prediction.

**Acknowledgements:** This work has been supported by the research training group GRK1660 of the German Research Foundation (DFG), by the German Federal Ministry of Education and Research (BMBF; GREWIS, 02NUK017G), and by the European Commission [EU; OPERRA, 604984, VIBRATO and DoReMi, under Grant FP7-249689].



# "Preclinical evaluation and imaging of immunoradiotherapy approaches"

**Gabriele Niedermann**

*Dept. of Radiation Oncology, University Clinics Freiburg, Germany*

Recent research has shown that certain forms of radiation therapy can induce tumor-specific T cells that contribute to local tumor control and also mediate systemic antitumor effects. Combination with classical T cell-directed immunotherapy can considerably enhance these effects. Preclinical experiments in tumor-bearing mice are important in the development of rational immuno/radiotherapy combinations. We study local and systemic effects (tumor control and tumor-specific T cell responses) of various immunotherapeutics (e.g., immune checkpoint inhibitors, T cell-recruiting antibodies, T cell-stimulating cytokines) in combination with immunogenic forms of tumor radiotherapy. We visualize the immunotherapeutics and their corresponding bound receptors by non-invasive imaging, e.g. positron emission tomography, in order to understand the mechanisms of action of combined immuno/radiotherapies. In addition, we use non-invasive imaging approaches to visualize the side effects of these combination therapies. In my talk, I will focus on the evaluation of combinations of hypofractionated tumor irradiation with PD-1 and CTLA-4 checkpoint blockers and with IL-2/anti-IL-2 complexes recently conducted by my group.

# Gut microbiota in radioimmunotherapy

**Conrad Rauber**, B. Routy, R. Daillère, P. Roberti, M. Vétizou, N. Waldschmitt, M. Chamailard, V. Cattoir, I. G. Boneca, B. Escudier, G. Zalcman and L. Zitvogel

*Institut de Cancérologie Gustave Roussy Cancer Campus (GRCC), Villejuif, France*

The tumor microenvironment is influenced by anticancer therapies, and even more so by those affecting the gut homeostasis. We reported that a deviated repertoire of the intestinal microbiome called « dysbiosis », caused by broad spectrum antibiotics compromised the efficacy of cyclophosphamide (CTX), an immunomodulatory alkylating agent exerting cytotoxic effects against cancer (Viaud, Science, 2013). Lately, we reported the importance of the gut microbiota in the efficacy of ipilimumab, a human monoclonal antibody targeting CTLA-4 (Vetizou, Science, 2015). Mechanisms underlying this gut-cancer axis in these two therapeutic contexts are different. CTX is responsible for disrupting the gut barrier integrity as well as intestinal homeostasis, allowing a NOD1/2-dependent translocation of several Gram-positive bacteria into secondary lymphoid organs. CTX breaks the intestinal tolerance towards the intestinal microbiota and leads to immunization of the host against some bacterial strains. We identified a Gram positive bacteria, *Enterococcus hirae*, which markedly modulates the intestinal and systemic immunity through the elicitation of bacterial-specific Th1 and pathogenic Th17 cells. Moreover, we have shown that *E. hirae* is capable of enhancing tumor-specific CD4+ and CD8+ T cell responses against candidate tumor antigens. Finally, *E. hirae* specific-memory Th1 immune responses selectively predicted longer progression-free survival in advanced lung or ovarian cancer patients treated with immunotherapy and chemotherapy (platinum salts- or CTX-based chemotherapy). Altogether, *E. hirae* represents a valuable probiotic against cancer, an oncomicrobiotic ameliorating the efficacy of the most common alkylating immunomodulatory compound. These findings have been extended to PD1/PDL-1 blockade, where we will show that antibiotics affect the survival of patients and where other oncomicrobiotics have been discovered in the context of PD1 blockade in lung and kidney cancers.

# Effects of Ionizing Radiation on Brain Metastasis-Associated Inflammation

M. Schulz, K. Niesel, A. Salamero-Boix, C. Woon Hyung, F. Rödel, E. Fokas, P. Harter, U. Pilatus and **Lisa Sevenich**

*Georg-Speyer-Haus, Frankfurt, Germany*

Brain metastases are the most common intracranial tumor in adults and diagnosis is associated with the shortest survival time compared to other sites of metastatic relapse with median survival times of only a few months. Tumor-associated inflammation is increasingly recognized as a critical regulator of primary tumor growth, metastatic dissemination and therapeutic response. Ionizing radiation (IR) remains one of the mainstays in the treatment of brain metastasis patients. In addition to direct effects of radiotherapy on cancer cells, it is known that therapeutic intervention with IR also affects tumor-associated stromal cells leading to increased recruitment, differential gene expression and altered effector functions in different tumor types. However, it is less well understood how IR affects inflammation in brain metastasis. The brain is a unique environment composed of highly specialized brain resident cells with restricted entry of immune and inflammatory cells from the periphery under physiological conditions. Thus the brain represents a particularly interesting tissue with respect to tumor- and therapy-induced inflammation. We use several experimental brain metastasis models to study the effects of IR on brain metastasis-associated inflammation. We found pronounced changes in the cellular composition of the tumor microenvironment together with altered gene expression signatures in brain-resident and recruited stromal cells as well as metabolic changes. Our data indicate that IR triggers inflammatory responses that can lead to the establishment of a cancer-permissive microenvironment in the brain. Interestingly, IR had different effects on cancer-associated stromal cells in brain metastasis derived from different primary tumor types. Our data emphasize the need for detailed understanding of the mechanisms that drive brain metastasis as well as the tumor type-dependent radio-response to establish novel therapeutic avenues for targeted- or immune therapies in combination with radiotherapy to maintain or induce anti-tumor responses against brain metastasis





# Session S01

## „Immune Response“

### **Ruth Muschel**

Oxford, United Kingdom

*„FGF2 is a Switch Controlling the Tumor Response to Radiation Through Macrophage Polarization“*

### **Benjamin Frey**

Erlangen, Germany

*„Pre-clinical and Clinical Hints for Immune Modulation by Low Doses of Ionizing Radiation“*

### **Amir Abdollahi**

Heidelberg, Germany

*„Radiation Induced Lung Fibrosis - a dynamic interplay between immune response, angiogenesis modulators and mesenchymal transition governing tissue remodeling“*

### **Ludwig Dubois**

Maastricht, Netherlands

*„Radiotherapy and immunocytokines: a perfect match for abscopal effects with long-lasting memory?“*

### **Roman Hennel**

Munich, Germany

*„Immunostimulatory effects induced by radiotherapy of breast cancer cells“*

### **Veronica Olivo Pimentel**

Maastricht, Netherlands

*„Radiotherapy causes long-lasting antitumor immuno-logical memory when combined with immunotherapy“*





# FGF2 is a Switch Controlling the Tumor Response to Radiation Through Macrophage Polarization

**Ruth Muschel**, J. Hong Im, K. Jones, J. Buzzelli, J. Kim and Y. Cao

*University of Oxford, Oxford, United Kingdom*

Irradiation of tumors can enhance anti-tumor immune responses. Myeloid cells are recruited to tumors by radiation. We found that macrophages recruited to radiated tumors in murine models expressed Fibroblast Growth Factor 2 (FGF2 or basic FGF), a potent angiogenic and fibroblast proliferative factor. We asked whether the FGF2 affected the tumor response to radiation. Administration of a high affinity, blocking antibody for FGF2 (GAL-F2) had no effect on the growth of unirradiated tumors. In contrast, GAL-F2 given after tumor irradiation led to either tumor cure (7/10) or prolonged growth delay (120-200 days) compare to irradiated control tumors (60 days)  $p < 0.05$ . The response to GAL-F2 correlated with the amount of FGF2 induced by radiation. To determine potential mechanisms for the anti-tumor effect, we examined the cellular infiltrates in the irradiated tumors. As expected, radiation of tumors recruited macrophages. Macrophages have different phenotypes, spanning a range from M1, a more inflammatory state to M2 the more protumoral. More macrophages from the irradiated tumors (compared to naïve macrophages or those from unirradiated tumors) had markers for protumoral M2 polarization. Blocking FGF2 with antibody in mice with irradiated tumors reduced macrophage recruitment overall and greatly decreased the proportion with markers for M2 polarization. To ask more directly whether FGF2 affected macrophage polarization, we turned to genetically altered mice unable to express low molecular weight FGF2, the secreted form of FGF2. Tumoral macrophages in these mice were enhanced in M1 inflammatory markers, but reduced in M2 markers, analogous to the changes in macrophages from mice with irradiated tumors after blocking FGF2. Tumors grew more slowly in the mice lacking low molecular weight FGF2, an effect both dependent and independent of a T cell immune response. In summary we have shown that radiation triggers the production of FGF2 in tumor macrophages. FGF2 by enhancing the M2 polarization of the tumor associated macrophages facilitates tumor growth in irradiated tumors. Blocking FGF2 reverses the M2 polarization and enhances the tumor response to radiation. These data suggest a potential strategy to improve outcomes after radiation by using an FGF2 blocking antibody.

# Pre-clinical and clinical hints for immune modulation by low doses of ionizing radiation

**Benjamin Frey**, L. Deloch, A. Harrer, P.-F. Rühle, O. Ott, T. Gryc, F. Rödel, C. Fournier, R. Fietkau and U. Gaipl

*Department of Radiation Oncology, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany*

Knowledge about effects of low doses of ionizing radiation on the organisms are not just since the Fukushima accident of great importance. In addition, low-dose radiation therapy (LDRT) has successfully been applied for the treatment of chronic inflammatory and painful degenerative diseases for decades. Therefore, research dealing with immune effects of low and moderate doses of ionizing radiation is growing. It has become evident, that also low doses of radiation are able to mediate systemic effects. Especially locally applied LDRT was shown to result in significantly reduced pain and enhanced mobility in patients, but the related immune mechanisms have been just fragmentarily analyzed. With diverse pre-clinical analyses, we identified activated macrophages as key target of LDRT. These innate immune cells which are key players in initiation and resolution of inflammation kept their functionality up to an irradiation with 2 Gy, but the secretion of cytokines was significantly altered by irradiation: In particular after irradiation of activated macrophages with a single dose of 0.5Gy significant reduced amounts of the inflammatory cytokine IL-1 $\beta$  and significant enhanced amounts of the anti-inflammatory cytokine TGF- $\beta$  were found in the macrophage supernatants. Further, irradiation with 0.5 Gy also induced apoptosis in fibroblast-like synoviocytes (FLS). The latter are in close proximity with activated macrophages in the inflamed joint. In the pre-clinical model system for rheumatoid arthritis (RA), the hTNF $\alpha$  tg mice, we observed that local irradiation of inflamed joints also impacted on bone homeostasis. Ex vivo, an increased mineralization of bone arranging osteoblasts was seen concomitant with enhanced apoptosis of bone resorbing osteoblasts after irradiation with 0.5Gy. No disadvantages of LDRT were observed in the non-inflamed, healthy system. We suggest LDRT as an additive treatment for chronic degenerative diseases (e.g. RA), since it impacts on bone homeostasis and inflammation. We initiated the IMMO-LDRT01 (NCT02653079) study aiming for the first time the analyses of the effects of locally applied low-dose RT on the peripheral immune system in a longitudinal section. We detected in a first round of patients a dynamic modulation of both, the innate and adaptive immune system by LDRT. After having completed the analyses of more patients, immune biomarkers for prognosis and prediction to further individually optimize LDRT will be obtained. The gained knowledge on the dynamic systemic immune modulation by low doses of ionising radiation might also be of value for radiation protection issues. In order to gain also more insight in immune processes guided by very low doses of irradiation, we also initiated the RAD-ON01 study. The latter demonstrated that distinct modulations of the peripheral immune system after radon spa therapy take place. Immune parameters correlated with pain reduction on the patients following radon spa and very low doses of ionizing radiation. We conclude that locally applied LDRT as well as radon spa result in dynamic modulations of the innate and adaptive immune system. We created a data pool for identification of immune biomarkers of radiation exposure. This can be used for optimizing LDRT (personalized medicine) and for monitoring of persons involuntary exposed to radiation (radiation protection issues).

**Support:** This work was supported by by the German Federal Ministry of Education and Research (BMBF; GREWIS, 02NUK017G), and by the European Commission [EU; OPERRA, 604984, VIBRATO and DoReMi, under Grant FP7-249689].

# Radiation induced lung fibrosis - a dynamic interplay between immune response, angiogenesis modulators and mesenchymal transition governing tissue remodeling

C. Zhou, M. Moustafa, J. Debus and **Amir Abdollahi**

*Division of Molecular and Translational Radiation Oncology, HIRO-NCRO, Heidelberg University and DKFZ, Heidelberg, Germany*

I will present data on systematic dose-response characterization of the radiation induced lung fibrosis (RILF) in C57Bl6 mouse model. These will include single- and fractionated whole thoracic irradiation series. A novel 3D-CT segmentation based fibrosis Index model is reported developed by Dr. Cheng Zhou in my lab. The FI formed the basis for detailed multi-scale characterization of the mechanisms governing RILF. Temporal dynamic and involvement of different immune response pathways and their implication are discussed. The impact of endogenous modulators of angiogenesis, the vascular integrity and the epithelial/endothelial to mesenchymal transition are highlighted. Finally, potential targets for reversal of these processes that were experimentally validated are presented towards development of next-generation mitigators of RILF.

# Radiotherapy and immunocytokines: a perfect match for abscopal effects with long-lasting memory

**Ludwig Dubois**, VO. Pimentel, D. Marcus, N. Rekers, N. Lieuwes, R. Biemans, D. Neri, J. Theys, A. Yaromina and P. Lambin

*Department of Radiation Oncology (MAASTRO), GROW - School for Oncology and Developmental Biology, Maastricht University Medical Centre, Netherlands*

Although radiotherapy (RT) is one of the major cancer treatment modalities to kill malignant cells, advanced-stage disease is often hard to control. Radiation-induced tumor cell death provides a plethora of pro-immunogenic effectors associated with inflammation. Although the potential for RT to generate anti-tumor immunity is apparent, the evidence that it does so in the clinical situation is limited. Stimulation of the immune system to assist in eliminating cancer cells within, and outside, the radiation field could be beneficial in advanced-stage or metastasized disease. Recently, it has been demonstrated that cancer patients have increased levels of cytotoxic T-cells after radiotherapy, making them eligible for immunotherapy. Interleukin-2 (IL2) is one of the essential cytokines for driving proliferation and differentiation of T-cells and NK-cells resulting in increased cytotoxic activity, eventually leading to tumor regression. Several studies have shown synergistic anti-tumor effects when combining RT with systemic IL2 administration, although this strategy was accompanied with severe adverse effects. Monoclonal antibodies, such as the human recombinant scFv fragment L19, have been designed as “targeting vehicle” for the selective delivery of immune-stimulatory cytokines to the tumor microenvironment while sparing normal tissue. The L19 antibody selectively localizes at the tumor neovascular fibronectin extra-domain B (EDB) positive sites following systemic administration and can serve as delivery vehicle for IL2. Our lab has combined single high-dose RT with systemic L19-IL2 administration in a number of murine xenograft models and found outstanding, long-lasting complete response rates mediated by cytotoxic CD8<sup>+</sup> T-cell (1,2) or NK-cell (3) activity depending on the tumor model. L19-IL2 is thus an immunocytokine with strong immune response enhancing properties in EDB-positive tumors. The combination therapy resulted also in anti-tumor immune effects outside the radiation field, an effect associated with CD4<sup>+</sup> T-cell response. Growth of secondary un-irradiated tumors was significantly delayed with even 20% cure. Similar results were found when irradiation was delivered in a fractionated manner, although without resulting in cures. An increased PD-1 expression on T-cells infiltrating these tumors suggests a more regulatory immunological phenotype after fractionated radiotherapy compared with a single high RT dose. Re-challenging cured animals with tumor cells did not result in tumor formation, associated with high CD127 expression. Our recent data show that radiotherapy combined with the immunocytokine L19-IL2 results in long-lasting complete response rates, also outside the radiation field (abscopal effect) and this effect is associated with a memory potential. (1) Zegers et al, Clin Cancer Research 2015, 21(5):1151-60 (2) Rekers et al, Oncoimmunol 2015, 4(8):e1021541 (3) Rekers et al, Radiother Oncol 2015, 116(3):438-42



# Immunostimulatory effects induced by radiotherapy of breast cancer cells

J. Krombach, **Roman Hennel**, N. Brix, M. Orth, G. Zuchtriegel, T. Vogl, C. Belka and K. Lauber

*Department of Radiation Oncology, Klinikum der Universität Muenchen, Germany*

**Purpose:** Radiotherapy is a cornerstone for the treatment of breast cancer. In addition to the standard fractionated regimens, ablative irradiation in form of intra-operative radiation therapy (IORT) is applied in breast cancer treatment. Thereby, high single (ablative) doses up to 20 Gy are administered into the former tumor bed directly after tumor resection. Mechanistically, the curative effect of ionizing radiation is considered to derive from the induction of tumor cell death by direct and indirect DNA-damage. Besides these cytotoxic effects, accumulating evidence supports a contribution of the immune system to local and systemic tumor control - particularly in the context of ablative radiotherapy. In the present study, we show that different radiotherapeutic treatment schedules vary in terms of their immunological consequences. **Methods:** Human HCC1937 breast cancer cells were subjected to fractionated (daily 2Gy) and ablative (single dose of 20Gy) radiotherapeutic regimens. Cell death induction, dendritic cell differentiation and T cell proliferation were analyzed by flow cytometry. Release of danger-associated molecular patterns (DAMPs) was measured by ELISA in cell-free culture supernatants. Endothelial cell activation was determined by quantitative fluorescence staining, qRT-PCR analyses, and multiplex cytokine arrays. The recruitment of monocytes was studied in vitro via trans-well migration assays and in vivo by the air pouch model. **Results:** Our data show that primary necrosis was induced in HCC1937 breast cancer cells especially after ablative irradiation. The cell-free culture supernatants of irradiated, necrotic HCC1937 cells attracted primary human monocytes in vitro and stimulated recruitment of different subsets of monocytic cells in the air pouch model. These included inflammatory monocytes, macrophages, and dendritic cells. Endothelial cells were activated after exposure to the cell-free supernatants of irradiated, necrotic HCC1937 cells. This activation was characterized by the upregulation of components critical for immune cell recruitment (adhesion molecules and cytokines) on the mRNA as well as on the protein level. Likewise, these supernatants stimulated enhanced expression of costimulatory molecules on dendritic cells, which translated into improved T cell priming. Classical DAMPs, such as HSP70, HMGB1, and S100A8/9, were found in high concentrations in the supernatants of ablatively irradiated HCC1937 cells, and currently their functional relevance is being investigated. **Conclusion:** Ablative irradiation of breast cancer cells exerts its immunostimulatory effect by induction of a necrotic form of cell death. DAMPs are released, which activate both endothelial and dendritic cells, resulting in leucocyte recruitment in vivo. Thus, necessary steps for the stimulation of an adaptive anti-tumor immune response could be observed after ablative irradiation, and further studies have to clarify to which extent this contributes to the therapeutic outcome.

[roman.hennel@med.uni-muenchen.de](mailto:roman.hennel@med.uni-muenchen.de)

# Radiotherapy causes long-lasting antitumor immunological memory when combined with immunotherapy

**Veronica Olivo Pimentel**, NH. Rekers, A. Yaromina, NG. Lieuwes, R., Biemans, WTV. Germeraad, EJ. van Limbergen, D. Neri, LJ. Dubois and P. Lambin

*Department of Radiation Oncology (MAASTRO), Maastricht University Medical Centre, The Netherlands*

**Introduction** Radiotherapy (RT) is one of the current standard of care treatments for cancer. It induces DNA damage lethal to cancer cells, but also immunogenic cell death by releasing tumor associated-antigens and damage-associated molecular patterns, thus enhancing immunity against cancer. Within our group, we have shown that the addition of the immunocytokine L19-IL2 to RT (single dose 10Gy) resulted in 75% of tumor remission in a heterotopic C51 murine colon carcinoma model. The immune system has the capacity of conferring protective immunity over a long period of time; the memory potential. We therefore investigated whether this combination therapy also resulted in a treatment-specific long-lasting immunological memory dependent on the interleukin 7 receptor (IL7R) – IL7 axis. **Material and methods** Balb/c mice were challenged with C51 tumor cells unilaterally and received different therapeutic schedules: 10Gy + L19-IL2, surgery + L19-IL2 or high single dose RT (40Gy) + vehicle. L19-IL2 was administered i.v (20 µg) 1, 3 and 5 days after irradiation or surgery. Control groups were mice without tumors receiving 10Gy on healthy tissue +L19-IL2 or not receiving any treatment (sham + vehicle). Mice who were cured after treatment were rechallenged with C51 tumor cells bilaterally after 150 days and tumor take was assessed. To investigate the role of the IL7R-IL7 axis, an in vivo blocking study of the IL7R (CD127) was performed in cured mice treated with 10Gy+L19-IL2. These mice were injected intraperitoneally with anti-CD127 (400 µg) or IgG isotype control one day before tumor rechallenge and every 48 hours for 3 weeks. Blocking capacity of the antibody was confirmed in peripheral blood by flow cytometry. The presence of central memory and effector memory T cells was assessed in peripheral lymphoid organs and blood. **Results** After rechallenge, 8 out of 12 mice did not show tumor take after the initial 10Gy + L19-IL2 and blockade of CD127 had no influence on this protective effect. Mice cured after surgery + L19-IL2 or high dose RT + vehicle showed significant ( $p<0.05$ ) less protective effects. Furthermore, control groups were all able to form tumours within 13 days. Immunological endpoint analyses revealed that mice able to reject tumour cells upon rechallenge have a significant high expression of CD44+CD127+ on CD8+ T in lymph nodes, spleens and blood. Moreover, mice able to reject tumour cells have a significantly high expression of central memory CD44+CD62L+ (7.3% [5.7-9.8] vs 2.0% [1.0-4.0],  $p= 0.01$ ) and effector memory CD44+CD62L- (36.3%, [34.5-42.8] vs 28.4% [17.7-40.3],  $p=0.01$ ) CD8+ T cells compared to other treatment groups. **Discussion** To obtain long-lasting protection associated with central and effector memory T cells against C51 tumours, prior tumor cure by the combination of radiotherapy and the immunocytokine L19-IL2 is the main requirement. Our data suggest that the IL7R-IL7 axis is associated with long-lasting anti-tumour responses but is not causative.



# Session S02

## „DNA Damage Response 1“

**Beate Volkmer**

Buxtehude, Germany

*„Epigenetic alterations in skin cancer tissue and UV-exposed skin“*

**Stephanie Hehlhans**

Frankfurt am Main, Germany

*„Nek1 depletion modulates apoptosis, DNA repair and radiation survival of 3D-cultured colorectal and cervix carcinoma cells“*

**Aashish Soni**

Essen, Germany

*„Increased DSB end resection causes enhanced formation of chromosomal translocations through Parp-1 dependent alt-EJ“*

**Klaudia Al-Refae**

Essen, Germany

*„Impact of Akt1 phospho-mutants on the cellular response to ionizing radiation“*

**Aadhya Tiwari**

Tuebingen, Germany

*„Y-box binding protein-1 stimulates repair of ionizing radiation-induced DNA double strand breaks in K-RAS(G13D)-mutated breast cancer cells“*

**Jagdish Paithankar**

Mangalore, India

*„Variations in radiation tolerance in life stages of Drosophila melanogaster provide clues to radiation tolerance mechanisms“*



# Epigenetic alterations in skin cancer tissue and UV-exposed skin

**Beate Volkmer**, I.-P. Chen and R. Greinert

*Elbe Kliniken, Stade/Buxtehude, the Netherlands*

Cutaneous Squamous Cell Carcinoma (cSCC) comprises the second frequent skin cancer worldwide. In 2013 in Germany about 41.000 cases have been diagnosed. The incidence is increasing since decades. Chronic sun exposure and exposure to artificial UV (e.g. in sunbeds) have been shown to be the main risk factors for induction and development of cSCC. Under certain conditions cSCC tends to metastasize at a low rate. However recent findings seem to show that occurrence of metastasized cSCC has been underestimated in the past. This might have important health consequences because metastasized cSCC have a very poor prognosis for the patient. We have been interested whether UV-radiation (UVA, UVB) or natural solar UV-exposure is involved in molecular steps promoting metastasis in cSCC. We concentrated, especially, on epigenetic modifications with a focus on differential miRNA expression. We had been able to show already, that UVA- and UVB-irradiation is able to induce UVA- and UVB-dependent differential expression in human skin keratinocytes. Now, we investigated the miRNA-expression patterns in SCC cell lines of different differentiation status and in cell lines of metastasized cSCC. Furthermore we compared differential miRNA expression in sun-exposed and unexposed human skin tissue and tissues from cSCC tumors. Differential miRNA expression was measured in a flow cytometry assay which is able to measure miRNA expression in up to 60 miRNAs at the same time (FireFly, AbCam). Hierarchical clustering of differential miRNA expression patterns show that sun-exposed and unexposed human skin tissue can be discriminated from cSCC tumor tissue by a group of about 20 miRNA (including miR-2013a-3p, a classical differentiation marker in human skin). Our analysis furthermore shows that different cSCC cell lines could be discriminated from cell lines derived from cSCC metastasis by the same set of miRNAs. In-silico analysis of gene targets for the miRNAs involved, identified a couple of important genes (e.g. p63, PTEN, FOXO3a, and others) which play key roles in skin cancer development and progression. Our data show that UV-dependent changes in miRNA expression levels are involved in skin cancer (cSCC) induction as well as in pathways involved in progression to metastasis. Further characterizing the special value of certain miRNAs in our group of miRNAs will help to detect biomarkers, on the miRNA level, for skin cancer risk and disease progression.

# Nek1 depletion modulates apoptosis, DNA repair and radiation survival of 3D-cultured colorectal and cervix carcinoma cells

**Stephanie Hehlhans**, T. Essary, J. Oppermann, F. Weipert, M. Löbrich, C. Rödel and F. Rödel

*Department of Radiotherapy and Oncology, Goethe-University, Frankfurt am Main, Germany*

**Introduction:** The Nek protein family member Never-in-mitosis A-related kinase 1 (Nek1) is involved in regulation of apoptosis, cell cycle and DNA repair. A recent study revealed Nek1 to phosphorylate Rad54 in late G2 phase thereby promoting the removal of Rad51 from the DNA and completion of DNA double-strand break repair by homologous recombination. In the present study, we investigated the effect of Nek1 depletion on radiation survival, DNA repair, cell cycle distribution and apoptosis in 3D grown colorectal and cervix carcinoma cells. **Methods:** Nek1 depletion in HCT-15 and SW480 colorectal cancer cells was accomplished by siRNA transfection while HeLa cells, stably transfected with a Doxycyclin-inducible shNek1 construct or a non-silencing control sequence (shCtrl), were treated with Doxycyclin for 5 days to induce shRNA expression. Subsequently, Nek1 knockdown and control cells were embedded in 0.5 mg/ml basement membrane extract (BME, Cultrex), irradiated 24 h thereafter (0 – 6 Gy, single dose) and analyzed for viability (MTT assay), cell cycle distribution, apoptosis induction (SubG1 phase, AnnexinV/7AAD staining, Caspase 3/7 activity), DNA double-strand break repair (gammaH2AX foci assay) and clonogenic radiation survival. **Results:** Nek1 depletion in combination with irradiation resulted in reduced viability, an increased number of Annexin V-positive and SubG1 phase cells and enhanced Caspase 3/7 activity compared to controls, while distribution of cells in G1, S or G2 phase was not modulated by Nek1. Moreover, Nek1 knockdown significantly enhanced the number of radiation-induced residual gammaH2AX foci and radiosensitized 3D-cultured cell lines. **Conclusion:** Our data strengthen the notion of Nek1 as a radiation resistance factor and potential molecular target for radiotherapy of colorectal and cervix carcinoma. Further in vivo studies will reveal the feasibility of such an approach for the treatment of cancer patients. **Acknowledgement:** This study was supported by the German Federal Ministry of Education and Research (BMBF; GREWIS: 02NUK017F) and the German Research Foundation (DFG Graduate school 1657).

# Increased DSB end resection causes enhanced formation of chromosomal translocations through Parp-1 dependent alt-EJ

**Aashish Soni** and G. Iliakis

*Institute of Medical Radiation Biology, University of Duisburg-Essen, Medical School,  
Essen, Germany*

DNA double strand breaks (DSBs) are the most deleterious among the lesions induced by ionizing radiation (IR). Prompt repair of DSBs is crucial for the overall fitness of a cell. Thus, various DSB repair pathways have evolved. c-NHEJ and HRR are the two main repair pathways, which if they fail to function properly are backed-up by error-prone alternative end joining (alt-EJ). DNA DSB end-resection is central to DSB repair pathway choice as cells move through replicative to post-replicative phases of the cell cycle. DSB end resection is required for error free HRR, but when this pathway fails to engage successfully it also enhances alt-EJ. DSB repair by alt-EJ can lead to the formation of chromosomal translocations, which are considered a hallmark of cancer. The mechanisms of shunting of a resected DSB towards error-free HRR or error-prone alt-EJ are crucial to pathway choice and require elucidation. In the present study we investigate the degree of DSB end resection as a contributing factor to translocation formation in repair proficient, as well as in mutants compromised in different DSB repair pathways. We investigate specifically cells irradiated in late S or G2 phase of the cell cycle, where all DSB repair pathways are functional. We will show that loss of control over resection at a DSB end leading to hyper-resection increases chromosomal translocation formation. Inhibition of resection in these mutants using small molecule inhibitors causes a marked decrease in translocation formation. Notably, translocations forming as a result of hyperresection are sensitive to Parp-1 inhibition, suggesting the involvement of alt-EJ in their formation. Acknowledgements: Supported by grants from BMBF & the DFG.



# Impact of Akt1 phospho-mutants on the cellular response to ionizing radiation

**Klaudia Al-Refae**, S. Oeck, G. Iliakis and V. Jendrossek

*Institute of Cell Biology, University Hospital Essen, Germany*

The survival kinase Akt participates in the regulation of essential subcellular processes, e.g. proliferation, growth, survival and apoptosis and is aberrantly activated in various human solid tumors. Akt has a documented role in promoting resistance against genotoxic stress including radiotherapy presumably by influencing the DNA damage response, DNA repair or both. We recently demonstrated that Akt1 is a direct target of DNA-PKcs-mediated phosphorylation in the presence of damaged DNA. Furthermore, to study the role of active Akt1 in the cellular radiation response and DNA repair we established murine prostate cancer cells (TRAMPC1) stably overexpressing various human Akt1-mutants including the clinically relevant Akt1-E17K mutant with increased membrane recruitment and activating phosphorylation at Threonine308 (T308) and Serine (S473), the phosphomimicking mutant Akt1-T308D/S473D as well as T308A, -S473A and T308AS473A phosphoablating mutants. We showed that overexpression of the dominant active Akt1-E17K mutant and the constitutively active phosphomimicking mutant Akt1-T308D/S473D significantly improved DNA repair and cell survival upon ionizing radiation (IR) whereas overexpression of Akt1-WT was without effect (Oeck et al., Sci Rep 2017 Feb 17;7:42700). Our novel data reveal that only expression of human Akt1-T308AS473A but not of the single phosphoablating mutants induces a significant delay in the kinetics of DNA repair in irradiated TRAMPC1 cells compared to Akt1-WT expressing TRAMPC1 cells as determined by the  $\gamma$ H2A.X-assay. However, this delay was not sufficient to cause a more pronounced reduction in long-term survival after irradiation compared to Akt1-WT expressing cells. Currently we explore potential differences in the ability of the various Akt1 phosphomutants to enter the nucleus and to interact with assumed nuclear Akt1 target proteins in cells before and after irradiation.

# Y-box binding protein-1 stimulates repair of ionizing radiation-induced DNA double strand breaks in K-RAS (G13D)-mutated breast cancer cells

**Aadhya Tiwari**, H. P. Rodemann and M. Toulany

*Division of Radiobiology and Molecular Environmental Research, Department of Radiation Oncology, Eberhard Karls University Tuebingen, Tuebingen, Germany*

**Introduction:** The Y-box binding protein-1 (YB-1) regulates majority of cancer hallmarks. For its functions, YB-1 needs to be phosphorylated at serine 102 (Ser-102), partially through the K-RAS/MAPK/ERK pathway, downstream to EGFR. K-RAS, in addition to activating MAPK/ERK pathway, upregulates the PI3K/Akt pathway. Here, we analyzed role of the K-RAS regulated signaling pathways on YB-1 phosphorylation in K-RAS wild-type (K-RASwt) and K-RAS(G13D)-mutated breast cancer cells. Likewise, the function of YB-1 in DNA double strand breaks (DSBs) repair and post-irradiation cell survival was investigated. **Methods:** Pharmacological inhibitors, blocking peptide, siRNA, shRNA and plasmid based overexpression approaches were used to study signaling pathways involved in YB-1 phosphorylation, the specific role of YB-1 in cell proliferation and effect of YB-1 on DSBs repair as well as on post-irradiation cell survival. Phosphorylation status of YB-1 in breast normal and tumor tissues from breast cancer patients was analyzed by immunofluorescence staining. **Results:** Irradiation and treatment with EGF stimulated YB-1 phosphorylation in K-RASwt breast cancer cells up to 24 hours after stimulation. K-RAS(G13D)-mutated cells presented a constitutively high level of phospho-YB-1 that was independent of irradiation and the EGF treatments. The constitutively high phosphorylation of YB-1 in K-RAS(G13D) cells occurred predominantly through the MEK/ERK pathway and was partially dependent on PI3K but independent of the activation and expression of the Akt1, Akt2 and Akt3 isoforms. Compared to single targeting of PI3K or MEK, dual targeting of both kinases synergistically inhibited YB-1 phosphorylation and attenuated expression of EGFR in K-RAS(G13D)-mutated cells. Similar to K-RAS-siRNA, both YB-1-siRNA as well as YB-1 blocking peptide impaired repair of radiation-induced DSBs. Thus, tumor cells transfected with YB-1-siRNA showed an increased radiation sensitivity. Moreover, tumor tissue from breast cancer patients presented an enhanced phosphorylation of ERK1/2 and YB-1 in association with over-expression of EGFR. **Conclusion:** In this study we demonstrated for the first time that MEK/ERK signaling is the predominant pathway by which K-RAS (G13D) mutation leads to constitutive phosphorylation of YB-1. Moreover P-YB-1 is involved in the repair of irradiation induced DNA-DSBs. Thus, we suggest YB-1 as a potential target in combination with radiotherapy to improve radiation response in breast cancer. **Funding:** This work is supported by Deutsche Forschungsgemeinschaft (DFG TO 685/2-1).

# Variations in radiation tolerance in life stages of *Drosophila melanogaster* provide clues to radiation tolerance mechanisms

**Jagdish G. Paithankar**, S. V Raghu and R. K Patil

*Department of Applied Zoology, Mangalore University, Mangalore, Karnataka state, India*

Humans are among the most radio-sensitive organisms which are a matter of concern. Discovery of high radiation resistance in some organisms have raised questions as to how high radiation resistance is achieved. Compared to the vertebrate, the invertebrates have high levels of radiation resistance. Insects were reported to be having high radiation resistance. The fruit fly *Drosophila melanogaster* is one such insect, adults of which are largely post-mitotic and have high levels of radiation resistance. *Drosophila* is emerging as a suitable model for radiation research due to a) It exhibits relatively high levels of radiation resistance, b) It provides genetic approaches to address physiological questions and c) It has different life stages during its life cycle, which keeps some parameters constant, while others vary. We have studied radiation tolerance in all life stages of *D. melanogaster*. From our study, we suggest that early pupae have high rates of cell division, which makes the organism radiation-sensitive at this stage. The Non-feeding 3rd instar larvae found highly radio-tolerant. To understand the mechanisms of radiation tolerance, the level of different antioxidants were tested and it is found to vary at different developmental stages. Some antioxidants did not correlate with the changes in radiation tolerance. Trehalose is a non-reducing sugar; known for vital role in guarding proteome. Trehalose levels found to vary in all the developmental stage. The protein carbonyl content varies in all life stages and it was highest in the early pupal stage. The trehalose level found lowest during the early pupal stage. Our experiments suggest that the high cell division rates at the pupal stage increase metabolism, DNA and protein synthesis, and the fall in trehalose level induces a higher rate of protein carbonylation. On the other hand, the NFTI larvae known for high radiation tolerance; showed high trehalose and low PC. Thus the organism exhibits highest and lowest sensitivity to radiation at early pupal and NFTI larval stage respectively. Thus, trehalose appears to be a key contributor in regulating radiation tolerance. The levels of catalase, GSH, GPx increased during the early pupal stage, whereas SOD and GST levels found decreased.



## Session S03

### „Tumor Response“

**Peter Huber**

Heidelberg, Germany

*„CTGF (connective tissue growth factor) in radiation tumor and normal tissue response“*

**Marc Vooijs**

Maastricht, Netherlands

*„Modeling tumour and normal tissue effects of combination treatments“*

**Heidi Lyng**

Oslo, Norway

*„Imaging hypoxia in prostate cancer“*

**Simone Moertl**

Munich, Germany

*„Exosomes from irradiated squamous head and neck cancer cells with altered protein cargo boost migration and chemotaxis-induced motility “*

**Sofia Ferreira**

Orsay Ville, France

*„Dbait and radiation treatments in pediatric brain tumors“*

**Emmy Rogakou**

Athens, Greece

*„20 years after the discovery of the  $\gamma$ -H2AX biomarker“*



# CTGF (connective tissue growth factor) in radiation tumor and normal tissue response

**Peter Huber**

*German Cancer Research Center (DKFZ), Heidelberg, Germany*

Connective tissue growth factor (CTGF, CCN2) is a matricellular protein which promotes invasion, adhesion, proliferation and angiogenesis in tumors including pancreatic carcinoma and glioblastoma. CTGF expression is correlated with higher tumor grade, pathology and worse patient survival. On the other hand CTGF is a central mediator of tissue remodeling and fibrosis (lung, skin, liver etc.) that has been reported to be an essential mediator for the fibrotic activity of TGFbeta, but can also act independently of TGFbeta. Here I describe that CTGF blockade by a monoclonal human antibody is an effective treatment for tumors alone and in combination with radiotherapy and also is an effective treatment for radiation-induced side effects such as lung fibrosis (e.g. Bickelhaupt et al., J Natl Cancer Inst. 2017 Aug 1;109(8)). The findings can be translated into the clinic and also constitute a deep link between tumors, inflammation and fibrosis.

# Modeling Tumour and normal tissue effects of combination treatments

**Marc Vooijs**

*Maastricht University, maastricht, The Netherlands*

In my talk i will discuss a small animal image guided radiotherapy platform for preclinical research into combination treatments for glioblastoma.



# Imaging hypoxia in prostate cancer

**Heidi Lyng**

*Oslo University Hospital, Norway*

Hypoxia is an adverse factor and associated with poor outcome of radiotherapy in prostate cancer. A non-invasive method to assess hypoxia at diagnosis could be used to stratify patients to different treatment options and is therefore highly warranted. PET imaging with hypoxia specific tracers has not been successful in this disease, and no other approaches have been developed so far. MRI is vital in treatment planning and monitoring of prostate cancer and an MRI based method to assess hypoxia could therefore translate rapidly into clinical practice. Since tumor oxygenation cannot be directly assessed by MRI, we have developed an algorithm where we combine images reflecting the underlying physiology causing hypoxia; i.e, the oxygen supply and consumption side in the tumor. The method is based on diffusion-weighted (DW) MRI with multiple b-values. We used a training cohort of 43 patients with intermediate or high risk prostate cancer to construct the method. All patients received the hypoxia marker pimonidazole prior to prostatectomy. We generated images reflecting fractional blood volume (fBV) and apparent diffusion coefficient (ADC) based on the data acquired with selected b-values. By comparing the fBV and ADC images with immunohistochemistry data in whole-mount sections from the surgery specimens, we showed that the images reflected tumor vascular density and cell density, respectively. The DW-MRI parameters fBV and ADC therefore seemed to visualize oxygen demand and supply in prostate cancer. An algorithm to combine the fBV and ADC images into hypoxia images was further developed, and validated in an independent cohort of 61 patients. In this talk, I will present our approach and discuss its potential usefulness in prostate cancer and other cancer types.

# Exosomes from irradiated squamous head and neck cancer cells with altered protein cargo boost migration and chemotaxis-induced motility

L. Mutschelknaus, O. Azimzadeh, J. Merl-Pham, SM. Huber, L. Edalat, V. Radulovic, S. Tapio, N. Anastasov, MJ. Atkinson and **Simone Moertl**

*Institute of Radiation Biology, Helmholtz Zentrum München, Germany*

Radiation is a highly efficient therapy in squamous head and neck carcinoma (HNSCC) treatment. However, there are indications that the radiation treatment itself may increase cell motility, thus influencing invasion capacity and the migration to local and distant sites. Recent evidence shows that cancer-cell-derived exosomes modify tumour cell movement and metastasis. In this study, we show that exosomes released from irradiated HNSCC cells promote migration of recipient cells. Molecular data in the recipient cells identified enhanced AKT-signalling, manifested through increased phospho-mTOR and MMP2/9 activity as underlying mechanism. AKT-inhibition in the recipient cells blocked the pro-migratory action of exosomes, suggesting AKT signalling as key player in exosome-mediated migration. Proteomic analysis of exosomes isolated from irradiated and non-irradiated BHY donor cells identified 39 up- and 36 downregulated proteins. In line with the observed pro-migratory effect of exosomes STRING process enrichment analysis assigned many of the deregulated exosomal proteins to cell motility and wound healing. Together, our findings demonstrate that exosomes derived from irradiated HNSCC cells confer a migratory phenotype to recipient cancer cells. This may be due to the transfer of radiation-regulated exosomal proteins that increase AKT signalling. We conclude that exosomes may act as a driver of HNSCC progression during tumour radiotherapy and are therefore attractive targets to improve radiation therapy strategies.

# Dbait and radiation treatments in Pediatric Brain tumors

**Sofia Ferreira**, C. Foray, C. Alapetite, P. Verrelle, F. Bourdeaut, F. Doz, O. Ayrault, F. Boussin, C. Pouponnot and M. Dutreix

*Institut Curie, Orsay, France*

Medulloblastoma is the most common and aggressive pediatric Central Nervous System cancer. Group 3 of the four medulloblastoma sub-molecular groups presents very poor prognosis and often metastasis, though bearing virtually no p53 mutations. Recurrent medulloblastoma are described to have DNA repair defects. Current treatments are highly effective, figuring radiotherapy as a great part of protocol treatments. However, pediatric patients often present significant neurocognitive, endocrine and psychological sequelae. Our project aims to identify new protocols using a DNA repair inhibitor: Dbait, to increase radiotherapy efficacy without additional adverse effects. Dbait are small molecules that inhibit all DNA repair pathways involved in single- and double-stranded DNA break repair. Dbait acts through over-activation of DNA-PK and PARP proteins and by promoting the hijacking of the DNA repair machinery away of the genomic therapies-induced damage. AsiDNA - a clinical form of Dbait - has demonstrated in clinic (DRIIM Phase I/II trial), to sensitize melanoma to radiotherapy without increased skin radiosensitivity. However, its toxicity and efficacy had not been yet addressed in developing brain and pediatric solid tumors. In vitro experiments have tested the efficacy of AsiDNA in combination with radiation in four different medulloblastoma cell lines. Noticeably, sensitivity to AsiDNA was independent of p53 status or sub-molecular group. AsiDNA showed an additive effect to radiation in all cell lines tested: radiation Lethal Dose 50% survival (LD50) showed an approximated twofold-decrease with the addition of AsiDNA prior radiation. As brain toxicity induced by radiotherapy is a major concern in pediatric patients, we studied the toxicity of the association of AsiDNA to irradiation in young murine models, of 10 days-old (P10) which have not completed brain development. A fluorescent form of AsiDNA(Cy5.5) showed the ability to cross the blood brain barrier and diffuse in brain without malignancy. Irradiation treatments used SARRP - a small beam photon-irradiator, in a way to irradiate merely brain rather than surrounding fragile tissues (e.g. salivary glands). We were able to define the lethal irradiation dose - 20Gy. Favorably, such set-ups have reproduced some adverse effects observed in pediatric brain-irradiated patients such as a decrease of 20.2% of circulating growth hormone 17 days after treatment and consequently an irreversible mean 2.3% reduction of femur size (mean values for 10Gy). Short- and long-term effects on brain are under analysis nonetheless, preliminary analyzes suggest that AsiDNA, alone or combined to radiation, show no additional toxicity in young murine models. Our results indicate that AsiDNA could have a radiosensitizing effect on Medulloblastoma cell lines and no toxicity in young murine model, revealing AsiDNA as a promising drug to maximize medulloblastoma treatments without additional adverse effects. Such preliminary results encouraged us to move forward with optimization of protocols for a pre-clinical study for an in vivo demonstration of enhanced efficacy of the combined treatments in medulloblastoma Cell line Derives Xenograft, that are now ongoing.

[sofia.morgadinho-ferreira@curie.fr](mailto:sofia.morgadinho-ferreira@curie.fr)

# 20 years after the discovery of $\gamma$ H2AX

**Emmy P. Rogakou**

*National and Kapodistrian University of Athens  
Athens, Greece*

In the past 20 years, the  $\gamma$ -phosphorylation of the histone H2AX (namely  $\gamma$ H2AX) has been established as a central epigenetic player in the DNA Damage Response, with specificity for Double-Strand Breaks (DSBs).  $\gamma$ -phosphorylation covers megabase-long domains in chromatin and extend both sides of the damage. This phosphorylation creates a “platform” on the chromatin fiber, where signal transduction factors interact with each other to activate and accelerate their kinetics.  $\gamma$ H2AX forms early after the generation of DSBs, and participates in both, the homologous and the non-homologous end joining DNA repair pathways.  $\gamma$ H2AX is considered as a guardian of the genome, as dysfunctions of the  $\gamma$ -phosphorylation lead to genomic instability. Respectively,  $\gamma$ H2AX levels are significantly increased in cells that exhibit genomic instability.  $\gamma$ H2AX is increased not only in cancer cells, but also in a cells that are in a precancerous stage, as genomic instability, characteristic in cancer cells, appears before cell transformation. Quantification of the  $\gamma$ H2AX signal to monitor DSBs is feasible by a spectrum of techniques. In addition, technologies based on antibodies against  $\gamma$ -phosphorylation has been shown to be both, specific and sensitive. Till now, a volume of evidence, that has been produced by different laboratories worldwide, support the notion that  $\gamma$ H2AX is an excellent epigenetic marker to detect double-strand breaks. Nowadays, translational research on the  $\gamma$ H2AX epigenetic biomarker is very dynamic, and is expected to develop further to cover several pathologies or therapies that involve an increased load of DSBs.



# Session S04

## „DNA Repair in Chromatin Context“

**Timothy Humphrey**

Oxford, United Kingdom

*„Histone H3K36 trimethylation, genome stability and cancer“*

**Claudia Rübe**

Homburg, Germany

*„Hair follicle stem cell fate is dependent on chromatin remodeling capacity following low-dose radiation“*

**Burkhard Jakob**

Darmstadt, Germany

*„Dynamics of DNA repair proteins in response to radiation induced DNA damage of different complexity“*

**Alexander Rapp**

Darmstadt, Germany

*„Identification of the elementary structural chromatin units of the DNA damage response“*

**Lovisa Lundholm**

Stockholm, Sweden

*„The role of chromatin in response to alpha and gamma radiation in breast cancer cells“*

**Nataša Anastasov**

Neuherberg, Germany

*„Non-coding RNAs as regulators of cellular epigenome and response to radiation“*



# Histone H3K36 trimethylation, genome stability and cancer

**Timothy Humphrey**

*CRUK MRC Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, United Kingdom*

Histone H3 lysine 36 (H3K36) methylation plays a central role in both maintaining genome stability and in suppressing tumorigenesis. In this respect, SETD2-dependent H3K36 trimethylation (H3K36me3) plays an important role in promoting homologous recombination (HR) repair of DNA double-strand breaks (DSBs). Further, H3K36me3 is also essential for DNA replication restart following replication stress resulting from checkpoint kinase inactivation. Accordingly SETD2-dependent H3K36me3 is frequently depleted in a number of cancer types, for which prognosis is poor. We recently exploited a conserved synthetic lethal relationship we identified in fission yeast to target H3K36me3-deficient cancers, using the WEE1 inhibitor, AZD1775, which is already in Phase II clinical trials. As a result of these studies, together with the development of a biomarker to detect H3K36me3 loss in patient tissue, the use of AZD1775 to target H3K36me3-deficient cancers is being adopted into clinical trials. Despite these advances, how H3K36me3 coordinates these distinct functions to maintain genome stability is unclear. Our current understanding of these roles, and how they may be exploited will be presented.

*[timothy.humphrey@oncology.ox.ac.uk](mailto:timothy.humphrey@oncology.ox.ac.uk)*



# Hair follicle stem cell fate is dependent on chromatin remodeling capacity following low-dose radiation

N. Schuler, S. Timm and Claudia E. Rübe

*Radiation Oncology, Saarland University, Germany*

The main function of the skin, to protect against the environment, is supported by the activity of different stem cell populations. The focus of this study was elucidating the coping mechanisms of stem cells against the stimulation of constant exposure to genotoxic stresses, both endogenous and exogenous, to ensure long-term function. Investigation of various mouse strains, differing in their DNA repair capacity, enables us to clarify fractionated low-dose irradiation (LDR)-induced consequences for different stem cell populations of the murine hair follicle in their physiological stem cell niche. Using microscopic techniques combined with flow cytometry we could show that LDR induces accumulation of persisting; pKu70-independent 53BP1-foci ("chromatin-alterations") in heterochromatic regions of the hair follicle stem cells (HFSCs). These remaining chromatin-alterations lead to varying stem cell consequences. On one side, CD34-positive HFSCs react by ATM-dependent, premature senescence, which is correlated with global chromatin compaction, whereby apoptosis is prevented by the activity of DNAPKcs. On the other side, highly damaged HFSCs seem to be sorted out of the niche by differentiation, transferring their chromatin-alterations to more proliferative LGR5-positive stem cells. The loss of basal HFSCs is then compensated by increased proliferation within the stem cell pool. Despite the initial success of these mechanisms in the maintenance of the stem cell population, the combined effect of the chromatin-alterations and the shift in stem cell pool composition, may lead to downstream long term functional loss of tissue or organs.

# Dynamics of DNA repair proteins in response to radiation induced DNA damage of different complexity

E. Janiel, G. Taucher-Scholz and **Burkhard Jakob**

*GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany*

The special physical and biological properties of accelerated charged particles are of high interest for the application in radiation therapy of solid tumours. In addition, charged particles are one of the major contributions of cosmic rays and thus their radiobiological properties important for risk estimation of human space exploration. The complexity of DNA lesions generated by charged particle radiation influences the employed repair pathways and thus the fidelity of repair. In case of high energetic particles (HZE) DNA damage of different complexity can be expected due to the radial dose profile of the traversing particles. Using live cell beamline microscopy, dynamic properties of the accumulation of double strand break (DSB) repair proteins like 53BP1 and NBS1 were studied. Single foci analysis revealed distinct differences between IRIF at track centre and off-track foci, which are characterised by a large distribution of delay times in their formation. Due to this variability, a global nucleus wide evaluation obscures the individual dynamics. To analyse protein dynamics after sparsely ionising radiation, we combined the beamline microscope with an x-ray tube. In single foci analysis, X-ray induced IRIF of 53BP1 showed a similar distribution of delayed formation as the delta-rays. We hypothesise that these delayed DSBs are not immediately produced, but generated during repair processing of non DSB lesions.

This work was partly funded by DFG graduate school GRK1657 and by BMBF grant 02NUK037A.

# Identification of the elementary structural chromatin units of the DNA damage response

**Alexander Rapp**, F. Natale, W. Yu, A. Maiser, H. Leonhardt, G. Taucher-Scholz and C. Cardoso

*Department of Biology, Technische Universität Darmstadt, Darmstadt, Germany*

Histone H2AX phosphorylation is an early signaling event triggered by DNA double strand breaks (DSBs) interfacing DNA damage response, chromatin organization and cell cycle checkpoint activation. To elucidate the elementary units of phospho-H2AX-labeled chromatin, we integrate super-resolution microscopy data of phospho-H2AX during DNA repair in human cells with genome-wide sequencing data and multiple genomic features. We identify phospho-H2AX chromatin domains in the nanometer range with median length of ~75 kbp. Correlation analysis with over 60 genomic features shows a time-dependent euchromatin-to-heterochromatin repair trend. After X-ray induced DSBs, phospho-H2AX-labeled heterochromatin exhibits DNA decondensation but retains its heterochromatic histone marks, indicating that chromatin structural and molecular determinants are uncoupled during repair. The phospho-H2AX nano-domains arrange into higher-order clustered structures of discontinuously phosphorylated chromatin, flanked by CTCF (CCCTC-binding factor). CTCF knock-down strongly impairs spreading of the phosphorylation signal throughout the 3D-looped nano-domains and decreases the number of clusters, as well as results in a decreased cell survival and a delayed DSB repair. Co-staining of phospho-H2AX with phospho-Ku70 or TUNEL-assay reveal that the clusters rather than the nano-foci represent single DSBs. We propose that each underlying individual chromatin loop is a microscopically distinct nano-focus, and clusters of phospho-H2AX decorated loops correspond to the so far identified phospho-H2AX foci, reflecting two dynamic yet distinct hierarchical organization levels of interphase chromosomes.

# The role of chromatin in response to alpha and gamma radiation in breast cancer cells

Lovisa Lundholm, M. Svetličič, H. Lisowska and A. Wojcik

*Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Sweden*

Chromatin structure is a factor in the sensitivity of cells to gamma radiation, but it has not been thoroughly studied in response to high linear energy transfer (LET) radiation such as alpha radiation. The focus of this study was to evaluate the relative importance of DNA damage induction and repair for sparsely ionizing gamma radiation which mainly damages the DNA by induction of free radicals, where compact chromatin protects the DNA, compared to densely ionizing alpha radiation where free radicals play a minor role due to its direct interaction with DNA and compact chromatin mainly would inhibit DNA repair. Pretreatment with the histone deacetylase inhibitor trichostatin A (TSA) for 18 h produced opposite results for the different radiation types in the aggressive, triple negative breast cancer cell line MDA-MB-231. TSA pretreatment before gamma radiation resulted in reduced clonogenic survival, while alpha radiation caused increased survival. An opening of chromatin at the selected TSA doses was confirmed by an increase in the acetylated lysine 9/14 of histone H3 (H3K9/14ac). Gamma H2AX foci displayed a trend towards an increase at 30 min and 3 h post gamma radiation upon TSA pretreatment, which is in line with an increased damage induction. On the other hand, damage induction at 30 min was similar but the number of foci did not fall at 3 h after alpha exposure upon TSA pretreatment. In conclusion, these data indicate a higher relative importance of DNA damage induction post gamma radiation, while a delayed but still more efficient repair is suggested as the net effect post alpha radiation in the state of opened chromatin.

# Non-coding RNAs as regulators of cellular epigenome and response to radiation

**Nataša Anastasov**, S. Richter, J. König, V. Radulovic and MJ. Atkinson

*Helmholtz Zentrum München - German Research Center for Environmental Health,  
Neuherberg, Germany*

The response to radiation recruits changes in the non-coding RNA expression dependent on dose-, time- and tissue type. Non-coding RNAs involving long non-coding (lncRNA) and microRNA (miRNA) families act through a multitude of interconnecting regulatory pathways. Our major goal is the understanding of the interrelationship between radiation exposure, non-coding RNA (ncRNA) genome and successful tumour therapy outcomes. We have shown that simple miRNA expression has some ability to predict outcomes, such as miR-21 or miR-221 characterization as biomarkers for breast cancer correlation with distant metastasis (Anastasov et al., 2012; Falkenberg et al., 2013). Furthermore, we have shown that suppressive epigenetic influence of specific lncRNA (PARTICLE) implicates an expanding role for such ncRNAs in global cellular methylation and intercellular communication in response to radiation exposure (O'Leary et al., 2015). Additionally, we have developed an innovative in vitro 3D microtissue model of cancer that reiterates the three-dimensional cellular physiology and dynamics of tumour tissue (Anastasov et al., 2015; Falkenberg et al., 2016). Three-dimensional (3D) microtissues are widely accepted as being more physiologically relevant than conventional 2D monolayer cell cultures, and can therefore help to generate more predictive data. Consequently we have begun to follow individual regulatory pathways responding to radiation, using 3D microtissue models of glioblastoma and breast cancer, in order to establish functional relevance of successful radiation treatment. Our results verified that miR-221 overexpression has the potential to promote G1 to S phase transition with resulting enhanced proliferation capacity and resistance to radiation treatment that was confirmed using 3D microtissue model. Radiation is directly changing the profile of non-coding genome expression including specific miRNA (miR-21; miR-221 etc.) or "stem cell" (CD44; Sox2 etc.) marker expression upon radiation treatment of tumour cells. In parallel migrative and invasive cellular characterization is going to be unravelled. With no doubt the long non-coding genome will continue to surprise and reveal unexpected layers of cellular regulatory complexity in the future radiation research.

## References:

1. Anastasov et al., Radiation Oncology 2012;
2. Falkenberg et al., Br J Cancer 2013;
3. O'Leary et al., Cell Reports 2015;
4. Anastasov et al., BMC Cancer 2015;
5. Falkenberg et al., Cancer Medicine 2016

[natasa.anastasov@helmholtz-muenchen.de](mailto:natasa.anastasov@helmholtz-muenchen.de)



## Session S05

### „Radiosensitizers“

**Gilles McKenna**

Oxford, United Kingdom

*„High-throughput screens for drugs to modify hypoxia“*

**Martin Pruschy**

Zurich, Switzerland

*„Targeting ADAM17 for radiosensitization“*

**Peter Sminia**

Amsterdam, Netherlands

*„The MAPK targeted agent MEK162 acts as radiosensitizer in glioblastoma therapy“*

**Simon Magin**

Essen, Germany

*„ATR dependent reactivation of suppressed alt-EJ in G0“*

**Mike Atkinson**

Neuherburg, Germany

*„Do non-coding RNAs play a role in the response to radiation?“*

**Bodo Laube**

Darmstadt, Germany

*„Impact of glutamate receptor mediated signaling on the induction and repair of DNA double-strand breaks in glioblastoma cells“*





# High-throughput Screens for Drugs to Modify Hypoxia

**Gillies McKenna**, L. Kunz-Schughart and G. Higgins

*Oxford Institute for Radiation Oncology, Oxford, United Kingdom*

Tumour hypoxia renders cancer cells resistant to cancer therapy, resulting in markedly worse clinical outcomes. Since net tumour oxygen levels are determined by the sum of the rate of oxygen delivery and the oxygen consumption rate (OCR) we sought to identify drugs that decrease the OCR of cancer cells and could be used as modifiers of tumour hypoxia in a clinical setting. We screened a library of 1,697 FDA-approved compounds. This screen identified a number of drugs which had the properties we sought. We will discuss a number of these with regard to their potential clinical utility concentrating in particular on the anti-malarial Atovaquone. For clinical purposes, we plan to concentrate on Atovaquone. Atovaquone is a ubiquinone analogue and is a drug primarily used to treat malaria and pneumocystis pneumonia. Atovaquone reduces the OCR by inhibition of mitochondrial complex III (the cytochrome bc1 complex) at pharmacologically achievable concentrations, alleviates hypoxia both in spheroids and in xenografted tumours, and causes a significant tumour growth delay in combination with radiation. Atovaquone rapidly decreases the OCR by more than 80% in a wide range of cancer cell lines at pharmacological concentrations. In addition, Atovaquone eradicates hypoxia in FaDu, HCT116 and H1299 spheroids. Similarly, it virtually eliminates hypoxia in FaDu and HCT116 xenografts in nude mice and causes a significant tumour growth delay when combined with radiation. We are now undertaking clinical studies to assess whether Atovaquone reduces tumour hypoxia in patients, thereby potentially increasing the efficacy of radiotherapy.

# Targeting ADAM17 for Radiosensitization

S. Bender, A. Sharma, A. Broggini-Tenzer and **Martin Pruschy**

*University Hospital Zurich, Zurich, Switzerland*

**Introduction:** The therapeutic response of ionizing radiation (IR) is imparted by genomic instability and DNA damage. However, IR also triggers intracellular signaling processes which lead to the secretion of various factors being involved in resistance mechanisms. Recently, we have shown that radiotherapy activates ADAM17 (A Disintegrin and metalloprotease domain 17) in NSCLC, which mediates IR-induced treatment resistance. Our new data point towards a link between ADAM17, chromatin remodeling via the histone methyltransferase SUV39H1 and the DNA damage response. **Materials and Methods:** Clonogenic survival and xenograft tumor growth delay assays were performed in response to IR in inducible shRNA-targeted tumor cell lines or in combination with small molecular agents. Chromatin remodeling and DNA damage signaling were investigated by immunofluorescence staining and Western blotting. Damaged DNA was analyzed with the comet assay, chromosomal aberrations by metaphase spreads and m-FISH. **Results:** Radiotherapy activates ADAM17 in NSCLC, which results in shedding of multiple survival factors, growth factor pathway activation, and IR-induced treatment resistance. Inducible-shRNA-mediated silencing of ADAM17 or targeting of ADAM17 with the small molecular inhibitor TMI-005 suppressed IR-induced shedding of these factors, downregulated ErbB-signaling in target cells and enhanced IR-induced cytotoxicity in vitro and in vivo. Targeting of ADAM17 interfered with chromatin remodeling, decreased the basal level of SUV39H1 and abolished a short-term increase of the SUV39H1 protein in response to irradiation. Surprisingly decreased amounts of residual  $\gamma$ H2AX were detected in ADAM17-depleted cells after irradiation despite their increased radiosensitivity in comparison to wildtype cells. At the same time, DNA damage signaling via Chk1 was impaired in ADAM17-deficient cells, implying a defect in the DNA repair machinery and pointing towards a potential mechanism of radiosensitization by ADAM17 targeting. **Conclusions:** Our findings demonstrate that IR significantly activates ADAM17, which results in shedding of survival factors, growth factor pathway activation and contributes to treatment resistance in NSCLC cells. Additionally, our data point towards a novel link between ADAM17, regulation of the chromatin state and the DNA damage response. We demonstrate that the impact of targeting ADAM17 is more pleiotropic than just diminishing ErbB signaling and provide a sound rationale for positioning ADAM17 inhibitors as radiosensitizers to improve the treatment of NSCLC.

# The MAPK targeted agent MEK162 acts as radiosensitizer in Glioblastoma therapy

R. Narayan , A. Gasol, J. van den Berg, B. Slotman, T. Würdinger, L. Stalpers, B. Baumert, B. Westerman, J. Theys and **Peter Sminia**

*Department of Radiation Oncology, VU University Medical Center, Amsterdam, The Netherlands*

**BACKGROUND** – Glioblastoma (GBM) is a highly aggressive and lethal brain cancer type. PI3K and MAPK inhibitors have been studied pre-clinically in GBM as monotherapy, but not in combination with radiotherapy, which is a key component of the current standard treatment of GBM. **METHODS** – GBM cell lines and patient representative primary cultures were grown as multicellular spheroids. Spheroids were treated with a panel of small molecule drugs including MK2206, RAD001, BEZ235, MLN0128 and MEK162 (binimetinib), alone and in combination with irradiation. Following treatment, spheroid growth parameters (growth rate, volume reduction and time to regrow), cell cycle distribution and expression of key target proteins were evaluated. In vivo, the effect of irradiation (3 x 2 Gy) without or with MEK162 (50 mg/kg) was studied in orthotopic GBM8 brain tumour xenografts with endpoints tumour growth and animal survival. **RESULTS** – The MAPK targeting agent MEK162 synergized with radiation as demonstrated by growth inhibition of spheroids. MEK162 down-regulated and dephosphorylated the cell cycle checkpoint proteins CDK1/CDK2/WEE1 and DNA damage response proteins p-ATM/p-CHK2. When combined with radiation this led to a prolonged DNA damage signal. In vivo data on tumour bearing animals demonstrated a significantly reduced growth rate, increased growth delay and prolonged survival time. In addition, RNA expression of responsive cell cultures correlated to mesenchymal stratification of patient expression data. **CONCLUSION** – The MAPK inhibitor MEK162 was identified as radiosensitizer in GBM spheroids in vitro and in orthotopic GBM xenografts in vivo. The data are supportive for implementation of this targeted agent in an early phase clinical study in GBM patients.

Funded by the Dutch Cancer Foundation (KWF), grant #VU2010-4874. A. Gasol was supported in part by the foundation STOPHersentumoren.nl, grant #2002657.

# ATR dependent reactivation of suppressed alt-EJ in Go

**Simon Magin** and G. Iliakis

*Institute of Medical Radiation Biology, University of Duisburg-Essen, Essen, Germany*

Unlike classical non-homologous end-joining (c-NHEJ), the activity of alternative end-joining (alt-EJ) shows cell cycle dependence in the processing of DNA double-strand-breaks (DSBs). The efficiency of alt-EJ is high in late-S and G2-phase of the cell cycle, but is markedly diminished in G1-phase. Notably, alt-EJ is almost fully suppressed when cells enter quiescence, either by serum deprivation or by growing into a plateau-phase. The mechanisms underlying these unique efficiency fluctuations remain unknown and are investigated here. DSB repair kinetics were measured by pulsed-field gel-electrophoresis (PFGE) after irradiation of cells with 20 Gy X-rays. Surprisingly, we found strong reactivation of alt-EJ in serum deprived human and murine c-NHEJ deficient cells upon treatment with the nucleoside analog 9- $\beta$ -D-arabinofuranosyladenosine (Vidarabine). In MEF Lig4<sup>-/-</sup> cells, this treatment not only restored alt-EJ to levels normally measured in cycling cells, but even brought rejoining to levels normally seen in wild-type MEFs. This effect could not be reproduced by treatment with any other nucleoside analog (e.g. Gemcitabine, Fludarabine, Cytarabine). Vidarabine treatment elicited pan-nuclear phosphorylation of H2AX, which was not due to apoptosis or other forms of cell-death. This pan-nuclear staining was strongly suppressed by inhibition of ATR (VE821), while inhibition of ATM was ineffective. Experiments using Lig4<sup>-/-</sup> / H2AX<sup>-/-</sup> MEFs generated using CRISPR/Cas9 technology showed that the Vidarabine effect on alt-EJ efficiency does not require  $\gamma$ -H2AX. Using 53BP1 as a surrogate marker for DSBs, we found that in the absence of Vidarabine, 53BP1 foci persisted for more than 24 h in cells exposed to 1 Gy. In cells treated with Vidarabine, 53BP1 foci decayed within 8h confirming the enhanced processing observed with PFGE. Strikingly, co-treatment with Vidarabine and VE821 strongly abrogated the Vidarabine-induced enhancement in alt-EJ, as measured either by PFGE or 53BP1 foci scoring. We speculated that activation of ATR after Vidarabine treatment may be related to ssDNA created by ectopic activation of end-resection in the serum deprived G1 cells. Although, we could not detect end-resection employing flow-cytometry and microscopy approaches, we hypothesize that Vidarabine treatment may act by inducing only limited resection, sufficient to promote ATR activation and alt-EJ. We conclude that treatment with Vidarabine, in an ATR-dependent way, counteracts the cell cycle dependent regulatory mechanisms that restrict alt-EJ. It remains to be elucidated how ATR becomes activated under these conditions, what is the role of DNA end resection, and which downstream targets are required. These studies will improve our understanding of the circuitry regulating alt-EJ and repair pathway choice in general in higher eukaryotic cells.

# Do non-coding RNAs play a role in the response to radiation?

**Michael Atkinson**, N. Anastasov, O. Azimzadeh, V. O'Leary, S. Mörtl, M. Rosemann, J. Smida and S. Tapio

*Institute of Radiation Biology, Helmholtz Zentrum München, Germany*

The currently accepted paradigm of radiation carcinogenesis postulates that cancer is initiated by failure to repair DNA damage in one or more critical driver genes in the hit cell, thereby conferring a clonal growth advantage to that cell. The mutations that deliver the essential hallmarks needed for development of cancer are assumed to arise in the progeny of the hit cell through genomic instability. The paradigm clearly does not apply to deterministic effects associated with cell killing or non-targeted cellular effects; nor can it be applied to the late-onset health effects of radiation exposure such as cataracts, cardiovascular disease, neurological impairment, diabetes or inflammation. All of the latter are typified by an absence of driver mutations and of clonal expansion. Given these incongruities we have reinvestigated the cellular events following irradiation using a combination of in vitro and in vivo model systems and high throughput proteomics. These studies show a persistent changes a set of metabolic processes in brain and heart tissues, influencing mitochondrial redox activity, inflammatory signalling, cytoskeletal structure etc. Our interest in how such alterations are maintained long after the initial insult has prompted us to investigate possible mechanisms. Our initial observations using endothelial cells as an in vitro model system revealed that microRNA processing was critical to cell survival after irradiation. We have identifies subsets of microRNAs whose cellular concentrations are regulated following exposure. Given the ability of microRNAs to regulate protein translation we have searched for possible target proteins. The targets of the up-regulated miR 525p and the down-regulated miR23a were shown to be proteins directly regulating cell survival. Thus increased miR525p suppressed translation of ARRB1 whilst miR23a down-regulation increased expression of XIAP. Intriguingly we have shown that microRNAs are cargo of the cell-survival promoting exosomes released locally and into the circulation by irradiated cells, possibly mediating non-targeted effects. A more sustained change in cellular phenotype is seen when long-non-coding RNA (lncRNA) transcripts are investigated. Here we observe that the transcriptional response to acute exposure includes up-regulation of a number of lncRNA species, including PARTICLE and TERRA. Both of these RNAs interact with the genome to induce epigenetic modification and consequent transcriptional regulation of selected regions of the irradiated cell genome. Transmission of lncRNA information by exosomal communication is again a possibility as irradiation increases PARTICLE content of circulating exosomes in radiation therapy patients.

# Impact of glutamate receptor mediated signaling on the induction and repair of DNA double-strand breaks in glioblastoma cells

**Bodo Laube**, A. Längle, H. Lutz and K. Rau

*Technische Universität Darmstadt, Darmstadt, Germany*

Originally it was believed that excitatory glutamatergic signaling is limited to neuronal cells in nervous systems. However this opinion changed since glutamate receptors have been proven to regulate proliferation and migration in neoplastic cells. Thus the neurotransmitter glutamate has been suggested as a new potential growth factor in tumor development. Glioblastoma multiforme (GBM) is one of the most common and aggressive malignant primary brain tumors in humans characterized by a high radio-resistance and a high degree of invasive growth. Mean survival time of patients with GBM is only 15 months with a high rate of recurrence even upon radiation therapy. However, recurrence of glioblastoma cells seems to be promoted by ionizing radiation (IR) whereas resistance to IR is due to an increased DNA repair capacity. Remarkably, GBMs secrete the neurotransmitter glutamate at high concentrations sufficient i) to stimulate proliferation, infiltration, and cell survival of the tumor cells and ii) to induce excitotoxic neuronal cell death in the surrounding tissue. The apoptotic cell death of neurons as well as the promoting effect of the high glutamate concentrations on tumor cells seems to depend on the spatial and temporal stimulation of different  $\text{Ca}^{2+}$ -permeable ionotropic glutamate-receptors (iGluRs) of the NMDAR-subtype. Subsequent activation of distinct signal transduction pathways (for example the cAMP-responsive element binding transcription factor (CREB)) by NMDARs is important for synaptic transmission, but also for cellular migration, and survival. In this study we show the expression of functional iGluRs in glioblastoma cell lines by patch-clamp techniques. To further investigate the role of the  $\text{Ca}^{2+}$ -permeable iGluRs in glioblastoma malignancy we examined DNA double-strand break (DSB) repair in human LN-229 cells after clinically relevant radiation doses of IR upon application of glutamate, glutamate receptor antagonists (MK-801) and the calcium chelator Bapta-AM. Radiation induced DNA DSBs were shown by phosphorylation of histone H2AX and 53BP1-foci visualized by immunofluorescence. DNA DSB repair upon radiation was significantly more effective in the presence of glutamate. In contrast, the NMDA receptor channel blocker MK-801 and the  $\text{Ca}^{2+}$ -chelator Bapta-AM impaired repair of DNA DSBs. Inhibition of  $\text{Ca}^{2+}$ -dependent transcription factor CREB by KG-501 impaired DNA repair in the same way, indicating a direct link between iGluR activation and DNA repair. In addition we examined the effect of glutamate and iGluR antagonists on cell survival and cell migration before and after IR. The results suggest that iGluR antagonists disrupting DNA repair, inhibiting cell migration and decreasing cell survival may constitute a novel optimization of therapeutic interventions. However, we found in addition that a subpopulation of cells, expressing the neural stem cell marker CD133 and supposed to drive tumor progression, show a formation of glutamate-induced DNA DSBs, prominently through NMDARs. In summary, we found in glioblastoma cells i) that glutamate improved the DNA damage response (DDR) of IR-induced DNA DSBs although activation of the NMDAR on its own resulted in DSBs and ii) that blocking NMDAR-mediated glutamatergic signaling resulted in a decreased cell survival and a sensitization to IR. In addition we can show that both glutamate and IR activate the CREB pathway and that the DDR is inhibited by CREB antagonists. Therefore we assume that, beyond its traditional role in neurons, NMDAR may be activated in GBMs inducing an autocrine glutamate signaling circuit with resultant stimulation of malignancy.



# Session S06

## „Radiation Protection“

### **Christian Streffer**

Essen, Germany

*„LNT-model for radiological protection: reasonable use and misuse “*

### **Wolfgang-Ulrich Müller**

Essen, Germany

*„Attributing health effects to ionizing radiation exposure and inferring risks “*

### **Ulrike Kulka**

Oberschleißheim, Germany

*„Research for radiation protection“*

### **Herbert Braselmann**

Neuherburg, Germany

*„A 9-feature genomic copy number signature is associated with radiation exposure in post-Chernobyl breast cancer“*

### **Niels Belmans**

Mol, Belgium

*„Age-related biological effects of dental cone-beam CT exposure“*

### **Christine Hellweg**

Cologne, Germany

*„Radiation protection for human space flights “*





# LNT-model for radiological protection: reasonable use and misuse

**Christian Streffer**

*Institute of Medical Radiation Biology, University Hospital Essen, Essen, Germany*

The concept of the linear dose response without a threshold (LNT) has been developed by the ICRP for “stochastic” radiation effects (genetic effects and cancer by ionising radiation) (ICRP 2007). In ICRP Recommendations from 2007 it is stated: “Use of this so-called linear-non-threshold (LNT) model is considered by the Commission to be the best practical approach to managing risk from radiation exposure and commensurate with the ‘precautionary principle’ (UNESCO, 2005). The Commission considers that the LNT model remains a prudent basis for radiological protection at low doses and low dose rates.” The LNT model is the basis for determining “effective dose”. This concept is apparently necessary to make the system of radiological protection practical. Nevertheless it has to be based on scientific grounds. ICRP (2007) also stated: “This averaging implies that the application of this approach is restricted to the determination of effective dose in radiological protection and, in particular, cannot be used for the assessment of individual risk.” The LNT model is often supported by epidemiological studies where it is frequently concluded that the observed data are best fitted by a linear dose response without a threshold. In these studies usually cancer is significantly increased after exposure to 100 mGy and higher and from these data an extrapolation down to very low doses below 100 mGy is done but quite often cancer rates deviate from a linear response relation in the low dose ranges. Unfortunately there no marker exists to conclude whether an individual cancer is caused by radiation exposure or by other exogenous or endogenous factors. It is certainly necessary to better understand the mechanism of the total cancer development in order to make the conclusion to approve or disprove the LNT concept. Considerable knowledge is available about radiation effects within hours, days or even weeks after exposure of which it is assumed that this leads to cancer (DNA damage, DNA misrepair, chromosomal damage, signal transduction, genomic instability etc.) but the cancers are usually recognized in the clinics only decades after the exposure especially in the low dose range. Thus there is a tremendous gap between these phases although a better understanding has been developed during recent years in some cancer entities. These data also show that certainly different mechanisms are valid for different cancer entities and this will apparently also influence the dose response after radiation exposure. The individual differences certainly also have to be considered. Finally it can be concluded that a dose response without a threshold is probably plausible only under the assumption that the stochastic effects develop from the one damaged, mutated or malignantly transformed cell. For the development of genetic, heritable effects this appears quite plausible but for the causation of cancer this is certainly an open question.

# Attributing health effects to ionizing radiation exposure and inferring risks

**Wolfgang-Ulrich Müller**

*Institute of Medical Radiation Biology, University Hospital Essen, Germany*

Ionizing radiation has the potential to cause various health effects. Among others: skin burns, epilation, sterility, cancer, leukaemia, cataracts, circulatory diseases, malformations, and mental retardation. Those effects are divided as far as possible into deterministic and stochastic for practical reasons in radiation protection. In particular, cancer, cataracts and circulatory diseases are very frequent, so that people will be diagnosed with those health problems quite often. In a number of cases the question is put forward, whether the health problem was caused by radiation. The answer is sometimes very difficult or even impossible to give. The answer is comparatively easy in the case of so-called deterministic effects, that is effects related to massive cell death and/or functional inactivation of many cells. In that case, the health effects observed like skin burns or epilation usually can be attributed to radiation or it can be stated that ionizing radiation was not responsible. This is possible, because the sequence of events of deterministic effects after radiation exposure is well known and can be used to relate the effect to radiation. Also, if it was a partial body exposure, the size of the radiation field is very informative. This is very different for so-called stochastic effects (cancer, leukaemia, hereditary diseases). One can attribute such effects to radiation, when populations of a considerable size are studied. But it is not possible to attribute them when individuals are affected. The major reason for this inability has to do with the fact that there is no biomarker known that indicates that this specific cancer was or was not caused by radiation. The only way to get some insight on the individual level is to calculate a probability of causation (better called “assigned share”). This probability is derived from studies of populations.

# Research for Radiation Protection

**Ulrike Kulka**

*Federal Office for Radiation Protection, Department of Radiation Protection and Health,  
Germany*

There is the necessity for radiation protection of humans, focussing on different backgrounds such as occupational, medical or environmental radiation exposure. While “ALARA” is the basic principle for handling ionising radiation, continuous research is required for optimisation of radiation protection by providing basic information to improve guidelines and legal regulations. Up to now there are various open questions regarding the effects of ionising radiation on humans and their environment and meanwhile institutions and initiatives on national and international level are co-operating in this field. Among the most pressing research areas for radiation protection are topics as ☐ individual radiation sensitivity (based on individual DNA or on group level such as age, gender, lifestyle, health stage) ☐ the effects of the dose and dose rate of ionising irradiation, especially low dose effects ☐ non cancer effects, such as cataracts and cardiovascular diseases. Also important in this regard is the easy access to research infrastructures and availability of education & training for the maintenance of competences in these fields. These topics are addressed and funded on national level by radiation protection agencies, research centres and special initiatives/organisations such as the KVFS in Germany. On European level platforms have been initiated such as MELOD (focussing on low dose effects), ALLIANCE (focussing on radio-ecology), NERIS (focussing on radiological and nuclear emergencies), EURADOS (focussing on dosimetry) and EURAMED (focussing on radiation protection in medicine). Within their activities is the identification and priority setting of research topics, and the interaction with funding agencies and authorities to its best.

# A 9-feature genomic copy number signature is associated with radiation exposure in post-Chernobyl breast cancer

C. Wilke, Herbert Braselmann, J. Heß, H. Zitzelsberger, S. Klymenko and K. Unger

*Research Unit Radiation Cytogenetics, Helmholtz Zentrum München, Munich, Germany*

Breast cancer is one of the most common cancers worldwide with more than one million women being affected per year. In addition to risk factors such as age and lifestyle, exposure to ionizing radiation is known to increase breast cancer risk. Further, DNA copy number alterations (CNA) are known to be involved in the process of cancer development. The main aim of our study was to detect a signature of genomic copy number changes allowing discrimination of radiation exposed and non-exposed breast tumours. CNA regions were determined by array comparative genomic hybridization (aCGH) from formalin-fixed paraffin-embedded (FFPE) breast cancer tissue samples (n=136) of 68 female Ukrainian patients that were exposed to radiation after the Chernobyl reactor accident and a matched set of 68 non-exposed Ukrainian patients. A genomic CNA signature was identified using multivariate logistic regression to establish a prediction model and a related risk score. For that purpose the data set was randomly split into a learning (n=68) and a validation set (n=68). Copy number changes in the model were selected by stepwise forward selection resulting in a signature containing 9 regions on chromosomal bands 7q11.22-11.23, 7q21.3, 16q24.3, 17q21.31, 20p11.23-11.21, 1p21.1, 2q35, 2q35, 6p22.2. For the validation set the related risk score was significantly associated with exposure to radiation ( $p < 0.05$ ). The risk score was not associated with any clinical characteristics of the patients such as hormone receptor status or known breast cancer markers, suggesting an independent association of the CNA signature with radiation exposure. The identified CNA signature may allow detection of radiation-induced breast cancers and pave the ground for specific identification and tailored treatment of secondary breast cancer.

# Age-related biological effects of dental cone-beam CT exposure

**Niels Belmans,** L. Gilles, M. Vranckx, I. Lambrichts, S. Baatout, R. Jacobs and M. Moreels

*Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium/Faculty of Medicine and Life Sciences, Biomedical Research Institute, Hasselt University, Belgium*

Cone Beam Computed Tomography (CBCT) is a multipurpose radiographic tool for diagnosis, treatment planning, follow-up and research in dental practice, mostly in the field of pediatric orthodontics. Like conventional CT, CBCT uses X-rays to generate anatomical images. Although CBCT is considered a low dose imaging modality, it is uncertain that using CBCT is completely without risk. Investigating these low dose effects is of particular interest in pediatric CBCT examinations, since children are known to be more radiosensitive than adults. As part of the European OPERRA-funded DIMITRA project the potential biological effects of CBCT both in vitro and ex vivo samples were investigated. The main focus is placed on pediatric patients, but adult samples are included to check for age-dependent differences. In the in vitro part low dose X-radiation-induced (0, 5, 10, 20, 50 and 100 mGy) effects were studied in dental stem cells (stem cells from the apical papilla, dental pulp stem cells and dental follicle stem cells) from three pediatric donors. DNA damage repair and repair kinetics were analysed by microscopical visualization of DNA double strand break (DSB) markers ( $\gamma$ H2AX/53BP1) 30 minutes, 1 hour, 4 hours and 24 hours after irradiation. For the ex vivo study oral mucosal cells and saliva samples were collected from consenting patients. DNA damage and repair kinetics are analyzed by microscopical visualization of DNA DSB markers ( $\gamma$ H2AX/53BP1) in exfoliated oral mucosal cells collected just before and after (30 minutes and 24 hours) CBCT exposure. Saliva was used in a pilot study to detect local changes in oxidative stress levels (8-OHdG secretion and total antioxidant capacity) in the oropharyngeal region and salivary glands induced by CBCT. Sample collection occurred just before and 30 minutes after CBCT exposure. Preliminary in vitro data show that there is a dose dependent increase in the amount of DNA DSBs 30 minutes and 1 hour post-irradiation for doses higher than 20 mGy. This damage is resolved 24 hours post-irradiation. These results are similar in three different stem cell types originating from three different pediatric donors. DNA damage analysis in oral mucosal cells from adult and pediatric patients reveals that no significant increases in the amount of DNA damage can be detected after CBCT examination. The amount of DNA DSBs is significantly higher in children than in adults before and 30 minutes after CBCT exposure. Finally, data from adult patients shows that 8-OHdG levels do not significantly increase in saliva after CBCT examination. The antioxidant capacity of saliva, however, decreases significantly in these patients. The results from pediatric patients show a significant increase in the amount of 8-OHdG after CBCT exposure and, contrary to the adult patients, a significant increase in total antioxidant capacity. In conclusion, preliminary data indicate that low dose X-rays induce increases in DNA damage in vitro, but CBCT examination does not lead to increased DNA damage in oral mucosal cells in adult and pediatric patients. Finally, pediatric patients show increased salivary 8-OHdG levels after CBCT examination combined with a slightly increased total antioxidant capacity, whereas adults show a significant decreased total antioxidant capacity. Our preliminary data indicate that adults and children react differently to CBCT exposure. By gaining more insight into the biological effects following CBCT exposure current guidelines for CBCT imaging can be adapted, leading to an improved radiation protection of the patient.

**Acknowledgements:** The DIMITRA project has received funding from the FP7-OPERRA project under grant agreement n°604984. Niels Belmans is the recipient of a UHasselt-SCK•CEN PhD grant.

# Radiation protection for human space flights

**Christine Hellweg,** C.Baumstark-Khan and T. Berger

*German Aerospace Centre (DLR), Institute of Aerospace Medicine, Radiation Biology  
Department, Cologne, Germany*

Space is a special workplace not only because of microgravity and the dependency on life support systems, but also owing to a constant considerable exposure to a natural radiation source, the cosmic radiation. Galactic cosmic rays (GCR) and solar cosmic radiation (SCR) are the primary sources of the radiation field in space. Whereas the GCR component comprises all particles from protons to heavy ions with energies up to  $10^{11}$  GeV, the SCR component ejected in Solar Energetic Particle events (SPE) consists mostly of protons, with a small percentage of heavy ions with energies up to several GeV. In low Earth orbit, the exposure to GCR is  $\sim 100$ - $250$  x higher compared to sea level. This factor rises to  $\sim 770$  x for travel in the interplanetary space according to recent measurements on a journey to Mars. On a six month mission to the International Space Station (ISS), astronauts accumulate radiation doses exceeding the terrestrial occupational annual limit of 20 mSv by far. Astronauts experience a chronic whole body exposure with single energetic particles (electrons, protons,  $\alpha$ -particles and heavy ions) of GCR. Contrary to other workplaces, the exposure on ISS or in future, on exploratory missions, continues after end of the working hours. The main concerns resulting from this exposure are increased risks of cancer, cataract, neurodegenerative effects and infertility. The effective dose as a pre-requisite for the radiation risk assessment was determined from organ doses measured within a human anthropomorphic phantom (MATROSKHA) which was exposed four times on the ISS. The ratio of organ to skin dose determined in these experiments allows estimation of the effective dose based on personal dosimeters of the astronauts. In interplanetary missions, in addition to the chronic, in average low dose GCR exposure at low dose rate, an acute whole body exposure to a high radiation dose at a high dose rate can occur during a SPE with the risk of acute radiation sickness. While shielding (e.g. in a radiation shelter) is effective against SPE protons and high dose exposures can be prevented by alerts based on active dose monitoring and sun activity data from satellites, the chronic GCR exposure cannot be shielded completely during space travel. Currently, radiation protection for astronauts is based on risk management including reduction of exposure (limiting mission duration, shielding of sleeping quarters) and risk surveillance by radiation monitoring (area monitoring, personal dosimeters, SPE alert). Age, gender, genetic predispositions and health and immune status are factors determining individual sensitivity and might be considered for crew selection for interplanetary missions. Ameliorative actions, including prophylactic treatment in order to lower the risk for chronic diseases, are under research and can be summarized as general recommendations for a healthy lifestyle. The treatment of acute radiation sickness encompasses e.g. administration of colony stimulating factors and symptomatic medication (antibiotics, anti-emetics, anti-diarrheic, anti-inflammatory drugs). Risk assessment for space radiation exposure is incomplete and many uncertainties concerning the biological effects of GCR remain. This is also reflected by the different exposure limits space agencies have set for their astronauts. To improve space radiation protection, active space radiation dosimeters, space weather prediction methods, and efficient shielding have to be developed and radiation measurements, including depth dose distribution in the human body, e.g. on the way to Moon and on the Moon surface are required. Mitigation of the effects of heavy ions is one of the most important challenges to be solved for the exploration of the solar system. The biological effects have to be further characterized and risk models should be updated accordingly.

[christine.hellweg@dlr.de](mailto:christine.hellweg@dlr.de)



# Keynote Lecture 3

**Simon Boulton**

London, United Kingdom

*„Mechanics of homologous recombination and its  
exploitation in cancer therapy“*



# Mechanics of homologous recombination and its exploitation in cancer therapy

**Simon J. Boulton**

*The Francis Crick Institute & VP Science Strategy, Artios Pharma Ltd., United Kingdom*

Homologous recombination (HR) is a highly conserved mechanism for the repair of DNA double-strand breaks and stalled or collapsed replication forks. HR is characterized by a remarkable homology search and strand exchange reaction catalysed by the RAD51 recombinase when it is assembled onto single-stranded DNA (ssDNA). Formation of a helical nucleoprotein filament allows searching for and invasion of homologous double-stranded DNA (dsDNA) to form joint molecules. Repair DNA synthesis from the 3' end of the invading strand then copies the correct sequence information from the intact duplex, before resolution of the joint molecules completes repair. Failure to efficiently execute HR repair is associated with genome instability and cancer development, as well as the severe congenital disorder Fanconi anemia. To ensure genome stability, HR is subject to both positive (eg. BRCA2, Rad51 paralogs) and negative regulation (eg. RTEL1, RECQ5) by co-factors and antirecombinases. Recent insights into the mechanism of action of the Rad51 paralogs and RTEL1 in controlling HR will be discussed. In addition, on going efforts to exploit HR-deficiencies in cancer will be presented with a focus on the development of first in class Pol-theta inhibitors.

***Simon.Boulton@crick.ac.uk***

# Plenary Session 2

## „DNA Damage Response and DNA Repair“

### **Markus Löbrich**

Darmstadt, Germany

*„ATRX promotes DNA repair synthesis and cross-over formation during homologous recombination“*

### **Heico van Attikum**

Leiden, Netherlands

*„Regulation and dynamics of DNA repair in a chromatin context“*

### **George Iliakis**

Essen, Germany

*„Logic and necessities in the repair of DNA double strand breaks in higher eukaryotes“*

### **Dik van Gent**

Rotterdam, Netherlands

*„Modulation of DNA repair enhances the ionizing radiation effect in anti-tumor treatment“*

### **Ester Hamond**

Oxford, United kingdom

*„Understanding and exploiting the hypoxia-induced DNA damage response“*





# ATRX promotes DNA repair synthesis and cross-over formation during homologous recombination

S. Juhász, A. Elbakry, A. Waizenegger, J. Spies and **Markus Löbrich**

*Darmstadt University of Technology, Darmstadt, Germany*

The chromatin remodeler ATRX (alpha-thalassemia/mental retardation X-linked) has crucial roles in preventing replication fork stalling, histone deposition and gene regulation. ATRX is frequently mutated in ALT-positive tumors which maintain telomere length by a specific form of homologous recombination (HR), termed alternative lengthening of telomeres. Surprising in this context, we demonstrate that ATRX-depleted human cells exhibit a defect in repairing exogenously-induced DNA double-strand breaks by HR. ATRX interacts with the HR protein Rad54 and operates downstream of the Rad51 removal step. DNA repair synthesis at exogenously induced DSBs was strongly diminished in ATRX-depleted cells suggesting that ATRX promotes repair synthesis during HR. Consistent with this model, ATRX interacts with PCNA and RFC-1, both of which are required for DNA synthesis. Moreover, ATRX depletion abolishes the formation of sister chromatid exchanges at DSBs revealing that it functions during HR pathways which involve cross-over formation. Since ATRX functions in unperturbed cells together with its chaperone DAXX to deposit the histone variant H3.3 in pericentromeric and telomeric regions, we next studied the role of DAXX and H3.3 during HR. Strikingly, DAXX- or H3.3-depleted cells exhibit identical HR defects as ATRX-depleted cells and both ATRX and DAXX function to deposit H3.3 during DNA repair synthesis. These findings suggest a model in which ATRX, DAXX and histone H3.3 constitute chromatin during DNA repair synthesis to promote extend repair synthesis and cross-over formation. Since cross-over formation is likely detrimental during HR by the ALT pathway, our findings provide an explanation for the frequent loss of ATRX, DAXX and H3.3 in ALT-positive tumors.

# Regulation and dynamics of DNA repair in a chromatin context

**Haico van Attikum**

*Leiden University Medical Center, Leiden, Netherlands*

Exposure of cells to ionizing radiation can lead to the formation of chromosomal DNA double-strand breaks (DSBs). These lesions provoke a multitude of cellular responses, including cell cycle checkpoints and DNA repair. Failure to repair DSBs can lead to cell death, as well as mutations and genome instability, which contribute to human diseases such as cancer. Efficient recognition and repair of DSBs, however, is complicated by the fact that genomic DNA is packaged in chromatin. The DNA repair machinery has to circumvent this barrier to gain access to the damaged DNA and repair the lesions. By using a cross-disciplinary approach that combines cutting-edge genomics with bioinformatics, genetics, biochemistry and high-resolution microscopy, we identified and characterized several chromatin-modifying enzymes (e.g. PARP1, CHD2 and RNF168) in DSB repair. This way we uncovered several novel mechanisms that regulate the repair of DSBs in chromatin. At the meeting, I will present some of our recent findings and indicate the current status of our work.

# Logic and necessities in the repair of DNA double strand breaks in higher eukaryotes

**George Iliakis**

*Institute of Medical Radiation Biology, University of Duisburg-Essen, Essen, Germany*

Eukaryotic cells respond to DNA damage by activating a network of biochemical pathways recognizing the damage and initiating responses leading to repair, apoptosis/autophagy or senescence. This network of responses is commonly referred to as the “DNA damage response” (DDR). Among the plethora of lesions generated in the DNA from various physical and chemical agents, DNA double strand breaks (DSBs) are the most severe inducing strong DDR. This strong response is explained by the documented fact that they bear high risk for cell death, or genomic alterations ultimately causing cancer. Cells of higher eukaryotes process DSBs utilizing at least three distinct repair pathways, homologous recombination repair (HRR), DNA-PK-dependent, classical non-homologous end-joining (c-NHEJ) and an alternative pathway (alt-EJ) thought to operate as backup to c-NHEJ and HRR. HRR relies on a sister chromatid as template to restore integrity in the DNA during the S- or G2-phases of the cell cycle, and is error-free on every count. c-NHEJ mediates fast joining of broken DNA ends to ensure chromosome integrity through a highly efficient and well-coordinated molecular machine, albeit without ensuring the restoration of DNA sequence at the DSB, or the joining of the original ends. Alt-EJ is slower than c-NHEJ and carries higher probability than c-NHEJ for sequence alterations at the junction and for the joining of unrelated DNA ends to generate chromosomal translocations. Thus, in a hierarchical categorization of the DSB repair pathways, alt-EJ will have the highest propensity for errors and HRR the lowest; therefore they are not equivalent options for the processing of a given DSB. This raises critical questions regarding the “logic” of repair pathway choice and makes selection on stochastic grounds unlikely. We will summarize results suggesting that cells evolved mechanisms allowing them to select error-free repair pathways as first instance in the repair of DSBs. Furthermore, we will provide evidence that the type of lesion particularly in the form of thermally labile DSBs, or DSB clusters, have profound effect on DSB repair pathway choice.

Work supported by grants from BMBF and BMWi.

# Modulation of DNA repair enhances the ionizing radiation effect in anti-tumor treatment

**Dik van Gent**, J. Nonnekens, T. G. Meijer, N. S. Verkaik, W. Zhang and P. ter Brugge

*Erasmus MC, Department Molecular Genetics, Rotterdam, the Netherlands*

Cancer is a disease of the genes, where an accumulation of several genetic alterations leads to uncontrolled cell growth. One of the early steps that transform a normal cell into a cancer cell is often a defect in repair of DNA damage. Such a DNA repair defect leads to increased genetic instability, which in turn shortens the time it takes to accumulate the various mutations that are necessary to develop into a full blown cancer. Interestingly, DNA damage is not only at the basis of carcinogenesis, but DNA damaging agents are also frequently used to combat cancer. The mechanistic background of this was originally thought to be a difference in proliferation between normal and cancer cells. However, this may be only part of the story and inherent DNA repair defects in tumors can probably (at least partially) explain tumor sensitivity differences. The DNA repair defect that originally caused the cancer may thus become the Achilles' heel of the tumor. One of the best known DNA repair defects is BRCA gene mutations in breast and ovarian tumors. This has become the first example of a DNA repair defect that can be combated by targeting another DNA repair enzyme, PARP1. PARP inhibitors can be used alone or in combination with other treatments in BRCA deficient tumors, but they can also sensitize tumor cells without BRCA mutations. We showed that peptide receptor radionuclide therapy combined with PARP inhibitors is very efficient to kill SSTR2 overexpressing neuroendocrine tumors and we expect that this principle will be useful in other tumors (e.g. PSMA positive prostate tumors). Also combinations with other inhibitors may be useful strategies to increase anti-tumor effect of targeted radionuclide therapy. Furthermore, we are developing functional assays to test DNA repair pathway deficiencies in tumor slices and organoids. After clinical validation of their predictive value, we expect that these assays will help to assign the most effective treatment to each individual patient.

# Understanding and exploiting the hypoxia-induced DNA damage response

**Ester Hammond**

*CRUK/MRC Oxford Institute for Radiation Oncology, Department of Oncology, The University of Oxford, Oxford, United Kingdom*

Exposure to severe levels of hypoxia, sometime referred to as radiobiological hypoxia, leads to the induction of the DNA damage response. As cancer cells in radiobiological hypoxia are significantly more resistant to radiation-induced DNA damage they negatively impact radiotherapy response. The aim of our work is to investigate the biological response and adaption by cancer cells to hypoxia, with the ultimate aim of developing new therapeutic strategies through the identification of critical molecular targets. Recently, we have demonstrated that the hypoxia-induced DNA damage response is a result of replication stress, due to diminished ribonucleotide reductase activity in the absence of oxygen. Despite the presence of replication stress and a DNA damage response in hypoxia, we have not observed the accumulation of DNA damage. This suggested that replication was maintained and hence replication forks protected in hypoxia. We have demonstrated that in response to hypoxia the stress-responsive small subunit of ribonucleotide reductase (RRM2B) is induced and that this protein has a number of attributes which afford it improved activity in the absence of oxygen. Consequently, loss of RRM2B leads to increased sensitivity to hypoxia. This study and the potential of targeting ribonucleotide reductase will be discussed.







# Session S07

## „DNA Damage Response 2“

### **Carsten Herskind**

Mannheim, Germany

*„A possible role of cell-cycle checkpoints in radioresistance and the shape of cell survival curves: Implications for lethal damage repair and dose rate effects“*

### **Wael Mansour**

Hamburg, Germany

*„Loss of PTEN-assisted G2/M checkpoint impedes homo-logous recombination repair and enhances radio-curability and PARP inhibitor treatment response in prostate cancer “*

### **Emil Mladenov**

Essen, Germany

*„CRISPR/Cas9 based model system to investigate the repair of DSB clusters with increased complexity“*

### **Michael Ensminger**

Darmstadt, Germany

*„Nek1 coordinates the repair of one ended DSBs with cell-cycle progression“*

### **Stefanie Mosel**

Essen, Germany

*„A novel role for the anti-apoptotic protein Survivin in DNA repair and replication“*

### **Ramon Lopez Perez**

Heidelberg, Germany

*„Towards a better understanding of the ultrastructural organization of DNA double-strand break repair foci“*



# A possible role of cell-cycle checkpoints in radioresistance and the shape of cell survival curves: Implications for sublethal damage repair and dose rate effects

**Carsten Herskind**, X. Liu, Y. Zhang, Q. Liu, L. Ma, E. Angelie, J. Liu, F. Wenz and M. R. Veldwijk

*Department of Radiation Oncology, Medical Faculty Mannheim, Mannheim, Germany*

**Purpose:** Radioresistance of tumours is a major obstacle to successful radiotherapy. Radioresistant cancer cells show typical shouldered cell survival curves with a shallow slope at low doses and downward curvature at higher doses. By contrast, primary normal skin fibroblasts and p53 wildtype lymphoblastoid TK6 cells show essentially linear survival curves with steeper initial slopes. According to conventional biophysical models this suggests that the fraction of intrinsically lethal lesions is lower in cancer compared with normal cells. Understanding the basis of this difference should help identify molecular targets in radioresistant cancer cells. The purpose was to test if progression through cell-cycle checkpoints may play a role in radioresistance and the shape of cell survival curves. **Materials and Methods:** Human U251 glioblastoma cells (homozygous for a common mutation in the p53 gene) and normal diploid skin fibroblast strain, GS4 (p53 wildtype), represented radioresistant cancer cells and normal cells, respectively. Human lymphoblastoid TK6 cells (p53 wildtype), TK6E6 (p53 suppressed by transduction of the HPV-E6 gene), and WTK1 (p53 mutant) were used to analyse the effect of p53 in related cell lines. Cellular radiosensitivity was measured by the colony formation assay. Cell-cycle progression and radiation-induced checkpoints were analysed by flow cytometry of BrdU pulse-labelled cells. Proliferating cells were identified by human antigen Ki-67, DNA double-strand breaks were quantified by gamma-H2AX foci, apoptosis by sub-G1 fraction of cells, and micronuclei by Hoechst staining. **Results:** Cell-cycle analysis of U251 glioblastoma showed a temporary (4-5h) arrest in G2/M but no radiation-induced G1/S arrest after irradiation with 2 Gy of 6 MV X-rays. Only approximately 20% of the cells were inactivated by this dose and the surviving cells continued to proliferate without evidence of permanent arrest or apoptosis. However, Ki-67 positive cells showed residual DSB repair foci and increased micronuclei levels 2-3 days after irradiation suggesting continued proliferation in spite of residual damage. By contrast, >70% of normal fibroblasts were inactivated by permanent cell-cycle arrest without residual DSBs after similar doses. This paradox suggested that progression through cell-cycle checkpoints may play a role in radioresistance. The role of p53 was studied in radiosensitive lymphoblastoid TK6 cells showing radiation-induced apoptosis. A temporary G1/S block in TK6 was abrogated in TK6E6 and progression through S phase was delayed. However there was little effect on radiosensitivity with only a slightly decreased linear slope of the survival curve in spite of less apoptosis than for TK6. Intriguingly, a fraction of the cells were permanently arrested after 2-3 cell divisions in both cell lines as determined by labeling experiments. By contrast, p53-mutated WTK1 cells are radioresistant with a shouldered survival curve and showed no G1/S arrest, little apoptosis, no permanent cell-cycle arrest, and continued to proliferate after a short G2/M arrest, mimicking the phenotype of U251. **Conclusions:** Taken together, these results support the hypothesis that cellular radioresistance at conventional fraction sizes of 2 Gy may be associated with a damage-tolerant G2/M cell-cycle checkpoint combined with a defect in a genomic stability checkpoint allowing cells to proliferate in spite of residual lesions. Since suppression of wild type p53 expression did not reproduce this phenotype, the defective checkpoint(s) may be associated with a dominant-negative mutation or a gain-of-function rather than loss-of-function of p53, or with mutation of a different gene. These findings have potential implications for dose rate effects related to sublethal damage repair and for the biological effects of high versus low doses. Further mechanistic studies into the role of cell-cycle checkpoints for radiation sensitivity are warranted.

[carsten.herskind@medma.uni-heidelberg.de](mailto:carsten.herskind@medma.uni-heidelberg.de)

# Loss of PTEN-assisted G2/M checkpoint impedes homologous recombination repair and enhances radio-curability and PARP inhibitor treatment response in prostate cancer

**Wael Mansour**, P. Tennstedt, J. Volquardsen , C. Oing, M. Kluth, R. Simon, C. Petersen, E. Dikomey and K. Rothkamm

*Lab of Radiobiology and Experimental Radiation oncology, University Medical Center  
Hamburg-Eppendorf*

Here we aimed to clarify the role of PTEN (Phosphatase and tensin homolog deleted on chromosome 10) in the repair of DNA double-strand breaks (DSB). We report that PTEN deficiency does not affect DSB repair via NHEJ but does contribute to HR. This is evidenced by PTEN depletion-mediated (i) inhibition of HR in a reported plasmid (ii) enhanced sensitivity to MMC or olaparib-mediated PARP inhibition and (iii) reduced RAD51 loading at X-ray-induced DSBs. No association was observed between PTEN status and RAD51 expression either in vitro in PTEN-deficient cells or in vivo in a tissue microarray of 1500 PTEN-deficient prostate cancer samples. Immunofluorescence and immunoblotting analyses demonstrated that PTEN depletion and sustained activation of AKT sequester CHK1 in the cytoplasm, thus impairing the G2/M checkpoint after irradiation, as evidenced by a significant increase in the mitotic index of cells after PTEN knockdown. We report for the first time that AKT inhibition recovers the G2/M checkpoint and restores HR efficiency in PTEN-depleted cells. PTEN loss is a marker for worse prognosis in prostate cancer. Here, we show for the first time that PTEN loss may predict for improved response of PC patients to radiotherapy. Further, given its contribution to HR, we provide evidence for the use of PTEN as a biomarker for predicting the response to PARP inhibitors as radiosensitizing agents in prostate cancer. Collectively, these data contribute to the understanding of PTEN's role in DSB repair via HR and implicate PTEN in maintaining genomic stability by delaying S- and G2-phase progression of damaged cells. This is achieved through regulating the cellular localization of CHK1, thus allowing time for DSB repair by HR before exit to mitosis. Furthermore, our findings are potentially of clinical importance for prostate cancer patients as they identify PTEN status in prostate cancer as a putative predictor of (i) radiotherapy response and (ii) response to treatment with PARP inhibitor olaparib alone or combined with radiotherapy.

# CRISPR/Cas9 based model system to investigate the repair of DSB clusters with increased complexity

**Emil Mladenov** and G. Iliakis

*Institute of Medical Radiation Biology, University Duisburg-Essen, Medical School, Essen, Germany*

From the variety of DNA lesions induced by ionizing radiation, double-strand breaks (DSBs), are known to provoke the most spectacular DNA damage responses affecting almost every aspect of the cellular metabolism. DSBs emerge through diverse mechanisms, and are classified in subgroups of different complexity. According to this classification, the simplest form is induced enzymatically, whereas the most complex comprises DSB clusters, which are expected to be induced mainly by high-LET radiation. Unrepaired or incorrectly processed DSBs severely affect cellular viability or result in genome instability and accelerated tumor genesis. To reduce such risks, cells of higher eukaryotes have evolved several DSB repair mechanisms differentiated by their diverse efficiency and discrete repair accuracy. Indeed, DNA-PKcs dependent non-homologous end joining (D-NHEJ), homologous recombination mediated repair (HRR), and alternative end-joining pathway (alt-EJ), operating as a backup (B-NHEJ), function in parallel to coordinate the repair of DSBs throughout the cell cycle and in different stages of cellular growth. Despite a large amount of data, revealing a role for all three repair pathways in elimination of DSBs with low complexity, there is limited information, of how DSB clusters are repaired in the contest of chromatin and what the consequences of their improper processing are. Moreover, DSB clusters generated during high-LET radiation exposure are expected to pose additional barrier to the current functions of all repair pathways, due to the general destabilization of the chromatin structure. However, direct test of this hypothesis requires specific biological systems. Specifically, CRISPR/Cas9 technology was utilized in order to induce DSB clusters of different complexity within the Exon 3 of a human HPRT locus and the fluctuations in mutation frequency at the corresponding locus were assessed as a function of increased DSB cluster complexity. Moreover, to determine the mechanisms involved in the repair on DSB clusters, the effect of small molecular compounds, inhibiting the activity of key DSB repair proteins (ATM, ATR, DNA-PKcs, PARP-1 and Rad51), was additionally validated. Our results clearly demonstrated that the increased DSB cluster complexity significantly increase mutations formations, which might be attributed to the involvement in the DNA repair process of the highly error-prone alt-EJ pathway.

# Nek1 coordinates the repair of one-ended DSBs with cell-cycle progression

**Michael Ensminger**, J. Spies, A. Cruz-Garcia and M. Löbrich

*Radiation Biology and DNA Repair, Darmstadt University of Technology, Germany*

In our recently published paper Spies et al. 2016 (Mol Cell. 2016 Jun 16;62(6):903-17.) we introduced the kinase Nek1 as a new factor for Homologous Recombination (HR), one of the two repair pathways to repair DNA double-strand breaks (DSBs). An important step during this process is the removal of Rad51 by Rad54 after strand invasion to allow DNA synthesis to start. We showed that Rad54 is activated by Nek1 via phosphorylation on Serin 572. If Nek1 is depleted or if the phosphorylation of Rad54 is inhibited, Rad51 cannot be removed and the HR process cannot continue. In addition to their role in repairing DSBs, HR factors also exert important functions in protecting stalled replication forks and their absence leads to degradation of newly synthesized DNA and genomic instability. Interestingly, Nek1 phosphorylates Rad54 specifically in the G2 phase of the cell cycle. This promotes Rad51 removal and allows the completion of HR. The absence of Rad54 phosphorylation during S phase, in contrast, prevents removal of Rad51 from stalled replication forks and ensures fork protection. Therefore, we concluded that Nek1 regulates Rad51 removal to orchestrate HR and replication fork stability. HR is the predominant pathway for dealing with one-ended DSBs arising from replication fork collapse. The observation that Nek1 activates Rad54 in a G2-specific manner raised one important question: How are one-ended DSBs repaired by HR if the removal of Rad51 is prevented during S phase? To address this question, we investigated the repair of DSBs induced at different times in S phase by the Topoisomerase 1 inhibitor Camptotecin (CPT) using the  $\gamma$ H2AX immunofluorescence analysis. After CPT treatment in early S phase, the number of  $\gamma$ H2AX foci remained at a constant level for several hours and only decreased at very late times. By staining with the S-phase marker PCNA, we monitored the transition of cells from S to G2 phase. Strikingly, the decline of foci numbers occurred at the same time when cells progressed from S to G2 phase. This result was observed after different concentrations of CPT and in different cell lines. In contrast, if cells were treated with CPT in late S phase, DSB repair was detected almost immediately. Finally, we observed that CPT-induced DSBs remained unrepaired when the transition to G2 phase was prevented by the replication inhibitor aphidicolin. Taken together, our results strongly support a new model for the repair of one-ended DSBs: The repair of such DSBs is initiated during S phase and early steps of HR like resection and Rad51 loading are performed in S phase. However, late steps of HR including Rad51 removal and DNA repair synthesis are postponed until cells have progressed to G2 phase. Thus, the HR process at one-ended DSBs is synchronized in a cell-cycle dependent manner and this synchronization is regulated by the G2-specific activation of Rad54 by Nek1.

# A novel role for the anti-apoptotic protein Survivin in DNA repair and replication

**Stefanie Mosel**, E. Schröder and S. Knauer

*Molecular Biology II, University Duisburg-Essen, Germany*

Survivin is a cancer-associated protein first identified as a member of the inhibitor of apoptosis protein (IAP) family and later shown to be a fundamental protein for genomic stability during mitosis. In cancer, a markedly increased Survivin expression is associated with tumor resistance against chemo- and radiotherapy, thus designating it an attractive therapeutic target. In contrast to its well-studied role during mitosis, little is known about the functional role during interphase, especially during replication. We could detect Survivin expression already during S phase, and thus significantly before its previously described stringently regulated increase during G2M transition. Functionally, knockdown of Survivin leads to a reduction in replication fork speed as investigated by DNA fiber assay analyses. During S phase, the chromosomal passenger complex (CPC) proteins Survivin, Aurora B kinase, Borealin and INCENP accumulate in distinct nuclear foci co-localizing with the replication associated protein PCNA. An in situ proximity ligation assay (PLA) revealed a direct interaction of PCNA and the CPC. Also co-immunoprecipitation analyses suggest a biochemical interaction of PCNA with the members of the CPC during S phase. Indeed, a potential PCNA binding motif could be identified within the inner centromere protein INCENP, resembling interaction sites already known for e.g. p21, the so-called PIP box motif. In sum, our data indicate a novel, previously unknown function for Survivin and the other CPC members apart from mitosis, once more underlining the pivotal role of Survivin in the context of genomic instability and tumorigenesis.



# Towards a better understanding of the ultrastructural organization of DNA double-strand break repair foci

**Ramon Lopez Perez**, E. Mladenov, V. Mladenova, KJ., Weber, J. Engelhardt, G. Iliakis and P. Huber

*CCU Molecular and Radiation Oncology, German Cancer Research Center, Heidelberg, Germany*

**Introduction** We have previously shown that carbon ion radiation-induced foci of the DNA double-strand break (DSB) marker  $\gamma$ H2AX are composed of several smaller subfoci, but their meaning is still unclear. Given that high linear energy transfer (LET) radiation is believed to induce complex DNA lesions, including DSBs in close proximity, it is possible that these subfoci represent individual DSBs. However, we have evidence that the smaller  $\gamma$ H2AX foci induced by low LET photons also consist of several (yet fewer) subfoci, despite that photon radiation is believed to generate isolated DSBs. Therefore, we have investigated the ultrastructure of  $\gamma$ H2AX and other DSB repair foci at isolated DSBs and DSB clusters of increasing complexity, using I-SceI restriction endonuclease based transfection constructs. **Methods** CHO cells were transfected with constructs containing I-SceI recognition sequences that allow induction of isolated DSBs or DSB clusters depending on the combination of I-SceI sites at specific distances and orientations (3 variants with 2 DSBs and one with 4 DSBs within ~500 bp of DNA). Transfection efficiency and clonogenic survival was tested and the remaining cells were fixed and stained for different DSB repair markers, including  $\gamma$ H2AX and pDNA-PKcs (Ser 2056). Samples were imaged using the two optical superresolution techniques stimulated emission depletion (StED) and single molecule localization microscopy (SMLM). **Results** The cells transfected with any of the constructs showed  $\gamma$ H2AX, as well as pDNA-PKcs foci consisting of several smaller subfoci. Compared to the constructs with 2 DSBs, the single-DSB construct induced smaller  $\gamma$ H2AX and pDNA-PKcs foci made up of lesser subfoci and the 4-DSB construct induced bigger foci of both markers that were composed of more subfoci. **Discussion** Our results support that the size of DSB repair foci (as detected with a conventional microscope) correlates with the number of DSBs at a certain distance range and are in principle agreement with the belief that high LET radiation causes more densely clustered DSBs than low LET radiation. The size of the  $\gamma$ H2AX, as well as pDNA-PKcs foci correlated with the number of subfoci they were composed of, but our data speak against the idea that each subfocus represents an individual DSB. Hence, it seems more likely, that these subfoci have a more general role for the organization of the DNA repair processes. Notably, our data also suggest that DNA-PK does not exclusively bind to the actual open ends of a DSB, where active repair takes place. It must be stated however, that our results are still preliminary.

# Session S08

## „Particle Radiation Modalities 1“

### **Beate Timmermann**

Essen, Germany

*„The pediatric program at the West German Proton Therapy Centre Essen (WPE): Aiming to reduce late effects and secondary malignancy induction“*

### **Thomas Schmid**

Neuherberg, Germany

*„Proton minibeam radiation therapy is a novel approach to minimize normal tissue damage“*

### **Sara Timm**

Homburg, Germany

*„Clustering of double-strand breaks following high-LET irradiation perturbs DNA repair and is associated with long-lasting chromatin decondensation“*

### **Katrien Konings**

Mol, Belgium

*„How do different radiation qualities affect the Hedgehog signaling pathway?“*

### **Ralf Kriehuber**

Jülich, Germany

*„Geno- and cytotoxicity of DNA associated Auger electron emitters“*

### **Elke Beyreuther**

Dresden, Germany

*„Analysing methods of animal irradiation experiments with deviations from prescribed dose“*



# The pediatric program at the West German Proton Therapy Centre Essen (WPE): Aiming to reduce late effects and secondary malignancy induction

T. Steinmeier, H. Thomas, C. Plass, S. Plaude, D. Geismar, M. Stickan Verfürth, C. Blase, G. Fleischhack and **Beate Timmermann**

*West German Proton Therapy Centre Essen (WPE), Essen, Germany; West German Cancer Centre, University Hospital Essen, Germany*

Background Proton beam therapy (PT) has experienced an increasing interest in pediatric oncology. It is an attractive modality in the treatment of tumors in contiguity to critical structures. The WPE offers modern PT since June 2013 and has experience with an outstandingly large cohort of pediatric patients in an interdisciplinary environment. We report results on feasibility, survival, tumor control and toxicity of PT in childhood cancer. Patients and Methods Between August 2013 and May 2017, 424 children aged from 0.9 to 17.9 years (median 6.0 years) were enrolled into the standardized prospective registry (KiProReg) at WPE. All children were either treated within the diagnosis-specific treatment guidelines of the multidisciplinary protocols of the Society for Pediatric Oncology and Hematology (GPOH), the International Society of Pediatric Oncology (SIOP) or according to the in-house standards which are based on recent studies and international standards. Diagnoses categories were central nervous system (CNS) tumors (81.6%), sarcomas (34.4%), nerve tumors (1.9%), head and neck tumors (1.7%), lymphomas (0.7%), and others (2.4%). The most common diagnoses were ependymoma (n=83), rhabdomyosarcoma (n=81), medulloblastoma (n=42) and AT/RT (n=29). Radiation sites were brain/head and neck (82.3%), pelvis (9%), spinal/paraspinal (8.5%) and thorax/abdomen (0.9%). Due to their young age, deep sedation was necessary in 64.9% of children. 67.2% of children presented with macroscopic residual tumor at time of PT. The median total dose was 54 Gy (range, 12-74 Gy). 195 (46%) of the children received concurrent chemotherapy. Baseline data, acute and subacute toxicities were classified according to CTCAE V 4.0. Results Median follow-up after first diagnosis was 1.5 years (range, 0.2-11.7 years). 347 patients (82%) showed disease control. Local, distant or both failure occurred in 37 (49%), 28 (37%) and 11 (14%) patients, respectively. 35 patients (8%) died so far due to local (n=15) or systemic progress (n=18). One patient died due to acute myeloid leukemia occurring during PT and one patient died due to multifactorial causes. PT was well-tolerated. During PT, new grade 3 toxicities in comparison to toxicities at baseline are exclusively observed in the organ classes blood and lymphatic (n=13), gastrointestinal (predominantly as oral mucositis) (n=9), general disorder class (n=3), anorexia (n=2) as well as skin and subcutaneous tissue (n=1). New documented grade 4 side-effects during PT were exclusively seen in the blood and lymphatic disorder class (n=4). All of them had received concomitant chemotherapy and one patient had also received craniospinal irradiation. Three months after PT, data on toxicity is available for 258 patients. Only a few patients showed grade 3 or 4 toxicities, predominantly in the blood and lymphatic disorder class (grade 3 n=4, grade 4 n=4). In addition, few grade 3 toxicities were observed in the fields of general disorder (n=1), anorexia (n=2) and hearing impaired (n=1). Long-term data after 12 and 24 months is available for 118 and 41 patients, respectively. One year after PT, grade 3 toxicities in the organ class blood and lymphatic disorders occurred in two patients. In one patient each, anorexia and lymphedema were reported. 24 months after PT, only two new grade 3 toxicities were documented (anorexia n=1, blood and lymphatic n=1). No grade 5 acute or late toxicities were observed at all. Conclusions Early data support good feasibility of PT in children at WPE. Early experiences are promising also in terms of tumor control. Patients require multidisciplinary care and treatments should be embedded in prospective studies and registries in order to collect prospective data on feasibility and tumor control. Patient referrals with regard to childhood cancer are increasing at our center. However, as for any other modern technique, long-term follow-up data is necessary to assess long-term outcomes.

[beate.timmermann@uk-essen.de](mailto:beate.timmermann@uk-essen.de)

# Proton minibeam radiation therapy is a novel approach to minimize normal tissue damage

**Thomas Schmid**, M. Sammer, J. Reindl, K. Ilicic, C. Greubel, D. Walsh, B. Schwarz, J. Wilkens, G. Dollinger and S. Combs

*Institute of Innovative Radiotherapy, Helmholtz Zentrum München, Neuherberg, Germany*

**Purpose** In Radiation Oncology, the maximum dose which can be delivered to a certain tumor is limited by the radiation induced damage in normal tissue surrounding the actual tumor. Proton minibeam radiotherapy aims to minimize normal tissue damage in the entrance channel while keeping tumor control through a homogeneous tumor dose due to channel widening with increasing track length. Acute side effects of proton minibeam irradiation were examined in an in-vivo mouse model to account for immune system, vasculature and higher complexity. In this study, partially widened proton minibeam sizes were simulated as they occur on their way to the tumor within the normal tissue. **Methods** A total of six different minibeam sizes were applied to the ear of Balb/c mice using 20 MeV protons. The average dose of 60 Gy was distributed in 4x4 minibeam sizes with beam sizes of  $\sigma = 0.09, 0.2, 0.31, 0.45, 0.56$  and  $0.9$  mm and a beam-to-beam distance of 1.8 mm. Inflammatory response, i.e. ear swelling and skin reactions, were monitored for 90 days following irradiation. **Results** The results show a link between the applied beam sizes and the dimension of acute side effects after irradiation. The largest beam sizes lead to significant ear swelling (up to 3-fold), erythema and desquamation 3-4 weeks after irradiation. With decreasing beam sizes, the maximum skin reactions were reduced until almost no ear swelling or other visible skin reactions to the irradiation could be detected. **Conclusion** Our results show that the tissue sparing effect of proton minibeam sizes is highest in the superficial parts of the beam channel whereas it is possible to create almost homogeneous irradiation in the tumor area, which could be used to obtain tumor control while sparing the surrounding healthy tissue. However, as even larger minibeam sizes have significantly reduced acute side effects than a broad beam, proton minibeam radiotherapy may offer various possibilities for new approaches in clinical proton and/or heavy ion radiotherapy. Supported by the DFG Cluster of Excellence: Munich-Centre for Advanced Photonics.

# Clustering of double-strand breaks following high-LET irradiation perturbs DNA repair and is associated with long-lasting chromatin decondensation

**Sara Timm**, Y. Lorat, B. Jakob, G. Taucher-Scholz and CE. Rube

*Department of Radiation Oncology, Saarland University, Homburg/Saar, Germany*

Background: High linear energy transfer (LET) radiotherapy offers superior dose conformity and biological effectiveness compared to low-LET radiotherapy, representing a promising alternative for radioresistant tumors. A prevailing hypothesis is that energy deposition along the high-LET particle trajectories induces DNA lesions that are more complex and clustered, especially in densely packed areas of chromatin, and are therefore more challenging to repair. However, the precise molecular mechanisms underlying the differences in radiobiological effects between high-LET and low-LET radiotherapies remain unclear. Material/methods: Human fibroblasts (interphase cells with homogenous chromatin density) were irradiated with high-LET carbon ions/calcium ions or low-LET photons. At 0.1, 0.5, 5 and 24h post irradiation, various proteins (53BP1, pATM, pKAP-1 and pKu70) involved in DNA damage response were visualized by immunofluorescence microscopy (IFM) and their DNA-damage pattern in the chromatin ultrastructure was characterized using transmission electron microscopy (TEM; immunogold-labelling). Results: IFM visualization of 53BP1 and pATM after low-LET irradiation revealed co-localizing foci and increasing foci areas over time. After 0.5h transient pan-nuclear signals of pATM and pKap-1 correlated, subsequently forming overlapping foci in heterochromatic regions after 5h. The high spatial resolution of TEM permitted the visualization of high-LET ion trajectories which were associated with local decondensed chromatin regions (DCRs) after 0.5h. These DCRs increased in number and size up to 5h and could still be visualized after 24h. In addition, repair proteins were visualized over time in the DCRs to investigate the analysis of local chromatin dynamics at the molecular level. Hereby, the accumulation of 53BP1 and pATM in increasing clusters over time was observed whereas pKap1 could only be detected after 5h in the heterochromatic border regions of the DCRs. While low-LET irradiation produced isolated DNA lesions, high-LET irradiation induced clustered DNA lesions with multiple DSBs along the heterochromatic periphery of the ion trajectories. In contrast, distinct chromatin dynamics such as the DCRs could not be detected after low-LET irradiation and most of the isolated DSBs were efficiently rejoined. Conclusions: The clustering of DSBs, especially in heterochromatin, following high-LET irradiation perturbs efficient DNA repair and is associated with long-lasting chromatin decondensation. These persistent changes in chromatin compaction may affect the structural and functional organization of the cell nuclei, leading to the greater biological effectiveness of high-LET irradiation versus that of low-LET irradiation.

# How do different radiation qualities affect the Hedgehog signaling pathway

**Katrien Konings**, A. Michaux, G. Nduva, S. Isebaert, K. Haustermans, S. Baatout and M. Moreels

*Radiobiology Unit, Institute for Environment, Health and Safety, Belgian Nuclear Research Centre (SCK•CEN), Belgium*

In recent years an increase is observed in the use of particle therapy (protons, carbon ions) for the treatment of cancer patients. This is in part because of the advantages of particles compared to X-rays, which include an increased biological effectiveness and improved sparing of healthy tissues. Metastasis is still an important cause of mortality in cancer patients and evidence shows that conventional radiotherapy could actually increase the formation of metastasizing cells. An important pathway implicated in the process of metastasis is the Hedgehog (Hh) signaling pathway. Recent studies demonstrated that activation of this pathway in response to photon exposure can lead to radioresistance and increased invasive and migratory capability of cancer cells. Currently, the impact of particle radiation on the Hh signaling pathway is unknown. In the present study the effect of different radiation qualities (photons, protons and carbon ions) on the Hh signaling pathway was investigated in prostate cancer cells (PC3) and paediatric medulloblastoma cells (DAOY). Experiments with X-rays were performed at SCK•CEN (Mol, Belgium) whereas experiments with protons were performed at the iThemba facility in South-Africa and with carbon ions at the Grand Accélérateur National d'Ions Lourds (GANIL) (Caen, France). Carbon ion irradiation was more effective in decreasing cell survival compared to X-rays (RBE10 = 1.9 for PC3 cells; RBE10 = 2.2 for DAOY cells). Preliminary RT-qPCR results demonstrated that genes involved in the Hh pathway were differentially affected after X-ray and carbon ion irradiation. In general carbon ion irradiation induced more pronounced changes in gene expression compared to X-ray irradiated samples. More specifically GLI3, SUFU and CCND1 had higher expression values at 24 h in carbon ion irradiated PC3 cells compared to the X-ray irradiated PC3 cells. For DAOY cells SHH, GLI2, CCND1 had higher expression values at 24 h after carbon ion irradiation compared to X-ray irradiated cells. Results from the Boyden chamber assay revealed that the migration capacity of DAOY cells decreased in a radiation dose dependent manner, with a more pronounced decrease of migration after proton and carbon ion irradiation compared to X-ray irradiation. The Hedgehog inhibitor GANT61 did not have a radiosensitizing effect in PC3 cells in combination with any of the radiation qualities. However, a radiosensitizing effect of GANT61 could be observed in the DAOY cells after carbon ion irradiation. In conclusion, exposure to low- and high-LET radiation induces cell line-dependent changes in the expression of several components of the Hh pathway. Moreover carbon ion irradiation affects the gene expression of the Hh pathway differently than X-rays, with carbon ions having a more pronounced effect on gene expression. Future experiments with Hh inhibitors will address whether modulation of the Hh pathway will differentially affect migration capacities of cancer cells after exposure to different radiation qualities.



# Geno- and Cytotoxicity of DNA-associated Auger electron emitters

**Ralf Kriehuber**, V. Dahmen, S. Schmitz, M., Unverricht-Yeboah and E. Pomplun

*Department of Safety and Radiation Protection, Research Center Jülich, Germany*

Theoretical considerations, Monte-Carlo simulations and experimental findings suggest that DNA-incorporated Auger electron emitters (AEE) cause primarily complex and clustered DNA lesions. It was previously shown that the shape of AEE-induced cell survival curves resembles that of High-LET irradiation and, therefore, poses the question of an increased biological effectiveness and a separate quality factor for Auger electrons. During electron capture or internal conversion an electron vacancy in an inner atomic shell is created. Filling the electron vacancy by a higher shell electron can initiate a process of non-radiative energy transmission, commonly termed as “Auger effect”. During the process numerous low-energy Auger electrons (up to 27 in the case of Iodine-125) with a short range are emitted leading to energy densities and free radical production in the close vicinity of the emitter exceeding that of a 5 MeV alpha-particle traversing the DNA double-helix. Experimental data demonstrates, that the cyto- and genotoxicity of AEE is comparable to low-LET radiation per unit dose when the AEE is exclusively located in the cytoplasm. However, in case of DNA- incorporation RBEs ranging from 5 – 9 are frequently reported. Employing the alkaline and neutral comet assay, the high DSB/SSB ratio of I-125-iododeoxyuridine derived from Monte-Carlo simulations could be experimentally confirmed. The unique properties of AEE and the possibility to target DNA in a sequence-specific manner using AEE-labeled Triplex-forming oligonucleotides (TFOs) enable to study the repair of complex DNA lesions at defined sites in more detail. A transgenic SCL-II p2RT strain carrying the stably integrated recoverable p2RT vector system harboring a specific triplex target sequence for TFO-p2RT will help to analyze the repair efficiency of complex DNA lesions regarding mutation frequency, mutation type and mutation localization.



# Analysing methods of animal irradiation experiments with deviations from prescribed dose

**Elke Beyreuther**, D. E. Passos, L. Karsch, S. Löck and J. Pawelke

*Helmholtz Zentrum Dresden – Rossendorf, Dresden, Germany; OncoRay – National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany*

**Introduction:** The development of new radiotherapy technologies is a long time process which requires proving the general concept although clinical requirements with respect to beam quality and controlled dose delivery may not yet be fulfilled. Exemplarily, the necessary radiobiological experiments with laser-accelerated ion beams, which promise to compact ion radiotherapy facilities, are challenged by low particle energy and fluctuating beam intensities delivered by currently available laser systems. The first issue was handled by establishing a small tumour model on mouse ear that allows full penetration by ~25 MeV proton beams [1], whereas the latter, i.e. the subsequent deviations of the delivered from the prescribed dose, should be considered mathematically. **Methods:** Based on tumour growth data and dose values obtained in a preceding in vivo trial comparing the biological efficacy of laser-driven and conventional LINAC electrons [2], different mathematical approaches to determine corresponding dose-response relationships were compared. During this experiment, the beam intensity fluctuations were not fully gathered by online dosimetry, which results in deviations of more than 10 % from scheduled dose as measured by retrospective absolute film dosimetry. Instead of classical averaging-per-dose point, which excludes animals with high dose deviations, multivariate linear regression, Cox regression and a Monte Carlo based approach were tested as alternatives to include all animals in statistical analysis. **Results:** The application of different mathematical approaches to the same set of experimental tumour growth data and dose values led to similar results, revealing a comparable radiobiological efficacy of laser-driven and conventional LINAC electrons. Although the inclusion of those animals that were previously excluded because of more than 10% dose deviation did not change the experimental conclusion, the new mathematical approaches allowed for including all animals in the analysis. Comparing the different approaches, multivariate linear regression and Cox regression were considered as most feasible for future analysis, since they were already implemented in commercial statistical software, like SPSS (IBM). **Conclusion:** The previously established small animal tumour model on mouse ear [1] together with the recently tested regression methods enable the investigation and evaluation of beams at new accelerators relative to their conventional equivalents despite their still limited beam stability, like laser-driven particle beams. The tested mathematical approaches allow for increasing the number of animals in analysis and therewith reduce the total number of animals in experiment with respect to the 3R of animal experimentation.

**Acknowledgement:** The work was supported by the German Government, Federal Ministry of Education and Research, grant nos. 03ZIK445 and 03Z1N511.

## **References:**

- [1] Beyreuther et al., PLOS One, 2017; 12: e0177428.
- [2] Oppelt et al., 2015; 54:155-166.

# Session S09

## „Intercellular comm./Bystander Effects“

**Carmel Mothersill**

Hamilton, Canada

*„Harnessing radiation induced bystander signalling mechanisms for radiotherapy“*

**Katalin Lumniczky**

Budapest, Hungary

*„Radiation-induced bystander effects in the haematopoietic system mediated by extracellular vesicles“*

**Géza Sáfrány**

Budapest, Hungary

*„Intracranial or local thorax irradiation-induced non-targeted effects in bone-marrow-derived endothelial progenitor cells of ApoE deficient mice “*

**Colin Seymour**

Hamilton, Canada

*„Low doses and non-targeted effects in Environmental Radiation Protection; where are we now and where should we go?“*

**An Aerts**

Mol, Belgium

*„Effect of ionizing radiation on intercellular communication in vascular endothelial cells“*

**Werner Rühm**

Neuherberg, Germany

*„Relevance of low dose and low dose rate research for radiation protection“*



# Harnessing radiation induced bystander signalling mechanisms for radiotherapy

**Carmel Mothersill**, M. Le, F. McNeill, A. Rainbow and C. Seymour

*McMaster University, Hamilton, Canada*

Recent efforts to discover the nature of the initial signal triggering the ionising radiation-induced bystander effect have led in two separate directions which appear to be mutually exclusive. Many groups have evidence that exosomes in transferred medium carry information in the form of miRNA which trigger responses in unirradiated cells. However our group have published several papers showing that no medium transfer is necessary and that a UVA signal generated as a result of secondary excitation decay following irradiation is able to trigger the bystander effect in unirradiated cells which are in separate sealed flasks. There are two possible explanations; either these are two alternative mechanisms or the exosomes are generated by the UV signal in both bystander and directly irradiated flasks. In order to test the latter hypothesis, we cultured HCT+/+ cells in medium containing exosome free serum and used our established protocols to generate bystander effects in unirradiated cells in separate flasks. The exosome fractions were harvested from both sets of flasks and from appropriate controls. These fractions were then added to new flasks of never exposed cells to see whether the bystander exosomes could in fact induce a bystander effect. The results suggest that bystander exosomes could indeed induce a bystander effect. Our conclusion is that secondary UV from excitation decay in irradiated cells may have an important role in triggering exosome production in bystander cells without the need for actual medium transfer. This new mechanism opens up the possibility of novel targets for use in radiotherapy.

# Radiation-induced bystander effects in the haematopoietic system mediated by extracellular vesicles

**Katalin Lumniczky**, T. Szatmári, D. Kis, E. Persa, E. Noémi, B. Anett, E. Kis and G. Sáfrány

*National Public Health Institute, Budapest, Hungary*

Radiation-induced bystander effects are the manifestation of radiation effects in cells not directly hit by radiation. The mechanisms governing radiation-induced bystander effects are still not entirely clarified. Extracellular vesicles (EVs) are small, membrane-coated bodies with complex RNA, microRNA and protein cargo released by the cells into the extracellular medium and thus are potential mediators of radiation-induced bystander effects. In order to investigate the role of EVs in radiation-induced bystander effects an in vivo study was designed. C57Bl/6 mice were irradiated with different doses of ionizing radiation, EVs were isolated from the bone marrow and injected into the tail vein of unirradiated mice. The effect of EV transfer was studied by comparing molecular and phenotypic changes of bone marrow cells and splenocytes of EV-recipient, bystander mice to the directly irradiated animals. Activation of the DNA damage response pathway in the spleen of the bystander animals as measured by  $\gamma$ -H2AX assay was comparable to the directly irradiated animals. Phenotypical changes in both the bone marrow and spleen of bystander animals were present, however they were restricted to certain cellular subpopulations. A significantly reduced hematopoietic stem cell pool in the bone marrow as well as CD4<sup>+</sup> and CD8<sup>+</sup> lymphocyte pool in the spleen was detected in bystander mice treated with EVs isolated from animals irradiated with 2 Gy. These alterations were comparable to changes present in the directly irradiated mice. The pool of TLR4-expressing dendritic cells was different in the directly irradiated mice, where it increased after 2 Gy and in the bystander animals, where it strongly decreased in a dose-independent manner. Total RNA was isolated from EVs derived from the bone marrow and plasma of directly irradiated mice and microRNA profiling was performed. A panel of differentially expressed miRNAs in the EVs isolated from irradiated mice with predicted involvement in pathways related to DNA damage repair, hematopoietic and immune system regulation, suggesting their participation in mediating radiation-induced bystander effects. In conclusion, we proved that EVs mediated certain radiation effects in the haematopoietic system of bystander mice and identified potential miRNAs carried by EVs which might be responsible for these effects.

**Funding:** This work was funded by the DoReMi and OPERRA EU-FP7 projects and by the National Research, Development and Innovation Office (grant agreement number: VKSZ\_14-1-2015-0021).

# Intracranial or local thorax irradiation-induced non-targeted effects in bone-marrow-derived endothelial progenitor cells of ApoE deficient mice

H. Hegyesi, N. Sándor, V. Léner, R. Elek, V. Lovas and **Géza Sáfrány**

*Division of Radiobiology and Radiohygiene, Public Health Directorate, National Public Health Institute, Budapest, Hungary*

**Introduction:** Endothelial cells might be involved in the late side effect of radiation therapy in atherosclerotic patients. Given the importance of radiotherapy in the cure of malignant diseases, it is a basic question to clarify how ionizing radiation influences endothelial progenitor pool and endothelial regeneration. Here we investigated/compared the variations of circulating endothelial progenitor cells (EPCs) and growth differentiation factor-15 (GDF-15) in C57Bl6 and atherosclerosis-prone ApoE KO mice. **Materials and Methods:** The head or the thorax of mice were irradiated with different doses of X-rays (0.1, 2 or 10 Gy), bone marrow and blood plasma cellular fractions were analyzed by flow cytometry at different time points following irradiation. **Results:** Circulating EPC counts in the blood were higher after medium and high dose cranial irradiation in ApoE deficient mice than in C57Bl6 mice 24 h post-irradiation, however at later time points the mobilization of these cells were observed only in the 10 Gy exposed group. This suggests that the radiation caused vascular damage induced the acute mobilization of EPCs. GDF-15 level - which is emerging biomarker of cardiovascular risk and disease - in the blood of irradiated mice were elevated only in the C57Bl6 mice at 24h. Local heart irradiation with 16 Gy also resulted in the elevation of bone marrow and circulating EPCs in ApoE mice 24 h after irradiation. Interestingly, an increased number of EPCs was also revealed 6 months after irradiation in ApoE mice. GDF-15 level raised up at 24h in both genotype of mice, but in the ApoE deficient mice the GDF-15 concentration was still elevated at 6 months post-irradiation. **Conclusions:** Increase in the EPCs suggests that the tissue damage due to radiation exposure activate the stem cell niche and allures EPCs in the peripheral blood for angiogenesis. The association of GDF-15 with early EPC mobilization is novel and warrants further investigations.

# Low doses and non-targeted effects in environmental radiation protection; Where are we now and where should we go?

Colin Seymour and C. Mothersill

*McMaster University, Hamilton, Canada*

The field of low dose radiobiology has advanced considerably in the last 30 years from small indications in the 1980's that all was not simple to a paradigm shift which occurred during the 1990's which severely dented the dose-driven models and DNA centric theories which had dominated until then. However while the science has evolved, the application of that science in environmental health protection has not. A reason for this appears to be the uncertainties regarding the shape of the low dose response curve, which lead regulators to adopt a precautionary approach to radiation protection. However the recent advances in preconditioning research suggest that one sided application of a precautionary principle may actually be doing harm and that a more flexible approach based on sound knowledge of basic mechanisms and factors driving variation in response may need to be considered. This paper will review low dose effects and mechanisms focussing on so called non-targeted effects which predominate at low doses. The aim will be to demonstrate just how variable low dose responses are, and how they are so dependent on context (including macro and micro-environment), underlying genetics, other environmental stressors and epigenetic responses. When aiming to protect, the approach is always to err on the side of caution, which currently means reducing exposure using the ALARA principle but what if the low dose exposure is beneficial? How can this be accommodated in regulatory systems especially if it only applies to subsections of the community or ecosystem or if the dose of highest benefit (DHB) is on a spectrum determined by other factors? The idea will be explored, that lessons can be learned from system level radiation protection approaches to non-human biota where populations or ecosystems not individuals are the target of protection efforts. Some of the experimental and modelling approaches being used to derive system level risk factors, may yield new concepts deserving of exploration in radiation protection in general.

# Effect of ionizing radiation on intercellular communication in vascular endothelial cells

R. Ramadan, E. Vromans, E. Decrock, S. Baatout, L. Leybaert and **An Aerts**

*Radiobiology Unit, Belgian Nuclear Research Centre (SCK•CEN), Mol, Belgium/  
Department of Basic Medical Sciences, Physiology group, Ghent University, Belgium*

**Introduction:** Medical applications of ionizing radiation (IR) have become widely used for diagnostic as well as therapeutic purposes. Emerging evidence indicates an excess risk of late occurring cardiovascular diseases, especially atherosclerosis, after IR exposure. IR induces cellular effects which may induce endothelial cell dysfunction, an early marker for atherosclerosis. In addition, intercellular communication through gap junctions (GJs) and hemichannels (both composed of connexins (Cxs)) may play a role in the development of atherosclerosis. However, the role of intercellular endothelial communication, particularly the role of Cxs, in radiation-induced atherosclerosis has never been described before. **Materials & methods:** Telomerase immortalized human Coronary Artery/Microvascular Endothelial cells (TICAE/TIME) were exposed to X-rays (0.1, 0.5 and 5 Gy). Several biological endpoints were investigated: Cx gene expression, Cx protein levels, GJ and hemichannel function. In addition, production of reactive oxygen species, cell death and inflammatory responses were assessed with or without applying a hemichannel blocker (TAT-Gap19). The statistical significance of difference ( $p < 0.05$ ) was evaluated with a Mann-Whitney T-test. **Results:** Exposure to IR induced acute and persistent upregulation of the pro-atherosclerotic Cx43 and downregulation of the anti-atherosclerotic Cx37 and Cx40 gene and protein levels in a dose-dependent manner. In addition, IR exposure increased GJ communication and hemichannel opening. Moreover, IR induced a dose-dependent increase in cell death, inflammatory responses (IL-6, MCP-1 and PECAM-1) and ROS production. These effects were significantly reduced in the presence of TAT-Gap19. **Discussion & conclusions:** An increase in intercellular communication after IR exposure may alter the transfer of IR damaging signals (ROS, cell death, inflammation) between the cells, resulting in an increase in endothelial cell damage. In addition, similar alterations in Cx expression levels have been reported in the literature in endothelial cells covering atherosclerotic plaques. Therefore, these results suggest the link between IR and atherosclerosis that need to be further investigated.

**Acknowledgements:** We would like to thank Dr. Ken Raj for TICAE cell line donation. Raghda Ramadan is supported by a SCK•CEN/Ghent University doctoral grant.



# Relevance of low dose and low dose rate research for radiation protection

**Werner Rühm**

*Helmholtz Zentrum München, Munich, Germany*

The current radiation protection system developed and recommended by the International Commission on Radiological Protection (ICRP) relies largely on data from the atomic bomb survivors in Hiroshima and Nagasaki, Japan, who were exposed to single acute doses at high dose rates. Because radiobiological evidence suggests that exposures to low dose rates are less harmful than those to high dose rates, and because it was thought that the dose response should be linear-quadratic rather than linear with dose, many national and international bodies have used a so-called Dose and Dose Rate Effectiveness Factor (DDREF) to estimate radiation risk at low doses and low dose rates. Recently, ICRP has formed a Task Group on this topic. The Task Group is currently reviewing the information available on molecular, cellular, animal and human epidemiological studies, and identifies any gaps in scientific knowledge. If considered necessary the TG produces scientific reviews on certain aspects relevant for the topic, initiates and performs research projects, and publishes the results in the peer-reviewed international journals. This paper describes the work of the Task Group and summarizes recent results obtained.



# Session S10

## „DNA Damage Response 3“

**Kerstin Borgmann**

Hamburg, German

*„CHK1-mediated replication fork stabilization confers radio-resistance in homologous recombination deficient tumor cells“*

**Claus Sørensen**

Copenhagen, Denmark

*„Novel DNA nuclease function governs cell fate decisions after ionising radiation“*

**Aurelie Vaurijoux**

Fontenay-aux-Roses, France

*„Persistent ionizing radiation-induced foci in human primary cells: transmission through cell division“*

**Fanghua Li**

Essen, Germany

*„Suppression of CtIP-controlled DNA end-resection impairs alt-EJ in quiescent cells“*

**Michael Hausmann**

Heidelberg, Germany

*„Nano-Probes and Localization Microscopy: A Pointilist View on Mechanisms of Radiation Response and DNA-Repair“*

**Moritz Frister**

Heidelberg, Germany

*„Ultrastructural studies on radiation-induced DNA double strand break repair foci with super-resolution microscopy“*



# CHK1-mediated replication fork stabilization confers radioresistance in homologous recombination deficient tumor cells

**Kerstin Borgmann**

*Laboratory of Radiobiology & Experimental Radiooncology, University Medical Center  
Hamburg-Eppendorf, Germany*

Failed protection of stalled replication forks to avoid genomic instability is a hallmark of homologous recombination (HR) deficiency and consequently cells are hypersensitive to DNA-damaging agents, including irradiation, poly(ADP-ribose)polymerase (PARP) inhibitors and Mitomycin C. Here we show that properly activated CHK1 protects cells from DNA damage at replication forks using DNA Fiber assay without restoring homologous recombination activity at DNA double strand breaks measured by plasmid reconstruction assay. Instead, its presence inhibits the recruitment of the MRE11 nuclease to stalled replication forks, which in turn protects nascent DNA strands from extensive degradation. Treatment of cells with siRNA and inhibitors against CHK1 and MRE11 confirmed that CHK1 protects nascent DNA at stalled replication forks after irradiation. Therefore our observations indicate that CHK1 inhibitors are eligible candidates to enhance the therapeutic ratio achieved by radiotherapy in the treatment of HR-deficient tumors.

# Novel DNA nuclease function governs cell fate decisions after ionising radiation

**Claus Sørensen**

*Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Copenhagen, Denmark*

Ionising radiation (IR) is widely used in the clinic to eradicate tumors through targeted DNA damage induction. The cellular responses to such genotoxic challenges include pathways that control cell cycle progression allowing time for repair or permanent cell cycle exit. We recently performed a number of genetic screens to unravel mechanisms underlying cell fate decisions after radiation. I will present our new results that identify a major role for DNA nucleases in actively shaping the proper response to radiation. Notably, we uncovered a new pathway that has an inbuilt homeostatic component allowing cells to fine-tune the response to genotoxic stresses. Our new work highlights the dynamic cellular response to IR, and we speculate that this pathway can be targeted to radiosensitize tumor cells.

*[claus.storgaard@bric.ku.dk](mailto:claus.storgaard@bric.ku.dk)*

# Persistent ionizing radiation-induced foci in human primary cells: transmission through cell division

**Aurelie Vaurijoux**, P. Voisin, A. Freneau, J. F. Barquinero and G. Gruel

*Institute of Radioprotection and Nuclear Safety, Fontenay-aux-Roses, Île-de-France, France*

Unrepaired DNA double-strand breaks (DSBs) induced by ionizing radiation are associated with lethal effects and genomic instability. After the initial breaks and chromatin destabilization, a set of post-translational modifications of histones occurs, including phosphorylation of serine 139 of histone H2AX ( $\gamma$ H2AX), which leads to the formation of ionizing radiation-induced foci (IRIF). DSB repair results in the disappearance of most IRIF within hours after exposure, although some remain 24 hours after irradiation. Their relation to unrepaired DSBs is generally accepted but still controversial. The goal of this work is to explore the characteristics of these persistent IRIF and their consequences on the cell behavior. Immunofluorescence labelling experiments were performed at different times after irradiation of primary human endothelial cells, with 4 MVp X-rays. The analysis of a large number of cells and foci allowed us to screen subpopulations of cells or foci through different characteristics, such as size, shape or cell cycle phase among others, and to weight their representativeness in the whole population of exposed cells. We observed persistent IRIF up to 7 days post-irradiation, and more than 70% of cells exposed to 5 Gy had at least one of these persistent IRIF 24 hours after exposure. Moreover we demonstrated that the presence of persistent IRIF does not permanently prevent cells from progressing through the cell cycle leading even to their transmission to the daughter cells. The frequency of these persistent IRIF was lower in daughter cells, in part due to asymmetric distribution of IRIF between some of them. We report a positive association between the presence of IRIF and the likelihood of DNA mis-segregation. Hence, the structure formed after the passage of a persistent IRI focus across the S and G2 phases may impede the correct segregation of the affected chromosome's sister chromatids. The ensuing abnormal resolution of anaphase might therefore cause the nature of IRIF in daughter-cell nuclei to differ before and after the first cell division. The resulting atypical chromosomal assembly may be lethal or result in a gene dosage imbalance and possibly enhanced genomic instability, in particular in the daughter cells.

# Suppression of CtIP-controlled DNA end-resection impairs alt-EJ in quiescent cells"

**Fanghua Li** and G. Iliakis

*Institute of Medical Radiation Biology, University of Duisburg-Essen, Medical School,  
Essen, Germany*

It is well known that classical non-homologous end-joining (c-NHEJ) is a major component of the DNA double strands break (DSBs) processing armamentarium of higher. However, cells with mutations in components of c-NHEJ still repair the majority of DSBs but with slower kinetics using an alternative end-joining pathway (alt-EJ) that is distinct from homologous recombination repair (HRR), and which operates as a backup not only to c-NHEJ but also to HRR. Using of short tracts of sequence homology (microhomologies) at the break site was demonstrated, which is an indication of DNA end resection requirement. Furthermore, reporter assays based on GFP-expression after processing of I-Sce I induced DSBs confirms key features of alt-EJ. It has been reported that Mre11 is required for the resection of as few as 20bp during alt-EJ, and similar conclusions have been drawn for CtIP and Nbs1, but it remains unknown whether DNA end resection is a general requirement for all alt-EJ events. Thus, there are reports for alt-EJ events that do not utilize, or strictly require, microhomologies. Indeed, a detailed comparison of microhomology length between wild type CHO-K1 cells and Ku80-deficient xrs5 cells found no striking differences, suggesting that alt-EJ can also function independently of microhomology. Actually, GFP-reporter assays integrating microhomologies in the construct (8 or 9 nt) are likely to overestimate their role in alt-EJ. Pulsed-field Gel Electrophoresis (PFGE) is another technique used widely to study alt-EJ in cells with defects in c-NHEJ. In the experiments using c-NHEJ mutants it is assumed that residual DSB processing reflects alt-EJ. A particularly intriguing characteristic of alt-EJ is the marked dependence on growth state: nearly entirely compromised in G0-phase, increases in G1-phase and then gradually further as the cell moves from G1- to G2-phase. The mechanism underlying this phenomenon is not known, but is very relevant for our understanding regarding DSB processing priorities set by the cell. Therefore, a tenable hypothesis is that the reduction of alt-EJ in quiescent cells derives from a suppression of the activity of resection apparatus. Indeed, it is widely accepted that resection in G1 is very limited. An important question in this regard is how is this inhibition mediated and what is the purpose of it teleologically speaking. In the present study, we address this question focusing on G1-phase of cells, when HRR cannot take place. It is possible to investigate the role of DNA end resection on alt-EJ rather specifically. We compare expression levels of CtIP, Mre11, Nbs1 and Rad50 in exponentially growing and plateau-phase cells, as these proteins are candidate components of the DNA end resection apparatus. We report that CtIP plays a key role in the regulation of alt-EJ, mainly by regulating DNA end resection, and that this occurs by adjustments both in protein expression, as well as in protein activity. CDKs take central roles in the regulation of CtIP activity, which facilitates its final activation by ATM. Collectively, the results indicate that limited DNA end resection is advantageous for alt-EJ, and reveal that CtIP is its main regulator.

# Nano-Probes and Localization Microscopy: A Pointillist View on Mechanisms of Radiation Response and DNA-Repair

**Michael Hausmann**, M. Krufczik, A. Hofmann, G. Pilarczyk, JH. Lee, A. Hausmann, DW. Heermann, F. Bestvater, H. Scherthan and G. Hildenbrand

*Kirchhoff-Institute for Physics, University of Heidelberg, Germany*

Novel light microscopic super-resolution techniques enable optical resolution down to about 10 nm even in 3D conserved cell nuclei. Localization microscopy, as one of these techniques, is based on the concept of using fluorescent labels that can be switched between two different spectral states (e.g. off/on) to achieve temporal isolation and thus spatial separation of molecular signals leading to pointillist images and quantitative structural parameter. By multi-colour localization microscopy molecular re-arrangements were investigated after exposure to ionizing radiation and during repair processes. These experiments include ErbB2-receptor arrangements in membranes, conformational changes of chromatin, and recruitment of repair proteins and repair foci formation under different radiation and repair conditions. After specific labelling by antibodies against heterochromatin or oligo-nucleotide nano-probing against ALU-repeats, network-like structures were detected and characteristic changes were elucidated after X-irradiation and during a time course of repair. The data indicate dose and repair process-dependent re-organisation of the different types of chromatin addressed. The recruitment of repair proteins and foci formation (e.g.  $\gamma$ -H2AX, MRE11) at DNA damage sites were analysed together with local compaction changes of their chromatin surroundings. The data show a dose-dependent early increase of the  $\gamma$ -H2AX DSB marker. During the early repair, the formation of dense  $\gamma$ -H2AX signals at dsDNA damage chromatin regions was increasing followed by continuous foci relaxation in the later repair phase. The spatial interaction between foci formation and local chromatin re-arrangements was shown. An analytic method was developed to characterize the local morphology by means of persistence topology. The advantage of using topological properties is an intrinsic scale invariance which allows the comparison of structures without restrictions to a certain length scale. Finally, a dose response was also observed by the spatial arrangement of ErbB2 receptors and their internalisation into the cytoplasm after X-irradiation. Since these receptors are involved in repair pathways, our measurements revealed radiation induced spatio-temporal modifications of these pathway endpoints. In conclusion, the investigations demonstrate the broad potential of localization microscopy in biological radiation research in order to better understand spatial re-arrangements of molecular complexes and mechanisms behind such radiation and repair response on the single cell level.



# Ultrastructural studies on radiation-induced DNA double strand break repair foci with super-resolution microscopy

**Moritz Frister**, R. Lopez-Perez, NH. Nicolay, J. Engelhardt, R. Vlijm and PE. Huber,

*DKFZ Heidelberg, Klinische Kooperationseinheit Molekulare Radioonkologie, Germany*

Introduction DNA double strand breaks (DSBs) and protein complexes associated with DSB-repair can be detected by fluorescent microscopy in the form of foci. Recent studies lead to the suggestion that foci which can be visualized by conventional microscopy are made up of subunits whose ultrastructural details might be significant for the recognition of DNA-damages and the function of DSB repair. But this ultrastructure remains largely unknown. Therefore, we applied superresolution microscopy to investigate the ultrastructure of DSB repair foci induced by several types of ionizing radiation. Methods MRC-5, CCD 841 (colon epithel) and HUVEC cells were irradiated with photons, electrons and carbon ions at biologically equivalent doses based on clonogenic survival assays (50% survival). The cells were fixed and stained with antibodies against  $\gamma$ H2AX (Ser 139), p53BP1, pDNA-PKcs (Ser 2056), pBRCA1 (Ser 1524) or RPA2/32. Foci were detected with conventional microscopy and the two optical superresolution techniques stimulated emission depletion (StED) microscopy and single molecule localization microscopy (SMLM). The ultrastructural kinetics of DSB recognition and repair complexes were correlated with the cell-cycle using EdU staining (S phase) and DAPI for classification. Results MRC-5, CCD 841 (colon epithel) and HUVEC cells showed relatively similar radiation sensitivities and a relative biological effectiveness (RBE) between 3,4 and 3,9 for C-12-ions compared to photons. For electrons, RBEs between 1,0 and 1,2 were detected. Radiation induced  $\gamma$ H2AX foci (as DSB-marker) and pDNA-PKcs foci (as a marker for non-homologous end joining) consisted of smaller subunits which could be observed in all cell-cycle phases and were located at defined areas. The number of  $\gamma$ H2AX-foci and their size were increasing with the biological equivalent dose and reached dose-dependent maxima between 30 minutes and two hours after irradiation. The size of  $\gamma$ H2AX-Foci also correlated with the number of pDNA-PKcs subunits in the same area. pBRCA1 foci (as a marker for homologous recombination) were only detectable in the S-phase. Further analyses are ongoing. Discussion Foci of  $\gamma$ H2AX, pDNA-PKcs and pBRCA1 all showed a so far unknown ultrastructure which depends on the cell-cycle phase and may have functional implications concerning DNA double-strand break repair. StED and SMLM microscopy obviously have the potential to help revealing these ultrastructural characteristics in contrast to conventional microscopy.



# Session S11

## „Radiation therapy 1“

**Marie Dutreix**

Orsay Ville, France

*„DNA repair inhibitors and radiotherapy “*

**Alexander Schramm**

Essen, Germany

*„Modulation of the DNA damage response by neurotrophin receptor signalling“*

**Heike Anders**

Munich, Germany

*„HSP90 inhibition: Sensitization of aggressive soft tissue sarcomas to radiotherapy by enhancing the extent and the immunogenicity of sarcoma cell death“*

**Justine Rudner**

Essen, Germany

*„USP9x-regulated Mcl-1 stability is important for prostate cancer survival in response to ionizing radiation“*

**Hua Jing**

Heidelberg, Germany

*„Combination of hypofractionated radiotherapy and IL-2/anti-IL-2 complexes and its theranostic evaluation“*

**Erika Zernickel**

Heidelberg, Germany

*„A synthetic lethality-based strategy for individual sensitization of lung cancer cell lines with vulnerability in the SWI/SNF complex to radiotherapy“*



# DNA repair inhibitors and radiotherapy

**Marie Dutreix**, L. Quero, P. M. Girard, S. Ferreira and M. Kozlac

*Unit CNRS3347- INSERM1021- université Paris-Saclay- Institut Curie, 91405 ORSAY,  
France*

DNA-damaging cytotoxic therapies including radiotherapy remain the mainstay of cancer management. However, perturbations in DNA repair pathways, that are frequent in cancers, alter the resistance of the affected tumors to DNA damaging treatments. Inhibition of DNA damage response may enhance the radiation therapeutic effects. Moreover, it may also be useful as a monotherapy when it achieves synthetic lethality, in which inhibition of a complementary DNA damage response pathway selectively kills cancer cells that have a defect in a particular DNA repair pathway. We compared efficacy in triple-negative Breast Cancer models of two different classes of DNA repair inhibitors: the poly(ADP-ribose) polymerase inhibitor (Olaparib) and the Double-strand break pathways inhibitor Dbait (AsiDNA). Molecular and cellular predictive markers were identified for both inhibitors classes and predict a potential benefit of their double combination with radiotherapy. Preclinical results confirm the interest of combining DNA repair inhibitors with radiotherapy in cancer treatment.

# Modulation of the DNA damage response by neurotrophin receptor signalling

I. Rudolf, V. Boron, G. Iliakis, A. Soni and **Alexander Schramm**

*University Hospital Essen, Germany*

High expression of the receptor tyrosine kinase TrkA/NTRK1 is associated with a favorable outcome in several solid tumors of childhood including neuroblastoma. By contrast, the highly homologous TrkB/NTRK2 receptor has been linked with resistance to radiation and anoikis. Here, we analysed the impact of TrkA/NTRK1 signaling on cell viability, checkpoint activation, and DNA repair capacity. For this purpose, SY5Y neuroblastoma cells with inducible TrkA/NTRK1 were used to shed light on the role of TrkA/NTRK1 in response to ionizing irradiation (IR). While no differences in DNA repair capacity following IR was found, activated TrkA/NTRK1 caused a G2 checkpoint deficiency at both low (1 Gy) and high doses (4 Gy) of IR. In a tightly controlled setting we confirmed that this effect was strictly dependent on activation of TrkA/NTRK1 by its ligand, nerve growth factor (NGF). Surprisingly, abrogation of IR-induced G2 block by TrkA/NTRK1 could not be rescued by ATM or ATR inhibition. Furthermore, cell viability was increased in TrkA/NTRK1-positive cells post-IR compared to vector control cells. TrkA/NTRK1-positive cells up-regulated PARP1 and showed significantly increased cell viability compared to TrkA/NTRK1-negative controls after PARP1-inhibition by Olaparib with and without irradiation. In conclusion, we here provide first evidence for a previously unrecognized function of NTRK signaling in checkpoint regulation and the response to IR.

# HSP90 inhibition: Sensitization of aggressive soft tissue sarcomas to radiotherapy by enhancing the extent and the immunogenicity of sarcoma cell death

**Heike Anders,** A. Ernst, H. Kapfhammer, M. Orth, N. Winssinger, C. Belka and K. Lauber

*Clinic for Radiotherapy, Ludwig-Maximilians-Universität (LMU), Muenchen, Germany*

Background: Radiotherapy is an essential part of multi-modal treatment for soft tissue sarcomas. Treatment failure is commonly attributed to radioresistance, but comprehensive analyses of radiosensitivity are not available, and suitable biomarkers or candidates for targeted radiosensitization are scarce. Methods: We systematically analyzed the intrinsic radioresistance of a panel of sarcoma cell lines, and extracted scores of radioresistance by principal component analysis (PCA). To identify molecular markers of radioresistance, transcriptomic profiling of DNA damage response regulators and HSP90 isoforms was performed. Their functional relevance was dissected by HSP90 inhibition and subsequent monitoring of client protein degradation,  $\gamma$  H2AX foci immunofluorescence, cell death, and clonogenicity analyses. Results: The expression levels of HSP90 and its clients ATM, ATR, and NBS1 revealed strong, positive correlations with the PCA-derived radioresistance scores. Their functional involvement was addressed by HSP90 inhibition, which preferentially sensitized radioresistant sarcoma cells and was accompanied by delayed  $\gamma$ -H2AX foci clearance and HSP90 client protein degradation. The induction of apoptosis and necrosis was not significantly enhanced, but increased levels of basal and irradiation-induced senescence upon HSP90 inhibition were detected. Finally, evaluation of our findings in the TCGA soft tissue sarcoma cohort revealed elevated expression levels of HSP90, ATM, ATR, and NBS1 in a relevant subset of cases with particularly poor prognosis, which might preferentially benefit from HSP90 inhibition in combination with radiotherapy in future. Conclusion: HSP90 inhibition by NW457 clearly has radiosensitizing properties in vitro and emerges as a promising strategy to improve the efficacy of radiotherapy – particularly in case of aggressive, radioresistant sarcoma. Expression levels of ATM, ATR, NBS1, and HSP90 isoforms appear to be valuable markers in this regard which might serve for future patient stratification. Analysis of a retrospective, in-house patient cohort should elucidate this in greater depth and confirm the translational potential for clinical treatment regimes. Furthermore, impairing DNA damage repair and changing the mode of cell death could favor tumor immunogenicity. Future studies have to evaluate these findings in vivo and should delineate the molecular and cellular mechanisms as well as the immunological consequences underlying HSP90 inhibitor-mediated radiosensitization of sarcoma.

# USP9x-regulated Mcl-1 stability is important for prostate cancer survival in response to ionizing radiation

**Justine Rudner**, S. Hogh-Binder, F. Wolfsperger, J. Hennenlotter, A. Stenzl, D. Klein, S. Huber and V. Jendrossek

*Institute of Cell Biology, University Hospital Essen, Germany*

Introduction: Anti-apoptotic Bcl-2 family members can contribute to radioresistance by interfering with apoptosis induction in response to ionizing radiation. Levels of these proteins are not only regulated at the transcriptional and translational level but also post-translationally. The anti-apoptotic Mcl-1 is a short-lived protein whose stability is closely regulated by proteasomal degradation. While ubiquitin ligases facilitate degradation, the deubiquitylating enzyme USP9x interferes with degradation by removing polyubiquitin chains from Mcl-1 thereby stabilizing this protein. Thus, stabilizing Mcl-1 by enhanced USP9x activity might contribute to radioresistance in tumor cells. Methods: USP9x and Mcl-1 levels in normal human prostate tissue samples as well as high grade and low grade prostate tumors were analyzed by immunohistochemistry. Further analysis was performed in LNCaP and PC3 human prostate cancer cell lines that were irradiated with 10 Gy. Protein levels were examined by Western Blot. Mcl-1 stability was calculated by analyzing Mcl-1 decline after incubating cells with cycloheximide. Apoptosis and cell death were examined by flow cytometry. Long-term effects were analyzed by colony formation assay. Knock-down of Mcl-1 or USP9x was achieved by transfecting cells with respective siRNA. Non-targeting siRNA was used as a transfection control. Results: USP9x and Mcl-1 immunoreactivity score increased during prostate cancer progression. Moreover, USP9x and Mcl-1 levels correlated significantly. Data bank analysis showed that high USP9x and high Mcl-1 expression levels were associated with decreased diseases-free survival in patients. While Mcl-1 levels were increased in response to ionizing radiation in LNCaP cells, Mcl-1 levels decreased in irradiated PC3 cells. The increased Mcl-1 levels in irradiated LNCaP cells were due to increased protein stability. However, both cell lines only moderately induced apoptosis in response to irradiation. While Mcl-1 knock-down resulted only in modest apoptosis induction, Mcl-1 knock-down clearly increased apoptosis levels in LNCaP cells, showing that survival of LNCaP cells but not PC3 cells strongly depend on Mcl-1. However, Mcl-1 knock-down in both cell lines increased radiation-induced apoptosis. Interestingly, USP9x knock-down in both cells only slightly increased background apoptosis, but significantly increased radiation-induced apoptosis and reduced Mcl-1 levels. Finally, USP9x knock-down significantly reduced clonogenic survival after irradiation. Conclusion: Anti-apoptotic Mcl-1 and deubiquitylating enzyme USP9x are upregulated during tumor progression and associated with a poorer prognosis. In addition, the data shows that USP9x can stabilize Mcl-1 levels in response to irradiation and increase sensitivity to ionizing radiation especially in LNCaP cells whose survival strongly depends on Mcl-1.

# Combination of hypofractionated radiotherapy and IL-2/anti-IL-2 complexes and its theranostic evaluation

**Hua Jing**, M. Hettich, M. Bartholomä and G. Niedermann

*Dept. of Radiation Oncology, University Clinics Freiburg, Germany*

Background: Combinations of radiotherapy (RT) and immune checkpoint inhibition (ICI) are effective in a fraction of patients with advanced malignancies. However, the majority of patients still do not respond. For these patients, and for patients with preexisting autoimmune diseases or ICI-induced autoimmune-like symptoms, alternative treatments are required. Combinations of RT with IL-2 can be very potent, but IL-2 has only a short half-life. It also stimulates immunosuppressive CD4<sup>+</sup> regulatory T cells and can lead to vascular leak syndrome, both depending on IL-2 binding to its high-affinity receptor CD25. To circumvent these disadvantages, we tested combination treatments of hypofractionated RT (hRT) and stable IL-2/anti-IL-2 complexes (IL-2c); the complexes prevent IL-2 binding to CD25 but preserve its binding to the low-affinity IL-2 receptor CD122. Material & Methods: Mice with established melanomas were treated with local hRT (2 × 12 Gy) and IL-2 or IL-2c. Besides tumor sizes and survival, the frequencies and quality of the tumor-specific T cells were assessed by flow cytometry. In addition, a novel PET tracer was developed for the detection of IL-2c and the ligand CD122. For this purpose, the IL-2c were conjugated with the chelator NOTA and further labeled with the radioisotope <sup>64</sup>Cu. Results: Treatment of mice harboring relatively large, established melanomas with hRT + IL-2c was superior to treatment with hRT + IL-2 or hRT alone. Treatment with IL-2c alone was not effective. The better activity of the hRT/IL-2c combination treatment was reflected by an enhanced number of tumor-specific CD8<sup>+</sup> cytotoxic T cells. The novel PET tracer allowed visualization of the whole-body distribution of the IL-2c and of the bound CD122 receptor. We also non-invasively visualized the side effects of IL-2c treatment by using different PET tracers and contrast-enhanced CT. Conclusions: We developed a novel combination treatment consisting of hRT and high-molecular-weight IL-2c, which resulted in long-term tumor control and sometimes cures in mice with large, established melanomas. Since similar high-molecular-weight complexes have recently been developed using humanized anti-human IL-2 antibodies, combination treatments with hRT could soon be evaluated in clinical trials. In addition, a novel PET tracer for the theranostic evaluation of the IL-2 complexes and their receptor has been developed by us.



# A synthetic lethality-based strategy for individual sensitization of lung cancer cell lines with vulnerability in the SWI/SNF complex to radiotherapy

**Erika Zernickel**, A. Sak, M. Groneberg and M. Stuschke

*Department for Radiotherapy, University Hospital Essen, Essen, Germany*

**Purpose:** The discovery of frequent mutations in various subunits of the mammalian SWI/SNF (mSWI/SNF) chromatin remodeling complex is an important finding from cancer genome profiling. The mSWI/SNF complexes consist of one of two mutually exclusive DNA-dependent ATPases, BRG1/SMARCA4 or BRM/SMARCA2, together with core and accessory subunits that function in mobilizing nucleosomes to regulate transcription, DNA replication and repair, and higher-order chromosome dynamics. Many lung cancers (10%-30%) show reduced or absent level of BRG1 expression and BRM depletion specifically kills BRG1-deficient tumor cells. Therefore BRM seems to be a critical and promising therapeutic target in BRG1-mutant cancers based on the concept of synthetic lethality. **Materials/Methods:** Expression of BRM was inhibited by transfection with siRNA in BRG1-deficient and BRG1-proficient cell lines. Radiation sensitivity in confluent (about 80% G1 and 10% S-phase), in exponential growing (about 40% G1 and 30 % S-phase) and in aphidicolin treated cells (about 80 % S-phase) was measured with clonogenic survival assay. In addition, tumor growth control has been measured after downregulation of BRM in combination with fractionated irradiation (1x 4 Gy/day or 2x 4 Gy/day) and single dose irradiation by using the mini-monolayer assay (Sak et al. Int J Radiat Oncol Biol Phys. 2012, 84: 492-9). In order to investigate the repair pathway immunofluorescence staining of Rad51, as a measure for homologous recombination repair (HRR) and  $\gamma$ -H2AX as well as 53BP1 foci as a measure for overall DSB signaling, were used. Additionally, we did colony assays after inhibition of the key enzyme of NHEJ pathway DNA-PK with Nu7441 (1  $\mu$ M) and Nu7026 (10  $\mu$ M) in combination with BRM knockdown in Brg1- mutated cells. **Results:** The results show (i) significantly reduced surviving fraction after BRM depletion specifically in Brg1- deficient cells but not in the proficient ones, (ii) significantly increased tumor growth control after depletion of BRM in BRG1- mutant cell lines but not in BRG1 wildtype cell lines after fractionated and as well as single dose irradiations in the plaque monolayer assay (iii) a significant increase of residual Rad51 foci at 24 h after irradiation in Brg1 deficient cells treated with si-BRM, without an effect on initial Rad51 foci formation at 4 h after irradiation, which was not due to an increase of S/G2 phase cells. In contrast to the HR, there is obviously no contribution of the NHEJ to the sensitizing effect after BRM depletion because no increase of initial and residual 53BP1 foci or  $\gamma$ H2AX was found. This was strengthened by the finding that the inhibition of the key enzyme of NHEJ pathway DNA-PK with Nu7441 (1 $\mu$ M) and Nu7026 (10 $\mu$ M) in combination with Brm knockdown in Brg1- mutated cells additionally increased siBrm effect. In addition, the results demonstrate that the radiosensitising effect of Brm depletion in Brg1 deficient cells increases in S-phase enriched cell populations. **Conclusion:** Overall, the results show that downregulation of BRM expression lead to an increase of radiation sensitivity specifically in BRG1-deficient cancer cell lines. The sensitizing effect increased in S-phase enriched cell population and was mainly due to dysregulation of homologous recombination repair. These findings can be used as a step stone for the development of targeted therapy options in Brg1 mutated cancer cells.



## Session S12

### „Predictive Assays/Biomarkers 1“

**George Don Jones**

Leicester, United Kingdom

*„Comet assay measures of cancer treatment cell sensitivity “*

**Nicolaas Franken**

Amsterdam, Netherlands

*„Reduced activity of DSB repair genes in prostate cancer patients with late normal tissue radiation-toxicity “*

**Pavel Lobachevsky**

Melbourne, Australia

*„ $\gamma$ -H2AX assay as a basis for prediction of individual radiosensitivity “*

**Dietrich Walsh**

Neubiberg, Germany

*„Real time imaging of ROS propagation after targeted mitochondrial irradiation“*

**Ulrike Schötz**

Munich, Germany

*„Biomarkers and targets for personalization of radiotherapy of HNSCC: CD44v6“*

**Michael Orth**

Munich, Germany

*„Mechanism and biomarkers predicting the outcome of taxane-based, concurrent radiochemotherapy in locally advanced lung carcinoma“*



# Comet assay measures of cancer treatment cell sensitivity

**George Don Jones**

*Leicester Cancer Research Centre, University of Leicester, UK*

Our genomic DNA is exposed to tens of thousands of damaging events per day and this continuous DNA damage, coupled with erroneous repair and the subsequent accumulation of mutations, is important in the development of several age-related diseases including cancer. However, for radiation and many chemotherapeutic drugs genomic DNA is the primary cellular target for the biological effects of these anti-cancer agents; indeed it is the DNA damage induced which is thought to be responsible for killing the of the cancer cells. Consequently, methods to assess DNA damage are important in studies of both carcinogenesis and cancer treatment. The alkaline comet assay (ACA), also known as single-cell gel electrophoresis (SCGE), has become a standard method for the detection of DNA damage and repair, both in vitro and in vivo. Previously<sup>1</sup>, we have shown that radiation-induced DNA damage, measured by ACA, correlates bladder cancer cell radiosensitivity in vitro. More recently<sup>2</sup>, we have shown that modified-ACA measures of cisplatin and mitomycin-C-induced damage also correlate bladder cancer cell chemosensitivity in vitro, with essentially the same rank order for chemosensitivity as for radiosensitivity. Furthermore, ACA studies of radiation-induced damage in different cell-DNA substrates (nuclei, nucleoids and intact parent cells) suggest that it is a feature retained in the prepared nucleoids that is responsible for the relative damage sensitivity of bladder cancer cells, suggestive of differences in the organisation of DNA within resistant vs. sensitive cells. We have also shown that ACA analysis of biopsies from bladder tumours reveal that reduced DNA damage sensitivity associates with poorer treatment outcomes; notably that tumours with a reduced damage response show a significant association with local recurrence of non-invasive disease and that reduced damage response was a better predictor of recurrence than the presence of high-risk histology in this cohort. In analogous studies<sup>3</sup> of comet assay measures of DNA damage as biomarkers of irinotecan response in colorectal cancer in vitro and in vivo, we show that irinotecan-induced DNA damage was detectable in two CRC cell-lines in vitro, with higher levels of immediate and residual damage noted for the more sensitive HT-29 cells. DNA damage was not detected in vivo, but was measurable in PBLs upon mitogenic stimulation prior to ex vivo SN-38 treatment. Results showed that those patients whose PBLs demonstrated higher levels of DNA damage following 10 h of SN-38 exposure ex vivo had significantly longer times to progression than those with lower damage levels (median 291 vs. 173 days,  $P = 0.014$ ). Consequently, with higher levels of irinotecan-induced initial and residual damage correlating with greater cell kill in vitro and a better in vivo clinical response, DNA damage measures may represent superior biomarkers of irinotecan effect compared to the more often-studied genetic assays for differential drug metabolism. Altogether, our studies demonstrate that mechanisms which govern treatment-induced DNA damage are both central to and predictive of cancer cell treatment sensitivity and identifies/substantiates the association between damage resistance and aggressive tumour phenotype.

## **References:**

1. AL Moneef et al., 2003, British Journal of Cancer, 89, 2271-2276.
2. Bowman et al., 2014, International Journal of Cancer, 134, 1102-11.
3. Wood et al., 2015, Cancer Medicine, 4, 1309-1321.

***gdj2@le.ac.uk***

# Reduced activity of DSB repair genes in prostate cancer patients with late normal tissue radiation-toxicity

B. van Oorschot, L. Stalpers and Nicolaas Franken

*Laboratory for Experimental Oncology and Radiobiology (LEXOR), Center for Molecular Medicine, University of Amsterdam, Amsterdam, the Netherlands*

Late normal tissue damage is a major drawback of cancer radiotherapy. It was found in a retrospective study of prostate cancer patients that late radiation toxicity was possibly related to a decreased repair of double strand break (DSB) as determined with the  $\gamma$ -H2AX foci assay (decay ratios of  $\gamma$ -H2AX foci: initial/residual  $\gamma$ -H2AX foci numbers) and reduced expression levels of DNA DSB repair genes. In a prospective cohort of 198 men irradiated for prostate cancer, DSB repair again determined with decay ratios of  $\gamma$ -H2AX foci and genome-wide expression profiles were examined in ex vivo irradiated lymphocytes. Patients were followed  $\geq 2$  years after radiotherapy, clinical characteristics were collected and toxicity was recorded using the Common Terminology Criteria (CTCAE) v4.0. Studied clinical factors were not correlated with late radiation toxicity. The  $\gamma$ -H2AX foci decay ratio was negatively correlation with toxicity grade, with a significant difference between patients with grade  $\geq 3$  and grade 0 toxicity ( $p=0.02$ ). The threshold foci decay ratio, determined in the retrospective study, correctly classified 23 of the 28 patients with grade  $\geq 3$  toxicity (sensitivity, 82%) and 9 of the 14 patients with grade 0 toxicity (specificity, 64%). Moreover, homologous recombination (HR) repair genes were less induced with increasing toxicity grade. The difference in fold induction of the HR gene-set was most pronounced between grade 0 and grade  $\geq 3$  toxicity ( $p=0.008$ ). Reduced responsiveness of HR-repair genes to irradiation and inefficient DSB repair correlate with severe late radiation toxicity. Using a decay ratio classifier, we could correctly classify 82% of the patients with grade  $\geq 3$  toxicity. These observations may be helpful to identify patients with a genetically enhanced risk of late radiation toxicity.

This research is sponsored by the Dutch Cancer Society, Koningin Wilhelmina Fonds (grants UVA 2008-4019 and UVA 2015-7820).

# Gamma-H2AX assay as a basis for prediction of individual radiosensitivity

**Pavel Lobachevsky**, T. Leong, P. Daly, J. Smith, N. Best, J. Tomaszewski, R. Martin and O. Martin

*Cancer Research Division, PeterMacCallum Cancer Centre, Melbourne, Australia*

Abnormally severe radiation toxicity effects emerging in a small percentage of cancer radiotherapy patients are well documented. It is generally accepted that increased intrinsic radiosensitivity accounts for these effects. Identification of patients with such increased radiosensitivity prior to commencement of radiotherapy has a potential for improvement of the treatment. A few approaches based on the evaluation of in vitro cellular radiation response have been suggested to develop a predictive radiosensitivity assay however none of them have progressed to clinical application. One of such approaches exploits the kinetics of elimination of gamma-H2AX foci in ex vivo irradiated blood lymphocytes, as a surrogate for DNA damage repair that represents an important factor in response to radiation. The utility of this approach for the prediction of radiosensitivity is questionable as follows from publications in this area. We therefore attempted to systematically evaluate a range of quantitative parameters describing gamma-H2AX foci kinetics aiming to develop an analytical tool and statistical criteria for discrimination of normal and radiosensitive patients. We analysed the gamma-H2AX response to ex-vivo irradiation of peripheral blood lymphocytes and plucked eyebrow hair follicles from 16 patients who developed severe late radiation toxicity following radiotherapy, and 12 matched control patients. From a range of studied quantitative parameters of the gamma-H2AX response, such as the fraction of unrepairable component, repair rate, ratio of foci number at 1 and 24 hr, and co-localisation efficiency with 53BP1 foci, the combination of the first two parameters, derived from non-linear regression analysis of foci repair kinetics, appeared to be the most powerful predictor of radiosensitivity. Our ROC (receiver operating characteristics) analysis suggests that the application of the classification criteria based on the combination of the unrepairable component and repair rate allows to discriminate individuals with increased radiosensitivity with a sensitivity of 80% at a specificity level 91% (9% false positive rate). We also introduce a visual representation of radiosensitivity status that allocates a position for each patient on a two-dimensional “radiosensitivity map”. Our analytical approach provides the basis for larger prospective studies involving evaluation of the radiosensitivity status prior to radiotherapy treatment to validate and further refine the algorithm.

# Real time imaging of ROS propagation after targeted mitochondrial irradiation

**Dietrich Walsh**, J. Reindl, K. Ilicic, C. Greubel, B. Schwarz, M. Sammer, T. E. Schmid  
and G. Dollinger

*Universität der Bundeswehr München, Neubiberg, Germany*

Introduction: Radiation induced reactive oxygen species (ROS) is one of the most investigated and least well understood phenomena in radiation biology. ROS has been linked to all kinds of damage and cellular responses in a wide variety of cellular processes. Due to the fast formation and interaction of ROS with cells, imaging of direct ROS induction is a technically complicated task. Mitochondria pose the single largest natural source of ROS in the cell and many of the aberrant processes triggered by ROS are linked to a change in mitochondrial activity. There are however very few investigations into the real-time monitoring and propagation of radiation induced ROS, especially on the mitochondrial level. Therefore the aim of the project is to irradiate mitochondria using the heavy ion microbeam SNAKE with a beam diameter of  $<1\mu\text{m}$  as already published [1] and then to analyze the effects of this targeted high-LET irradiation on ROS production and propagation. Methods: To investigate the generation and propagation of radiation induced ROS after targeted mitochondrial irradiation, fluorescence sensors for superoxide ( $\text{O}_2^-$ ) and  $\text{H}_2\text{O}_2$  were used. The irradiation and imaging was performed concurrently so the production and propagation of the radiation induced ROS could be tracked in situ. MCF-7 cells were irradiated with varying numbers of carbon ions to detect the radiation induced ROS. Results: Real time imaging of the targeted mitochondrial irradiation shows a sharp and short burst of ROS. The length and intensity of the burst relates directly to the amount of ions applied to the mitochondria (30-1000 Cpp). Lower carbon ion numbers ( $<60$ ) show little or no response. The initial superoxide burst is only detected in the targeted mitochondria and not in the rest of the cell. Propagation to non-irradiated neighbouring cells was not observed. Conclusion: We have shown that high-LET particle induced ROS can be imaged in real time concurrently to irradiation enabling us to induce and investigate highly localized ROS production in mitochondria. The results indicate that mitochondrial ROS production is a very fast and highly dynamic process and that larger carbon ion numbers than expected ( $>100$ ) are needed to induce a visible initial burst of superoxide in mitochondria. Superoxide propagation to neighboring cells or throughout the cell was not detected.

[1] D.W.M. Walsh et al. Scientific Reports accepted March 2017

# "Biomarkers and targets for personalization of radiotherapy of HNSCC: CD44v6"

**Ulrike Schötz**, M. Orth, M. Selmansberger, J. Schuster, B. Stegen, J. Heß, K. Unger, H. Zitzelsberger, C. Belka and K. Lauber

*Department of Radiotherapy and Radiooncology, Philipps-University Marburg, Germany;  
Department of Radiotherapy and Radiation Oncology, Ludwig-Maximilians-University,  
Munich, Germany*

For locally advanced head-and-neck squamous cell carcinoma (HNSCC), 5-year overall survival rates are still limited to approximately 50%, and therapeutic failure is mainly attributed to therapy resistance. Suitable markers that predict the treatment outcome – apart from the HPV status – are so far not available. Therefore, the aim of the study was to identify suitable predictive and/or prognostic markers for stratification of HNSCC patients and potential therapeutic targets in a preclinical setting. A promising group of candidates we identified are transcripts of the surface receptor CD44 with the variant exon v6 (CD44v6). The methodological approach was to evaluate the clinical relevance of the potential marker by screening of retrospective cohorts followed by an in silico fine tuning to identify transcript variants. Subsequently, in vitro analyses of the radiation response in established HNSCC cell lines were performed in order to characterize the underlying mechanisms and to identify druggable targets. Finally, an in vivo evaluation was carried out in an orthotopic mouse xenograft model. In a clinical setting, we examined CD44v6 mRNA expression levels in surgical specimen of a cohort of 96 HNSCC tumor patients undergoing adjuvant radiochemotherapy. Elevated CD44v6 expression levels were associated with impaired overall survival. The results were validated using RNA-seq data from TCGA. Moreover, TCGA data allowed us to identify one specific transcript variant of the receptor to be mainly responsible for poor outcome in patients. In vitro, we observed a significant positive correlation between clonogenic survival and CD44v6 mRNA and protein expression in a panel of 7 established HNSCC cell lines. Functionally, RNAi silencing of CD44v6 expression sensitized cells to irradiation. This was mainly due to an increase in irradiation-induced senescence and alterations in the expression of secreted factors. In an orthotopic xenotransplantation model, CD44v6 silenced and non-silenced cells were inoculated into the floor of the mouth of nude mice. Upon tumor engraftment, mice were treated with 2x 6 Gy or left untreated. First results indicate more pronounced tumor growth delay in CD44v6 silenced tumors, and thus a better therapeutic response towards radiation therapy. Our study identifies CD44v6 as a crucial driver of therapy resistance in a subset of HNSCC. We are currently investigating the underlying mechanisms in depth in order to evaluate CD44v6's potential as biomarker and/or therapeutic target in personalized radiotherapy of HNSCC in greater detail.



# Mechanism and biomarkers predicting the outcome of taxane-based, concurrent radiochemotherapy in locally advanced lung carcinoma

**Michael Orth**, K. Unger, U.Schötz, C. Belka and K. Lauber

*Department of Radiation Oncology, Ludwig-Maximilians-University Munich, Germany*

Concurrent radiochemotherapy implementing taxanes as radiosensitizing agents has become a crucial option for the treatment of various cancer entities at locally advanced stages. While the therapeutic synergism between these two treatment modalities has been well documented during the past twenty years, the mechanism behind this synergism remained mostly elusive, thus precluding the identification of any mechanism-based marker that would be of predictive value for this particular treatment regimen. We show that clinically relevant doses of Paclitaxel, the prototype taxane, stimulate a tripolar kind of mitotic spindle formation leading to chromosomal fail-segregation onto supernumerary daughter cells, aneuploidy and cell death. The fact that this mechanism is also described by a recent report investigating the anti-neoplastic effects occurring inside the tumors of breast cancer patients after the systemic administration of Paclitaxel (Zasadil et al. (2014), Sci Transl Med 6(229), 229ra43), calls the ancient envision of the mode of action exhibited by Paclitaxel and, most likely, taxanes per se, consisting in facilitation of a persistent cell cycle arrest during somatic cell division into question. We now show that this distinct mode of action that is based upon the induction of tripolar spindle formation is also interlinked with Paclitaxel-mediated radiosensitization of tumor cells, in a manner depending on the overexpression of the proto-oncogenic mitotic protein kinase Aurora A (AURKA) and its cofactor TPX2. In accordance, reducing the expression levels of TPX2 by RNA interference not only rescues bipolar mitotic spindle formation and thus bipartite cell division, but it also attenuates the radiosensitizing effects of Paclitaxel. We confirmed these data using the TCGA lung adenocarcinoma patient cohort thereby finding that accelerated expression levels of AURKA and TPX2 are associated with a specifically improved overall survival upon taxane-based radiochemotherapy, but not upon non-taxane-based radiochemotherapy or sole radiotherapy. Therefore, our data provide novel insights into the mechanism that underlies taxane-mediated radio sensitization of tumor cells and also identify the first mechanism-based markers that are of predictive value for taxane-based radiochemotherapy.



# Keynote Lecture 4

**Marco Foiani**

Milan, Italy

*„An integrated ATR, ATM and mTOR-mechanical network  
controlling nuclear plasticity and cell migration“*

# An integrated ATR, ATM and mTOR-mechanical network controlling nuclear plasticity and cell migration

**Marco Foiani**

*IFOM (FIRC Institute of Molecular Oncology) and University of Milan, Italy*

ATR and ATM control chromosome integrity, chromatin dynamics and cell cycle events. mTOR shares similarities to ATR and ATM and coordinates nutrient sensing pathways and cytoskeleton dynamics. ATR, ATRIP and Chk1 associate to the nuclear envelope during S phase and prophase, and in response to mechanical stimulation of the plasma membrane to coordinate chromatin and nuclear envelope dynamics. This pathway is influenced by mTOR, actin dynamics and calcium levels. We visualized the morphology of the nucleus in ATR and CHK1-defective cells and found aberrant condensation events and nuclear envelope anomalies that contribute to envelope invaginations, micronuclei formation and chromosome fragmentation. We identified key ATR co-factors implicated in the coordination between chromatin dynamics and nuclear envelope metabolism. Using mechanobiology approaches we measured the stiffness of wild type, ATR, ATM, CHK1 and mTOR defective cells and found significant differences that influence cell plasticity and interstitial migration. These and other observations implicate ATR, ATM and mTOR in the control of genome integrity, nuclear dynamics, cell plasticity and migration and suggest the existence of an integrated mechanical network involving different PI3-kinases.

# Plenary Session 3

## „Mechanisms of Radiation Effects“

### **Thanos Halazonetis**

Geneva, Switzerland

*„A vertebrate DNA replication completion checkpoint shows similarities to the spindle checkpoint“*

### **Christian Reinhardt**

Cologne, Germany

*„ATM restoration in vivo induces lymphoma regression through cell autonomous and non-cell autonomous mechanisms“*

### **Björn Schumacher**

Cologne, Germany

*„Genome stability in aging and disease“*

### **George Garinis**

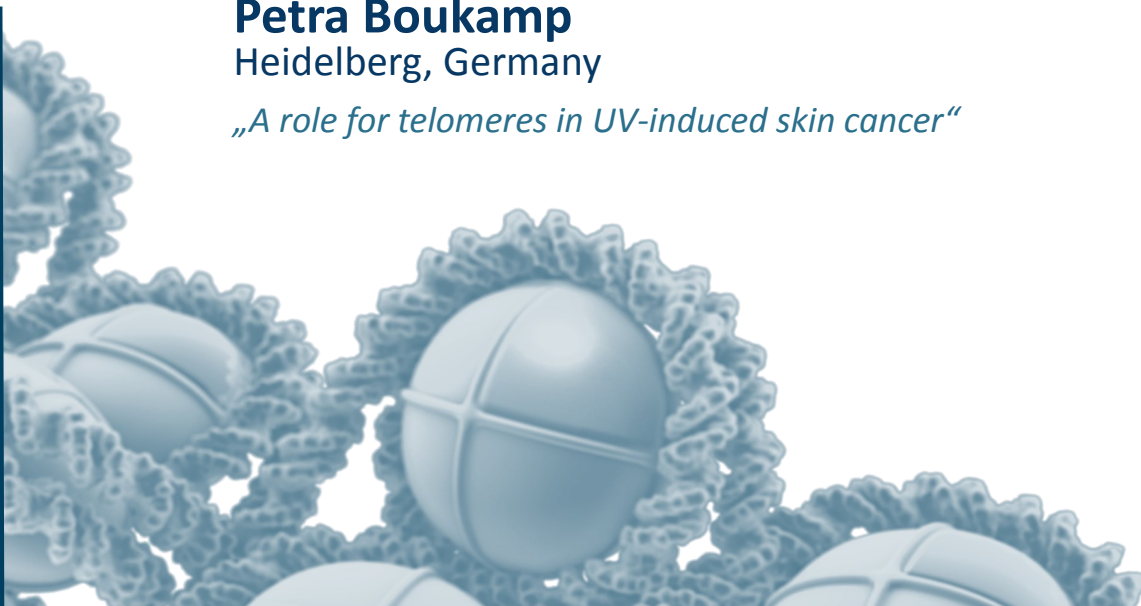
Heraklion, Greece

*„Nucleotide excision repair: from persistent DNA damage to chronic inflammation“*

### **Petra Boukamp**

Heidelberg, Germany

*„A role for telomeres in UV-induced skin cancer“*





# A vertebrate DNA replication completion checkpoint shows similarities to the spindle checkpoint

K. Sobkowiak, M. Kohzaki, F. Huber, S. Emamzadah, L. Tropia, R. Bohm, S. Hiller and  
**Thanos Halazonetis**

*University of Geneva, Switzerland*

Cell cycle checkpoints have been identified that monitor all the critical steps during cell division, except for completion of DNA replication. We examined the hypothesis that Rev7/Mad2L2, a protein with sequence similarity to the Mad2 spindle assembly checkpoint protein, functions in a DNA replication completion checkpoint. Chicken and human cells lacking Rev7 entered mitosis prior to completing DNA replication. The three-dimensional structure of Rev7 bound to Rev3 shows similarities to the structure of Mad2 bound to Mad1 and, as in the case of Mad2, binding of Rev7 to its ligand was associated with a major conformational change. We propose that human cells have a checkpoint that monitors completion of DNA replication and that this checkpoint has similarities to the spindle assembly checkpoint.

# Atm restoration in vivo induces lymphoma regression through cell autonomous and non-cell autonomous mechanisms

**Christian Reinhardt**

*Clinical and Molecular Oncology Department of Internal Medicine, University Hospital  
Cologne, Cologne, Germany*

Germline mutations in Ataxia Telangiectasia Mutated (ATM) cause Ataxia-telangiectasia, which is characterized by cerebellar ataxia and cancer predisposition. In addition, ATM is frequently inactivated in various human tumors. Given its role in genome maintenance, it is unclear whether ATM loss acts as an instigating lesion promoting acquisition of additional oncogenic mutations, or whether continued absence of ATM is critical for preserving the cancerous state. We generated a reactivatable Atm allele, which phenocopies Atm-knockout mutants prior to reactivation. We show that Atm reactivation in B- and T cell lymphomas induces lymphoma regression through cell autonomous and non-cell autonomous mechanisms, suggesting that continued Atm deficiency is required for the maintenance of pre-existing lymphomas. Our data further indicate that impaired immune surveillance contributes to cancer predisposition in A-T patients.

# Genome stability in aging and disease

**Björn Schumacher**

*University of Cologne, Cologne, Germany*

Aging is the principle risk factor for multiple chronic diseases including cardiovascular disease, dementia, cancer, chronic kidney disease, arthritis, and osteoporosis. The causal contribution of DNA damage in driving the aging process has become evident in a variety of progeroid syndromes that are caused by defects in DNA repair systems such as nucleotide excision repair (NER). Transcription-coupled (TC-) NER defects lead to growth retardation and premature ageing in Cockayne syndrome (CS) patients, while global-genome (GG-) NER mutations lead to highly skin cancer prone Xeroderma pigmentosum (XP). To understand the causes for the distinct outcomes of genome instability we have employed the simple metazoan *C. elegans* as model. DNA damage that persists in (postmitotic) somatic tissues leads to activation of the insulin-like growth factor signalling (IIS) effector DAF-16. The FoxO transcription factor DAF-16 enables developmental growth amid persistent DNA lesions and promotes maintenance of differentiated tissues through enhanced tolerance of DNA damage that accumulates with aging. Somatic tissues also respond to germline DNA damage that induces systemic stress resistance (GDISR) that is mediated by the innate immune system and executed through elevated activity of the ubiquitin proteasome system (UPS). We propose that GDISR elevates somatic endurance to extend reproductive lifespan when germ cells require time to reinstate genome stability before resuming offspring generation. To gain comprehensive insights into response mechanisms to persistent DNA damage we performed a proteomics, phosphoproteomics, and lipidomics analysis of NER deficient *C. elegans*. We find striking similarities to proteomes of aging animals underscoring the causal contribution of DNA damage responses to the aging process. We discern a signalling network revolving around IIS as important response pathways to DNA damage. Moreover, we find dampened fatty acid metabolism and marked induction of autophagy, which becomes essential for the animals to withstand the DNA damage. The multi-layered reprogramming leading to DNA damage tolerance and the GDISR systemically promote functional maintenance of the organism challenged by genome instability and might therefore open important new avenues for preserving functional integrity of cells and tissues during aging.



# Nucleotide Excision Repair: from persistent DNA damage to chronic inflammation

E. Goulielmaki, A. Ioannidou, K. Stratigi, G. Chatzinikolaou and **George Garinis**

*Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece*

Nucleotide Excision Repair (NER) represents the best known example of how a set of DNA repair factors previously known only to safeguard genome stability also function in controlling decisive steps in mammalian development and disease. Indeed, NER factors are now known to function in epigenetic regulation of gene expression, nuclear receptor signaling, the reprogramming of pluripotent stem cells, and in postnatal mammalian growth. Here, we will present evidence of how the accumulation of irreparable DNA lesions in our genome triggers chronic inflammatory responses leading to tissue degeneration and metabolic abnormalities in patients and mice with congenital defects in NER.

# A role for telomeres in UV-induced skin cancer

**Petra Boukamp**, A. Bort, D. Krunic and C. Leufke

*German Cancer Research Center, Heidelberg, Germany*

The incidence of skin cancer is increasing worldwide and cutaneous squamous cell carcinomas (SCCs) are associated with considerable morbidity and mortality, particularly in immunosuppressed individuals ('carcinomatous catastrophe'). Besides ultraviolet (UV)-indicative mutations, chromosomal aberrations are also prominent which are believed to contribute to skin cancer progression through chromosomal gains and losses and with that combined changes in the expression profile. As telomeres are essential in preserving chromosome integrity, we propose that UV-induced telomere erosion as well as UV-induced aberrant spatial telomere distribution and organization contribute to genomic instability and thus allow for chromosomal aberrations that additionally contribute to skin cancer initiation and progression.



# Session S13

## „Particle Radiation Modalities 2“

### **Lorenzo Manti**

Naples, Italy

*„Enhancement of clinical proton biological effectiveness by beams of proton-boron fusion reaction“*

### **Andrzej Wojcik**

Stockholm, Sweden

*„X-rays and  $\alpha$ -particles interact in inducing DNA damage in U2OS cells“*

### **Alexandros Georgakilas**

Athens, Greece

*„Uniting ionizing radiation, DNA damage and immune response through an integrative experimental and in silicoanalysis“*

### **Georgia Terzoudi**

Athens, Greece

*„Biological effectiveness of protons in comparison to  $\alpha$ -particles and accelerated C-ions as measured through induction and repair kinetics of chromosome damage in G0-lymphocytes by means of the PCC-assay“*

### **Charlot Vandevoorde**

Cape Town, South Africa

*„Assessment of out-of-field DNA damage and the impact of neutron RBE on secondary cancer risk in paediatric proton therapy“*

### **Marco Durante**

Trento, Italy

*„Ground-based research for space radiation protection“*



# Enhancement of clinical proton biological effectiveness by means of proton-boron fusion reaction

**Lorenzo Manti**, P.A.G. Cirrone, F. Cammarata, G. Cuttone, L. Giuffrida, G. Korn, D. Margarone, A. Minopoli, G. Petringa, A. Picciotto, P. Pisciotta, G. Russo and V. Scuderi

*Dipartimento di Fisica “E. Pancini”, Università Federico II di Napoli & Istituto Nazionale di Fisica Nucleare, Sezione di Napoli, Naples, Italy*

Accelerated proton beams is one of the fastest growing modalities in the fight against cancer worldwide [1]. Compared to conventional radiotherapy by photons or electrons, protontherapy offers a superior ballistic precision in confining most of delivered dose to the tumour, thereby increasing normal tissue/organs-at-risk sparing due to the inverted depth-dose exhibited by charged particles. However, therapeutical protons are mostly a low-LET radiation; hence, they are of no avail towards radioresistant cancers. Conversely,  $^{12}\text{C}$  ions are amenable at overcoming tumour cell resilience by virtue of their greater RBE. Unfortunately, economic and radiobiological issues currently hinder a wider clinical application of these ions. Thus, enhancing proton RBE is desirable. To this intent, we exploited the  $^{11}\text{B}(\text{p},\alpha)^2\alpha$  nuclear fusion reaction [2] to generate high-LET alpha particles with a clinical proton beam. The elegant idea of Proton-Boron Capture Therapy (PBCT) as first suggested by Yoon et al. [3], is similar, in principle, to that of the well-known approach using alpha particles from the  $^{10}\text{B}$ -neutron reaction (BNCT) to locally radiosensitize tumours. The cross section for the p- $^{11}\text{B}$  (PB) reaction peaks for proton energies reached within a clinical SOBP, rendering in principle less critical the need for the differential uptake of boron drugs by cancer cells as required in BNCT. Additionally, the intrinsic emission of prompt gamma-rays from the p-B reaction can be potentially exploited for on-line treatment verification [4]. To date, no experimental proof-of-principle demonstration of PBCT radiobiological gain has been carried out. To maximize the reaction rate, we used one of the BNCT commonly used boron carriers, sodium borocaptate (BSH), with natural boron content rather than in its  $^{11}\text{B}$ -enriched formulation. To demonstrate in vitro PB-mediated enhancement of tumour cell killing by protons, DU145 prostate cancer cells were irradiated with the clinical 62 MeV proton beam at LNS-INFN at mid-SOBP depth (calculated LET 5 keV/micron) and assayed for clonogenic survival. To assess the role of radiation quality in DNA damage induction we analysed structural chromosome aberrations (CA) in the non-tumorigenic MCF-10A cell line by means of calyculin-A induced PCC and FISH painting. A concentration of 80 ppm (parts-per-million) was chosen for BSH, in whose presence cells were pre-treated for 6-8 hrs and then irradiated. Whereas BSH alone did not induce significant cytogenetic damage, we recorded a significant increase in cellular lethality and occurrence of CA in irradiated BSH-treated samples compared to controls exposed to protons only. Notably, from survival data, a Dose-Modifying Factor of about 2.2 was calculated. Moreover, BSH caused a marked shift in the spectrum of radiation-induced CA yielding a greater frequency of complex-type aberrations, typical of high-LET radiation exposure, at each given proton dose. Altogether these data strongly suggest that the  $^{11}\text{B}(\text{p},\alpha)^2\alpha$  reaction leads to an augmentation of proton biological effectiveness via the production of high-LET alpha particles and therefore they represent the first experimental demonstration that PBCT may be a viable strategy to potentiate protontherapy clinical efficacy.

## References:

1. Particle therapy facilities under construction. [www.ptcog.ch](http://www.ptcog.ch)
2. H.W. Becker et al, Z. Physik A - Atomic Nuclei, 327, 341-355 (1987)
3. D.-K. Yoon et al, Appl Phys Lett, 105, 223507 (2014)
4. G. Petringa et al., JINST 12, C03059 (2017)

# X-rays and $\alpha$ -particles interact in inducing DNA damage in U2OS cells

A. Sollazzo, B. Brzozowska, L. Cheng, S. Haghdoost, H. Scherthan and **Andrzej Wojcik**

*Stockholm University, MBW Department, Stockholm, Sweden*

The survivors of atomic bomb explosions in Hiroshima and Nagasaki are monitored for health effect within the Life Span Study (LSS). The LSS results represent the most important source of knowledge about cancer effects of ionizing radiation and they form the basis for the radiation protection system. One uncertainty connected to deriving universal risk factors from these results is related to the problem of mixed radiation qualities. The atomic bomb explosions generated a mixed beam of the sparsely ionizing gamma radiation and densely ionizing neutrons and what is not taken into consideration is the problem of a possible interaction of the two radiation types in inducing biological effects. The existence of such interaction would suggest that the application of risk factors derived from the LSS to predict cancer effects after exposure to pure gamma radiation (such as in the Fukushima prefecture) leads to an overestimation of risk. In order to analyze the possible interaction of radiation types a mixed beam exposure facility was constructed where cells can be exposed to sparsely ionizing X-rays and densely ionizing alpha particles. U2OS cells were used, which are stably transfected with a plasmid coding for the DNA repair gene 53BP1 coupled to a gene coding for the green fluorescent protein GFP. Induction and repair of DNA damage which are known to be related to cancer induction were analyzed. The results suggest that alpha particles and X-rays interact, leading to cellular, and possibly cancer effects not predictable based on assuming simple additivity of the individual mixed beam components.

# Uniting ionizing radiation, DNA damage and immune response through an integrative experimental and in silico analysis

**Alexandros G. Georgakilas**, Z. Nikitaki, I. V. Mavragani, A. Pavlopoulou, V. Gorgoulis, O. A. Martin, C. E. Hellweg, G. Pantelias, G. Terzoudi and G. Iliakis

*DNA Damage laboratory, Physics Department, National Technical University of Athens, Athens, Greece*

Exposure to ionizing radiation (IR) induces a variety of cellular responses initiated by the DNA damage sensors proteins like ATM/ATR, activation of DNA damage response (DDR) and DNA repair (R) and later apoptosis and inflammatory or immune response pathways (1). Current evidence suggests that the cell handles these radiation-induced damage effects as something distinct from the ones induced by the endogenous replication stress (2). The signature of IR i.e the induction of complex and usually repair resistant DNA damage consisting of a variety of DNA lesions like double strand breaks (DSBs), single strand breaks (SSBs) and oxidized bases/abasic sites in a small DNA volume of a few cubic nm, it is expected to exert also the so called ‘non-targeted’ or systemic effects (3). Our groups have focused in this study, on the induction and processing of complex DNA lesions in different human cellular systems and with radiations of different LET. Specifically human cell lines MCF7, HepG2, A549, MO59K/J were used and radiation qualities of increasing LET, that is gamma-, X-rays 0.3-1 keV/mum, alpha-particles 116 keV/mum and 36Ar ions 270 keV/um. Using gamma-H2AX or 53BP1 foci staining as DSB probes, we calculated a DSB apparent rate of 5-16 DSBs/cell/Gy decreasing with LET. A similar trend was measured for non-DSB oxidized base lesions detected using antibodies against the human repair enzymes 8-oxoguanine-DNA glycosylase (OGG1) or AP endonuclease (APE1), that is damage foci as probes for oxidized purines or abasic sites, respectively (4). In addition, using colocalization parameters previously introduced by our groups, we detected an increasing clustering of damage for DSBs and non-DSBs. The complexity of DNA damage was found to increase with LET. In addition, through in silico analysis we have searched for the associate of DNA damage response proteins with the immune system and the generation of a systemic response in the organism. In this presentation, I will first present experimental evidence on how the mammalian cell or organism is expected to respond to complex DNA damage induction i.e. the signature of IR and primary ‘danger signal’. At second, I will discuss our knowledge of how a growing tumor in mice can lead to an increase of local oxidative and inflammatory markers, followed by decreased proliferation and increased presence of senescent cells indicated ongoing oxidative and genotoxic stress at distant sites similar to IR. Last but not least and in the light of our recent bioinformatics work, I will present our findings of the close relationship between DDR network proteins, inflammatory and immune response suggesting the necessity of a holistic approach in such cases of exogenous stress like IR (5).

**Acknowledgements:** This work has been supported by DAAD grant “DNA Damage and Repair and Their Relevance to Carcinogenesis” (No 57339330) and RESEARCH PROJECTS FOR EXCELLENCE IKY/SIEMENS awarded to I.V.M and A.G.G.

**References:**

1. Pateras, I.S., et al. (2015), *Pharmacol Ther*, 154, 36-56.
2. Nikitaki, Z., et al. (2015), *Frontiers in chemistry*, 3, 35.
3. Nikitaki, Z., et al. (2016), *Semin Cancer Biol*, 37-38, 77-95.
4. Nikitaki, Z., et al. (2016), *Free Radic Res*, 50, S64-S78.
5. Georgakilas, A.G. et al. *Cancer Lett.*, 368, 164-172



# Biological effectiveness of protons in comparison to $\alpha$ -particles and accelerated C-ions as measured through induction and repair kinetics of chromosome damage in G0-lymphocytes by means of the PCC-assay

**Georgia Terzoudi**, R. Cherubini, D. Zafiropoulos, L. Sarchiapone, A. Pantelias, V. De Nadal, L. Baggio, G. Pantelias

*Laboratory of Health Physics, Radiobiology & Cytogenetics, National Centre for Scientific Research "Demokritos", Athens, Greece*

There is an increasing interest in the use of high-LET radiation as a new promising cancer treatment modality. Particularly, protons offer a substantial clinical advantage over conventional photons because of the unique depth-dose characteristics of protons, which can be exploited to achieve significant reductions in normal tissue doses. Moreover, this unique characteristic allows escalation of tumour doses improving thus local control and survival while reducing toxicity and improving quality of life. However, studies on the effectiveness and risks of protons on normal tissue are relatively sparse. Data on relative biological effectiveness based on cytogenetic damage vary significantly due to different exposure conditions and the complications introduced by cell cycle kinetics and the difficulties of damaged irradiated cells to reach mitosis. In the present work, G0 human lymphocytes isolated from whole blood were exposed to a proton beam with an incident energy of 2.2 MeV and incident LET of 28.5 keV/ $\mu$ m in the dose range 0-6 Gy. To overcome the cell cycle kinetics issues following irradiation, the initial number and repair kinetics of excess chromosome fragments were investigated using the fusion premature chromosome condensation (PCC) methodology. The results obtained show that, following proton irradiation, there is a significant increase in the initial excess chromosome fragments when compared to those obtained by  $\gamma$ -rays using a Co-60 irradiator, as well as by alpha particles and accelerated Carbon-12 ions. For alpha irradiation, with particle energy 4.70 MeV at the cell surface entrance and LET at 92 keV/ $\mu$ m, a Curium-244 source was used, and for Carbon-12 irradiation the ions were accelerated at 56.5 MeV with LET at 295 keV/ $\mu$ m. The results revealed as well differences in the repair kinetics as quantified by the number of residual un-rejoined fragments after 2, 6, 12 and 24 hours post irradiation incubation at 37°C. The distribution of residual G0-PCC breaks as well as the mean break number for protons differed from those for  $\alpha$ -particles and C-ions and all were significantly higher when compared to those obtained for  $\gamma$ -rays. This result reinforces the notion that the LET- dependent structure in the irradiated lymphocytes is reflected in the repair processes. The different RBE values obtained for protons,  $\alpha$ -particles and C-ions, based on chromatin breakage and formation of chromosomal aberrations, are indicative of their toxicity and crucial for micronuclei formation and chromothripsis, which are at the basis of both cell death and radiation induced carcinogenesis. At present, work is in progress to test the hypothesis that using protons a significant reduction in normal tissue doses proximal and distal to the target volume is achieved.

# Assessment of out-of-field DNA damage and the impact of neutron RBE on secondary cancer risk in paediatric proton therapy

**Charlot Vandevoorde**, P. Beukes, E. de Kock, S. Chiriotti, A. Parisi, M. De Saint-Hubert, L. Tran, D. Prokopovich, A. Rosenfeld and J. Slabbert

*NRF iThemba LABS, Somerset West, South Africa*

The clinical application of proton therapy (PT) increased substantially over the last few years. The growing interest in PT is due to the high degree of dose conformation and the lower integral whole-body dose of protons compared to conventional photon radiotherapy (RT), which result in a reduction of side effects. However, despite the dose sparing properties of protons, they do have the potential to produce unwanted dose outside the primary field due to stray radiation, including secondary neutrons. Dose deposited outside the primary field may increase the risk of secondary malignancies, which is of particular importance for paediatric patients, known to be more susceptible to develop radiation-induced secondary cancer after RT. It is anticipated that the secondary neutron doses are only a fraction of the treatment dose, but low neutron doses have been well established to have a high biological effectiveness and potential for carcinogenesis. At present, there exists considerable uncertainty on how the relative biological effectiveness (RBE) for neutrons varies with dose and neutron energy, and whether the current RBE models and associated weighting factors are even appropriate for cancer risk estimation following PT. Previous studies measured and simulated the dose deposited outside the primary field by using a variety of techniques and detectors, however no radiobiological evaluation has been reported so far. Therefore, a measurement campaign was set up at NRF iThemba LABS to quantify the DNA damage attributable to secondary stray radiation in paediatric PT at different lateral and longitudinal positions outside the proton field. Radiobiology measurements were supported by (micro)dosimetry measurements to determine the relative contributions from neutron, gamma and scattered charged particle doses. Whole blood samples (2.0 ml) from two donors were irradiated in test tubes at 6 locations in Perspex sleeves positioned in a water tank. Four fixed positions outside the primary proton field were used (10mm, 35mm and 60mm from the lateral field edge and one downstream position 30mm behind the distal field edge, all at 85mm depth), in addition to two reference positions in the field (at 30mm depth along the entrance plateau and at 85mm depth, which corresponds to the middle of the SOBP). All out-of-field positions lie within the diameter of the head of a 5y-old child (average 150mm). Dosimetric measurements were performed at the same positions with neutron bubble detectors (Bubble Technology Industries), Li6 and Li7 enriched thermoluminescent dosimeters (TLDs) and a silicon-on-insulator microdosimeter (MicroPlus™ Probe). Irradiations were performed with a modulated clinical 200 MeV proton beam (range 100mm, collimator aperture 30mm and SOBP 31mm) and output factors (Gy/monitor unit) were measured with a T2 ionisation chamber. Whole blood samples were exposed to 0.2, 0.4, 0.6, 0.8 and 1 Gy doses at the six different positions and the micronucleus (MN) assay was performed to evaluate mutagenic effects of the secondary out-of-field stray radiation. Dose response curves for the out-of-field and reference positions were analysed and corresponding RBE values were calculated and will be presented. The results of the (micro)dosimetric measurements will also be presented, including  $Y_d$ ,  $\langle Q \rangle$  and Equivalent Dose. The measured neutron doses and RBE values for secondary stray radiation in passive scattering PT will be critically discussed, in particular for children, since the reduction of secondary cancer risk is in fact one of the principal reasons for the shift from photon-based therapy towards PT in paediatric oncology. Acknowledgement: We wish to thank the Physics Advisory Committee (PAC) of NRF iThemba LABS for support and beam time allocation.

# Ground-based research for space radiation protection

**Marco Durante** and C. La Tessa

*Trento Institute for Fundamentals Physics Applications, National Institute for Nuclear Physics, Italy*

Cosmic radiation is generally acknowledged as a potential showstopper for the human exploration of the Solar system. Research on protection from the harmful protons and HZE ions in the space radiation spectrum is urgently needed to reduce the uncertainty on the risk and to develop suitable countermeasures. Most issues can be addressed with physics and biology experiments at high-energy particle accelerator. We will report recent results from the NASA Space Radiation Health Program at the Brookhaven National Laboratory in the USA and plans for the next ESA Investigations of Biological Effects of Radiation (IBER) program.

# Session S14

## „Radiation Toxicity and Carcinogenesis“

**Christophe Badie**

Didcot, United Kingdom

*„Tracking pre-leukemic live cells in murine radiation-induced leukaemia“*

**Neeraj Kumari**

Delhi, India

*„IL-6 protects cells from radiation induced cell death by activating anti-oxidant defence and STAT-3 mediated pro-survival signalling“*

**Mieke Verslegers**

Mol, Belgium

*„Investigating congenital eye defects in prenatally-irradiated mice and the efficacy of folic acid as a radio-protective agent“*

**André Claude Mbouombouo Mfossa**

Mol, Belgium

*„Mechanistic analysis of radiation induced microcephaly in mice“*

**Emma Coninx**

Mol, Belgium

*„The hippocampus: a target for premature aging after early-life irradiation“*

**Paul Schofield**

Cambridge, United Kingdom

*„Mechanism and biomarkers predicting the outcome of taxane-based, concurrent radiochemotherapy in locally advanced lung carcinoma“*



# Tracking pre-leukemic live cells in murine radiation-induced leukaemia

T. Verbiest , R. Finnon , N. Brown , L. Cruz-Garcia , P. Finnon , G. Manning , E. Ross ,  
CL. Scudamore and **Christophe Badie**

*Public Health England, United Kingdom*

The hematopoietic cells of the bone marrow are amongst the most sensitive cells in the body to the damaging effects of ionising radiation. Although initial effects include cell toxicity, the most important long-term outcome is leukaemia. Leukaemia development is a genetically complex, multistep process and the target cells for leukaemogenesis are widely held to be hematopoietic stem and progenitor cells (HSPC). However, little is currently known of the characteristics, numbers and responses of these cells and the underlying mechanisms of radiation leukaemogenesis remain largely unidentified. Murine models are indispensable in studying leukaemogenesis with no in vitro system satisfactorily mimicking this process. The CBA mouse model of radiation-induced acute myeloid leukaemia (rAML) is well characterised with close histopathological similarity to that of the human disease. In this model, two molecular ‘hits’ have been identified. The first is the well characterised radiation-induced dose-dependent chromosome 2 deletion (Del2) with the loss of a copy of the essential haematopoietic transcription factor Sfpi1/PU.1. The second hit is a specific Sfpi1/PU.1 point mutation on the second allele. We have established a unique chromosome 2 Sfpi1-GFP/Rosa-mCherry mouse model harbouring both constructs, positioned in the radiation-induced Chr2del to study both initiation and progression of radiation-induced leukaemogenesis. We hypothesized that continued blood sampling of mice exposed to radiation prior to rAML presentation should provide a better understanding of progression of leukaemogenesis and clonal expansion, allowing the identification of pre-leukaemic hematopoietic cells. Following radiation exposure, CBA Sfpi1GFP/mCherry mice presented with expanding clones of pre-leukemic hematopoietic cells harbouring Del2, identified by the lack of either GFP or mCherry fluorescence. Using pyrosequencing in sorted bone marrow cells, we were able to identify the presence of Sfpi1 point mutations within sub-populations of these pre-leukemic cells, identifying for the first time the presence of such cells within a living animal and being able to characterise the clonal evolution. Interestingly, we also described an apparent gender difference in the phenotype of the pre-leukemic cells and in the presentation of the actual leukaemia, suggesting a possible difference in leukemic target cell within the hematopoietic stem cell compartment between male and female mice. In conclusion, this model allowed us to identify and characterise HSPC at risk for leukaemia development following radiation exposure in asymptomatic mice. Further studies using this mouse model will help to further characterise the key molecular mechanisms involved in the initiation and progression of radiation-induced leukaemia.

[christophe.badie@phe.gov.uk](mailto:christophe.badie@phe.gov.uk)

# IL-6 protects cells from radiation induced cell death by activating anti-oxidant defence and STAT-3 mediated pro-survival signalling

**Neeraj Kumari**, Y. Rai, D. K. Sah, B.S. Dwarakanath, A. Das and A. N. Bhatt

*Institute of Nuclear Medicine and Allied Sciences, Timarpur, Delhi, India; Department of Biotechnology, Delhi Technological University, Delhi, India*

IL-6 is known to induce radio-resistance in cancer cells by regulating multiple signaling pathways including apoptosis, survival, proliferation, angiogenesis, and, most importantly, the metabolism. Thereby, acting as a major obstacle in radiotherapy. IL-6 also protects normal cells like cardiomyocytes, hepatocytes, retinal ganglion, spleen and neuronal cells from oxidative stress, ischemia/reperfusion injury and other stress induced cell death. Therefore, we tested the hypothesis, if IL-6 treatment before irradiation will confer radio-resistance in normal cells and protect them from radiation induced cell death. We used the pleiotropic IL-6 as a prophylaxis for radiation exposure in hematopoietic (Raw264.7), fibroblast (NIH3T3) and intestinal epithelial (INT407) cell lines. We found a significant increase in cell survival (1.3 fold in INT407 cells to 1.4 fold in Raw264.7 cells) after IL-6 treatment prior (2hr.) to radiation exposure. Further, we found that IL-6 reduces radiation induced cell death/apoptosis. The result of IL-6 mediated reduction in radiation induced apoptosis is substantiated by the levels of pro and anti apoptotic proteins. The pro-apoptotic proteins (Bax, p21, p53, PARP and caspase 3 cleavages) was found to be down regulated while anti apoptotic proteins (Mcl-1, Bcl2, Bclxl) was upregulated in IL-6 treated groups. It was observed that STAT-3 was phosphorylated within 15 min. after IL-6 treatment and inhibition of STAT-3 phosphorylation using JSI-124 abrogates the IL-6 mediated protection from radiation induced cell death. Further we observed, IL-6 also induces the Nrf2 expression and maintains the antioxidant status of the cells. In our study we found increased levels of GSH SOD, and reduced ROS, lipid peroxidation and protein carbonylation. Therefore, our study suggests that IL-6 protects the cells from radiation induced cell death by inducing antioxidant defense system and STAT-3 mediated pro-survival signaling.

# Investigating congenital eye defects in prenatally-irradiated mice and the efficacy of folic acid as a radio-protective agent

K. Craenen, Mieke Verslegers, L. Craeghs, J. Buset, S. Baatout, L. Moons and A. Benotmane

*Belgian Nuclear Research Centre SCK-CEN, Radiobiology Unit, Mol, Belgium*

Previous epidemiological and animal studies indicated that exposure to high or moderate doses of ionizing radiation at early embryogenesis can lead to severe congenital defects such as exencephaly and microphthalmos (reduced eye size). Even though the teratogenicity of ionizing radiation has been observed in previous studies, there has been only limited effort to ascertain the molecular mechanisms underlying radiation-induced defects. As a first objective, we investigated the molecular mechanisms in the developing mouse embryo following acute X-irradiation (1.0 Gy) at embryonic day (E) 7.5, which we identified as a model for radiation-induced microphthalmos and exencephaly. RNA analysis using microarray and RT-qPCR uncovered an altered gene expression of PAX6, Wnt2b, Tyr and BMP4, which are all involved in the early embryonic melanogenesis pathway, both at E9 (early optic cup development) and at E11 (lens-detachment phase). Indeed, a Fontana-Masson staining of the melanin pigment demonstrated a noticeable lack of pigmentation at E11 following X-irradiation, and an irregular melanin distribution at E18. Of note, many of the genes identified in our study were already considered causal for microphthalmos and/or exencephaly by previous studies that used a variety of mutant mouse models. From a radiation protection point of view, radiotherapy of pregnant women is commonly discouraged. However, in urgent cases clinical staff may find that the benefits of irradiation outweigh the risks. Currently, there are no known radio-protectants to protect the unborn child from developing congenital defects. Here for, as a second objective, we investigated the radioprotective role of folic acid, a key factor in DNA synthesis and proliferation and a known antioxidant, in radiation-induced defects such as microphthalmos. To test this, C57/Bl6J mice were fed three different FA-enriched diets (3.5 mg/kg (control), 10 mg/kg and 20 mg/kg FA), starting one week before coupling. Of note, we observed no apparent toxic effects of FA supplementation. Interestingly, FA fortification resulted in a significant decrease of radiation-induced microphthalmos, exencephaly, agnathia and open-eye phenotypes in E18 fetuses irradiated with 1.0 Gy at E7.5. However, no dose response could be observed with the double fortification, suggesting that elevating the FA dose beyond the suggested dose in mice (10 mg/kg) holds no added value. As an overall conclusion, we have established that the melanogenesis pathway is a key player in the development of radiation-induced microphthalmos. In addition, folic acid was found to serve as a potent radioprotectant in preventing several radiation-induced congenital anomalies.



# Mechanistic analysis of radiation-induced microcephaly in mice

**André Claude Mbouombouo Mfossa**, M. Verslegers, S. Baatout, M. A. Benotmane, D. Huylebroeck and R. Quintens

*Radiobiology Unit, Belgian Nuclear Research Centre, Mol, Belgium / Laboratory of Molecular Biology, Department of Development and Regeneration, KU Leuven, Belgium*

Prenatal exposure to moderate and high doses of ionizing radiation (IR) during embryonic brain development in humans and mice can result in microcephaly. Previous work has shown that the overall changes in gene expression in the embryonic mouse brain shortly after radiation exposure are very similar to those observed in several other genetic mouse models of microcephaly and to Zika virus-infected human neural progenitor cells. These changes, most of which being mediated by the tumor suppressor p53, may therefore underlie the observed developmental defects. Among these genes, we have identified novel p53 targets, some of which are not yet functionally characterized. Unlike classical p53 targets, they are extensively enriched during embryonic brain development, as well as during neuronal maturation suggesting that they are functionally required for proper embryonic brain development and neuronal differentiation. The purpose of this research is to gain more insight into the role of p53 and some of these currently uncharacterized candidate targets, in brain development and the response to ionizing radiation. Currently, our focus is on D630023F18Rik, one of these novel p53 target genes. We found that several short splice variants are induced in a p53-dependent manner after IR both in primary cortical and hippocampal neurons, neuron progenitor cells and the embryonic brain. Activation of these short variants results from binding of p53 to an alternative promoter of the gene. Both long and short variants are preferentially expressed in the brain (and kidney) compared to other tissues and are highly induced during mouse brain development (in vivo) as well as during maturation of primary cortical and hippocampal neurons (in vitro). Overexpression of the long isoforms in Neuro-2a cells indicates that they code for proteins of about 25 kDa. Further investigations to assess the exact sequence of the radiation-induced transcript revealed a novel exon expressed in radiation-responsive variants. The spatio-temporal localization of the different transcripts by in situ hybridization is currently ongoing. In parallel, possible functions of the gene are being investigated using overexpression and knockdown strategies in different cell types. Furthermore, to illustrate the global role of p53 during brain development, we have generated mice in which the p53 gene is specifically knocked out at the stage of early neurogenesis in the dorsal forebrain. These mice will be used to evaluate morphological and functional effects of prenatal radiation exposure in comparison to wild-type mice.

# The hippocampus: a target for premature aging after early-life irradiation

**Emma Coninx**, M. Neefs, S. Baatout, L. Moons, R. Quintens and M. Verslegers

*Radiobiology Unit, Institute for Environment, Health and Safety, Belgian Nuclear Research Centre (SCK•CEN), Mol, Belgium; Neural Circuit Development and Regeneration Research Group, Department of Biology, University of Leuven, Leuven, Belgium*

The human developing brain is highly sensitive to ionizing radiation exposure [1]. Follow-up studies of atomic bomb survivors in Hiroshima and Nagasaki [2] and pediatric patients receiving cranial radiotherapy, have reported cognitive defects at adult age, respectively after pre- and postnatal irradiation exposure. The latter even suggested the presence of Alzheimer's disease (AD) hallmarks long after receiving radiotherapy [3, 4]. The long-term radiation-induced changes show a remarkable similarity with age-related hallmarks, including reduced neurogenesis, neuro-inflammation, oxidative stress and DNA damage [5, 6]. Still, the mechanisms underlying the radiation-induced defects to the developing brain remain poorly understood. Previous investigations in our laboratory have convincingly identified the hippocampus as a primary target for radiation-induced defects to the developing brain. Following prenatal irradiation with 1-Gy X-rays, an aberrant neurogenesis in the hippocampal dentate gyrus [7] and a reduction in hippocampal long-term potentiation was noted in adult C57BL/6J mice. This radiation-induced hippocampal impairment was confirmed in 90-week old mice that had been prenatally irradiated, by a clear decrease in hippocampal volume and impaired hippocampal-dependent cognition. Our current goal is to evaluate hippocampal dysfunctionality after early-life irradiation, possibly leading to accelerated brain aging and AD pathology. To this end, we optimized an in vitro aging model using primary mouse hippocampal neurons. Maturing neurons will be irradiated with 1.8-Gy X-rays, after which the effect on cellular aging will be established by examining neuronal connectivity, senescence, amyloid beta (A $\beta$ ) and p-tau depositions, etc. This in vitro model will be complemented with in vivo studies using triple transgenic (3xTg-AD) mice predisposed to developing AD and showing accelerated aging. After 1.8-Gy irradiation (single or fractionated) of ten-day-old 3xTg-AD mice, cellular aging and AD pathology will be investigated with a focus on the hippocampal region. In all, our results will give better insights into possible radiation-induced aging and neurodegeneration after X-ray exposure to the developing brain, which will ensure a better protection of the unborn child and a better follow-up of children receiving cranial radiotherapy.

## References:

1. Verreert, T., et al., *Neural Plast*, 2016. 2016: p. 1243527.
2. Otake, M. and W.J. Schull, *Int J Radiat Biol*, 1998. 74(2): p. 159-71.
3. D'Ambrosio, D.J., et al., *J Neurooncol*, 2007. 85(1): p. 77-9.
4. Schuitema, I., et al., *J Clin Oncol*, 2013. 31(27): p. 3378-88.
5. Richardson, R.B., (Albany NY), 2009. 1(11): p. 887-902.
6. Hernandez, L., et al., *Aging Cell*, 2015. 14(2): p. 153-61.
7. Verreert, T., et al., *J Neurodev Disord*, 2015. 7(1): p. 3.

# The STORE database; a platform for sharing experimental and epidemiological data and resources in radiobiology

**Paul Schofield**, U. Kulka, M. Gruenberger, M. Birschwilks, C. Adelman and B. Grosche

*University of Cambridge, Cambridge, United Kingdom*

The STORE data-sharing platform was originally conceived and developed under EC FP7 funding between 2009-12 following the success of the ERA project which successfully mobilised large amounts of legacy data from large-scale radiation exposures into a freely accessible database. Since then STORE has been an integral part of the MELODI strategy for data sharing, supported by the BfS, and now part of the CONCERT infrastructure. We report here recent key developments in the technical infrastructure of STORE and plans for using STORE for sharing data for specific applications in biodosimetry emergency preparedness, radioecology, and with major institutions outside Europe. The sharing of data and biomaterials from publicly-funded experimental radiation science adds enormous value to the original investment and improves reproducibility and accountability. Moreover, sharing yields substantial scientific added value through re-analysis and facilitation of new investigations. These issues have been recognised by the European Commission, and under Horizon2020 there is an ultimate intention to mandate sharing of critical primary data from all funded projects. CONCERT will support this policy and will require the sharing of key data from CONCERT funded project calls. This is particularly important for the data underlying publications, but in many cases it is important that large datasets that are collected as part of a project may also be shared. STORE provides a platform for the controlled sharing of data, publicly and within defined groups of users, and is the only available datasharing platform that is truly data-type agnostic. Data is discoverable through comprehensive standardised metadata and citeable through accession numbers and unique digital object identifiers (DOI). STORE moved into the BfS computing environment in March 2016, benefiting from the stability and security of the BfS, and is now accessible on [www.storedb.org](http://www.storedb.org). STORE is registered with Re3Data and Biosharing, and has its details in the MIRIAM registry of biological databases at EBI, generating unique and persistent data identifiers. New applications and partnerships are being developed on top of the STORE platform, for example a large collection of cytogenetic images as part of the activities of RENEB, and a resilient platform for data sharing and exchange to be used in response to a radiological emergency. Recently STORE has undertaken to archive and distribute radioecology data and will provide access to the JSHARE database of mouse radiation exposure experiments from the National Institute for Radiation Sciences in Japan. Generally, STORE will act as portal to any other database related to radiation research, together with pointers to radiation biology-associated infrastructures and biological collections. Access and deposits are free of charge and users are encouraged to use the resources and contact STORE for any advice or assistance they might need.



# Session S15

## „Normal Tissue Response 1“

**Florian Wirsdörfer**

Essen, Germany

*„Loss of CD73 prevents macrophage accumulation and phenotype changes in radiation-induced lung fibrosis“*

**Noëlle Mathieu**

Fontenay-aux-Roses, France

*„Bowel radiation injury: complexity of the pathophysiology and promises of cell and tissue engineering“*

**Shwetaanjali Nimker**

Delhi, India

*„Dose-dependent morphological and biochemical alterations in the peripheral blood erythrocytes of gamma-irradiated Swiss albino mice“*

**Arjan van Dijk**

Bilthoven, Netherlands

*„A mechanistic model for radiation induced atherosclerosis“*

**Sylwia Kabacik**

Didcot, United Kingdom

*„Ionising radiation increases permeability of endothelium through ADAM10-mediated cleavage of VE-cadherin“*

**Lorena Giuranno**

Maastricht, Netherlands

*„Improving lung cancer outcome by reducing normal lung toxicity“*



# Loss of CD73 prevents Macrophage Accumulation and Phenotype changes in Radiation-Induced Lung Fibrosis

S. de Leve, **Florian Wirsdörfer**, F. Cappuccini, A. Schütze, AV. Meyer, K. Röck, LF. Thompson, JW. Fischer, M. Stuschke and V. Jendrossek

*Institute of Cell Biology (Cancer Research), University Hospital Essen, Germany*

While radiotherapy is an integral part of cancer therapy, pneumonitis and fibrosis constitute dose-limiting side effects of thorax and whole-body irradiation. So far, the contribution of immune cells to disease progression is largely unknown. Here we aimed to study the role of CD73/adenosine-induced changes in the myeloid compartment in radiation-induced lung fibrosis. C57BL/6 wildtype (WT) or CD73 knockout (CD73<sup>-/-</sup>) mice received a single dose of whole thorax irradiation (WTI, 15 Gy). Myeloid cells were characterized in flow cytometric, histological and immunohistochemical as well as RNA analyses. We observed a dramatic loss of alveolar macrophages (AM) at 3 weeks post-irradiation in WT and CD73<sup>-/-</sup> mice that reconstituted at 6 to 12 weeks post-irradiation. Radiation-induced lung fibrosis in WT mice was associated with a phenotypic switch of AM towards an alternatively activated phenotype characterized by increased expression of the anti-inflammatory macrophage mannose receptor (MMR). Further, macrophages accumulated in organized clusters and expressed pro-fibrotic mediators during the fibrotic phase. In irradiated CD73<sup>-/-</sup> mice no clusters of alternatively activated macrophages were found. We speculate that accumulation of alternatively activated macrophages in organized clusters represents the origin of fibrotic foci after WTI and is driven by a cross-talk between CD73/adenosine signaling and the hyaluronic acid system.

# Bowel Radiation Injury: Complexity of the pathophysiology and promises of cell and tissue engineering

**Noëlle Mathieu**, L.Moussa, C. Demarquay, V. Monceau, A. Lapierre, P. Weiss, M. Benderitter and A. Semont

*IRSN, France*

Statement of the Problem: Bowel radiation injury is an insidious disease associated with substantial morbidity and mortality. Moreover, it's an increasing problem as more patients receive radiotherapy and survive longer after their tumor treatment. Bowel radiation injury results from the treatment of several cancers by radiotherapy in which normal colorectal tissues are present in the irradiation field. The clinical expression of bowel complications associated to radiotherapy resembles chronic bowel disease of other etiologies. However, recent studies have identified differences and specialists have proposed that complications following pelvic radiotherapy should be recognized as a "new disease" by Andreyev et al. The growing number of cases declared every year highlights the importance of understanding the mechanisms involved and of finding effective therapies. There is no unified approach for the assessment and treatment of this disease partly due to insufficient knowledge about the mechanism involved in the development of bowel radiation injury. However, unresolved inflammation is hypothesized to have an important role in late side effects. We used an experimental model of radiation proctitis developed in rats that reproduces severe colonic mucosal damages and fibrosis similar to those observed in patients treated by radiotherapy (1). Findings: Our studies demonstrated the involvement of inflammation and immunity in colorectal damages induced after localized irradiation. We also evaluated the benefit of immunomodulatory mesenchymal stromal cells isolated from adipose tissue (Ad-MSC) to reduce late side effects (2-3). We demonstrated a therapeutic benefit on different crucial functions of the colon and determined pleiotropic action mechanisms of the cell therapy treatment. Our studies also identified targets to potentiate the therapeutic effect of Ad-MSC. We have thus highlighted a biomaterial-assisted MSC therapy to alleviate colonic radiation-induced damage (4). Conclusion & Significance: Our studies provide evidence for the potential of Ad-MSC to limit radiation effects on the colon and could open new perspectives in the treatment of other radio-induced diseases.

## References:

1. Sémont A (2013), PLoS One. 29;8(7).
2. Bessout R (2014), Mucosal Immunol. 7(3):656-69.
3. Bessout R (2015), J Pathol. 237(4):435-46.
4. Moussa L (2017), Biomaterials 115:40-52

# Dose-dependent morphological and biochemical alterations in the peripheral blood erythrocytes of gamma-irradiated Swiss albino mice

**Shwetanjali Nimker**, V. Singh, K. Sharma, JS. Kumar, V. Kumar, R. Saraswathy and S. Chandna

*Division of Natural Radiation Response Mechanisms, Institute of Nuclear Medicine and Allied Sciences, Timarpur, Delhi, India*

Haematopoietic cells are extremely sensitive to radiation damage amongst all the cell lineages, even at relatively low level of exposure. While lymphocytes/leucocytes readily undergo radiation-induced apoptosis, other cell lineages also suffer radiation-induced damage to varying levels. The red blood cells (RBCs) too show significant radiation response, although erythrocytopenia may not set in at doses known for leukocytopenia. Like other cells, RBCs also rely on  $\text{Ca}^{2+}$  dependent signalling during differentiation from precursor cells.  $\text{Ca}^{2+}$  is a universal signalling molecule involved in regulating cell cycle and fate, metabolism and structural integrity, motility and volume. Intracellular  $\text{Ca}^{2+}$  levels in the circulating human RBCs take part not only in controlling biophysical properties such as membrane composition, volume and rheological properties, but also physiological parameters such as metabolic activity, redox state and cell clearance. In this study using flow cytometry, we observed a dose-dependent increase in erythrocytes' intracellular calcium ion levels and reactive oxygen species following irradiation at 1Gy-10Gy. Since the calcium-calmodulin complex plays a key role in the regulation of cytoskeletal stability, these intracellular changes in red blood cells also affect several membrane proteins including spectin, actin, and 4.1R, together with tropomyosin, tropomodulin, adducin, dematin, p55. These changes in membrane proteins lead to abnormal cell morphology of erythrocytes. Our study shows that different doses of gamma radiation could cause various degrees of damage to the erythrocyte membrane. After whole body irradiation, the proportion of normal erythrocytes decreased while that of abnormal ones increased in a dose-dependent manner, especially at doses above 2Gy. The abnormal erythrocytes mainly observed were echinocytes, spherocytocytes besides other aberrant shapes. We also observed that increased intracellular calcium levels mediate several  $\text{Ca}^{2+}$  dependent processes inside the cell including changes in cell volume and morphology (high MCHC, spherical index); appearance of calpain-induced band 3 cleavage fragments; increase in oxidative stress or unusually high met-Hb; membrane lipid peroxidation; ATP depletion due to hyperactivation of PMCA; increase in inter-RBC aggregability; and increase in PS externalization. Detailed results of post-irradiation changes in red blood cells observed by microscopy and flow cytometry will be presented.



# A mechanistic model for radiation induced atherosclerosis

A. Kloosterman, T. van Dillen, H. Bijwaard, Arjan van Dijk, S. Heeneman, S. Hoving, F. Stewart and F. Dekkers

*National institute for public health and the environment (RIVM), Bilthoven, the Netherlands*

Patients who have undergone radiotherapy are known to be at an increased risk of developing vascular disease, but the biological mechanisms involved are not fully understood. Furthermore, it remains unclear at which stages in the development of disease radiation can act. Mechanistic models may improve insights that contribute to answers to these questions. We have developed a mathematical model which aims to describe the biological processes involved in the development of radiation-induced atherosclerosis. The model is based on two consecutive processes: a probabilistic, dose-dependent plaque initiation process, followed by deterministic plaque growth. This model has been tailored to experimental data from ApoE<sup>-/-</sup> mice exposed acutely to 0, 8, or 14 Gy X-ray doses at the Dutch National Cancer Institute (NKI). ApoE<sup>-/-</sup> mice were chosen since atherosclerosis in this mouse strain partly mimics the process of atherosclerosis as it occurs in humans. Our analysis indicates that in an acute exposure scenario, ionizing radiation contributes to the initiation of new plaques. In this setting, we found no evidence for an effect of radiation on the growth of existing plaques.

# Ionising radiation increases permeability of endothelium through ADAM10-mediated cleavage of VE-cadherin

**Sylwia Kabacik** and K. Raj

*Radiation Effects Department, Centre for Radiation, Chemical and Environmental Hazards,  
Public Health England, Chilton, United Kingdom*

The association between ionising radiation (IR) exposure and risk of cardiovascular diseases (CVD) is well documented, but the underlying mechanism is still poorly understood. As atherosclerotic plaques are the most common cause of CVD, we investigated the effects of IR on one of the critical parameters for atherosclerotic plaque formation – endothelium permeability to macromolecules. We used endothelial cells from human coronary artery as a model of the endothelial layer. Our results show that exposure of this endothelial layer to IR increased its permeability to macromolecules of various sizes in a dose-dependent manner. Immunofluorescence analysis revealed disruption of cell junctions caused by decreased amounts of two junction proteins, one of which is vascular endothelial cadherin (VE-cadherin). The reduction in the level of this protein was not due to diminished transcription but to protein processing instead. We observed a radiation dose-dependent increase in the cleavage of VE-cadherin by ADAM10. This was not mediated through the canonical VEGF route but was instead accompanied by intra-cellular calcium release. Importantly, inhibition of ADAM10 activity rescued IR-induced permeability. Our observations demonstrate that exposure to IR activates ADAM10 to cleave VE-cadherin leading to augmented endothelium permeability; a feature that can lead to the development of atherosclerotic plaques and increase the risk of cardiovascular disease.

# Improving lung cancer outcome by reducing normal lung toxicity

**Lorena Giuranno**, EM. Roig, R. Iannone, C. Wansleebe and M. Vooijs

*Maastricht University, Netherlands*

Lung cancer is the leading cause of cancer death. New treatments that complement standard chemoradiation and surgery are urgently needed. Deregulation of the NOTCH signaling pathway is associated with poor outcome and treatment resistance in patients and in preclinical models (Theys et al., 2013) suggesting NOTCH signaling as a novel therapeutic target. Treatment outcome is limited by dose-limiting side-effects which negatively affect tumour control and quality of life. Reducing side-effects may improve tumor control by dose-escalation and treatment-time. What is currently lacking are primary human lung tissue models that enable robust evaluation of normal tissue effects prior to clinical studies. Therefore we established and characterized primary lung organoids and air liquid interface system (ALI), pseudo-stratified cultures derived from primary human bronchial epithelial cells (PBEC) from 3 different donors. In these cultures basal cells proliferate and differentiate into ciliated and mucous/secretory cell types resembling the human bronchus. We investigated the consequences of blocking NOTCH using the pan-notch g-secretase inhibitor (DBZ 1uM) alone and when combined with irradiation (2, 4 Gy) and evaluated early and late response to radiotherapy when combined with NOTCH inhibition at different time-points. Using immunofluorescence, western blot and qPCR we found that basal cells (p63, CK5) cease proliferation (Ki67, EdU) at day 21 and mucous cell differentiation (Muc1/5ac) precedes ciliary differentiation (Ac-Tub) by 5 days starting at day 10 and both complete at day 28. Proliferation decreases overtime but inhibiting NOTCH in differentiated cells at day 21 increases p63+ proliferation alone and even more when combined with radiotherapy. In all 3 donors NOTCH inhibition increases p63+basal progenitors and ciliated cells and decreases mucous cells alone and when combined with radiation. We observed increased pATM and pCHK2 12h post irradiation when NOTCH signaling was inhibited.  $\gamma$ H2AX staining shows no difference 1h post irradiation but reduced DNA breaks 24h post irradiation when NOTCH was inhibited. We are further studying how NOTCH inhibition affects the survival/expansion of progenitor cells evaluating immediate and long-term DDR and repair effects. These data support the use of such normal patient tissue for predictive toxicity screening of combination treatments and disclose important novel interactions between NOTCH inhibition and radiotherapy.



## Session S16

### „Low Dose Radiation Effects“

**Horst Zitzelsberger**

Munich, Germany

*„Radiation markers in breastcarcinogenesis “*

**Claudia Fournier**

Darmstadt, Germany

*„Immune-related, low dose effects in adipose and other joint cells and tissue “*

**Roel Quintens**

Mol, Belgium

*„Prenatal irradiation as a potential inducer of premature differentiation during corticogenesis “*

**Maria Gomolka**

Oberschleißheim, Germany

*„Gene expression changes in former uranium workers of the sag/sdag wismut “*

**Claudia Dalke**

Neuherberg, Germany

*„A lifetime study in mice – assessing several biological endpoints after exposure to low doses of IR“*

**Daniela Hladik**

Neuherberg, Germany

*„Low-dose radiation exposure leads to irreversible changes in the proteome of the murine hippocampus “*



# Radiation markers in breast carcinogenesis

**Horst Zitzelsberger**, C. Wilke, J. Hess, S. Klymenko, V. Chumak, A. Walch, M. Selmansberger, H. Braselmann, F. Fend, H. Bösmüller and K. Unger

*Research Unit Radiation Cytogenetics, Helmholtz Zentrum München, Munich, Germany*

Ionizing radiation is a well-known risk factor for the development of breast cancer. However, radiation-specific molecular markers and mechanisms as they have been reported for thyroid cancer are largely unknown in this highly prevalent tumor entity. Therefore, the aim of our study was to investigate different molecular levels in tumor samples from radiation-exposed female clean-up workers and non-exposed controls from a cohort of post-Chernobyl breast cancers. We investigated differential miRNA and protein expression and the occurrence of genomic copy number alterations in the exposed and the control group. We were able to identify radiation-associated alterations in the discovery cohort, which subsequently could be confirmed in an independent validation cohort. This includes expression of hsa-miR-26b-5p, which was associated with radiation exposure in both cohorts. Moreover, a downregulation of the TRPS1 protein which is a transcriptional target of hsa-miR-26b-5p, was associated with radiation exposure. Since TRPS1 overexpression is common in sporadic breast cancer, its observed downregulation in radiation-associated breast cancer warrants clarification of the specific functional role of TRPS1 in radiation-induced carcinogenesis. For this purpose, the impact of TRPS1 expression on the transcriptome was characterized in two radiation-transformed breast cell culture models after siRNA-knockdown. Deregulated genes upon TRPS1 knockdown were associated with DNA-repair, cell cycle, mitosis, cell migration, angiogenesis and EMT pathways. Furthermore, we identified interaction partners of TRPS1 from the transcriptomic correlation networks derived from gene expression data on radiation-transformed breast cell culture models and also from data on sporadic breast cancer tissues provided by the TCGA database. The genes correlating with TRPS1 in the radiation-transformed breast cell lines were primarily linked to DNA damage response and chromosome segregation, whilst the transcriptional interaction partners in the sporadic breast cancers were mostly associated with apoptosis. Thus, upregulation of hsa-miR-26b-5p and downregulation of TRPS1 in radiation-associated breast cancer tissue samples suggests these molecules representing radiation markers in breast cancer.

# Immune-related, low dose effects in adipose and other joint cells and tissue

K. Shreder , F. Rapp , A. Maier, J. Wiedemann , U. Gaigl , B. Frey , G. Kraft and  
**Claudia Fournier**

*GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany*

Patients suffering from chronic inflammatory bone disorders, i.e. osteoarthritis and rheumatoid arthritis are classically treated by anti-inflammatory and pain relieving drugs. Also low dose irradiation is used, either as an alternative or complementary to a pharmacological treatment. More than 40,000 patients per year, suffering from chronic inflammatory bone diseases undergo repeated exposures to radon or to photons (low dose radiotherapy, LDRT). Functional improvement of the inflamed joints and pain relieve are reported, but especially for radon exposure the cellular and molecular basis is widely unknown. To explore this and to contribute to a scientific evaluation of risk and benefit of radon exposure is the goal of the research activities of the GREWIS-consortium. The estimated doses delivered during radon exposure to the whole body are very low, are below 2mSv. We hypothesized that a biological effect of such low doses arises from an accumulation of radon and its decay products at specific tissue sites. The major dose contribution of radon exposure comes from  $\alpha$ -particles, which are emitted during radon decay; and the distribution of the dose deposited by  $\alpha$ -particles in the tissue is heterogeneous and can be up to 0.5 Gy for a traversed cell. To find these putative “hot spots” of radon exposure, part of our work is dedicated to measure diffusion and solubility of radon in different tissues. We have constructed a radon chamber where we can expose different tissues under defined conditions. Our results confirm former studies that the solubility of radon in fat tissue is higher compared to muscle tissue. Based on this result, we hypothesized that potential target cells of radon exposure, which drive chronic inflammatory diseases, are also located in fatty tissues. This idea is consistent with our results from the RAD-ON01 patient study, where we measured in the serum of radon treated patients a long-term decrease of inflammatory factors (adipokines), typically released by fatty tissue. We also observed a decrease of bone degradation markers in serum samples and a changed activation of immune cells. As in the joint the articular fat pad is located in close vicinity to bone, cartilage and bone-related vasculature, we assume a local influence of radon exposed fat pad on the inflamed joints and the bone, synovial, cartilage and immune cells involved in the disease. In our ongoing in vitro and ex vivo investigations, we did not detect pronounced changes in adipocytes after irradiation. However, adipokines activate the cartilage degrading synovial fibroblasts, and we found that irradiation inhibits partially this inflammatory activation. This suggests that a decreased adipokine level inhibits the inflammatory action of synovial fibroblasts in the joints, but other cells of the fatty tissue than adipocytes are responsible for the changed release of adipokines after radon treatment in patients.

Funded by BMBF grant No. 02NUK017 (GREWIS).

[c.fournier@gsi.de](mailto:c.fournier@gsi.de)

# Prenatal irradiation as a potential inducer of premature differentiation during corticogenesis

**Roel Quintens**, B. Hubrecht, R. Benotmane, S. Baatout and M. Verslegers

*Belgian Nuclear Research Centre, Mol, Belgium*

The developing brain is particularly sensitive to radiation-induced defects. For example, impaired cognition and a reduction in brain size (microcephaly) have been demonstrated in both human and animal studies. Short-term effects of in utero irradiation that lead to these functional deficits are being explored, but a complete understanding of the underpinning mechanisms is still missing. A previous study suggested that irradiation of the embryonic mouse brain results in the activation of a p53-dependent differentiation program. Since it is well known that premature neuronal differentiation can result in microcephaly, we aim to investigate whether this process also occurs in prenatally irradiated brains. In this study, we investigated brain development in mice irradiated at embryonic day 11. As expected, we observed a significant increase in DNA damage shortly after X-ray exposure (0.1 Gy and 1.0 Gy), accompanied by a massive increase in apoptotic cells at 6 h and 24 h post-irradiation. Furthermore, a transient G2/M cell cycle block at 1 h and 2 h post-irradiation was noted in neocortical cells irradiated with 1.0-Gy X-rays, in contrast to 0.1-Gy irradiated cells. Of interest, Pax6 and Dcx immunostainings unveiled an increase in the proportion of postmitotic maturing neurons in the neocortex after 1.0-Gy irradiation, indicative for premature neuronal differentiation. To study the underlying cause in more detail, we performed stainings for Tight junction protein 1 (Zo1). Zo1 is essential for attachment of glial stem cells to the apical membrane and for modification of their cleavage plane orientation, to initiate neuronal differentiation. We found a clear disruption of Zo1 expression at the ventricular lining both in 0.1- and 1.0-Gy irradiated brains as compared to sham-irradiated controls, indicating that radiation affects glial-to-neuronal transition. Finally, irradiation of Neuro2a neuroblastoma cells with 8.0 Gy reduced their proliferative capacity and induced neurite outgrowth, which could be prevented by prior treatment with the p53 inhibitor alpha-pifithrin. In all, our results suggest that prenatal irradiation indeed leads to premature differentiation of cortical neuron progenitors, which may contribute to the observed microcephalic phenotype. Whether this mechanism is mediated by the transactivating properties of p53, is currently being investigated using mice in which p53 is knocked out in the Emx1 lineage of telencephalic progenitors. Acknowledgement: This work is financially supported by the Research Foundation - Flanders (G0A3116N).



# Gene expression changes in former uranium workers of the sag/sdag wismut

**Maria Gomolka**, K. Nieselt, S. Poths, M. Bonin, G. Johnen, M. Lehnert, D. Taeger, U. Kulka and D. Samaga

*AG-SG1.2 Biologische Strahlenwirkungen, Biologische Dosimetrie, Bundesamt für Strahlenschutz, Neuherberg, Germany*

The Wismut cohort is the world largest cohort of uranium miners exposed to radon and radon progeny as well as long-lived radionuclides and external gamma irradiation. In 2008 the BfS initiated biobanking of blood samples from former high and low exposed workers. Together with collected medical and exposure data, this material represents a unique resource for radiation research. To investigate the long-term effects of radiation on transcriptional profiles a total of 200 blood samples of Wismut miners (only males) were investigated by using whole genome microarrays. Current smokers and cancer cases were excluded. Cumulative exposure to total absorbed red bone marrow dose for the high exposed workers (n=100) ranges from 170 mGy to 668 mGy and was below 30 mGy for the age and smoking matched low exposed workers (n=100). In a first stage of the study high quality expression data could successfully be generated for 130 RNA samples. By elaborated bioinformatic methods, a gene signature of 101 genes was identified, which allowed discrimination between high and low radiation dose. In the subsequent validation procedures 30 highly exposed and 29 low exposed subjects were compared and the gene signature was validated positively with a sensitivity of 100% and a specificity of 75%. This is the first study to show validated gene expression changes in uranium workers 20 to 30 years after radiation exposure. However, further research is needed to rule out other factors influencing the gene expression pattern.

# A lifetime study in mice – assessing several biological endpoints after exposure to low doses of ionizing radiation

**Claudia Dalke**, S. Kunze, F. Neff, U. Rößler, M.-C. Ung, S. M. Hölter, K. Unger, S. Tapio, U. Kulka and J. Graw

*Institute of Developmental Genetics, Helmholtz Zentrum München, Munich, Germany*

There is a desperate need for quantitative data to judge the effects of low dose ionising radiation. In a lifetime study with mice as a mammalian animal model we analysed radiation effects on the eye, especially the ocular lens and other organs, as well as the body weight, tumour rate and locomotor, sensorimotor and cognitive function. Young adult mice (10 weeks) of different genetic constitution (wild type and *Ercc2*<sup>+/-</sup>) were whole body irradiated with a single low dose of ionising radiation (0, 63, 125 or 500 mGy) at a low dose rate (63 mGy/min). Lens opacification was analysed monthly by Scheimpflug imaging and the retinal fundus and thickness was analysed by OCT. At different time points (4 and 24 hours, 4, 12, 18 and 24 months post irradiation) mice were sacrificed and a set of organs were analysed histologically. Behaviour tests were performed 4, 12 and 18 months post irradiation. Over a follow up period of 24 months after irradiation the lens opacification showed a significant dose-dependent effect; nevertheless it was a subtle increase, which is not clinically relevant. A significantly altered survival rate and a dose-dependent risk for several types of tumours were indicated by pathological screening. Also the analysis of some behaviour tests showed significant dose-dependent radiation effects. We demonstrated clear long term effects after exposure to low doses of ionising radiation, but the ocular lens does not seem to be one of the most radiation-sensitive tissues as supposed so far.

# Low-dose radiation exposure leads to irreversible changes in the proteome of the murine hippocampus

**Daniela Hladik**, O. Azimzadeh, C. von Törne, S. Hölter-Koch, K. Unger, F. Theis, M. Gomolka, J. Graw, M. J. Atkinson and S. Tapio

*Institute of Radiation Biology, Helmholtz Zentrum München, German Research Centre for Environmental Health GmbH (HMGU), Neuherberg, Germany*

The impact of low-dose radiation on the human brain attracts attention due to the increasing use of ionizing radiation in diagnosis and therapy. Whilst high doses are known to induce cognitive impairment little is known about the effects of low doses on brain function. Our study has investigated the long term alterations to the proteome of the hippocampus of mice following an acute low dose whole body exposure. In addition to wild type animals we also studied litter mates heterozygous for Ercc2 mutation. The ERCC2 protein has an important function in transcription-related DNA repair. Mutations in this protein have been associated with radiation sensitivity and neurodegeneration. The mice were irradiated at the age of 10 weeks with doses between of 0 - 0.5 Gy (dose rate 0.063 Gy/min). Quantitative proteomic analysis of the hippocampal tissue was performed 18 and 24 months post-IR by LC-MS/MS using label free methodology. Data was evaluated with INGENUITY software and validated by immunoblotting and functional assays. The proteome analysis indicated long-term effects of low-dose exposure on the hippocampus of both the wild type and the ERCC2+/- mutant mice. Canonical pathway and network analysis showed that proteins were significantly deregulated along to several pathways that are connected to the PI3K/AKT signalling. This pathway and its downstream signaling is indispensable for normal brain function and maintenance of neuronal cells, especially in stress response. They are also involved in synaptic plasticity and build-up of neuronal structures. Validation of the changes by analysis of the central protein AKT showed that radiation increased the phosphorylation of Ser473 in both wild-type and mutant mice, whereas the effect on downstream targets of AKT differed between the wild type and mutant mice. In the wild type group exposure to low-dose radiation affected protein synthesis and gene expression downstream of the AKT pathway, whereas in the ERCC2+/- mice AKT pathway activation more predominantly influenced cellular proliferation and apoptosis. These observations indicate that low doses of radiation are able to irreversibly alter normal neuronal function. Trends towards deficits in learning and memory were observed in behavioural testing. Such knowledge will be important in order to balance the risk against benefit in the use of medical radiology. Supported by the German Federal Ministry of Education and Research (FKZ 02NUK045C)



# Session S17

## „Predictive Assays/Biomarkers 2“

### **Gabriel Pantelias**

Athens, Greece

*„The use of Premature Chromosome Condensation for elucidating the mechanism underlying chromothripsis and for early triage biodosimetry in cases of large-scale exposures“*

### **Malte Kriegs**

Hamburg, Germany

*„Kinomic profiling in radiation oncology: strategies towards personalized molecular targeting“*

### **Sarah Meneceur**

Dresden, Germany

*„Are residual  $\gamma$ -H2AX foci predictive for radiosensitivity? Evaluation of in vivo irradiated tumors and of ex-vivo irradiated patient biopsies“*

### **Cläre von Neubeck**

Dresden, Germany

*„Using  $\gamma$ -H2AX Foci as potential predictors for individual radiosensitivity“*

### **Judith Reindl**

Neubiberg, Germany

*„Using DNA DSB protein clusters as a potent marker for biological microdosimetry on high-LET particle tracks in human cells“*

### **Kristian Unger**

Neuherberg, Germany

*„Prediction models in radiation oncology “*



# The use of premature chromosome condensation for elucidating the mechanism underlying chromothripsis and for early triage biodosimetry in cases of large-scale exposures

A. Pantelias, G. Terzoudi and **Gabriel Pantelias**

*Laboratory of Health Physics, Radiobiology and Cytogenetics, National Centre for Scientific Research "Demokritos", Athens, Greece*

Combination of next-generation DNA sequencing, single nucleotide polymorphism array analyses and bioinformatics has revealed the striking phenomenon of chromothripsis, described as complex genomic rearrangements acquired in a single catastrophic event affecting one or a few chromosomes. It is postulated that mechanical stress causes chromosome shattering into small lengths of DNA, which are then randomly reassembled by DNA repair machinery. A number of hypotheses have been proposed to explain chromothripsis, including ionizing radiation, DNA replication stress, breakage–fusion–bridge cycles, micronuclei formation and premature chromosome condensation (PCC). In the present work, we provide experimental evidence on the mechanistic basis of chromothripsis and on how chromosomes can get locally shattered in a single catastrophic event. We first show that PCC at repairing or replicating DNA sites induces the mechanical stress needed for chromosome shattering to ensue. Then, we demonstrate that such mechanical stress and chromosome shattering can also occur in chromosomes within micronuclei or asynchronous multinucleate cells when primary nuclei enter mitosis. Following an aberrant mitosis, chromosomes could find themselves in the wrong place at the wrong time so that they may undergo massive DNA breakage and rearrangement in a single catastrophic event. The fate of chromosomes in micronuclei, following asymmetrical cell divisions and misalignment of chromosomes, was observed throughout the cell cycle using PCC and FISH analysis with PNA centromeric and telomeric probes. Our results support the hypothesis that PCC triggers shattering and chromothripsis in chromosomes or chromosome arms still undergoing DNA replication or repair in micronuclei or asynchronous multinucleate cells, when main nuclei enter mitosis. Furthermore, the PCC methodology was used for the development of a rapid, minimally invasive approach for biodosimetry in cases of large-scale exposures. For this purpose, the standard PCC procedure was adapted and lymphocytes from blood volumes of 50-150µl were successfully fused with CHO mitotic cells in 96-well Deepwell plates of 2ml. Such a micro-PCC assay is a high priority for early triage in radiation emergencies, as it allows a simultaneous dose-estimation for hundreds of blood samples and a cost-effective analysis of radiation-induced excess PCC fragments using Giemsa stain. Interestingly, the morphology of the lymphocyte Prematurely Condensed Chromosomes (PCCs) obtained with the micro-PCC assay was of identical quality to that obtained using the standard PCC assay. When overexposed individuals are identified during early triage using the micro-PCC assay, their dose estimates can be further determined based on the accurate scoring of dicentric and centric ring chromosomes in lymphocyte PCCs. To this end, the micro-PCC assay can be combined with fluorescence in situ hybridization (FISH) using simultaneously centromeric/telomeric (C/T) peptide nucleic acid (PNA) probes.

# Kinomic profiling in radiation oncology: Strategies towards personalized molecular targeting

**Malte Kriegs**, L. Bußmann, N. Struve, L. Gleißner, K. Hoffer and K. Rothkamm

*University Medical Center Hamburg-Eppendorf, Germany*

After X-irradiation, the matter of cell survival and cell death depends on the detection of DNA damages and subsequent processes like cell cycle check-point regulation, DNA repair and induction of apoptosis. These processes are influenced by different signal transduction pathways, especially pathways that rely on protein kinases. Because in tumors some of these kinase cascades are frequently hyper-activated causing radioresistance, targeting of protein kinases is a common strategy to radio-sensitize tumor cells. But, inhibition of protein kinases can only be effective, if the targeted kinase is really (hyper-) active in the individual tumor, either irradiation independent or activated by X-irradiation. Therefore, individual analysis of kinase activity is essential to specify targeted therapy which uses clinical available inhibitors as well as to identify new targets. Because a great many kinases are potentially involved explorative strategies are of advantage. So far, most studies use the expression of a certain kinase as a surrogate marker for its activity, mainly analyzed by immunohistochemistry, which does support exploratory research. Here we present different explorative approaches for the analysis of protein phosphorylation and kinase activity in tumor cell lines and patient derived biopsies (tumor & normal tissue), including SH2-Profilig, peptide arrays (PamSystem®) and mass spectrometric approaches. Using these techniques, we can clearly show that tyrosine kinase signaling in HNSCC tumors differs significantly from normal tissue but also from HNSCC cell lines. Furthermore, the studies also reveal that kinase (EGF-receptor) over-expression does not consequently reflect increased kinase activity in tumor biopsies and that X-irradiation has heterogeneous effects on kinase activity, such as activating but also inhibitory effects. We also demonstrate that kinase activity-profiling methods might be used to predict the response of a certain tumor cells towards specific inhibitors also in the context of radiosensitization. Finally, we have shown that explorative mass spec approaches can successfully be applied to explore the relationship between EGF-receptor targeting and DNA repair inhibition. Taken together, we think that the precise and individual characterization of kinase activity with or without X-irradiation is crucial for an effective use of targeting agents in combination with X-irradiation. To reach this goal kinomic profiling and explorative proteomic approaches are highly promising tools, not only in basic but also for translational research especially for the unveiling of predictive biomarkers.

# Are residual $\gamma$ H2AX foci predictive for radiosensitivity? Evaluation of in vivo irradiated tumors and of ex vivo irradiated patient biopsies

**Sarah Meneceur**, C. von Neubeck, V. Gudziol, S. Löck, M. Krause and M. Baumann

*OncoRay – National Center for Radiation Research in Oncology, Faculty of Medicine and  
University Hospital Carl Gustav Carus, Technische Universität Dresden, Helmholtz-  
Zentrum Dresden - Rossendorf, Dresden, Germany*

Ionizing radiation leads to DNA damages - including double strand breaks (DSB) - which are detected by DNA repair proteins. Upon DSB formation, the histone variant H2AX is phosphorylated, giving rise to  $\gamma$ H2AX foci. It has previously been shown that residual  $\gamma$ H2AX foci (24 h post irradiation) negatively correlate with the local tumor control in head and neck squamous cell carcinoma (HNSCC) xenografts after 4 Gy irradiation in vivo [1]. Additionally, it is well known that the tumor microenvironment plays a critical role in cellular response to radiation. In this study, we propose to analyze residual  $\gamma$ H2AX foci in established HNSCC models in a dose and microenvironment dependent manner. Besides, we translated the  $\gamma$ H2AX foci assay to an ex vivo setting with HNSCC patient derived biopsies. Five HNSCC cell line derived xenografts (FaDu, UT-SCC-14, UT-SCC-5, SKX, SAS) were subcutaneously transplanted on the hind leg of NMRI nude mice. When the tumor reached a size of 7x7 mm, BrdU (viability marker) and pimonidazole (hypoxia marker) were injected, and the mice were randomly distributed to 5 treatment arms (untreated control, 2 Gy, 4 Gy, 6 Gy, 8 Gy). The mice were sacrificed 24 h post irradiation, and the tumors were excised, fixed and embedded in paraffin (FFPE). Consecutive paraffin sections of the tumors were stained for BrdU and pimonidazole on the one hand and for  $\gamma$ H2AX on the other hand. GammaH2AX foci were manually counted in oxic areas at a maximal distance of 45  $\mu$ m from the closest perfused vessel. In an attempt to transfer the assay to a clinical setting, patient derived HNSCC biopsies were ex vivo cultivated for 24 h including 4 h of pimonidazole and BrdU treatment, subsequently irradiated with 0 – 8 Gy and fixed after 24 h. Statistical analysis confirm that residual  $\gamma$ H2AX foci linearly increase in a dose dependent manner in FaDu, SKX, UT-SCC-5 and UT-SCC-14 ( $p < 0.01$ ). However, in the very radioresistant model SAS, no linear increase of foci with dose could be observed, implying too low doses for radiation response evaluation or very high repair capacity of the model. The slope ( $s$ ) of the dose response curve of the radiosensitive model SKX [ $s = 1.06$ ] significantly differed from the more radioresistant models (FaDu [ $s = 0.25$ ], UT-SCC-5 [ $s = 0.31$ ] and UT-SCC-14 [ $s = 0.42$ ]) underlining the potential of the slope of the dose response curve to assess radiosensitivity. Interestingly, in the five analyzed models, the TCD50, 30fx, 6wk and the TCD50SDclamp (tumor control dose) negatively correlate with residual  $\gamma$ H2AX after 6 Gy or after 8 Gy only (Spearman's rho,  $p < 0.05$ ). In the ex vivo irradiated patient derived tumor biopsies, slopes ranging from 0.13 to 0.82 could be measured. This result suggests patient specific repair capacities - which are of particular interest for future treatment individualization. Altogether, this study further supports the use of  $\gamma$ H2AX foci as a promising biomarker to predict radiosensitivity and might contribute to the individualization of cancer treatment in the near future.

**Acknowledgement:** This work was supported by a grant of the Federal Ministry of Education and Research (BMBF 02NUK035C).

**Reference:** [1] Koch et al., Radiother. Oncol. 108:434–39 (2013)

[sarah.meneceur@uniklinikum-dresden.de](mailto:sarah.meneceur@uniklinikum-dresden.de)



# Using $\gamma$ H2AX Foci as potential predictors for individual radiosensitivity

**Cläre von Neubeck**, S. Meneceur, T. Rassamegevanon, R. Hiemann, A. Elimport, S. Löck, V. Gudziol, D. Roggenbuck, M. Krause and M. Baumann

*OncoRay - National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden and German Cancer Consortium (DKTK), Partner Site Dresden, Dresden, Germany;*

The tumor response to radiation therapy differs among patients even when tumors of the same entity are compared influencing the therapy outcome and therewith patient survival. It is of high clinical relevance to predict the tumor's response to radiation before onset of therapy to allow for a treatment individualization e.g. radiation dose escalation/ combination therapy to increase survival in case of radio-resistant tumors or radiation dose de-escalation to spare radiation-induced side effect without compromising the therapy outcome in case of radio-sensitive tumors. Among the variety of DNA damages induced by radiation, DNA double strand breaks have the highest lethal potential for the affected cell. Early in DNA DSB repair, the histone variant H2AX is phosphorylated ( $\gamma$ H2AX) in the vicinity of the DSB giving rise to repair foci. The newly formed DNA repair protein  $\gamma$ H2AX is therefore of great interest for developing a predictive biomarker for radiation response. In our previous publications, we could show that  $\gamma$ H2AX foci can reliably be measured in tumor sections of in vivo irradiated xenografted human head and neck squamous cell carcinoma (HNSCC) [1, 2]. Thereby, the tumor micromilieu assessed by the proliferation marker BrdU and the hypoxia marker pimonidazole critically influences the number of  $\gamma$ H2AX foci. A significant negative correlation of  $\gamma$ H2AX foci with historical data of the local tumor control (TCD50) could only be found in oxic regions of the tumor in a maximum distance of 45  $\mu$ m from the nearest perfused vessel [2]. Adaptation of the  $\gamma$ H2AX foci assay to a clinically applicable ex vivo irradiation set-up based on tumor biopsies showed the feasibility to culture the specimens under standard condition and measure residual  $\gamma$ H2AX foci in xenograft and patient derived biopsies 24 h post irradiation [3]. The slopes of the  $\gamma$ H2AX foci dose response curves thereby resemble the known clinical radio-responsiveness of the tumor entity [3, 4]. Following the question of the representativeness on an individual tumor specimen for the whole tumor, multiple equally treated biopsies from HNSCC xenografts were analyzed for  $\gamma$ H2AX foci and subjected to a linear mixed-effects model showing significant intra-tumoral heterogeneities in-between biopsies and within a particular sample [5]. In order to prove the clinical relevance of  $\gamma$ H2AX foci as predictive biomarker, a preclinical and a clinical study was initiated to furnish evidence that the slope of the dose response curve correlates with TCD50 values and clinical parameters (e.g. outcome, tumor grade, stage), respectively. So far, a significant negative correlation could be found for  $\gamma$ H2AX foci following 6 Gy and 8 Gy and the clinical relevant fractionated TCD50 (Spearman's rho,  $p < 0.05$ ). An automated foci analysis has been developed to increase the samples throughput, reduce the inter-observer variabilities and to further develop the biomarker  $\gamma$ H2AX foci into a diagnostic usable tool. Overall, our results further support clinical relevance of  $\gamma$ H2AX foci as predictive biomarkers for individual radiosensitivity holding the potential of treatment individualization.

**Acknowledgement:** This work was supported by grants of the Federal Ministry of Education and Research (BMBF 02NUK035C) and the Federal Ministry for Economic Affairs and Energy (BMWi 16KN041835).

**References:** [1] Menegakis et al., Radiother. Oncol. 100:137-44 (2011), [2] Koch et al., Radiother. Oncol. 108:434-39 (2013), [3] Menegakis et al., Radiother. Oncol. 116:473-79 (2015), [4] Menegakis et al., Radiother. Oncol. 116:480-5 (2015), [5] Rassamegevanon et al. submitted 2017

# Using DNA DSB protein clusters as a potent marker for biological microdosimetry on high-LET particle tracks in human cells

**Judith Reindl**, J. Huber, C. Greubel, M. Sammer, B. Schwarz, C. Siebenwirth, D. W. M. Walsh, A. A. Friedl and G. Dollinger

*Institut für angewandte Physik und Messtechnik, Universität der Bundeswehr München, Germany*

Ionizing-radiation induces double-strand breaks (DSB) with varying local density and complexity depending on its LET (Linear Energy Transfer). Based on the clinical relevance of the enhanced relative biological effectiveness of high-LET particle-irradiation, it is of great importance to study the influence of radiation quality on the number of induced DSB. Also current evidence indicates that proteins responsible for detection and repair of DSB cluster in different structural and/or functional domains around the damage[1]. The aim of this study is to count every single DSB that is induced by high-LET irradiation. We counted the number of ionizing-radiation induced DNA-PKcs foci (IRIF) in human HeLa cells 2-5min after damage induction. Irradiation was performed at the ion-microprobe SNAKE using high-LET 20MeV lithium ( $\text{LET}=116\text{keV}/\mu\text{m}$ ), 27MeV carbon ions ( $\text{LET}=500\text{keV}/\mu\text{m}$ ) and low-LET 21MeV protons ( $\text{LET}=2.6\text{keV}/\mu\text{m}$ ). Imaging was performed using super-resolution STED microscopy, with a resolution of 105nm. The IRIF show an average size of  $(190\pm 10)\text{nm}$  that allows separation of DSB by such small distances, i.e. in high LET ion tracks. Every DSB was labelled by a DNA-PKcs IRIF as proven by counterstaining with 53BP1 after low-LET proton irradiation where separation of individual DSB is in most cases larger than the 53BP1 gross size of about 0.6nm. This enables counting of densely-packed DSB in high LET particle tracks due to the small IRIF size. Lithium ions produce  $(2.85\pm 0.10)\text{IRIF}/\mu\text{m}$  track length. This is in good accordance to the prediction of the  $(2.7\pm 0.4)\text{DSB}/\mu\text{m}$  induced by 33MeV lithium ions by Monte-Carlo-based PARTRAC simulation[2]. For carbon ions  $(4.35\pm 0.16)\text{IRIF}/\mu\text{m}$  were counted, which does not match the prediction of  $(10.2\pm 2.2)\text{DSB}/\mu\text{m}$ . Instead of using single DSB for the simulation in PARTRAC, 150nm clusters which represent the size of the DNA-PKcs IRIF were used. The predicted number of clusters is  $(3.3\pm 0.3)\text{DSBclusters}/\mu\text{m}$ , which is somewhat lower but close to the measurements. We conclude that for the first time it is possible to perform biological microdosimetry for particles with  $\text{LET}\leq 116\text{keV}/\mu\text{m}$  by counting the number of DNA-PKcs IRIF. For particles with much higher LET the size of the DNA-PKcs clusters is still too large for individual DSB separation.

## References:

- [1]Reindl et al.;Sci.Rep. (2017)7:40616.
- [2]Hauptner et al.;Ion Beam Science (2006):59-85.

# Prediction models in radiation oncology

M. Niyazi, L. Schneider, D. Piehlmaier, D. Fleischmann, R. de Bin, C. Legrand, AL. Boulesteix, M. Selmansberger, K. Lauber, U. Ganswindt, H. Zitzelsberger, M. Henke, C. Belka, J. Hess and **Kristian Unger**

*Department of Radiation Oncology, University Hospital, LMU Munich, Germany*

Cancer is a therapeutic challenge and treatment regularly consists of a multi-disciplinary approach involving surgery, chemotherapy and radiotherapy. However, there is huge variation between patients with regard to therapy response and identification of patients who are likely not responding adequately to therapy is the prerequisite for tailored treatment approaches. This requires prognostic models which are built from molecular and clinical data in combination with clinical outcome data available for individual patients. In addition to traditional predictive modelling approaches that only make use of either clinical data or one single level of molecular data alone for the construction of a prognostic molecular signature we also establish a multilevel predictive modelling approach. If available, this approach allows employing different levels of data accessible for the same patient resulting in one single prediction rule. We conducted single-level prediction on glioblastoma (GBM) and head and neck squamous cell carcinoma (HNSCC) and for the latter also multilevel prediction was performed. For GBM a prognostic 4-miRNA signature was built predicting overall survival in standard-of-care treated patients and which was validated in two independent cohorts. The signature is independent of age, sex, IDH1 status and most importantly also of MGMT promoter methylation status which is the only relevant established molecular prognostic factor in GBM. In combination with MGMT promoter methylation status the miRNA signature allows differentiating three prognostic groups in GBM to be potentially considered in a tailored treatment approach. For HNSCC we were able to identify a 5-miRNA signature predicting freedom from recurrence that was identified in the multi-centre cohort and which was independently validated in a monocentric cohort. In addition, we generated a prognostic 4-gene signature in HNSCC identified in a radiotherapy treated subcohort of the TCGA HNSCC dataset which was independently validated in a monocentric cohort. For HNSCC we also generated a prognostic multi-level signature including elements from clinical data, genomic copy number data and miRNA expression data. In all, we established a framework that allows efficient generation of prognostic single-level and multi-level signatures which we were able to show for radiotherapy treated GBM and HNSCC cohorts. The presentation will give an introduction on the methodology of single-level and multilevel predictive modelling and will also present our most recent results on the topic.



## Session S18

# „Radiation Therapy/Stem Cells“

**Lisa Wiesmüller**

Ulm, Germany

*„The MLL breakpoint cluster region: a target for chemo- and radiotherapy “*

**Daniel Cullen**

Dublin, Ireland

*„Identification of prostate cancer patients at risk of late radiotoxicity following radiation therapy using Raman spectroscopy“*

**Daniel Lopez**

Bogota, Colombia

*„Effect of NIS expression in response to treatment with external beam radiotherapy in a colon carcinoma cell line“*

**Mozhgan Dehghan Harati**

Tübingen, Germany

*„Akt isoforms differently regulate ALDH activity and the expression of stem cell markers in cancer cells“*

**Sylvia Ritter**

Darmstadt, Germany

*„Ionizing radiation alters the differentiation potential of human embryonic stem cells “*

**Yannick Saintigny**

Caen, France

*„Multimodal treatments of radioresistant glioblastoma stem cells: emerging effective tryptic of Temozolomide, PARP inhibitors and particle therapy“*



# The MLL breakpoint cluster region: a target for chemo- and radiotherapy

**Lisa Wiesmüller**, B. Gole, M. Rall, D. Kraft and C. Fournier

*Department of Obstetrics and Gynecology, Ulm University, Germany*

The breakpoint cluster region of the MLL gene (MLLbcr) is frequently rearranged in therapy-related and infant acute leukemia, but the destabilizing mechanism is poorly understood. We recently proposed that DNA replication stress results in MLLbcr cleavage via endonuclease G (EndoG) and represents the common denominator of chemo- and radiotherapy-induced destabilization of the MLLbcr. We performed a siRNA screen for new factors involved in MLL rearrangements employing an enhanced green fluorescent protein-based reporter system. We identified ten factors acting in line with EndoG in MLLbcr breakage including activation-induced cytidine deaminase (AID). Further analysis connected AID and EndoG in MLLbcr destabilization via base excision repair (BER) components. Notably, inhibition of the core BER factor Apurinic-apyrimidinic endonuclease 1 protects against MLLbcr cleavage in tumor and human cord blood-derived hematopoietic stem/progenitor cells (HSPCs), harboring the cells of origin of leukemia. Notably,  $\gamma$ -ray treatment of HSPCs, i.e. the cells of leukemia origin, causes pronounced and specific breakage of the MLLbcr as compared to other sites in the genome. DNA double-strand break repair measured adjacent to the leukemia-associated MLL breakpoint cluster sequence in reporter lines revealed radiation dose dependency of potentially rearrangements underscoring the risk of leukemia-induction by radiation treatment.

# Identification of prostate cancer patients at risk of late radiotoxicity following radiation therapy using Raman spectroscopy

**Daniel Cullen**, A. Maguire, J. Bryant, D. Medipally, J. Armstrong, M. Dunne, A. Meade, O. Howe and F. Lyng

*Dublin Institute of Technology, Dublin, Ireland*

The success of radiation therapy in tumour control depends on the total dose given but the tolerance of the normal tissues surrounding the tumour limits this dose. It is not known why some patients develop radiation toxicity, and currently, it is impossible to predict before treatment which patients will experience adverse effects as a result of radiotherapy. An assay to predict risk of radiation toxicity would guide the selection of treatment modality to reduce this risk in high risk patients or allow dose escalation in low risk patients to improve tumour control. Individualised cancer treatment would improve quality of life by minimising late radiation toxicity in high risk patients and by improving treatment response in low risk patients. This study aimed to evaluate Raman spectroscopy for identification of cancer patients at risk of late radiation toxicity following radiotherapy. Twenty prostate cancer patients enrolled on a radiotherapy trial who showed severe late toxicity in follow up and a matched set of twenty patients who showed no/minimal toxicity in follow up were identified. Blood samples were acquired, cultured and irradiated in vitro. In parallel, DNA damage was assessed post-irradiation using the  $\gamma$ H2AX assay and G2 chromosomal radiosensitivity assay. Raman spectra were acquired from lymphocytes. High sensitivity and specificity in classifying patients on the basis of treatment toxicity was achieved.

# Effect of NIS expression in response to treatment with external beam radiotherapy in a colon carcinoma cell line

**Daniel López**, L. González, F. Castillo-Rivera, M. Pinzón, L. del Riesgo, T. Pourcher, R. Garzón and A. Ondo

*School of Medicine, Universidad Nacional de Colombia, Bogota, Colombia; Biochemistry and Biotechnology Research Group, School of Medicine, Universidad del Rosario, Bogota, Colombia.*

NIS protein (sodium/iodine symporter) is a glycoprotein that is specifically expressed in organs that metabolize iodide, especially in thyroid gland where it plays a very important role in the biosynthesis of thyroid hormones. Its ability to transport iodide and iodide's radionuclides has allowed it to be used as a diagnostic and therapeutic tool in thyroid cancers. Due to its great function, several authors have studied the possibility to use NIS in other types of extrathyroidal cancers; for this purpose, the use of gene therapy can lead to the expression of NIS protein in tissues that commonly do not have this protein. Experimental results has shown that cancer treatment with metabolic radiotherapy has been effective and have diminished adverse events after the therapy. Nevertheless, the evidence has also shown that some tumors are not completely destroyed. For this reason, it should be important to combine with another therapy, i.e. radiotherapy. In order to evaluate the effectiveness of treatment with external ionizing radiation as a complementary therapy to NIS-based gene radiotherapy, the response to ionizing radiation was evaluated on a Colon Carcinoma Cell line, stably transfected with the nis gene (HT29-NIS). Firstly, survival curves were developed with clonogenic assays in order to evaluate the survival of cells treated with ionizing radiation. The results showed that NIS cells were more radiosensitive compared to the parental cell line. Based on these results the DNA damage was assessed through comet assay, showing more DNA damage in cells with NIS transporter and increased activation of DNA damage response assessed with the quantification of gamma-H2AX foci. Finally, in order to analyze the possible alteration on the REDOX cell status, we evaluated the levels of reactive oxygen species with a fluorescent probe and the expression levels of Nrf-2 with western blot analysis. These results showed that both cell lines (parental and cells with NIS protein) can handle the oxidative stress decreasing ROS, but parental cells activate antioxidant response more efficiently, with the nuclear translocation of Nrf-2 protein. These results demonstrate that in the cell line with NIS transporter, the protein seems to be a radiosensitivity factor that would facilitate the combined treatment of cancer with metabolic radiotherapy and external beam radiotherapy.



# Akt isoforms differently regulate ALDH activity and the expression of stem cell markers in cancer cells

**Mozhgan Dehghan Harati**, HP. Rodemann and M. Toulany

*Division of Radiation Biology & Molecular Environmental Research, Dept. of Radiation Oncology, University of Tübingen; German Cancer Consortium (DKTK), partner site Tübingen and German Cancer Research Center (DKFZ) Heidelberg, Germany*

Cancer stem cells (CSC) are proposed to be one of the major causes of resistance to ionizing radiation. Previous reports demonstrate that accelerated DNA repair is one of the underlying mechanisms of radioresistance of CSC. The PI3K/Akt pathway is the major survival pathway in cancer cells that is frequently upregulated in human tumors. Several studies have shown the link between the PI3K/Akt signaling pathway and the biology of CSC. Akt is the major kinase downstream to PI3K and consist of three isoforms. In the present study we investigated role the Akt isoforms on aldehyde dehydrogenase (ALDH) enzyme activity and the expression of the CSC markers Nanog, Bmi1, Sox2 and Oct4 and in tumor cells from different origin. Human breast cancer cell lines HBL-100, MCF-7 and MDA-MB-231 as well as colon carcinoma cell line HCT116 cells were used. ALDEFLUOR assay was applied to measure level of ALDH activity. Pharmacologic inhibition of ALDH and an ALDH specific siRNA approach were applied to analyse effect of ALDH activity on post-irradiation cell survival. Knockdown/knockout approaches were used to study effect of the Akt1, 2 and 3 isoforms on ALDH activity and expression of various CSC markers. ALDH activity was highly expressed in HBL-100 cells while it was not detectable in MDA-MB-231 cells. In HBL-100 cells, inhibition of ALDH activity by N,N-diethylaminobenzaldehyde (DEAB) induced radiosensitization. In this cell line, knockdown of Akt isoforms by siRNAs inhibited ALDH activity by 65% (Akt1), 61% (Akt2) and 46% (Akt3). This inhibition was associated with radiosensitization of the cells. In the HCT116 colon carcinoma cell line, knockout of Akt1 but not of Akt2 led to the inhibition of ALDH1 activity by about 53%. However, in MDA-MB-231 cell line, which does not present ALDH activity, DEAB as well as siRNA against ALDH1 induced radioprotection. In this cells, shRNA knockdown of Akt1 but not of Akt2 or Akt3 downregulated the expression of Bmi1 and Nanog. Moreover, neither the expression of Sox2 nor of Oct4 was affected by Knockdown of either the Akt isoforms. This study demonstrates that ALDH activity and expression of other stem cell markers can be regulated differently through different Akt isoforms. In addition, reduction of ALDH activity mediated by knockdown of specific Akt isoforms also affects clonogenic activity of the tumor cells. Currently we are investigating the underlying mechanism(s) involved in the heterogeneous effect of Akt isoforms on CSC markers and cellular radiosensitivity of tumor cells in vitro. Acknowledgment: This project is supported by grants from the Deutsche Forschungsgemeinschaft, (RO 527/7-1 and TO 685/2-1).

# Ionizing radiation alters the differentiation potential of human embryonic stem cells

**Sylvia Ritter**, O. Arrizabalaga, E. Nasonova, M. Mayer, C. Thielemann and IS. Schroeder

*GSI Helmholtz Center, Biophysics, Darmstadt, Germany*

To date, little is known about how ionizing radiation impacts the earliest stages of human embryonic development although ionizing radiation is a natural part of our environment and is increasingly used in medicine for diagnosis and therapy. Current risks estimates are based on few epidemiological data and on animal experiments mainly performed with mice and rats. An ideal in vitro model to examine the radiation effects on early embryonic development are human embryonic stem cells (hESC) exploiting their ability to self-renew and to generate all cell types of the body (i.e. of the three germ layers). In the present study we examined the effects of X-ray exposure (1 and 3 Gy) on WA09 (H9) hESC. Cell cycle progression, apoptosis, cytogenetic damage and stem cell signaling were analyzed up to four days after exposure. At early post-irradiation times ( $\leq 24$ h) X-rays induced a transient G2/M arrest, apoptosis and cytogenetic damage. Additionally, a significant downregulation of stem cell signaling markers was detected. Specifically, the expression of activin receptors was significantly affected. Subsequent analyses 4 days post-irradiation showed that the progeny of irradiated cells nearly resumed to control levels. As activin receptors play an important role in stem cell maintenance and are also essential for the differentiation of hESC to definitive endoderm (DE) that will ultimately give rise to inner organs such as liver, lung and pancreas, we examined the ability of surviving hESC to form DE. Differentiation was initiated 4 days post-irradiation. At the subsequent days the expression of typical DE markers (Foxa2<sup>high</sup>/Sox17<sup>high</sup>/Sdf1<sup>low</sup>) was analyzed by quantitative PCR revealing a lower differentiation capability of the progeny of irradiated cells compared to the sham-irradiated controls (for details see [1]). To give a full picture, we also examined the ability of surviving hESC to generate cardiomyocytes or neural stem cells as an example of the mesodermal and ectodermal lineage, respectively. Consistently, these experiments showed that the progeny of X-ray irradiated cells maintain their differentiation capacity. Yet, the efficiency was lower than in sham-irradiated cells and/or the functionality was impaired as judged by electrophysiological studies. Currently, the underlying mechanisms are investigated in more detail. Altogether, our data demonstrate that ionizing radiation has a prominent and long-lasting effect on the inherent properties of surviving hESC. Obviously, their differentiation capacity is impaired affecting all three germ layers although the extent may vary. These alterations may explain the developmental retardations, organ malformations, or prenatal death observed after exposure to ionizing radiation.

This work was funded by the German Ministry of Education and Research (Grant 02NUK025 and 02NUK034C).

## References:

[1] Luft S, Arrizabalaga O, Kulish I, Nasonova E, Durante M, Ritter S, Schroeder IS. Stem Cells Dev. 2017;26(5):341-352

[s.ritter@gsi.de](mailto:s.ritter@gsi.de)

# Multimodal treatments of radioresistant glioblastoma stem cells: emerging effective tryptic of Temozolomide, PARP inhibitors and particle therapy

**Yannick Saintigny**, P.Lesueur, E. El-Habr, MP. Junier, D. Stephan, F. Chevalier and H. Chneiweiss

*CIMAP (CEA/CNRS/ENSICAEN/UNICAEN), Caen, France*

Glioblastoma is a radioresistant tumor with a high level of intra-field recurrence usually surrounded of high risk complications healthy tissues. Concurrent Temozolomide exposure associated with ionizing irradiation followed by adjuvant Temozolomide alone is standard treatment for glioblastoma patients (STUPP protocol) with unfortunately, a poor prognosis still remaining. Indeed, glioblastoma stem cells promote radioresistance and may be the source of tumor recurrence after radiation. However, recent studies highlight a replication dependent radiosensitization effect of PARP inhibitor which could be exploited for specifically target tumor tissue with slight or no effect on surrounding healthy tissues. Poly (adenosine diphosphate-ribose) polymerase (PARP) is a family of enzymes involved in a wide number of cellular processes, including DNA replication, transcription, repair and cell death. Since a decade, it has been shown that PARP-inhibitor could sensitize radioresistant cells alone or in combination with unrepaired damages because of mutated genes as BRCA1 (synthetic lethality). Particle therapy is an innovative technique of radiation therapy using High-LET carbon ions. It has been successfully used in the treatment of tumors resistant to conventional radiation-therapy due to a higher biological effectiveness compared to low-LET radiations. The purpose of this project was to determine (i) an enhancement ratio of STUPP protocol by using high LET (Carbon ions) radiation instead of low LET (X-rays) and PARP inhibitors and, (ii) a specific effect on glioblastoma stem cells. Results show a clear impact of a treatment combining Temozolomide, PARP inhibitors and particle therapy on cell viability and proliferation. Moreover, we analyze cell cycle distribution and replication. Finally, we sequence both glioblastoma stem cell lines used for a 69 genes panel involved in DNA repair in order to seek correlation with our findings. Taken together, our data show undoubtedly an beneficial impact of the tryptic on the control of glioblastoma stem cells.



# Keynote Lecture 5

**Thomas Helleday**

Stockholm, Sweden

*„Novel regulators of IR-induced DNA repair and dNTP generation at DNA damage sites, and implications for cancer treatments“*

# Novel regulators of IR-induced DNA repair and dNTP generation at DNA damage sites, and implications for cancer treatments

**Thomas Helleday**

*Science for Life Laboratory, Division of Translational Medicine and Chemical Biology,  
Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm,  
Sweden*

The 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) is an important enzyme in the glycolysis, serving to maintain a high glycolytic flux via synthesis of the allosteric activator fructose-2,6-biphosphatase. PFKFB3 has high expression in tumors compared to normal tissue and its depletion reduces tumor growth. It has recently been shown that PFKFB3 supports proliferation via its nuclear localization, however its nuclear function is not entirely clear. Here we show for the first time that PFKFB3 forms irradiation-induced foci and colocalizes with RRM2, to generate dNTPs in an ATM-dependent manner. PFKFB3 depletion impairs homologous recombination (HR) and non-homologous end-joining (NHEJ), delays recovery from radiation-induced cell cycle arrest, consistent with that we observe an up-regulation of PFKFB3 in radiation-resistant cancer patients. We further report and validate a novel potent and selective PFKFB3 inhibitor KAN0437757 that inhibits DNA repair and ultimately reduces cancer cell growth in vivo. Thus we establish a novel role for PFKFB3 in DNA repair and PFKFB3 inhibition as an attractive approach to decrease resistance to therapeutically induced DNA breaks.

# Plenary Session 4

## „Clinical Translation of Radiation Biology“

### **Penny Jeggo**

Sussex, United Kingdom

*„Double-strand breaks repair disorders“*

### **Wolfgang Dörr**

Vienna, Austria

*„Stem cells for amelioration of radiation-induced oral mucositis: preclinical studies“*

### **Verena Jendrossek**

Essen, Germany

*„Adaptation to the adverse tumor environment - towards novel combined treatment strategies“*

### **Franz Rödel**

Frankfurt am Main, Germany

*„PD-1/PD-L1, CD8 and FOXP3 expression and human papilloma virus load in patients with anal squamous cell carcinoma treated with chemoradiation: Rationale for immunotherapy“*

### **Rob Coppes**

Groningen, Netherlands

*„Use of organoid cultures in the study of radiation response and regeneration“*





# Double strand break repair disorders

**Penny A. Jeggo**

*Genome Damage and Stability Centre, Life Sciences, University of Sussex, Brighton, United Kingdom*

The response to DNA double strand breaks (DSBs) involves pathways of DSB repair and a distinct signaling response that is not essential for DSB repair but enhances accuracy of the process. The major DSB repair pathway is DNA non-homologous end-joining (NHEJ) and ataxia telangiectasia mutated (ATM) lies at the core of the signaling response. Patients with mutations in most components of NHEJ have been identified. ATM-deficiency confers ataxia telangiectasia (A-T). Mutations in other proteins involved in the ATM signaling have also been described. I will focus on NHEJ deficiency in patients with a brief comparison and discussion of signaling deficient patients. NHEJ deficiency confers clinical features that including clinical radiosensitivity, immunodeficiency, microcephaly and growth delay, and cancer predisposition. The identification of these patients, coupled with mouse models of NHEJ deficiency, have provided insight into developmental roles of NHEJ. I will discuss insight into the basis underlying microcephaly in these patients, which has emerged from studies using mouse models. I will compare these features to those observed in A-T and the other signaling defective disorders and evaluate explanations for the different outcomes. Finally, I will consider how our understanding of NHEJ has helped us provide optimal care for such patients, providing a translational benefit of our work.



# Stem cells for amelioration of radiation-induced oral mucositis: Preclinical studies

**Wolfgang Dörr**, R. Noack, A. Pickert, A. Piro, A. Siegemund and J. Haagen

*Applied and Translational Radiobiology, Dept. Radiotherapy, Medical University Vienna  
and Dept. Radiotherapy and Radiation Oncology, Medical Faculty Carl Gustav Carus,  
Technical University of Dresden, Germany*

The dominating process underlying the radiation response of oral mucosa is the impairment of cell production in the germinal tissue compartments and consequently an insufficient compensation of the ongoing cell loss at the surface. This imbalance eventually results in complete mucosal denudation and ulceration. Therefore, replacement of proliferating epithelial (stem) cells by circulating bone marrow stem cells may represent an option for amelioration of radiation-induced oral mucositis. An increase in circulating stem cells in the present study was facilitated either through transplantation of bone marrow (BMT) thus including both haematopoietic and mesenchymal stem cells, mesenchymal stem cell transplantation (mSCT) or by stem cell mobilisation (SCM) from the bone marrow by GM-CSF or AMD3100. After single dose irradiation of mouse tongue mucosa, only SCM with a maximum number of circulating stem cells at various days, but not BMT at the same days was effective in reducing the incidence of mucosal ulceration. This discrepancy may be attributed to the longer time interval over which the number of circulating stem cells is increased after mobilisation in contrast to transplantation. With fractionated irradiation over 1 week (days 0-5), an effect of BMT before the onset of radiotherapy or at the end of the treatment week, but not during the week (day 1), was observed. Similar results were achieved for 3 weeks of fractionation. SCM with one week of fractionation increased the mucosal tolerance in all protocols, but with 3 weeks of fractionation, again, a lack of efficacy was found for a maximum mobilisation effect during the first treatment week. In conclusion, stem cell therapy can compensate about 20-30 % of the fractionated radiation dose, except for administration during the initial period of fractionated irradiation. First mechanistic studies suggest that the effect is not based on clonal expansion and trans-differentiation of the circulating stem cells within the epithelium. Individual cells migrate into the irradiated tissue (as has been shown by transplantation of male cells into female mice) and serve as a source of locally active substances, or such substances are secreted within the circulation. The basis for the lacking effect of stem cell therapy during the initial phase of radiotherapy remains unclear. Vascular changes, such as changes in the endothelial expression of adhesion molecules (e.g. ICAM-1) may prevent homing of the cells in the irradiated area. Alternatively epithelial changes, like altered receptor expression (e.g. EGFR), may inhibit transmigration of the stem cells during this period.

# Adaptation to the adverse tumor microenvironment - towards novel combined treatment strategies

**Verena Jendrossek**

*Institute of Cell Biology (Cancer Research), University Hospital Essen, Germany*

Radiotherapy and chemotherapy are part of standard treatment concepts for various solid human tumors. However, adverse conditions in the tumor microenvironment such as limited nutrient availability, hypoxia, acidosis and an activated tumor stroma shape genetic, epigenetic and pathway alterations that not only promote the acquisition of malignant traits of cancer cells but also enhance resistance of cancer cells to genotoxic therapies. Hence researchers aim to gain an improved understanding of specific vulnerabilities of cancer cells in their adverse microenvironment in order to define new therapeutic targets for overcoming microenvironment-mediated radiation resistance without increasing the toxic effects of ionizing radiation on normal tissues. For example, we and others showed that chronic severe hypoxia/reoxygenation stress drives complex metabolic reprogramming of cancer cells involving amongst others increased antioxidant defense. Interestingly, tolerance to severe hypoxia/reoxygenation stress was associated with increased resistance of these cells to ionizing radiation that could be targeted by specific small molecule metabolic inhibitors. The presentation will highlight knowledge on context-specific metabolic preferences of cancer cells and discuss cellular and molecular factors that may link altered cancer cell metabolism to the cellular radiation response and radiation resistance and may therefore be suited to counteract microenvironment-mediated tumor cell radiation resistance and thus improve the therapeutic gain of radiotherapy

Supported by grants of the DFG (GRK1739/2) and the Deutsche Krebshilfe (110355).

# PD-1/PD-L1, CD8 and FOXP3 expression and human papilloma virus load in patients with anal squamous cell carcinoma treated with chemoradiation: rationale for immunotherapy

**Franz Rödel**, P. Balermipas, D. Martin, E. Fokas and C. Rödel

*Department of Radiotherapy and Oncology, Goethe-University, Frankfurt am Main, Germany; German Cancer Research Center (DKFZ), Heidelberg, Germany; German Cancer Consortium (DKTK) partner site: Frankfurt, Heidelberg, Germany*

**Purpose:** There is growing evidence that the level of tumor-infiltrating lymphocytes (TIL), most pronounced cytotoxic CD8(+) T cells, in line with programmed cell death 1 (PD-1) and its ligand (PD-L1) emerge as valuable prognostic marker in a multitude of tumor entities. In the present investigation, we examined the prognostic relevance of the immune markers, FOXP3+ Tregs and phosphorylated Caspase-8 (T273) in patients with anal squamous cell cancer treated with chemo- radiotherapy (CRT) also in the context of human papilloma virus 16 (HPV16) DNA load and p16INK4a expression. **Patients and methods:** The baseline histochemical expression of the markers as mentioned before was correlated with clinicopathologic characteristics, and cumulative incidence of local failure, disease-free survival (DFS) and overall survival (OS) in pretreatment biopsies of 150 patients uniformly treated with 5FU/Mitomycin-C based CRT. **Results:** After a median follow-up of 40 months (1-205 months), the 5-year cumulative incidence of local failure and DFS was 19.4% and 67.2%, respectively. Strong immune marker expression was significantly more common in tumors with high HPV16 viral load. In multivariable analysis, high CD8+ and PD-1+ TILs expression predicted for improved local control ( $p=0.023$  and  $p=0.007$ , respectively) and DFS ( $p=0.020$  and  $p=0.014$ , respectively). Moreover, high p16INK4a ( $p=0.011$ ) and PD-L1 ( $p=0.033$ ) expression predicted for better local control, whereas high FOXP3+Tregs ( $p=0.050$ ) and phosphorylated Caspase-8 ( $p=0.031$ ) expression correlated with superior DFS. Female sex and high HPV16 viral load correlated with favorable outcome for all three clinical endpoints. **Conclusion:** The present findings provide, for the first time, a robust explanation for the favorable clinical outcome of HPV16-positive patients harboring strong immune cell infiltration. These findings may be relevant for treatment stratification with immune PD-1/PD-L1 checkpoint inhibitors to complement CRT and should be explored in a clinical trial.

# Use of organoid cultures in the study of radiation response and regeneration

**Rob Coppes**

*Departments of Cell Biology and Radiation Oncology, University of Groningen, University Medical Centrum Groningen, The Netherlands*

The response of normal tissues to irradiation is mainly determined by the survival and regenerative potential of the tissue stem cells, and modulated by inflammatory processes, vasculature damage and altered neuronal innervation and fibrosis. Interestingly, transplantation of tissue specific stem cells has been shown to restore tissue homeostasis and prevent late radiation effects. Moreover, the sparing of localized stem cells was predicted to preserve salivary gland function in patients treated for head and neck cancer. Interestingly, mounting evidence indicates that cancer stem cells might contribute to the poor prospects. Recently, we and others have developed methods to culture patient specific organ and tumour stem cell containing organoids (tissue resembling structures). These organoids contain all the tissue/tumor lineages and the tissue/tumor stem cells, as indicated by their secondary organoids self-renewal potential and regeneration/regrowth potential and offer the opportunity to investigate tissue and patient specific assessment of the response of stem cells to (chemo-) radiotherapy. Stem cell survival curves and DNA DSB repair kinetics indicate that the response of organoids to different radiation qualities may differ from tissue to tissue, especially in the low dose regions typically delivered to the normal tissue outside the planning target volume. Therefore, organoids cultures could be used to investigate the mechanism of differences in response of normal and tumour stem cells to irradiation and exploit these for personalized optimisation of (chemo-) radiation treatment and prediction of treatment response.





## Session S19

# „Normal Tissue/Stem Cells Response“

**Diana Klein**

Essen, Germany

*„Radioprotection of normal lung tissue by mesenchymal stem cell therapy“*

**Lara Barazzuol**

Groningen, Netherlands

*„A coordinated DNA damage response activates adult quiescent neural stem cells in a niche-position and age-dependent manner“*

**Insa Sigrid Schröder**

Darmstadt, Germany

*„Ionizing radiation negatively affects cardiac differentiation of pluripotent stem cells“*

**Felix Meyer**

Hamburg, Germany

*„Interplay of DNA repair and stem-like phenotype determines the sensitizing effect of CHK1, RAD51 and PARP1 inhibition in TNBC“*

**Danuta Galetzka**

Mainz, Germany

*„Molecular karyotyping and DNA methylation pattern analysis in primary fibroblasts of patients with childhood cancer“*

**Harry Scherthan**

Munich, Germany

*„DNA damage in leukocytes after internal exposure to beta and alpha emitters“*



# Radioprotection of normal lung tissue by mesenchymal stem cell therapy

**Diana Klein**, A. Wieseemann, J. Steens and V. Jendrossek

*Institute of Cell Biology (Cancer Research), University Hospital Essen, Essen, Germany*

Radiotherapy (RT) plays a key role in cancer treatment. The ultimate goal of radiation therapy is to reduce or eliminate tumor burden while sparing normal tissues from long-term injury. However, local recurrence of primary tumors and distant metastasis are the leading causes of death in many cancer patients. Herein the high intrinsic sensitivity of normal tissues to ionizing radiation often precludes the application of curative radiation doses. As an example radiation-induced pneumonitis and fibrosis constitute the dose-limiting side effects of therapeutic and accidental thorax or whole-body irradiations. No causative radioprotective treatments are available to date and current research efforts are aimed to protect the normal tissue during RT and thus to possibly increase the radiation dose. With the use of the mouse model of radiation-induced pneumopathy, we could show that the barrier function of the endothelium of the lung is temporarily impaired (acute phase) as a result of the irradiation. This is associated with an increased permeability of these vessels and consequently an increased extravasation of immune and circulating tumor cells. As a long-term complication a loss of the endothelial cells is observed, which are accompanied by the development of the fibrosis. As a therapeutic option in the sense of a radioprotection, mesenchymal stem cells were administered to the animals immediately after irradiation (within the acute, inflammatory phase). For this, on the one hand, MSCs were used which were traditionally isolated from the bone marrow (BM-MSC), on the other hand, vessel-resident MSCs isolated from the aorta (Ao-MSC). Herein we hypothesized that therapeutic application of VW-MSC might be particularly well suited for the radioprotection of endothelial cells because of their tissue-specific action. Interestingly, the radiation-induced vascular hyperpermeability could be significantly reduced by the therapeutic application of the MSCs after irradiation. An ultrastructural analysis of the lungs of irradiated and MSC-treated animals confirms significant improvements in the morphological state of the endothelia. An increased infiltration and extravasation of (pre-metastatic) immune cells could also be limited. A therapeutic application of the MSCs after irradiation could also counteract the significantly increased extravasation of circulating tumor cells and subsequent metastasis without promoting the progression of existing metastases. Moreover, a therapeutic use of these cells in the long-term experiment counteracts a loss of endothelial cells and fibrosis progression. The high protective potential of the vessel wall-resident MSC compared to the stem cells isolated from the bone marrow is due to the tissue-specific effect of the Ao-MSCs. In summary, adoptive transfer of MSCs early after irradiation counteracts radiation-induced vascular damage and EC loss as late adverse effects. Our results are of direct clinical relevance, since they contribute to an improved understanding of the mechanisms of a main dose-limiting side effect of radiotherapy. This is a necessary step in the development of protective treatment strategies.



# A coordinated DNA damage response activates adult quiescent neural stem cells in a niche-position and age-dependent manner

**Lara Barazzuol**, L. Ju and P. Jeggo

*Department of Radiation Oncology, Department of Cell Biology, University Medical Center Groningen, Groningen, The Netherlands*

Long-lived adult stem and progenitor cells are at risk of accumulating DNA mutations that can contribute to oncogenesis and accelerated aging. In this study, using the mouse brain as a model, we define the functional consequences and mechanisms with which adult neural stem cells (NSCs) and their progeny respond to radiation-induced DNA damage within the sub-ventricular zone (SVZ). Exploiting recent evidence into regional differences within the SVZ, we spatially mapped apoptosis, DNA repair capacity and proliferation along the dorso-ventral axis of the SVZ of wild type and ataxia telangiectasia mutated (Atm<sup>-/-</sup>) mice in response to 2 Gy X-rays. We show that progenitors and neuroblasts, in contrast to NSCs, undergo radiation-induced apoptosis. This differential response is cell type-dependent and is not the result of quiescence status, senescence induction or differences in DNA repair capacity. We additionally show that ATM-dependent apoptosis together with proliferation arrest and neuroblast differentiation drive quiescent NSC activation allowing repopulation of the SVZ. In addition to the adult brain, we examined the DNA damage response of the neonatal SVZ at postnatal day 5, of importance for assessing the higher sensitivity to radiation-induced carcinogenesis. Radiation-induced apoptosis at P5 was overall higher than in the adult SVZ; however, the neonatal SVZ displays a lack of proliferation arrest and as a consequence repopulation occurs more rapidly from damaged progenitors and neuroblasts. These niche-position and age-dependent differences in the DNA damage response of adult NSCs and their progeny may have important implications in both radiotherapy treatment planning and radiation protection.

# Ionizing radiation negatively affects cardiac differentiation of pluripotent stem cells

S. Nitsch, F. Braun, J. Kunz, S. Ritter and **Insa Sigrid Schroeder**

*GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany*

Ionizing radiation can induce cardiotoxicity that is a health concern not only during the earliest steps of embryogenesis when the heart forms, but also in paediatric and adult patients that have been subjected to radiation therapy. Despite their frequent use, animal models fail to faithfully recapitulate human heart development, physiology, and pathology due to inter-species differences in biological pathways and ion channels. In contrast, human embryonic stem cells (hESCs) can be used to generate in vitro engineered heart tissue mirroring the in vivo situation in a reliable and accurate way. Therefore, we used the WA09 (H9) hESC line and its subline H9-hTnnTZ-pGZ-D2 (H9-cTNT), a cardiac reporter cell line that drives GFP expression under the cardiac troponin T (cTnT) promoter allowing the dynamic fluorescent tracking of cardiomyocyte generation to study the effect of ionizing radiation on cardiac development. Using a previously published protocol [1], both cell lines reproducibly generated contractile cardiac clusters that expressed typical markers for mesoderm (Brachyury), cardiac progenitors (Tbx5, Nkx2.5) and cardiomyocytes (e.g. cTnT, Mlc2a, MYH6/7, NPPA) albeit with H9-cTNT showing higher expression than H9 hESCs. Irradiation with 1 Gy X-rays 7d prior to differentiation initiation was performed to examine the impact of radiation exposure to surviving hESCs. It led to a marked decrease in the mRNA expression of all cardiac markers analyzed and reduced function judged by the video-based analysis of the beating frequency. Preliminary data also suggest a negative impact at a dose of 0.1 Gy X-rays. Analyses of the beating frequencies also revealed that the cardiomyocytes derived from irradiated hESCs never reached the beating rates of their non-irradiated counterparts. The underlying mechanisms are currently investigated. Our results indicate that even after a recovery from immediate radiation impacts such as apoptosis and DNA damage, the exposure to ionizing radiation leads to an altered differentiation behaviour in the hESC progeny. In summary, the radiation-induced impairment may indicate a negative impact on the positional and temporal patterning of the cardiomyocytes during early development in vivo. [1] Kadari et al., Stem Cell Rev and Rep 2015, 11:560 This work was funded by the German Ministry of Education and Research (Grant 02NUK025A) and the HGS-HIRe PhD programme.

# Interplay of DNA repair and stem-like phenotype determines the sensitizing effect of CHK1, RAD51 and PARP1 inhibition in TNBC

**Felix Meyer**, S. Becker, A. Niecke, S. Werner, C. Peitzsch, L. Hein, A. Dubrovskaya, H. Wikmann, K. Rothkamm and K. Borgmann

*Laboratory of Radiobiology and Experimental Radiooncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

Breast cancer comprises a heterogeneous group of tumors of whom 20% are categorized as triple-negative (TNBC). Important biological characteristics and potential therapeutic targets of TNBC include high proliferation, a basal-like and mesenchymal phenotype and a defect in the DNA repair pathway Homologous Recombination (HR), which feeds the observed elevated chromosomal instability in these tumors. TNBCs show an enrichment of cancer stem cells and therapy resistance. This project aims to develop treatment intensification strategies based on the simultaneous exploitation of the HR-deficiency and the stem-like phenotype, using specific inhibitors for RAD51, CHK1 and PARP1 in combination with irradiation. Expression of HR-related (RAD51, BRCA1, PTEN, CHK1, MRE11, ATR, ATM) and stem-like factors (ZEB1, E-Cadherin,  $\beta$ -Catenin, ALDH1) as well as HR functionality (via RAD51 foci, MMC-sensitivity and plasmid reporter assay) were determined in the TNBC line MDA-231 WT, its two sublines preferentially metastasizing to brain (BR) or bone (SA) and in the luminal BC line MCF7. DNA replication (fiber assay) and migration assay were also tested. Radiosensitivity and the radiosensitizing effect of different inhibitors was analyzed by colony assay and correlated to the CIN in the METABRIC database. Distinct differences in the expression of HR-related proteins were observed, with an elevated expression of CHK1, MRE11 and ATM in BR and SA relative to WT and MCF7. BR and SA showed a typical stem-cell like protein expression profile, together with a higher migration capacity, increased HR-capacity, resistance against MMC and less DNA damage, in line with an HR-proficient phenotype. After irradiation no advantage in survival for the BR and SA cell lines was observed, suggesting that not HR, but superordinate CHK1 mediated DDR promotes radioresistance. This was confirmed by a distinct radiosensitization after CHK1i; the most radioresistant WT cell line was most strongly sensitized by an EF=3. That effect also showed up in replication-processes, the higher the EF the stronger the inhibitory effect on replication. The effect of other inhibitors on radiosensitivity is currently being investigated. A second promising target is RAD51, because a METABRIC analysis (952 TNBCs) showed that in TNBC with high CIN RAD51 and CHK1 are significantly stronger expressed than in TNBC with low CIN. In conclusion the results presented here show that DNA repair and a stem-like phenotype are closely intertwined in determining resistance to tumor therapy of TNBCs with high CIN.

# Molecular karyotyping and DNA methylation pattern analysis in primary fibroblasts of patients with childhood cancer

**Danuta Galetzka**, S. Zahnreich , M. Hess, A. Wichmann, M. Endres, A. Maierhofer, J. Flunkert, T. Hankeln, C. Spix, T. Haaf, M. Marron and H. Schmidberger

*Department of Radiation Oncology and Radiation Therapy, Universität Mainz, Mainz, Germany*

Malignancies in children are unlikely to be due to environmental hazards or unhealthy lifestyle. Instead, genetic factors may play a predominant role for most malignancies in children. The successful treatment of childhood cancer by chemotherapy and radiotherapy has been associated with a 4-6-fold elevated risk for second primary malignancies in later life, compared with the general population. In particular, modulation or dysregulation of DNA repair- and cell cycle-associated genes may contribute to second primary cancer predisposition. With the help of the German Childhood Cancer Registry, we recruited a cohort of 23 individuals who survived a childhood malignancy and then developed a second primary cancer (2N) and 23 carefully matched controls who survived a childhood cancer without developing a second malignancy (1N). Cancer free patient from the department of accident surgery and orthopaedics recruited in the KIKME study (Cancer in Childhood and Molecular Epidemiology) served as controls (0N). Donors were matched according to age and sex (N0, N1, N2), year of first primary diagnosis and type of first primary malignancy (N2 and N1). Aiming to identify new candidate loci for cancer susceptibility factors we compared the genome-wide SNP DNA profiles (CGH-Analysis), DNA methylation (EPIC-Chip) and RNA-Seq signatures (NGS) after radiation in primary fibroblasts derived from our cohort in G0/G1-phase of the cell cycle. The cells were treated with 2, 5 and 8 Gy and harvested after 15 min, 2h and 24h. We identified in cancer patients microdeletions and microduplications, spanning in total 38 Genes including known tumor genes as SLC2A3 and GSTT2, candidate genes as ZNF630 and ZDHHC11 and genes or loci with yet unknown assignment as MIR570 or DDTL, which apparent response to radiation treatment in healthy controls after controlling the false discovery rate ( $FDR < 0.05$ ). 16 genes are unique for the 2N group whereas 12 genes were only present in the 1N group. An overlap of 10 genes is present in both groups. The results are now compared to the genome-wide methylation profiles. In addition, genomic Q-PCR, RT-PCR and bisulphite pyrosequencing experiments for a subset of conspicuous gene loci will be carried out to validate our data. Our results may reveal novel genes or factors particularly involved in initial steps of tumorigenesis or second cancer susceptibility.

***Danuta.Galetzka@unimedizin-mainz.de***

# DNA damage in leukocytes after internal exposure to beta and alpha emitters

**Harry Scherthan**, S. Schumann, U. Eberlein and M. Lassmann

*Bundeswehr Institute of Radiobiology affiliated to the Univ. of Ulm, Munich, Germany*

Application of ionizing radiation (IR) in clinical therapeutic procedures is the major source of man-made radiation exposure. While high dose and dose rate exposures with penetrating sparsely ionizing radiation can induce acute radiation sickness, incorporation of radionuclides often leads to protracted internal low dose irradiation at a low dose rates. We studied the induction of DNA double strand damage by enumeration of colocalizing foci of phospho-histone ( $\gamma$ ) H2AX and the DNA damage sensor protein 53BP1 in the chromatin of in-vitro and in-vivo exposed leukocytes and noted a dose-dependent DSB focus induction in leukocytes exposed in solution as well as in nuclear medicine patients. By exposing a defined volume of blood with diluted radionuclides we generated an in vitro DSB focus calibration-curve for the  $\beta^-$  emitters I-131 and Lu-177. For these two beta emitters a linear regression fitted to the data showed a good correlation between the number of IR-induced foci (RIF) per cell and the absorbed doses to the blood. Testing the high energy  $\beta^+$  emitter Ga-68 with this setup revealed similar results but at a ~23% reduced slope of the Ga-68 calibration-curve relative to the values obtained for the  $\beta^-$  emitters. Since the mean positron energy emitted by Ga-68 (829 keV) is significantly higher compared to the mean electron energy of I-131 (182 keV) and Lu-177 (134 keV), it seems possible that the different energy deposition patterns may lead to an altered DSB foci yield. Currently, we conduct further experiments with different radionuclides to clarify an energy dependency of DSB induction. In contrast to low LET irradiation, the transit of high LET alpha particles through cells and their nuclei cause tracks of closely spaced ionization events, leading to complex DNA damage including clustered DSBs that may lead to chromothryptic type of translocation series. Alpha-particle-induced DNA damage can be directly visualized by  $\gamma$ -H2AX DNA damage tracks traversing nuclear chromatin. We will report on the progress in establishing an in-solution calibration curve using DNA damage foci and  $\gamma$ -H2AX particle tracks after in-vitro exposure to solutions containing the alpha emitter Ra-223 that is currently used for treating patients with metastatic prostate cancer. Support: DFG

# Session S20

## „Low Dose Radiation Effects/Countermeasures“

### **Carmen Rios**

Rockville, United States

*„Expediting the drug development process by repurposing licensed products – a NIAID/RNCP viewpoint“*

### **Serge Candéias**

Grenoble, France

*„Low dose radiation accelerates ageing of the T cell receptor repertoire in mice“*

### **Michael Abend**

Munich, Germany

*„Pre-exposure gene expression in baboons with and without pancytopenia after radiation exposure“*

### **Johanna Mirsch**

Darmstadt, Germany

*„Efficient DSB repair after low irradiation doses requires a critical cellular radical level“*

### **Lisa Deloch**

Erlangen, Germany

*„Low-dose irradiation has an impact on bone metabolism and reduces fibroblast-like synoviocytes in dependence of the inflammatory state“*

### **Vijay Singh**

Bethesda, United States

*„Gamma-tocotrienol as a promising radiation counter-measure for acute radiation syndrome: Efficacy and biomarkers“*



# Expediting the drug development process by repurposing licensed products – A NIAID/RNCP Viewpoint

**Carmen Rios**, D. Cassatt, A. DiCarlo, M. Satyamitra, L. Taliaferro and C. Hackett

*National Institute of Health: National Institute of Allergy and Infectious Diseases,  
Bethesda, USA*

The possibility of a radiological or nuclear incident is of increasing concern. Media attention focused on threats involving radioactive sources that could be breached or lost, the potential for accidents in nuclear power facilities and possible detonation of a nuclear device, highlight the need to address this national security issue. To date, only two drugs - Neupogen® and Neulasta® (Amgen) - have been approved by the U.S. Food and Drug Administration (U.S. FDA) to mitigate hematopoietic injuries that could result from radiation exposure during a mass casualty incident. These drugs were already licensed for treatment of chemotherapy-induced neutropenia, and the company relied on a wealth of clinical data, as well as adequate and well-controlled animal studies, to support approval. The process of developing new drug candidates and advancing them toward licensure is a scientifically complex, time-consuming and expensive process. There is an urgent need to find new drug development strategies that reduce the time frame, decrease costs and improve success rates. Drug repurposing is one possibility. Products already approved by the U.S. FDA for other indications have been tested in humans and there is a record of toxicity, potency and pharmacologic parameters. Because repurposing builds upon previous research and development efforts, new candidate therapies could advance much faster through the drug development pathway. The goal of the Radiation Nuclear Countermeasures Program (RNCP) of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), is to ensure the government stockpiling of safe and efficacious candidate therapies to treat radiation injuries. Repurposing drugs may help countries reach this goal more efficiently.



# Low dose radiation accelerates ageing of the T cell receptor repertoire in mice

**Serge Candéias**, J. Mika, P. Finnon, T. Verbiest, R. Finnon, N. Brown, S. Bouffler, J. Polanska and C. Badie

*CEA, Fundamental Research Division, Laboratory of Chemistry and Biology of Metals,  
UMR5249 CEA-CNRS-UGA, Grenoble, France*

While the biological effects of high dose ionizing radiation on human health are well characterized, the consequences of low dose radiation exposure remain poorly defined, even though they are of major importance for radiological protection. Lymphocytes are very radiosensitive, and radiation-induced health effects may result from immune cell loss and/or immune system impairment. In order to decipher the mechanisms of effects of low doses, we analyzed the modulation of the T-cell receptor gene repertoire in mice exposed to a single low (0.1 Gy) or high (1 Gy) dose of radiation. High throughput T-cell receptor gene profiling was used to visualize T lymphocyte dynamics over time in control and irradiated mice. Radiation exposure induces “aging-like” effects on the T-cell receptor gene repertoire, detectable as early as 1 month post exposure and for at least 6 months. Surprisingly, these effects are more pronounced in animals exposed to 0.1 Gy than to 1 Gy, where partial correction occurs over time. Importantly, we found that low dose radiation effects are partially due to the hematopoietic stem cells impairment. Collectively, our findings show that acute low dose radiation exposure specifically results in long term alterations of the T lymphocyte repertoire.

This work has been supported by the European Commissions (DoReMi, European Atomic Energy Community's Seventh Framework Program (FP7/2007-2011) under grant agreement n°249689), by National Science Centre grant HARMONIA 4 n°2013/08/M/ST6/00924 (JP) and by MNiSW grant 02/010/PMB16/0052 for co-financed international project (JP, JM). Calculations were carried out using infrastructure of GeCONil (POIG.02.03.01-24-099).

# Pre-Exposure Gene Expression in Baboons with and without Pancytopenia after Radiation Exposure

M. Port, F. Herodin, M. Valente, M. Drouet, R.Ullmann, M. Majewski and **Michael Abend**

*Bundeswehr Institute of Radiobiology, Munich, Germany*

Radiosensitivity differs in humans and likely among primates. The reasons are not well known. We examined pre-exposure gene expression in baboons (n = 17) who developed haematologic acute radiation syndrome (HARS) without pancytopenia or a more aggravated HARS with pancytopenia after irradiation. We evaluated gene expression in a two stage study design where stage I comprised a whole genome screen for messenger RNAs (mRNA) (microarray) and detection of 667 microRNAs (miRNA) (real-time quantitative polymerase chain reaction (qRT-PCR) platform). Twenty candidate mRNAs and nine miRNAs were selected for validation in stage II (qRT-PCR). None of the mRNA species could be confirmed during the validation step, but six of the nine selected candidate miRNA remained significantly different during validation. In particular, miR-425-5p (receiver operating characteristic = 0.98; p = 0.0003) showed nearly complete discrimination between HARS groups with and without pancytopenia. Target gene searches of miR-425-5p identified new potential mRNAs and associated biological processes linked with radiosensitivity. We found that one miRNA species examined in pre-exposure blood samples was associated with HARS characterized by pancytopenia and identified new target mRNAs that might reflect differences in radiosensitivity of irradiated normal tissue.

# Efficient DSB repair after low irradiation doses requires a critical cellular radical level

**Johanna Mirsch**, N. Lengert, R. Weimer, B. Drossel and M. Löbrich

*Radiation Biology and DNA repair, TU Darmstadt, Germany*

Double-strand breaks (DSBs) are the most lethal DNA lesions induced by ionizing radiation and their efficient repair is crucial to limit the carcinogenic risk. The DNA repair response after low dose irradiation is of special interest as such exposures are common in our daily life. We utilized the high sensitivity of the  $\gamma$ H2AX-foci assay and observed that human fibroblasts fail to repair DSBs efficiently after very low doses of X-rays (less or equal than 10 mGy). However, the repair efficiency was increased in cells pre-treated with low concentrations of hydrogen peroxide, suggesting that this induces a response, which is required for the repair of radiation-induced DSBs at low radiation doses (Grudzenski et al. 2010, PNAS 107:14205-10). One interpretation of this finding is that a certain cellular radical level is required to efficiently activate the repair machinery. To test this hypothesis, we asked if the DSB repair capacity at low doses can be further diminished when cells are treated with a radical scavenger prior to irradiation. Using a customized semi-automated foci counting method for human fibroblasts based on co-localising  $\gamma$ H2AX-53BP1-foci signals, we quantified DSBs in over 1.5 million cells after irradiation with doses of 5-50 mGy. We observed a decreased DSB repair capacity after doses of 5-10 mGy, which was further decreased in cells pre-treated with the radical scavenger N-Acetylcystein (NAC). To address the in vivo relevance of this finding, we analyzed mice irradiated with 10 mGy and treated or not treated with NAC. The repair of DSBs after low dose irradiation was severely limited in all analysed tissues except the lung, an organ which is expected to have a higher cellular radical level. Strikingly, the efficient repair capacity in the lung was reduced to that of the other organs in mice pre-treated with NAC. These data fully consolidate our cellular studies and suggest that the cellular radical level is critical for efficient DSB repair at low doses. Since radical stress can activate repair kinases such as ATM, further studies will investigate which repair kinases might not be fully activated after low dose exposure.

# Low-dose irradiation has an impact on bone metabolism and reduces fibroblast-like synoviocytes in dependence of the inflammatory state

**Lisa Deloch**, A. Derer, R. Fietkau, B. Frey and U. S. Gaipl

*Department of Radiation Oncology, Universitätsklinikum Erlangen, Germany*

Background: Rheumatoid Arthritis (RA) is a chronic, progressive, inflammatory autoimmune disease that mainly affects the joints. Its main features are synovial inflammation followed by cartilage and bone destruction. Even though there are various successful treatment options available, a good proportion of patients does not respond properly or has to reduce their medication. Using the human TNF $\alpha$  transgenic (hTNF $\alpha$  tg) mouse model in various ex vivo and in vivo settings we already obtained hints that low-dose radiotherapy (LD-RT) might be a valuable therapy option for RA patients. However, the mode of action of LD-RT within the joint in dependence on the inflammatory state is still unknown. Thus, we compared the impact of LD-RT on inflammatory cells derived from hTNF $\alpha$  tg mice with healthy, wild-type-derived ones. Methods: For all experiments cells derived from either hTNF $\alpha$  tg or C57Bl/6 littermates mice were used. Bone marrow cells were differentiated into osteoclasts (OC) and mineralization properties of isolated osteoblasts (OB) derived from calvarias were examined. Murine fibroblast-like synoviocytes (FLS) were isolated from the joints of the hind feet and investigated with regards to their inflammatory phenotype. All of these cell types which play a major role in RA were exposed to LD-RT with a dose of 0.1Gy, 0.5Gy, 1.0Gy, and 2.0 Gy. Results: LD-RT reduced cell growth and enhanced apoptosis in both inflammatory and healthy FLS, starting from a dose of 0.5Gy. Likewise, starting at a dose of 0.5Gy, LD-RT reduced the numbers of differentiated inflammatory OCs, the numbers of non-inflammatory OC were even increased after exposure of the bone marrow cells to 0.1Gy. Additionally, a dose of up to 0.5Gy had no impact on selected OC differentiation markers on mRNA level in healthy OCs, while in inflammatory ones Acp5, catK, c-fos and RANK expression was increased starting from a dose of 0.1Gy on. OB-mediated bone mineralization was increased only after exposure to 0.5Gy in inflammatory OBs, while in healthy ones a significant increase was observed after exposure to 0.1Gy and 0.5Gy. Conclusion: LD-RT of in particular 0.5Gy resulted in attenuation of inflammation by inducing FLS apoptosis and had a protective impact on bone homeostasis in inflammatory OCs and OBs of hTNF $\alpha$  tg mice (RA setting). Of note is that in synopsis no disadvantages of LD-RT were observed in the non-inflamed, healthy system: While OC numbers were increased after 0.1Gy, also an increased OB activity at 0.1Gy could be observed, balancing bone homeostasis. We conclude that LD-RT is a treatment alternative or addition to classical RA therapy that mediates its positive effects via osteoimmunological mechanisms. Acknowledgement: Supported by the German Federal Ministry of Education and Research (GREWIS, 02NUK017G).

# Gamma-tocotrienol as a promising radiation countermeasure for acute radiation syndrome: Efficacy and biomarkers

**Vijay Singh**

*Armed Forces Radiobiology Research Institute, USUHS, Bethesda, MD, USA*

Gamma-tocotrienol (GT3) is one of the eight isomers (tocols) of vitamin E and appears to be one of the most promising radioprotective tocols. GT3 has been demonstrated to increase survival in rodents, through ameliorating the radiation-induced injuries of the hematopoietic and gastrointestinal systems. When administered 24 h prior to irradiation, GT3 significantly protected irradiated mice and induced high levels of G-CSF. Injection of a G-CSF neutralizing antibody to the GT3-treated mice resulted in complete neutralization of G-CSF and abrogation of its radioprotective efficacy in murine model. Similar observations were made with several radiation countermeasures. GT3 mobilized progenitors from bone marrow to peripheral circulation and mobilized progenitors mitigated radiation injury in lethally irradiated mice. Recently, GT3 was evaluated in nonhuman primates (NHPs) for its efficacy against lethal doses of radiation. GT3 was administered subcutaneously 24 h before irradiation and its efficacy was tested without supportive care. Various biomarkers were identified using microRNA analysis, transcriptomics, and metabolomics studies. Results demonstrated that the GT3 treatment significantly decreased the duration and severity of neutropenia and thrombocytopenia in irradiated NHPs. GT3 administered in one dose was comparable to multiple G-CSF and two PEGylated G-CSF administrations in combination with supportive care, in terms of improving radiation-induced neutropenia and thrombocytopenia. GT3 modulated several metabolomics biomarkers in serum and mitigated radiation-dependent transcriptomic changes within the fronto-limbic circuit in NHPs. Serum concentrations of miR-30a, miR-126, and miR-375 correlated with the radioprotective efficacy of GT3, that is, these miRNAs in the GT3-treated and irradiated NHPs resembled the unirradiated animals. These three miRNAs could be used as biomarkers of GT3 efficacy in protecting animals from the radiation impact because their profiles after GT3 treatment converged with those observed in the pre-irradiation macaques. Our studies indicate that GT3 is a promising radiation countermeasure and there are several promising biomarkers for its efficacy. Currently, GT3 is under advanced development with the support from Congressionally Directed Medical Research Program for humans against the potentially lethal effects of radiation exposure.



## Session S21

# „Modelling of DNA Damage Responses“

### **Michael Scholz**

Darmstadt, Germany

*„Modelling the impact of cell cycle dependent repair mechanisms and repair deficiencies based on chromatin loop structure and replication status“*

### **Werner Friedland**

Neuherberg, Germany

*„Modelling of DNA damage by light ions from radiotherapy-relevant energies down to stopping“*

### **Andrea Ottolenghi**

Pavia, Italy

*„From track structure to systems biology: How many roads must a man walk down ...“*

### **Mario Pietro Carante**

Pavia, Italy

*„Full predictions of cell death and chromosome damage along hadrontherapy dose profiles by the BIANCA biophysical model“*

### **Kohei Sasaki**

Sapporo, Japan

*„A simulation study for both of the targeted and untargeted effect on the uniformly irradiated cells“*

### **Thomas Friedrich**

Darmstadt, Germany

*„Which spatial dimensions of radiation damage interaction are relevant for the high effectiveness of ions?“*



# Modelling the impact of cell cycle dependent repair mechanisms and repair deficiencies based on chromatin loop structure and replication status

P. Günther, A. Hufnagl, S. Lerchl, T. Friedrich and **Michael Scholz**

*GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany*

Cell cycle dependent radiosensitivity and the impact of repair deficiencies are tightly linked as a consequence of the varying availability of different repair pathways throughout the cell cycle. We have shown that the cell cycle dependent variation of sensitivity after photon irradiation can be modelled based on a classification of DSB according to their clustering within chromatin loop structures and the replication status of the chromatin loop [1]. A key feature of this model is the distinct assignment of different repair pathways, i.e. non-homologous end joining (NHEJ) and homologous recombination (HR), to unreplicated and replicated loops, respectively. This model concept can thus also be used to analyze the impact of repair deficiencies of the major repair pathways. In our contribution, by comparing model calculations with further experimental data we show that the model correctly predicts the range of variation in sensitivity between cell lines with repair deficiencies in either the NHEJ, the HR or simultaneously in both the NHEJ and HR pathways. Furthermore, the variation of RBE between these different cell lines after high-LET radiation can be explained. In addition we discuss the “inverted” cell cycle dependence of radiosensitivity after high-LET radiation, namely the increased sensitivity in S-phase, as compared to the decreased sensitivity in S-phase after low-LET radiation. Such an inversion is also expected according to model predictions for very high doses of photon radiation (>20 Gy). The implications on the shape of dose response curves for asynchronous cell populations will be described. [1] Hufnagl A et al., DNA Repair 27:28-39 (2015)



# Modelling of DNA damage by light ions from radiotherapy-relevant energies down to stopping

**Werner Friedland**, P. Kunderát, E. Schmitt, J. Becker, A. Ottolenghi, G. Baiocco, S. Barbieri and M. Dingfelder

*Department of Radiation Sciences, Helmholtz Zentrum München, Neuherberg, Germany*

Radiation effects of ions over a wide range of energies are of paramount interest in particle radiotherapy (RT). The treatment planning has to account primarily for the distribution of the deposited dose. For ions also the increased relative biological effectiveness (RBE) has to be taken into account; clear understanding of RBE is essential for optimal use of ion RT. Radiation transport model calculations have proven their suitability for dose determination in ion RT on spatial scales down to mm whereas the RBE of ion radiation qualities is essentially related to damage distributions on nm- and  $\mu\text{m}$ -scales; in this domain track-structure based simulations are the method of choice. At low energies (i.e. in Bragg peak regions), however, the availability of cross sections has limited such calculations to energies above about 1 MeV/u; simulations below this limit have been performed for H, He and C only. To overcome this limitation, the biophysical Monte Carlo modelling tool PARTRAC [1] has been extended by a new cross-section scaling scheme [2]. It accounts for the effect of charge-changing processes, and yields track structures with linear energy transfer (LET) values corresponding to established standards down to energies around 10 keV/u where interactions with nuclei in the target material become important. Initial DNA damage has been determined in models of human lymphocyte and fibroblast nuclei for H, He, C, N, O and Ne ions with 0.25 – 256 MeV/u energy and scored in terms of double-strand break (DSB) sites, DSB clusters and their complexity [3]. For the lowest energies, where ion's stopping power largely varies along the transport distance, the DNA damage pattern has been analyzed differentially in dependence on local LET. The calculated DSB yields are in agreement with DNA fragmentation measurements [4]. H and He are more effective in producing DNA lesions than heavier ions at the same LET. For ions heavier than He, yields of DSB sites are independent of ion type but LET-dependent, with maximal RBE (related to fast protons) of about 2.2 around 200 keV/ $\mu\text{m}$ . Corresponding yields of DSB clusters show a slightly ion-dependent maximum around 500 keV/ $\mu\text{m}$  with very high RBE of about 30. The complexity of DSB clusters is ion-specific and increases up to the highest LET values. At very low energies, the DSB yields on the distal side of the Bragg peak are lower than at the same LET on the proximal side. The calculations have recently been supplemented by corresponding studies for Li, Be and B ions. For these ions the damage yields are in general between the results for He and C, but more short fragments may be formed by these ions than by lighter or heavier ones. As a new endpoint for comparison with foci experiments, yields of DSB and DSB clusters merged on scales of the order of 100 nm have been determined; they show an ion-type independent maximum around 30 keV/ $\mu\text{m}$  LET not more than 40% above the low-LET limit. Calculations on DNA damage response including induction of chromosomal aberrations are underway; results will be reported at the meeting. The performed mechanistic simulations provide a unified picture on ion-induced DNA damage. They help elucidate the role of DNA damage clustering on nano- and micrometer scales and their impact on biological effects of radiation. **Acknowledgement:** Supported by EURATOM FP7 (grant agreement n° 249689) and German BMBF (funding no. 02NUK031C). **References:** [1] Friedland et al 2011 Mutat Res 711:28-40 [2] Schmitt et al 2015 Radiat Prot Dosim 166:15-18 [3] Friedland et al 2017 Sci Rep 7:45161 [4] Höglund et al 2000 Int J Radiat Biol 76:539-547

# From track structure to systems biology: How many roads must a man walk down ...

**Andrea Ottolenghi**, G. Babini, G. Baiocco, S. Barbieri and J. Morini

*Physics Department, University of Pavia, Pavia, Italy*

Systems Biology approaches aim to identify, integrate and mathematically model properties emerging across the temporal and spatial scale (in cells, tissues, organs, etc.), which might not be directly measured or explained from the study of the system as sum of its single parts. With the rapidly increasing production of a great deal of biological data, thanks to the new “omics” technologies (e.g. Next Generation Sequencing), the possibilities to understand biological systems as a whole are increasing day after day. Nowadays, all different molecular layers within the cells can be deeply investigated (genomics, transcriptomics, proteomics, etc), allowing a complete reconstruction of the metabolic and cellular networks, and their stimuli-induced specific perturbations, more easily than before. During the last decade more and more efforts have been devoted to the application of Systems Biology approaches to Radiation Biology, relying on the awareness that full knowledge of the response to radiation of a biological system cannot be acquired with a pure reductionist approach. On the contrary, a holistic approach is needed to understand radiation-induced perturbations of the system acting as a whole, determined by the synergic sum of all the interacting elements. Parallel to these developments, special attention is being paid in the radiation research community to pursue and renew investigations based on track structure modeling, tracing back radiation effects to physical and chemical processes leading to initial damage (typically to the DNA, but extended also to other targets) and its time evolution (e.g. DNA repair processes, chromosomal aberration etc.). In the course of the years, this approach has maintained its potential in offering an insight into the dependence on the radiation quality, on the dose rate, and, more in general, into the shape of the dose-effect curve, particularly at low doses. Coordinated actions are essential to bring together results and activities which can be ascribed to these two approaches: the success of a multiscale approach lies indeed in our capability not to lose sight of any of the scales involved, each to be investigated with the needed level of details, but all to be described in a language as common as possible, thus guaranteeing the chance of heralding major advancements in the field of Radiation Biology.

# Full predictions of cell death and chromosome damage along hadrontherapy dose profiles by the BIANCA biophysical model

**Mario Pietro Carante**, J. Tello, M. Bernal and F. Ballarini

*University of Pavia and INFN, Pavia, Italy*

An improved version of a biophysical model called BIANCA (Biophysical ANALysis of Cell death and chromosome Aberrations) [F Ballarini and MP Carante, Radiation Physics and Chemistry 2016, 128] was developed within the INFN project ETHICS, and applied to calculate and predict radiation-induced cell death and chromosome aberrations, with focus on radiation types and energies used in hadrontherapy. The model, implemented as a Monte-Carlo simulation code, is based on the following assumptions: i) radiation induces DNA “Cluster Lesions” (CLs), where by definition a CL is a critical lesion able to produce two independent chromosome fragments; ii) chromosome fragment un-rejoining, or distance-dependent mis-rejoining, gives rise to chromosome aberrations; iii) certain aberrations (dicentric, rings and large deletions) lead to cell death. The yield of radiation-induced CLs, which for each radiation quality (i.e. particle type and energy) is adjusted by comparison with experimental data from the literature, is the main model parameter. The second, and last, adjustable parameter is the probability,  $f$ , that a chromosome fragment remains un-rejoined. Good agreement was found between simulation outcomes and experimental data both for a radioresistant and for a normal cell line, thus allowing to produce a database of CL yields for different particle types and LET. For a given particle type, the increase of the CL yield (expressed as CL/micron) with LET was fitted by a linear-quadratic function; this made it possible to establish a one-to-one correspondence between LET and CL yield, and thus to perform full predictions of cell death and chromosome aberrations in principle for any LET value within the range covered by the experiments, including LET values for which no experimental data were available for comparison. The model was then applied to calculate the dependence of these biological effects on depth for SOBP profiles available in different centres, including the CATANA facility at INFN-LNS in Catania, where proton treatment of eye melanoma is performed [Carante M.P. and Ballarini F. Front. Oncol. 6:76 2016], and the CNAO hadrontherapy centre in Pavia. In particular, for protons the biological effectiveness was found to increase in the distal region, and high levels of damage beyond the distal dose fall-off were observed. In line with other studies, these results suggest that assuming a constant RBE along a proton SOBP may be sub-optimal. A generalization of this approach is ongoing within the MC-INFN project, which foresees an interface of the BIANCA model with the FLUKA radiation transport code to provide a biological output in terms of cell death and chromosome damage, in addition to physical dose distributions. In the meanwhile a further model refinement is in progress, consisting of implementing and testing different functions (a negative exponential and a Gaussian) to describe the so-called “proximity effects”. The results obtained so far for gamma rays suggested that, at least for lymphocytes, this approach leads to a better agreement reproduction of the experimentally-observed ratios of acentric rings and interstitial deletions to centric rings (G-ratio). Acknowledgements: this work was partially supported by INFN under projects ETHICS (P.I. L. Manti) and MC-INFN/FLUKA (P.I. P. Sala). M. Bernal research activity was supported by “Conselho Nacional para o Desenvolvimento Científico e Tecnológico” (CNPq) in Brazil

[mariopietro.carante01@ateneopv.it](mailto:mariopietro.carante01@ateneopv.it)

# A simulation study for both of the targeted and untargeted effect on the uniformly irradiated cells

**Kohei Sasaki**, Y. Matsuya, Y. Yoshii, T. Sanada, Y. Yaegashi and H. Date

*Faculty of Health Sciences, Hokkaido University of Science, Japan*

The aim of this investigation is to make clear the responses both of targeted and untargeted effect on the uniformly low dose irradiated cells by demonstrating the behavior of the cell-to-cell signaling in silico and comparing with the reported experimental results. We developed a stochastic model for the mixed cell population which included cells both of traversed and not traversed by the ionizing radiation. The model involves couple of signaling pathways: the gap junction intercellular communication (GJIC), and diffusion of extracellular signals such as cytokine. The conditions assumed here correspond to the experiment by Hu et al. (2007), in which human skin fibroblasts (AG1522) are plated onto a rectangular dish (10 x 6 mm<sup>2</sup>) and a confluent monolayer is formed. A quarter of cells is exposed to  $\alpha$ -particles from an 241Am source with a dose of 10 mGy. The shielded culture is divided into three equal areas defined as BY1, BY2 and BY3. The assumptions underlying this simulation are described as follows: (1) virtual confluent cells are formed by the Voronoi tessellation and randomly seeded in a 10 x 6 mm<sup>2</sup> rectangle, (2) the cell-to-cell signals generated by irradiated cells immediately spreads over the adjacent cells through gap junctions when a cell contacts with other cells, (3) the cells that accept the signal become activate and cause DNA double-strand breaks (DSBs) with a probability defined as PDSB, (4) the signaling via GJIC has a range of 5  $\mu$ m, (5) the extracellular signals are also generated by IR cells and diffuse in the cell culture medium through Brownian motion, where the diffusion coefficient is estimated at 108 nm<sup>2</sup>s<sup>-1</sup> from a mass of cytokine, (6) when a extracellular signal comes within a sphere of 25  $\mu$ m radius about the center of a untargeted cell, the signal disappears and DSBs are induced in the cell nucleus, (7) DSBs are repaired with a time-dependent rate that is determined by a simple numerical model and  $\gamma$ -H2AX foci data. For analyzing the spatiotemporal kinetics of inducing DSBs, we calculated the number of DSB positive cells in each area after irradiation. The fractions were compared with the experimental results. In the diffusion simulation of signals, the results raised the possibility that extracellular signals cause about 10% of DSBs in the IR area but rarely contribute to early untargeted effect in the distal areas, BY2 and BY3. In conclusion, the present model was able to reproduce the DNA damage kinetics on the targeted and untargeted cells.

# Which spatial dimensions of radiation damage interaction are relevant for the high effectiveness of ions?

**Thomas Friedrich**, C. Greubel, K. Illicic, S. Girst, J. Reindl, M. Sammer, C. Siebenwirth, T. Schmid, M. Scholz and G. Dollinger

*GSI Helmholtz Center, Darmstadt, Germany*

The enhanced action of high LET radiation in comparison to high energy photon radiation, often expressed as the relative biological effectiveness (RBE), is widely exploited in radiotherapy and radiobiology. It is frequently reported to be reasoned in the condensed energy deposition along ion tracks within the biological target, where the proximity of DNA lesions results in more complex, more severe damage. The spatial dimensions of such proximity have been largely considered, but up to now no general conclusion about the importance of different possible scales is established. We report here on results gathered in a consortium project aiming to investigate the dependence of RBE on mechanisms on different spatial scales, both experimentally and by a model study: Experimental data gained with microbeam focused spots of low LET protons, and high LET Lithium and Carbon ions provide evidence that the size of the DNA (~ nanometer), the size of DNA chromatin loops (~ micrometer) and the size of cell nuclei (~ 10 micrometer) all have an impact on the radiation damage as measured with an survival assay of CHO-K1 hamster cells. For instance, strong focusing of low LET protons to  $\mu\text{m}$  sized spots considerably enhances the effect, and the spots reveal properties of high LET particles. However, carbon ions still provide a higher effectiveness, indicating relevance of the extremely high doses within a few nm of their track structure. On the theory side, the local effect model (LEM) which assumes lesion interaction mechanisms on these scales was used to compare corresponding RBE model predictions with the experimental data. The experiment and the independent model calculations are in excellent agreement. This demonstrates that these different coexisting spatial scales are relevant for a consistent understanding of high LET radiation damage. Likewise, the results indicate an interpretation of the underlying mechanisms as DSB formation (nm scale), DSB interaction ( $\mu\text{m}$  scale) and hit statistics (10 nm scale). Finally, at hand of the experiments and model simulations insight in the relative importance of these mechanisms for the RBE is given.

Supported by the BMBF within project 'LET-Verbund' (02NUK031).

A decorative graphic on the left side of the page, featuring a light blue DNA double helix structure. Several spherical, light blue protein-like structures are attached to the DNA strands. The graphic is positioned vertically along the left edge of the page.

# Poster Session 1



<b>P1</b>	<b>Gabriel Adrian</b> Lund, Sweden	<i>"A standardization method to compensate for cell-density dependent radioresistance in colony formation assays"</i>
<b>P2</b>	<b>Vidhula Ahire</b> Amsterdam, The Netherlands	<i>"Investigating Parp-inhibition as an adjunct treatment to enhance cervical cancer thermo-sensitivity to radiochemotherapy"</i>
<b>P3</b>	<b>Anne Bravard</b> Paris, France	<i>"ATM- dependent induction of PRNP protects neuronal cells against Ionizing Radiation"</i>
<b>P4</b>	<b>Nikko Brix</b> Munich, Germany	<i>"Dimensionality reduction of clonogenic survival data to systematically identify molecular targets for radiosensitization in breast cancer"</i>
<b>P5</b>	<b>Martin Bucher</b> Neuherberg, Germany	<i>"Genome instability and DNA repair capacity in radiation sensitive children"</i>
<b>P6</b>	<b>Helmut Bühler</b> Bochum, Germany	<i>"The efficiency of irradiation of glioblastoma cells is enhanced synergistically by the combination of chloroquine and PARP-inhibitor"</i>
<b>P7</b>	<b>Antonina Cebulska-Wasilewska</b> Warsaw, Poland	<i>"Coexisting factors influence on Low and High Dose of Iodine-131 Impact on Individual Susceptibility to Ionising Radiation and Biomarkers of Health Risks"</i>
<b>P8</b>	<b>I-Peng Chen</b> Buxtehude, Germany	<i>"Distinct microRNA expression patterns in sun-exposed, sun-protected human skin and SCC tumor tissue"</i>
<b>P9</b>	<b>Lei Cheng</b> Stockholm, Sweden	<i>"DNA damage and repair after a combined exposure to alpha particles and X-rays"</i>
<b>P10</b>	<b>Francois Chevalier</b> Kalyani, India	<i>"Radio-sensitization of chondrosarcoma cells by PARP-inhibitors"</i>
<b>P11</b>	<b>Noami Daems</b> Mol, Belgium	<i>"Hybrid gold nanoparticles coated with organic polymers and antibodies as a platform for cancer theranostics: Cytotoxicity assessment"</i>
<b>P12</b>	<b>Marie Davidková</b> Prague, Czech Republic	<i>"Radiomodifying features of organometallic compounds"</i>
<b>P13</b>	<b>Sara Sofia Deville</b> Dresden, Germany	<i>"Synemin is a novel co-regulator of the radiation-induced DNA damage response in head and neck cancer cells"</i>



<b>P14</b>	<b>Angela Diana</b> Oxford, United Kingdom	<i>"Proteomic profiling of ECM colorectal cancer after irradiation"</i>
<b>P15</b>	<b>Klaus Dittmann</b> Tuebingen, Germany	<i>"Nuclear EGFR Regulates Stability of VEGF Signalling-Associated mRNAs in Response to Radiation"</i>
<b>P16</b>	<b>Bastien Doix</b> Louvain-la-Neuve, Belgium	<i>"How immunogenic cell death induced by anticancer photodynamic therapy can enhance radiation-induced immune response"</i>
<b>P17</b>	<b>Yasin Bahadir Erol</b> Essen, Germany	<i>"Effect of inhibiting Mre11 on the radiosensitivity of NSCLC cell lines after concurrent treatment with cisplatin and ionising radiation"</i>
<b>P18</b>	<b>Roser Esplugas</b> Reus, Spain	<i>"Effect of radiotherapy on circulating miRNAs-146a, -155, -221 and -222 levels in women with breast cancer"</i>
<b>P19</b>	<b>Florian Frohns</b> Darmstadt, Germany	<i>"Analysis of DNA repair pathways in rod photoreceptor cells of nocturnal and diurnal animals"</i>
<b>P20</b>	<b>Ana Gasol Garcia</b> Amsterdam, Netherlands	<i>"Nanocarrier delivery of the radiosensitizer MEK162 in brain tumour therapy"</i>
<b>P21</b>	<b>Paulo Godoy</b> Stockholm, Sweden	<i>"Targeting cellular antioxidant system to sensitize glioma stem cells to acute and chronic irradiation"</i>
<b>P22</b>	<b>Giovanna Granata</b> Oxford, United Kingdom	<i>"Identification of novel tumour specific determinants of radiosensitivity using a CRISPR Whole Genome screen"</i>
<b>P23</b>	<b>Debora Grasso</b> Brussels, Belgium	<i>"Cancer radiosensitivity under metabolic control"</i>
<b>P24</b>	<b>Torsten Groesser</b> Roskilde, Denmark	<i>"A new efficient cancer treatment by combining ablation techniques and Auger electron therapy"</i>
<b>P25</b>	<b>Christine Hansel</b> Essen, Germany	<i>"Targeting improved antioxidant defense and dNTP sanitization is effective in eradicating cancer cells adapted to acute or chronic anoxia/re-oxygenation stress"</i>
<b>P26</b>	<b>Anja Heselich</b> Darmstadt, Germany	<i>"Influence of posttranslational modifications on radiation-induced DNA damage repair processes"</i>

<b>P27</b>	<b>Eva Hirschmann</b> Erlangen, Germany	<i>"Radiation and checkpoint blockade in the treatment of metastases"</i>
<b>P28</b>	<b>Anup Kainthola</b> New Delhi, India	<i>"Trichostatin A mitigates radiation induced gastrointestinal syndrome and bacterial translocation in gut"</i>
<b>P29</b>	<b>Anna Katsiki</b> Athens, Greece	<i>"Challenges and problems while using data from NCBI Gene Expression Omnibus (GEO) to detect gene expression profiles – a case study regarding NSCLC cell lines treated with IR"</i>
<b>P30</b>	<b>Tamara Kazimova</b> Zurich, Switzerland	<i>"Identification of biologically active factors in ionizing radiation regulated secretome"</i>
<b>P31</b>	<b>Maria Koźlak</b> Orsay, France	<i>"Mechanism of action of Dbait, an original class of DNA repair inhibitor."</i>
<b>P32</b>	<b>Adam Krysztofiak</b> Essen, Germany	<i>"The membrane-targeted antineoplastic alkylphosphocholine erufosine modulates the cellular radiation response by interfering with the lipid compartment"</i>
<b>P33</b>	<b>Wei-Chun Lee</b> Dresden, Germany	<i>"Identification of beta 8 integrin as novel determinant of pancreatic cancer cell radioresistance"</i>
<b>P34</b>	<b>Ahmed Allam Mohamed</b> Freiburg, Germany	<i>"c-MET as potential target for radio-sensitization in pancreatic cancer"</i>
<b>P35</b>	<b>Ryosuke Mori</b> Hokkaido, Japan	<i>"A model analysis to estimate the number of DNA double-strand breaks in cells exposed to X-rays"</i>
<b>P36</b>	<b>Michael Orth</b> Munich, Germany	<i>"Exploiting novel combined-modality approaches for the treatment of highly aggressive, invasive pancreatic ductal adenocarcinomas"</i>
<b>P37</b>	<b>Michael Orth</b> Munich, Germany	<i>"HSP90 inhibitors as radiosensitizers: Lessons from different tumor models"</i>
<b>P38</b>	<b>Norman Reppingen</b> Darmstadt, Germany	<i>"Toward the pharmacological augmentation of radiotherapy"</i>
<b>P39</b>	<b>Muhammad Assad Riaz</b> Essen, Germany	<i>"Combined effects of metformin and cisplatin on radiation sensitivity in non-small cell lung cancer"</i>

<b>P40</b>	<b>Diana Savu</b> <i>Bucharest-Magurele, Romania</i>	<i>"Cellular differentiation exacerbates UV-radiation sensitivity in vitro in a human dopaminergic neuronal model"</i>
<b>P41</b>	<b>Johannes Schulte-Pelkum</b> <i>Izmir, Turkey</i>	<i>"AKLIDES® cell damage: development of an automated cell-based system for the quantitation of 8-OHdG damage"</i>
<b>P42</b>	<b>Noemí Serra</b> <i>Reus, Spain</i>	<i>"Exposure to low-doses of ionizing radiation and mercury during neonatal development modifies the liver and kidney function in mice"</i>
<b>P43</b>	<b>Efe Cumhur Sezgin</b> <i>Tübingen, Germany</i>	<i>"Antagonizing the CXCR4 chemokine receptor by AMD070 radiosensitizes HPV-negative human head and neck squamous cell carcinoma (HNSCC) lines"</i>
<b>P44</b>	<b>Maria Souli</b> <i>Athens, Greece</i>	<i>"Applying Broadband Dielectric Relaxation Spectroscopy (DRS) for the biophysical analysis of mammalian tissues under a variety of environmental stresses"</i>
<b>P45</b>	<b>Marcus Unverricht-Yeboah</b> <i>Jülich, Germany</i>	<i>"Comet Assay analysis of DNA strand breaks in human cells after exposure to the DNA-incorporated Auger Electron Emitter Iodine-125"</i>
<b>P46</b>	<b>Arjan van Dijk</b> <i>Bilthoven, The Netherlands</i>	<i>"Reverse engineering the historical trend in risky UV-exposure from trends in cancer"</i>
<b>P47</b>	<b>Tatjana Vatter</b> <i>Tübingen, Germany</i>	<i>"Methadone interferes with the stress response of irradiated glioblastoma cells"</i>
<b>P48</b>	<b>Aurelie Vaurijoux</b> <i>Fontenay-aux-Roses, France</i>	<i>"Study of DNA damage signaling and its fate induced by low and high energy X-rays"</i>
<b>P49</b>	<b>Mohd Yasser</b> <i>Essen, Germany</i>	<i>"Prompt DNA double strand breaks (pDSBs) yield and acquired epithelial to mesenchymal transition (EMT): A potential association in determining DNA damage response"</i>

# A standardization method to compensate for cell-density dependent radioresistance in colony formation assays

**Gabriel Adrian** and C. Ceberg

*Lund University, Skane University Hospital, Department of Clinical Sciences Lund, Oncology and Pathology, Lund, Sweden*

**Background** We recently investigated a rescue-like effect in colony formation assays (CFA), causing a gradual radioresistance with increasing cell density (submitted article). In CFA the cell density varies with radiation dose, and thereby, signaling mediated effects may compromise the validity of the measured outcome. The induced radioresistance was more prominent at higher doses, and at 12 Gy a 3.7-fold increase in surviving fraction (SF) was found. This cell-density dependent radioresistance raise problems for in vitro determinations of radiation response. **Methods** Based on the CFA outcome for two cell lines (melanoma MM576 and prostate cancer DU-145) with varying cell densities and irradiation doses, we created a standardization method to compensate for the unavoidable cell density dependent radioresistance. A simple mathematical procedure enabled the estimation of a "50-colony-CFA". Briefly, the method approximates a linear relationship of the effect for each dose, and calculates the number of seeded cells needed to achieve 50 colonies. **Results** With the standardized "50-colony-CFA" the diverging SF-results could be stabilized. In the original data for DU-145, D0 and Dq-values varied between 2.4-3.4 Gy and 1.8-2.4 Gy, respectively. A normalized "50-colony-CFA" yielded D0 = 2.6 Gy and Dq = 1.8 Gy. **Conclusion** CFA comprises signaling mediated effects influencing the radiation response. To account for these unavoidable effects, and to enhance the validity of in-vitro experiments, a simple mathematical standardization method is introduced. Hereby, the variation in CFA-outcomes due to cell density dependent radioresistance can be adjusted for.

# Investigating Parp-inhibition as an adjunct treatment to enhance cervical cancer thermo-sensitivity to radiochemotherapy

**Vidhula Ahire**, Oei, R. Ten Cate, H. M. Rodermond, H. P. Kok , J. Crezee and N. A. P. Franken

*Laboratory for Experimental Oncology and Radiobiology (LEXOR), Center for Experimental Molecular Medicine; Department of Radiotherapy, Academic Medical Center (AMC), Amsterdam, The Netherlands*

Introduction: DNA double strand breaks (DSBs) are a cytotoxic type of DNA lesion caused by ionizing radiation and chemotherapeutic drugs. The crucial pathways for repairing such lethal damages are the homologous recombination (HR) and non-homologous end joining (NHEJ) repair pathway which involves proteins like BRCA2, Rad51 and Ku70 , DNA-PKcs proteins respectively. Thermo-therapy interferes with the HR pathway by deactivating BRCA2. Cisplatin (cDDP) works by interrupting the NHEJ pathway. And, adding a Parp-inhibitors(Parp-i) , blocks the activity of Parp1 , which is an essential protein in the NHEJ, back-up NHEJ and the single strand break repair mechanisms. The purpose of this study is to enhance the thermal sensitivity of therapy resistant cervical cancer cells with the support of Parp-i, which would consequently augment their response to radio- and / or chemotherapy in clinics. Methods: Cervical cancer cells were treated with Parp-i, thermo-therapy was given at 42°C, with radiation doses (0-8Gy) and cDDP. Cell reproductive death was determined by the clonogenic assay. Comet assay was performed to measure the DNA damage, and the DNA double strand breaks were analysed by the  $\gamma$ H2AX staining. To interpret the mechanisms of action expression levels of different DNA repair and apoptotic proteins were investigated by the western blotting. Results: The combinatorial treatment of Parp-i and thermo-therapy increased the efficacy of radio and chemotherapy as compared to thermo-radiotherapy and thermo-chemotherapy. It significantly decreased the reproductive cell death, increased DNA damage and  $\gamma$ H2AX phosphorylation which was retained even after 24hr as observed in the comet assay and  $\gamma$ H2AX staining. Western blotting revealed in the understanding the mechanism of action of Parp-i induced thermo-sensitivity. Conclusion: Thermal sensitivity of cervical cancer to radiotherapy and chemotherapy is significantly enhanced by Parp-i incorporation.

## References:

- [1] Arlene L. Oei, CM van Leeuwen , Vidhula Ahire, Rosemarie ten Cate, Lukas J.A. Stalpers, Johannes Crezee, H. Petra Kok and Nicolaas A.P. Franken. Oncotarget 2017.
- [2] Arlene L. Oei, Vidhula Ahire, Nicolaas A.P. Franken et.al. (Accepted April 2017, IJH)

**Financial support :** The Dutch Cancer Foundation (UVA 2008-4019 and UVA 2012-5540)

## "ATM- dependent induction of PRNP protects neuronal cells against Ionizing Radiation"

J. Bernardino-Sgherri, C. Dehen, F. Auvré, D. Busso , G. El Masri<sup>1</sup>, A. Lioustko, O. Etienne, F. Boussin, J. P. Radicella and **Anne Bravard**

*CEA, Paris, France*

While the role of the pathological form of the prion protein (PrP<sup>Sc</sup>) is well documented, the physiological function of the normal protein (PrP<sup>C</sup>) is still unclear. PrP<sup>C</sup> was shown to be expressed at high levels in tumors particularly resistant to chemo/radiotherapy suggesting that it could be involved in protecting cells against genotoxic agents. According to this hypothesis, recent work performed in our laboratory showed for the first time an unexpected role of PrP<sup>C</sup> in protecting neuronal cells from the toxic effect of the alkylating agent MMS through stimulation of the Base Excision Repair pathway (Bravard et al., NAR 2015; 43:904-16). The goal of the present study was to establish whether PrP<sup>C</sup> could also have a role in protecting neuronal cells from ionizing radiations and to elucidate the underlying mechanisms. We showed that down-regulation of PrP<sup>C</sup> results in the sensitization of mouse neuronal cells to ionizing radiation, both in vivo and in vitro. This feature was also observed in the human neuroblastoma cell line SH-SY5Y. In these cells, radiation exposure led to a rapid and dose-dependent increase in PrP<sup>C</sup> expression. Surprisingly, the transcriptional activation of the PRNP gene by IR appeared to be entirely dependent on the ATM kinase activity. Finally, by using luciferase assays with different constructs of the PRNP promoter, we were able to identify an AP1 consensus sequence as responsible for the activation of PRNP after irradiation. Taken together, these results unveil a new role for the prion protein as a key player in the DNA damage response pathway activated by ATM to protect cells from ionizing radiation induced toxicity and point to PrP<sup>C</sup> as a potential target to limit tumor resistance to radiotherapy.

# Dimensionality reduction of clonogenic survival data to systematically identify molecular targets for radiosensitization in breast cancer

**Nikko Brix**, R. Hennel, C. Belka and K. Lauber

*Department of Radiation Oncology, Klinikum der Universität München, Germany*

**Introduction:** With approximately 70,000 new cases per year in Germany, breast cancer is the most common malignancy in women. Together with surgery and chemotherapy, the majority of patients is undergoing radiotherapy. While stratification by clinicopathological parameters – such as hormone receptor and Her2 expression – is part of the clinical routine, biomarkers for tumor radioresistance and targets for radiosensitization are currently not available. The colony formation assay represents a versatile tool to analyze cellular radiosensitivity in vitro making it indispensable for the identification of factors involved in tumor cell radioresistance. As an alternative to the linear-quadratic model, we propose a novel approach of dimensionality reduction to fully exploit the information obtained from clonogenic survival assays which allows, for instance, correlation with gene expression data. **Methods:** Clonogenic survival of 13 breast cancer cell lines (BCCs) and normal human mammary epithelial cells (HMECS) upon irradiation with 0-6 Gy was analyzed in colony formation assays. The data derived thereof were subjected to linear-quadratic fitting and principal component analysis (PCA) to extract scores of radioresistance for each cell line. Next, mRNA expression levels of more than 60 DNA damage response (DDR) regulators were measured by qRT-PCR. In order to identify predictors of radioresistance and potential targets for radiosensitization, mRNA expression levels were correlated with the PCA-derived radioresistance scores. **Results:** Among the 14 cell lines analyzed, strong differences in clonogenic survival were observed. Using the linear-quadratic model, very high goodness-of-fit levels were obtained ( $R^2=0.957-0.999$ ). However, obvious differences in radiosensitivity between several cell lines were not revealed by the respective  $\alpha/\beta$  values which failed to reflect the overall steepness of survival curves. Data reduction by PCA allowed the extraction of radioresistance scores. Notably, more than 60% of the variance in the dataset was covered by the first PC which was nearly equally loaded by all measured variables (SF1, 2, 4, and 6). Correlation of radioresistance scores with mRNA expression levels of DDR regulators identified potential predictors of radioresistance. Target validation using RNA interference and selection of suitable pharmacological inhibitors are ongoing. **Conclusion:** Dimensionality reduction by PCA is a suitable method to extract scores of radioresistance from clonogenic survival datasets which can be correlated with other types of data, such as mRNA expression levels. This approach facilitates the identification of DDR regulators which may be further validated as potential biomarkers of radioresistance and/or targets for radiosensitization.



# Genome instability and DNA repair capacity in radiation sensitive children

**Martin Bucher**, U. Roessler, U. Oestreicher, U. Kulka, D. Samaga, D. Endesfelder, G. Dückers, P. Lankisch, A. Borkhardt, H.J. Kirlum, CE. Rübe, E. Meese, S. Hornhardt and M. Gomolka

*Department of Radiation Protection and Health, Federal Office for Radiation Protection, Neuherberg, Germany*

Aim of the project is to identify radiation-sensitive AT patients by means of the radiation induced foci assay and two established cytogenetic approaches: the highly sensitive mFISH technique and dicentric assay. We want to investigate how reliable the single or combined biological endpoint detects the individual radiation sensitivity. For this purpose we used Ataxia telangiectasia (AT) patients as a proper sample group to define the radiation reaction. AT is an autosomal recessive multiorgan disorder based on a mutated ATM gene. Besides neurodegeneration and immunodeficiency AT causes chromosomal instability, cancer predisposition and abnormal sensitivity to ionizing radiation. The ATM gene product plays an important role in signalling of DNA double-strand breaks (DSB) and phosphorylates many proteins involved in DNA-repair, like the highly conserved histone variant H2AX. However, an incorrect ATM protein leads to defects in the DNA damage response, unresolved DSB and chromosomal aberrations as a marker for genomic instability. DNA repair capacity and chromosomal aberrations were analysed in a collective of 8 young AT patients (from 2-18 years of age) with confirmed ATM mutation or clinical phenotype in comparison to a control group of 10 healthy children (2-19 years of age). After 1 Gy in vitro  $\gamma$ -irradiation of isolated peripheral lymphocytes endogenous, induced DNA damage and residual foci were detected by the accumulation of radiation induced foci (RIF) of  $\gamma$ -H2AX. Repair kinetics and DNA repair capacity were evaluated. Total chromosomal aberrations were analysed by mFISH technique and dicentric aberrations by giemsa-staining. Performance of these techniques in respect to time exposure, effort and material input was compared. With both cytogenetic techniques we could detect the radiation sensitive phenotype of the AT patients compared to the control group. In contrast this group difference is not obvious in the  $\gamma$ -H2AX assay. Evaluation of RIF of  $\gamma$ -H2AX shows for AT patients on the one hand some individuals with high numbers of foci per cell and on the other hand some individuals with a much lower induction. In addition healthy individuals can repair induced DNA damage after in vitro irradiation pretty good. In contrast, AT children show poor DNA damage repair with individual differences. It seems to be that not all AT patients are able to phosphorylate H2AX properly after irradiation. In summary, genome instability as detected by the cytogenetic assays is a reliable general biomarker for radiation sensitivity. DNA damage induction and DNA repair as detected by H2AX phosphorylation seems to depend on the individual underlying mutation.

This project is supported by the Federal Ministry of Education and Research, Grant 02NUK035D and by EPI-CT EU-Grant FP7-EURATOM-FISSION 97571.

[mbucher@bfs.de](mailto:mbucher@bfs.de)



## The efficiency of irradiation of glioblastoma cells is enhanced synergistically by the combination of chloroquine and PARP-inhibitor

**Helmut Bühler**, D. Milanovic, B. Priesch-Grzeszkowiak, A. Kochanneck, P.Nguemgo-Kouam, T. Hero and I. A. Adamietz

*Klinik für Strahlentherapie und Radio-Onkologie, Ruhr-Universität Bochum, Germany*

Background: In 2006 Sotelo et al. showed in a small clinical study that the OS of glioblastoma patients was significantly enhanced by the malaria drug chloroquine (chq), when given in addition to standard therapies. For cultured glioma cells in vitro we could show that this chq-effect is probably due to an increase in radio sensitivity of the cells. In the present study we investigated if the addition of a PARP inhibitor might increase this chq-effect further. Methods: Chq and/or PARP-inhibitor ABT-888 in doses of 0 or 10  $\mu$ M were added to U343 glioblastoma cells prior to irradiation with 2 Gy photons (5Gy/min). Intracellular ROS were analyzed by fluorescence using the indicator carboxy-dichloro-fluorescein-acetate. DS-breaks were visualized by staining of gamma-H2AX-foci. Clonogenic survival of the cells was tested via colony forming and proliferation with a MTT-test. Results: Intracellular ROS were enhanced by 26 % and DS-breaks by 19 % after the addition of chq. The colony formation was reduced to  $62.5 \pm 4.6$  % by chq and to  $53.6 \pm 1.2$  % by ABT-888. The combination of both resulted in a remaining clonogenic survival of only  $16.1 \pm 0.7$  % compared to the untreated controls. The inhibiting effect on proliferation was much lower with a remaining  $69 \pm 3.0$  % after a combined treatment with both agents. Conclusions: Our data show that chq is a potent radiosensitizer and that its efficiency can be enhanced by inhibiting the DS repair with a PARP-inhibitor. The much lower effect on proliferation indicates that cytotoxicity might be a minor reason for the observed effects of the compounds.

# Coexisting factors influence on Low and High Dose of Iodine-131 Impact on Individual Susceptibility to Ionising Radiation and Biomarkers of Health Risks

**Antonina Cebulska-Wasilewska**, M. Krzysiek, G. Krajewska and P. Krajewski

*Central Laboratory for Radiological Protection, Warsaw, Poland; Institute of Nuclear Physics, Polish Academy of Sciences, Krakow, Poland*

Iodine-131 is often used in thyroid diagnostics and therapy. External and internal exposure to radioiodine can also lead to molecular and cellular damage in peripheral blood lymphocytes. Aim of this study was to explore influence of coexisting factors on low and high doses of Iodine - 131 impact to variability between individual radiosensitivities of various subjects. Reported earlier<sup>1</sup> study groups consisted of 30 individuals free of thyroid diseases, 41 patients exposed diagnostically to low doses of I-131 and 37 hyperthyroidism patients exposed therapeutically to high doses. The standardized DNA repair competence assay was used to test efficacy of the fast DNA repair process in G0 cells. On cellular levels, cytogenetic preparations were made in fresh blood samples before and after challenging cells in vitro with high X-rays dose. Frequency of sister chromatid exchanges (SCE) and percentage of cells with significantly elevated numbers of SCE (HFC), were used as cytogenetic biomarkers associated to homologous recombination (HR) and compared to radiosensitivity evaluated from cytogenetic biomarkers of cancer risk (unstable chromosome aberrations and micronucleus frequency in binucleated cells). 65 samples of isolated lymphocytes were also chosen, randomly from the basic groups under the study, for XRCC polymorphisms tests. Strong variability between individuals is observed in all investigated groups in biomarkers detected in lymphocytes, both, before and after challenging in vitro with high X-ray dose. Nevertheless, in the group of patients exposed to diagnostic (low) I-131 doses, the efficiency of post challenging fast repair is significantly higher than in unexposed control group, and linked to notably decreased cytogenetic damage. However, five weeks after administration of therapeutic (high) Iodine-131 doses, significant increases are observed of post challenging not repaired damage; DNA and cytogenetic. Obtained results show slight dependence on gender and family predisposition to cancer, and significant dependence on polymorphism in XRCC1(399), XRCC1(399), XRCC13(241) genes. While enlarging polymorphism study might be useful, though, model of short-term biomarker battery is proposed, applicable for triage and prediction of health risk from any IR exposure.

1.Cebulska-Wasilewska A., et al, (2017) Retrospective biological dosimetry at low and high doses of radiation and radioiodine impact on individual susceptibility to ionizing radiation. *Genome Integrity*, Vol.8.1, pp. 2-12.

## Distinct microRNA expression patterns in sun-exposed, sun-protected human skin and SCC tumor tissue

I-Peng Chen, S. Henning, B. Volkmer and R. Grinert

*Mol Cell Bio, Derma, Hospital Buxtehude, Germany*

UV irradiation, a human carcinogen, is the main risk factor for the induction of skin cancers especially for the squamous cell carcinoma (SCC). With yearly 87 000 new cases SCC is the second most frequently occurring cancer in Germany. Though most of the SCC cases are treatable, for about 2% of the SCCs there is a bad prognosis due to metastasis. UV-induced genetic changes in SCC have been well documented in the past years and recent studies have in addition demonstrated the significance of epigenetic modifications for the pathogenesis of SCC. The regulation of SCC through microRNAs is, however, still insufficiently investigated. Particularly, the role of miRNAs in the transition from the excessively sun-exposed skin to SCC in-situ and further to metastasized SCC is far from being understood. Using „Firefly Particle Technology“, a flowcytometry-based quantification method (Abcam) we have determined the expression of 23 cancer related miRNAs in sun-exposed, sun-protected skins and in SCC tumor tissues. The SCC tumor samples showed distinct miRNA expression profile from that of sun-protected and sun-exposed skins. When compared to the sun-protected skins 14 miRNAs are differentially expressed in SCC. Three of them are up-regulated (miR-21, miR-31 and miR-155) and 11 are down-regulated (e.g. miR-23a, miR-23b, miR-29c, miR-101, miR-203a). Due to their cancer supporting/promoting ability miR-21 and miR-155 have been well accepted as onco-miRs and are found to be highly upregulated in SCC. miR-203a is a skin differentiation marker which expression counteracts the cancer development. This miRNA showed a more than 10-times reduction in SCC tissue. Sun-exposed skin samples, however, did not show a distinct miRNA expression profile when compared to sun-protected skin. Interestingly some of the identified miRNAs (miR-23a, miR-23b, miR-29c) overlapped with the UV-responsive miRNAs that have been detected in our group, previously. Global DNA hypomethylation, an important epigenetic characteristic for cancers, could also be detected in SCC tumors. The reduction of the genome-wide DNA methylation seemed to be correlated with the transcriptional repression of the DNA methyltransferase 1, DNMT1. This might suggest a critical role of DNMT1 in the pathogenesis of SCC. We further determined these epigenetic alterations in 7 SCC-derived cell lines which are often used in SCC studies. Surprisingly only a small overlap of the alterations between the cell lines and the SCC tumor tissues was detected. This discrepancy might reflect the different nature of the systems (more artificial versus more native). The responsiveness of the identified miRNAs/methylation changes to UV/sunlight and a possible epigenetic regulation of SCC upon UV will be discussed.

# DNA damage and repair after a combined exposure to alpha particles and X-rays

Lei Cheng, A. Sollazzo, L. Lundholm, B. Brozowska, S. Haghdoust and A. Wojcik

*Stockholm University, Sweden*

Under many situations, people are exposed to mixture of high linear energy transfer (LET) and low LET ionizing radiation (IR). To estimate the biological effect induced by combined IR is important for human radiation protection. Previous studies showed both additive and synergistic effect of combined IR of different LET and the outcome seemed to be dependent on the experiment setup. Earlier studies from our lab indicated a synergistic effect in cells exposed to mixed beams of alpha particles (high LET) and X-rays (low LET). But the mechanism of synergism is unclear until now. The purpose of our present research is to analyze the effect of combined action of alpha particles and X-rays on DNA damage and repair, and DNA repair response protein activation, in order to more closely investigate the mechanism of the observed synergism. A dedicated mixed-beam exposure facility used in the experiments is installed and characterized at the Stockholm University, which allows simultaneously exposure of cells to <sup>241</sup>Am alpha particles and X-rays under controlled temperature conditions. Experiments were done with human peripheral blood mononuclear cells (PBMC), where we previously saw synergism at the level of cytogenetic damage. Initial level of DNA damage and the kinetics of DNA repair were analyzed at the level of individual cells with standard alkaline comet assay, which can measure the total DNA damage of single strand breaks (SSBs), double strand breaks (DSBs), incomplete excision repair sites, cross links and alkali labile sites. The activation of proteins involved in the DNA repair after exposure, including phosphorylated p53 (S15), pATM (S1981), and pDNA-PKcs (S2056), were tested by western blot. Both the initial level of DNA damage and DNA repair kinetics indicated a synergistic effect of mixed beams detected by comet assay. After 1 h incubation following 2 Gy of different exposures (X-ray, alpha particles and mixed beams), mixed beam caused the highest expression of phosphorylated p53 (S15) compared to X-rays ( $p < 0.01$ ) and alpha particles ( $p < 0.05$ ) and also highest expression of pATM (S1981) compared to X-rays ( $p < 0.05$ ) and alpha particles ( $p < 0.07$ ), which indicate a synergistic effect. But the expression of pDNA-PKcs (S2056) showed an additive effect. At 3h post-radiation, all of the three phosphorylated proteins showed a synergistic effect of mixed beams, although the increasing level of pATM induced by mixed beams was not significantly higher than X-ray and alpha particles. The level of pKu70 (S5) 3h post-radiation was tested by western blot too. With a high expression level in control, the level of pKu70 showed gradient increase after exposure of 2 Gy x-ray, alpha particles and mixed beams respectively. The preliminary study in DNA damage response gene expression also showed a trend of an increased expression induced by mixed beams compared to single X-ray or alpha particle exposure. Mixed beams might induce not only more initial total DNA damage, but also higher level of damage complex, compared to single type of radiation, in order to trigger stronger

## Radio-sensitization of chondrosarcoma cells by PARP-inhibitors

U. Ghosh, JB. Austry, FP. Cammarata, P. Pisciotta , GAP. Cirrone, P. Lesueur, Y. Saintigny and **Francois Chevalier**

*Department of Biochemistry & Biophysics; University of Kalyani, India*

Chondrosarcoma is a malignant tumor arising from cartilaginous tissue and presenting radio- and chemo- resistances to conventional treatments. The main treatment first consists to a surgical resection, which may conduct to severe disabilities for the patient; in addition, this procedure may not be possible for inoperable locations such as the skull base. Carbon-ion irradiation (hadron-therapy) is proposed as an alternative treatment, due notably to a higher biological effectiveness and a better ballistic as compared with conventional radiotherapy with X-Rays. This study aimed to examine the cellular responses of chondrosarcomas to conventional radiotherapy and hadrontherapy, associated with PARP-inhibitors in order to better understand the biological effects of combined treatments. Three human chondrosarcoma cell lines of different grades and displaying differential radio-sensitivities, were irradiated with photons, proton or carbon ions, in association with different PARP inhibitors. To better understand the PARP-inhibition process, we first analyzed Poly-ADP ribose chains formation using western blotting, and we observed a maximum of signal after irradiation with CH2879 cells. As attempted, clonogenic assays confirmed the better biological effectiveness of carbon ion over X-Rays; the resistance to radiation of each cell line was calculated from the corresponding survival curves. PARP-inhibitors increased radio-sensitivity, with a factor depending of the dose and irradiation quality (1). Apoptosis was increased following treatments, as observed by flow cytometry and western blotting experiments. This study demonstrates the capacity of PARP-inhibitors in radio-sensitizing chondrosarcoma cells, using conventional photon irradiation as well as using proton beam and carbon beam irradiation. 1. Lesueur, P., Chevalier, F., Austry, J.-B., Waissi, W., Burckel, H., Noël, G., Habrand, J.-L., Saintigny, Y., and Joly, F. (2017) Poly-(ADP-ribose)-polymerase inhibitors as radiosensitizers: a systematic review of pre-clinical and clinical human studies. *Oncotarget* 5,

# Hybrid gold nanoparticles coated with organic polymers and antibodies as a platform for cancer theranostics: Cytotoxicity assessment

**Noami Daems**, S. Li, O. Fichera, K. Van Hoecke, S. Baatout, T. Cardinaels, C. Michiels, S. Lucas and A. Aerts

*Radiobiology Unit, Institute for Environment, Health and Safety, SCK•CEN, Belgian Nuclear Research Centre, Mol, Belgium/ Unité de Recherche en Biologie Cellulaire (URBC)-NARILIS, University of Namur, Belgium*

In cancer radiotherapy, gold nanoparticles (AuNPs) have emerged as promising radiosensitizers, which accumulate in the tumor and are believed to increase the effectiveness of external beam radiotherapy by local production of reactive oxygen species (ROS) and secondary electrons upon irradiation. At UNamur, 5 nm gold nanoparticles coated with an organic shell of polyallylamine are produced by plasma vapour deposition (AuNPs@PPAA) (1). Optionally, the AuNPs@PPAA can be conjugated to anti-EGFR antibodies (Cetuximab) (mAb-AuNPs@PPAA) which actively target EGFR-overexpressing cancer cells in vitro and in vivo (2-3) and for which in vivo biodistribution studies have demonstrated a significant accumulation in the liver and the spleen. Therefore, the cytotoxicity profile of the gold nanoparticles should be investigated properly in healthy cell types prior to use the mAb-AuNPs@PPAA in clinical applications. Human kidney (HK-2) cells and telomerase-immortalized microvascular endothelial (TIME) cells were studied as first examples of healthy cells. We performed MTS tetrazolium cytotoxicity assays after 3 hours of incubation and live cell imaging with apoptotic markers Annexin V and caspase 3/7 during 48 hours of incubation with AuNPs@PPAA. The nanoparticle concentrations tested ranged from 8  $\mu$ M to 254  $\mu$ M of gold. The MTS cytotoxicity assay resulted in a significant dose-dependent reduction of cell viability in TIME cells. In contrast, an increased tetrazolium-to-formazan conversion was seen in HK-2 cells, which could represent an enhanced enzymatic activity as a response to AuNPs@PPAA. During live cell imaging, a concentration of 254  $\mu$ M of gold resulted in an increase of Annexin V and caspase 3/7 signaling after 10 and 8h in HK2 and TIME cells, respectively. Lower concentrations, even down to 8  $\mu$ M, also lead to apoptosis in TIME cells after longer incubation times. In the future, we will assess the cellular AuNPs@PPAA uptake by means of ICP-MS and TEM. Furthermore, ELISA and western blot can identify which underlying pathways may be modulated by the AuNPs@PPAA. Oxidative stress induced by exposure to AuNPs@PPAA will be evaluated by means of ROS measurements. Finally, the toxicity evaluation will be completed with studies testing a liver cell line and we will investigate if the AuNPs@PPAA are able to activate splenic macrophages. Experiments will be repeated with mAb-AuNPs@PPAA.

**Acknowledgements:** N Daems is supported with a 2-years FRIA PhD grant of F.R.S-FNRS (National Fund for Scientific Research, Belgium).

## References:

- (1) Moreau N, et al. Plasma Process Polym. 2009;6(1):S888-S892
- (2) Marega R, et al. J Mater Chem. 2012;22(39):21305-21312
- (3) Karmani L, et al. Contrast Media Mol Imaging. 2013;8(5):402-8

[noami.daems@sckcen.be](mailto:noami.daems@sckcen.be)

## Radiomodifying features of organometallic compounds

**Marie Davidková**, D. Reimitz, O. Mestek, J. Pinkas and J. Kočíšek

*Department of Radiation Dosimetry, Nuclear Physics Institute of the CAS , Prague, Czech Republic*

Experimental study is exploring combined effect of [Ru( $\eta^6$ -p-cymene)Cl<sub>2</sub>(1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decanephosphine)] (RAPTA C) or [cis-diamminedichloridoplatinum(II)] (CDDP) and ionizing radiation on initial DNA damage. DNA plasmids pBR322 were incubated in water solution with studied organometallic compounds. The samples were then irradiated by Co-60 gamma radiation and yields of single and double DNA strand breaks were determined using agarose gel electrophoresis. It has been previously proposed that the synergic effect of organometallic compounds and radiation is based on the interaction of secondary low energy electrons with DNA adducts. To distinguish the contribution of OH radicals or electrons to DNA-adduct damage induction, analogue experiments in presence of different concentrations of OH radical scavenger tris(hydroxymethyl)aminomethane (tris) were performed. When irradiating plasmid DNA in solution containing free organometallic molecules, no combined effect was observed, indicating that the contribution to DNA damage caused by products of radiolysis of RAPTA C or CDDP is negligible in comparison to the damage caused to DNA by products of water radiolysis. After binding to DNA, CDDP adducts with DNA strongly enhance the damage in a good agreement with the results of previous studies. RAPTA C adducts act radio-protective at low doses of OH radical scavenger tris and show no combined effects at higher tris levels. Radioprotectivity of RAPTA C is therefore primarily caused by enhanced resistance of RAPTA C modified DNA against the damage induced by radicals. Further studies with model Pt and Ti compounds are in progress to better elucidate the synergic effects on the molecular level. Acknowledgement:

The work was supported by the Czech Science Foundation (Grants No. 16-10995Y and P108/12/G108).



# Synemin is a novel co-regulator of the radiation-induced DNA damage response in head and neck cancer cells

**Sara Sofia Deville**, S. Förster and N. Cordes

*OncoRay – National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany and Helmholtz-Zentrum Dresden - Rossendorf, Dresden, Germany*

Introduction: Focal adhesion proteins (FAPs) have been shown to essentially contribute to cancer cell therapy resistance. Based on our previous finding that integrins partially control DNA repair processes, we here aim at characterizing the function of FAPs in the DNA damage response. Among others, we identified Synemin, an intermediate filament protein, as novel DNA repair regulator and highly potential novel cancer target in head and neck squamous cell carcinoma (HNSCC). Methods and materials: A novel 3D High Throughput esiRNA Screen (3DHTesiRNAs) using (3D)-laminin-rich extracellular matrix (Ir-ECM) was established. Screening for residual double strand breaks (DSBs) and clonogenic radiation survival was performed in UTSCC15-pEGFP-53BP1 HNSCC cells upon esiRNA-mediated FAPs knockdown and X-ray exposure (6 Gy). The top 2 targets were validated in a panel of 10 3D Ir-ECM HNSCC cell cultures regarding  $\gamma$ H2AX/53BP1 foci and clonogenic survival. Immunostaining and 3D chromatin fractionation (CF) of Synemin prior and post irradiation (IR) were performed. Upon Synemin knockdown, DNA repair assay for NHEJ and HR as well as Western Blotting for protein expression and phosphorylation were employed. Results: Among a number of interesting novel targets found in our 3DHTesiRNAs, Synemin turned out as novel determinant of HNSCC radiosensitivity. Synemin silencing led to radiosensitization of 3D HNSCC cell cultures. Intriguingly, we showed that Synemin knockdown resulted in a 40% reduction in NHEJ without affecting HR. Concomitantly, phosphorylation of ATM Ser1981, DNA-PKcs Ser2056 and c-Abl Tyr412 were diminished relative to controls. Associated with these observations, we found a dramatic Synemin accumulation in the perinuclear area, which is accompanied by an increased interaction of Synemin with chromatin. Conclusion: Our data indicate the interfilament protein Synemin as a new important determinant of DNA repair and radioresistance in HNSCC cells. Ongoing research is focusing on evaluating the molecular mechanism how Synemin participates in NHEJ and chromatin organization.



## Proteomic profiling of ECM colorectal cancer after irradiation

**Angela Diana**, A. Yuzhalin and R. Muschel

*University of Oxford, Oxford, United Kingdom*

Radiotherapy plays a central part in curing cancer. For decades, most research on improving treatment outcomes has focused on modulating radiation-induced biological effects on cancer cells. Recently, has been better understood that components within the tumour microenvironment have pivotal roles in determining treatment outcomes. However, it is not well understood what features of the ECM proteome ( called matrisome ) contribute to this response. Here we describe a method for extracting the ECM from a colorectal cancer animal model using decellularisation and biochemical enrichment. We analysed resulting extracellular matrices by quantitative label free biochemical enrichment and identified a number of potential ECM signatures involved in irradiation response in colorectal cancer.

# Nuclear EGFR Regulates Stability of VEGF Signalling-Associated mRNAs in Response to Radiation

**Klaus Dittmann**, Mayer, S. Czemm, S. Huber and H. P. Rodemann

*Division of Radiobiology and Molecular Environmental Research, University of Tuebingen, Germany*

Cell membrane-associated EGFR is activated and translocated into a perinuclear/nuclear localization in response to exposure to stress or EGF treatment and is subsequently found in complex with mRNAs. Hypoxia, treatment with cisplatin or EGF, increased mRNA amount within this complex, whereas radiation failed to induce a quantitative change. Gene array analysis identified approximately 3000 different mRNA species enriched or reduced at nuclear EGFR compared to control IgG. Functional annotation indicated that under the top scoring KEGG-pathways was, "VEGF/HIF-1 signalling pathway". RT-PCR of mRNAs involved in VEGF/HIF-1 signalling showed a radiation-induced increase in mRNA expression for 52 mRNA species out of 84 in response to irradiation. For 43 of these 52 mRNAs (80%), a reduced amount of mRNA was observed in complex with nEGFR. This suggests, that binding to nEGFR correlates to mRNA destabilisation. Furthermore, radiation induced increase in VEGF-signalling associated mRNAs is abolished in presence of the EGFR inhibitor Erlotinib. To further elucidate a role of nEGFR in RNA-destabilisation, we performed a nEGFR IP and tested for presence of the proteins GW182, AGO2, PABPC1 and cNOT1, all constituents of the cNOT-deadenylase complex. We identified all four proteins in complex with nuclear EGFR. Blockage of EGFR kinase by Erlotinib reduced AGO2 phosphorylation at residue Y393 and increased cNOT deadenylase activity. This observation suggests that EGFR and AGO2 can act as negative regulators of cNOT activity. Consequently, we observed an increased expression of mRNAs associated with VEGF/HIF-1 signalling, when these mRNAs are released from complex with nEGFR. Since specificity of mRNA-deadenylation by cNOT is due to miRNAs associated guidance, we characterized miRNAs in complex with nEGFR. We identified several thousands of different miRNA species. To further elucidate the role of miRNAs and EGFR kinase activity in this context, we selected the miRNA Hsa-mir-1180p5, which targets the VEGF/HIF-1 signalling associated mRNA coding for NFATC4. We selected this miRNA, since NFATC4 mRNA is increased in response to irradiation and Hsa-mir-1180p5 is the only miRNA identified in complex with nEGFR, which targets NFATC4 mRNA. We transfected cells with an inhibitor of Hsa-mir-1180p5 or pre-treated cells with Erlotinib. Indeed, Hsa-mir-1180p5 knockdown increased and Erlotinib treatment decreased NFATC4 protein expression. NFATC4 protein expression decreased cloning efficiency and increased radio-sensitivity of A549 and FaDu tumour cells. In summary, our data suggest a new role for EGFR in response to irradiation. The membrane associated tyrosine-kinase receptor is activated and translocated to a nuclear / perinuclear location in response to irradiation. In complex with cNOT deadenylase and AGO2 protein, EGFR-kinase activity regulates stability of mRNAs involved in VEGF/HIF-1 signalling by miRNA guided deadenylation. We suggest a two-step regulation, where in a first step cNOT deadenylase is directed to specific mRNAs guided by miRNA and in a second step NOT activity is controlled by AGO2 in a EGFR kinase dependent manner.

The work was supported by a grant of Deutsche Forschungsgemeinschaft [Di402/9-2]

[klaus.dittmann@uni-tuebingen.de](mailto:klaus.dittmann@uni-tuebingen.de)

## How immunogenic cell death induced by anticancer photodynamic therapy can enhance radiation-induced immune response

**Bastien Doix** and O. Feron

*Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Louvain-la-Neuve, Belgium*

Immunotherapy is making its way to the forefront of malignancy treatment. Yet current immunotherapies are mainly surfing on the checkpoint inhibitors hype, the effects of which being mostly due to the relief of immunosuppression at the tumor site. In parallel, the newly conceptualized immunogenic cell death (ICD) though, achieved with specific chemotherapy like mitoxantrone but also Photodynamic Therapy (PDT), describes cancer cell death with a direct activation of the immune system. Cancer cells dying in such an immunogenic fashion actually release molecules and proteins (the so-called DAMPs or damage associated molecular patterns) able to attract dendritic cells (DCs), prime them with tumor associated antigens and promote their maturation. These DCs can then trigger lymphocytes, especially Cytotoxic T Lymphocytes (CTL) to destroy tumor cells both at the primary and at metastatic sites. In this project, we aim to examine whether PDT could contribute to stimulate the anti-tumor innate and adaptive immune response associated with radiotherapy. Abscopal effects of radiotherapy (i.e., systemic response at distance from irradiated sites) indeed support the occurrence of radiation-induced enhancement of the host immune system (although this effect may not be sufficient per se to eradicate tumors). We have recently developed a new photosensitizer (termed OR141) to be used for anticancer PDT (Pinto et al., Oncogene 2016). OR141 preferentially accumulates in the ER compartment where it generates high concentration of singlet oxygen  $^1O_2$  upon light activation. Consecutive oxidative stress in the ER was shown to induce an Unfolded Protein Response (UPR) and to kill cancer cells. We now document that PDT-induced cell death is associated with the induction of DAMPs, including the translocation of calreticulin at the plasma membrane of dying cells (ecto-CRT) and to the release ATP and HMGB1 in the extracellular compartment. We also show that co-culture of bone marrow-derived dendritic cells (BM-DCs) with cancer cells killed by OR141-PDT leads to phagocytosis of these cells and to the maturation of the dendritic cells (identified as CD11c<sup>+</sup> MHCII<sup>+</sup> CD80<sup>+</sup> CD86<sup>+</sup> by flow cytometry) whereas co-culture with cancer cells dying in a non-immunogenic manner (i.e., freeze-thaw protocol) did not drive any maturation of said DCs. Finally, anticancer vaccination could be obtained by injecting mice with syngeneic tumor cells killed with OR141-PDT before challenging them in the opposite flank with live cancer cells. The effects of the combination of OR141 treatment with radiotherapy will be discussed and first results documenting PDT-driven stimulation of radiation-induced immune response will be presented.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement N° 642623

[bastien.doix@uclouvain.be](mailto:bastien.doix@uclouvain.be)

# Effect of inhibiting Mre11 on the radiosensitivity of NSCLC cell lines after concurrent treatment with cisplatin and ionising radiation

**Yasin Bahadir Erol**, A., Sak, MA. Riaz, M. Groeneberg, M. Melnikova, J. Thomale and M. Stuschke

*Strahlenklinik, Universitätsklinikum Essen, Germany*

**Purpose:** The standard therapy prescribed for advanced stage lung cancer is the high-dose radiotherapy with concurrent cisplatin-containing chemotherapy. However, one of the major clinical limitations in cancer chemotherapy is the development of cisplatin (CP) resistance which decreases its effectiveness in the treatments of patients with non-small cell lung cancer cell lines (NSCLC) and it is influenced by many factors such as DNA repair. **Methods:** Human NSCLC cell lines, more resistant to cisplatin (A549, H1299A) and more cisplatin sensitive (H460, H661) were used. Cells were treated with 10µg/ml cisplatin for 4h and exposed to IR (2Gy, 4Gy) and analysed the functional status of the different DSB repair pathways. In addition, the repair protein Mre11, one of the consisting proteins of MRN complex, was inhibited by small molecule inhibitor mirin, which prevents MRN-dependent activation of ATM and inhibits Mre11-associated exonuclease activity. The effect on the radiosensitisation of CP in combination with or without mirin treatment before ionization radiation (IR) was evaluated. Cell proliferation, clonogenic survival assay, cisplatin Pt-[GG] intrastrand adduct formation and radiation induced repair foci (γ-H2AX, 53BP1, Rad51) were used to characterize the effects of treatments. **Results:** Cisplatin treatment significantly reduced radiation induced γ-H2AX and Rad51 foci formation in H460 as well as in A549 cells. Inhibition of MRN complex with mirin significantly inhibited NSCLC cell proliferation in a concentration and time-dependent manner. The combination treatment of mirin/cisplatin and IR exhibited the highest antiproliferative effect compared to IR alone, cisplatin/IR or Mirin/IR. The concurrent use of Mirin/cisplatin and IR was also analysed by clonogenic survival. Cisplatin treatment resulted in a significant radiosensitization of H460 and H661 but not of A549 and H1299 cells. However, the combination of mirin with cisplatin further enhanced the sensitivity of NSCLC cells to IR. At molecular level, Mirin increased residual Pt-[GG] adduct formation in DNA 24 h after CP treatment by about 10% in H460 cells. **Conclusion:** The present data show that cisplatin can significantly inhibit signalling of radiation induced DSB, the mechanism of which mostly relies on perturbation of higher-order chromatin organization by cisplatin induced DNA intrastrand cross-links. Inhibition of Mre11 with mirin potentiates the effect of combined radiotherapy and CP-treatment in NSCLC cell lines.

## Effect of radiotherapy on circulating miRNAs-146a, -155, -221 and -222 levels in women with breast cancer

**Roser Esplugas**, N. Serra, M. Bellés, V. Linares, M. Arenas and JC. Vallvé

*Physiology Unit, School of Medicine, Rovira i Virgili University, Reus, Spain; Laboratory of Toxicology and Environmental Health, School of Medicine, Rovira i Virgili University, Reus, Spain*

Breast cancer is one of the most important neoplasia among women. Radiotherapy reduces the risk of local recurrence and the risk of breast cancer mortality. Unfortunately, one long-term side effect of this treatment is the development of cardiovascular disease several years later. Recent studies have determined that circulating microRNAs (miRNAs) are modulated in disease states. MiRNAs are a large class of evolutionarily conserved small non-coding RNAs that regulate gene expression at the post-transcriptional level by targeting the 3' untranslated region of mRNA transcripts; thus inhibiting mRNA translation. MiRNAs-146a, -155, -221 and -222 are involved in the progression of atherosclerosis and cardiovascular pathogenesis. Hence, the aim of our study was to evaluate the modulation of these miRNAs expression in response to radiotherapy treatment in women with breast cancer. Blood samples of 135 women with breast cancer were collected pre- and post-radiotherapy (3 months later). After miRNA isolation and Reverse Transcription, the levels of miRNAs-146a, -155, -221 and -222 were measured at these two time-points by real-time PCR. Delta Ct method was used in order to quantify miRNA expression, employing the miRNA-U6 as housekeeper. Then, paired t-test was performed for the statistical analysis. On one hand, the results exhibited a significantly positive correlation between miRNAs expression pre- and post-radiotherapy (Pearson correlation coefficient was above 0.89 for all bivariate correlations). Furthermore, miRNA-155 presented the lowest expression in plasma pre- and post-radiotherapy. The expression of miRNAs-146a, -221 and -222 significantly increased in both pre- (17%, 33% and 66%, respectively) and post-treatment (17%, 34% and 68%, respectively) when compared to miRNA-155. Moreover, it was shown that circulating levels of all selected miRNAs were enhanced post-radiotherapy. miRNAs-221 and -222 had the higher increase (26%,  $p=0.0015$  and 25%,  $p=0.0014$ ; respectively), whereas miRNA-146a rose 10% ( $p=0.0159$ ) and miRNA-155 augmented 5% without statistical significance ( $p=0.1100$ ). On the other hand, we showed significantly inverse correlation between pre-radiotherapy miRNAs expression and age. Finally, associations of all miRNA expression with dyslipidemia and cigarette smoking were observed. While dyslipidemic patients had significantly low levels of miRNA than non- dyslipidemic, smokers presented less levels of miRNA post-radiotherapy treatment than non-smokers. In conclusion, radiotherapy enhanced the expression of circulating miRNAs-146a, 155, 221 and 222 in women with breast cancer. This increase together with other risk factors might be associated with the development of future cardiovascular pathologies.

# Analysis of DNA repair pathways in rod photoreceptor cells of nocturnal and diurnal animals

**Florian Frohns**, A.Frohns and M. Löbrich

*University of Technology, Radiation Biology and DNA Repair, Darmstadt, Germany*

Therapeutic gene targeting applications, based on the selective induction of DNA double strand breaks (DSBs) in the mutated gene and its repair by the DNA repair pathway "homologous recombination" (HR) represent a new possibility for the cure of monogenic disorders. But since most cells of the body are postmitotic and DNA repair via HR is suppressed in those cells, the usage of those strategies seems to be restricted to the small population of cycling cells. Nevertheless, recent studies show that the suppression of HR in postmitotic / G1 cells is reversible by genetic manipulations, which probably enables therapeutic gene targeting in postmitotic tissues in the future. Thus, a better understanding of DNA damage signalling, DNA DSB repair pathways and their mutual suppression in specific tissues and cell types is essential to overcome the current hurdles for the establishment of this new therapeutic approach. In the past, mostly mice have been used to gain that knowledge. Many monogenic disorders have been described that lead to the degeneration of photoreceptor (PR) cells of the retina, which is associated with irreversible sight loss. Gene targeting applications in those cells have become a promising approach to cure these disorders. Therefore, studies of the DNA damage signalling in this cell type are highly relevant. However, for two reasons the mouse does not seem to represent an appropriate model system to analyze DNA damage signalling in PR cells: Due to their unique chromatin organization, which is probably due to their nocturnal lifestyle, the DNA damage response in PRs shows striking differences to that of other neuronal cell types. These differences include - amongst others - a unique repair defect of radiation induced DNA DSBs. Thus, it is unclear whether the findings in mice can be transferred into the PRs of humans, which are a diurnal species. Up to now, nothing is known about the DNA damage response and repair in PR cells of diurnal animals. In order to answer that question we compared the DNA damage response and repair capacities of PR cells in four different species including pigs and humans. Our results provide new insights into the repair defect of PRs in nocturnal animals, but also show severe differences in the accumulation of DNA repair related proteins at DSBs and DSB repair kinetics between diurnal and nocturnal PRs. Thus, in order to gain a better understanding of the DNA damage response in human PRs, diurnal animals - preferably the pig - have to be used as animal models instead of mice in the future. This will increase the chance to successfully develop gene targeting strategies in PR cells for the cure of monogenic blinding disorders.

## Nanocarrier delivery of the radiosensitizer MEK162 in brain tumour therapy

**Ana Gasol Garcia**, R. de Kruijff, A. Van der Meer, R. Narayan, P. Slangen, G. Torrelo Villa, B. Slotman, E. de Vries, A. Denkova and P. Sminia

*Department of Radiation Oncology, Vanderbilt University Medical Center, Amsterdam, The Netherlands*

**Purpose:** To investigate the use of polymeric nanocarriers (polymersomes) for localized delivery of radiosensitizing agents, in combination with radiotherapy, enabling the drug to cross the blood brain barrier to treat brain tumours. **Introduction:** Despite aggressive therapy, the survival of patients with a glioblastoma multiforme (GBM) is still poor. When combining radiotherapy with anti-cancer agents, we face with the problem of the blood brain barrier (BBB). Polymeric nanocarriers conjugated with a ligand (glutathione, GSH) hold potential to pass the BBB through receptor mediated endocytosis and therefore are an auspicious strategy for the delivery of drugs to brain tumours. In a previous study from our group, the MAPK inhibitor MEK162 (binimetinib) was identified as radiosensitizer in glioma spheroids in vitro and in an orthotopic brain tumour model in vivo. Here, we studied the efficacy of the loading of MEK162 in polymersomes (with and without GSH conjugation), their uptake in U87 glioma 3D spheroids and vascular endothelial cells and inhibition of key target proteins. **Materials and Methods:** In vitro model: human U87glioma spheroids. Treatments: Radiation (4 Gy / 5 x 2 Gy), MEK162 1 uM alone or loaded in (glutathione conjugated) polymersomes. Endpoints: Long-term spheroid growth assay. Western Blot as a readout of drug activity. BBB model: confocal microscopy to trace the uptake of fluorescent labelled polymersomes in human vascular endothelial cells. **Results:** Exposure of U87 spheroids to free MEK162 and MEK162 loaded in polymersomes resulted in a 13 days growth delay time (GDT) in MEK162 loaded nanocarriers and 18 days GDT in MEK162 relative to controls. Radiation alone resulted in a GDT of 6 day, which was further prolonged to 11 days by additional MEK162 and 10 days for MEK loaded nanocarriers. The data demonstrate that both free MEK162 and MEK162 delivered via polymersomes follow the same inhibition trend. Protein expression shows that p-ERK, the substrate of MEK, is not phosphorylated at 24 hours, neither after exposure to free MEK162 nor to MEK162 loaded polymersomes. New loading method (adding water to solvent) appeared to be able to encapsulate with a loading efficiency of 38% compared to the 8% of the previous method (adding solvent to a water solution). In the BBB in vitro model, we demonstrated differential cellular distribution in endothelial cells after uptake of non- and glutathione conjugated polymersomes probably hinting that these polymersomes are being uptaken by a different mechanism and possibly would undergo receptor mediated transcytosis. **Conclusion:** Our data show: (1) Efficient loading of MEK162 in polymersomes (2) Their uptake in human glioma cells (3) Inhibition of the target protein (p-ERK) (4) Differential uptake of conjugated polymersomes in endothelial cells. Taken together, the treatment strategy of the delivery of radiosensitizers via polymeric nanocarriers yields promising perspective in the treatment of GBM patients. **Supported by [www.STOPHersentumoren.nl](http://www.STOPHersentumoren.nl)**

[a.gasolgarcia@vumc.nl](mailto:a.gasolgarcia@vumc.nl)



# Targeting cellular antioxidant system to sensitize glioma stem cells to acute and chronic irradiation

**Paulo Godoy**, E. Sakamoto-Hojo and S. Haghdooost

*The Wenner-Gren Institute, Stockholm University, Sweden; Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo, Brazil*

Glioblastoma (GBM) is a malignant brain tumor with poor outcome and is very resistant to all types of treatments. Glioma stem cell (GSC) presents an enhanced antioxidant defense compared to their serum-differentiated counterparts and is resistant to chemo- and radiotherapy. We propose to analyze the expression of some important antioxidant proteins/expressed genes in undifferentiated and differentiated GSC exposed to chronic and acute doses of ionizing radiation. The highly expressed proteins in undifferentiated GSCs will be targeted by shRNA to increase the radiation effects. Several analyses will be performed, such as oxidative stress (8-oxo-dG detection and measurement of oxidized/reduced glutathione ratio), cell differentiation ability (stemness and differentiation markers), and self-renewal ability (sphere formation assay and clonogenic survival) on irradiated or sham-irradiated silenced GSCs or their differentiated counterparts. Our hypothesis is that reducing the antioxidant capacity of GSCs and increasing ROS production by chronic and acute low dose irradiation will reduce GSCs functionality by increasing differentiation and decreasing self-renewal ability. For the GSC culture, we use the U87MG GBM cell line cultured as neurospheres on neural stem cell medium, NSCM (DMEM F12, b27, EGF and FGF2), for three passages and then grow them in monolayer on collagen I coated flasks. Culturing GSC cells in differentiation media (DMEM F12 + 10% SBF) for 1 week resulted in differentiated cells. Initially, GSC was seeded in NSCM and after 1 week, the formed neurospheres were counted, dissociated and reseeded again. This process was repeated 2 more times during 3 weeks under chronic radiation (0, 1.4, 4.1, 12 and 24 mGy/h). In parallel, the cell proliferation was measured weekly by counting GSC (grown on collagen I flasks) and differentiated cells irradiated or sham-irradiated by using a cell counter, for 3 weeks. We observed a significant decrease in the number of neurospheres after week 3 at 4.1 and 12 mGy/h ( $p \leq 0.05$ ) and already after week 2 ( $p = 0.023$ ) for 24 mGy/h. We observed similar proliferation rates for exposed differentiated cells. We also compared the sensitivity to acute radiation of GSC and differentiated cells by analyzing neurospheres and clonogenic survival, respectively after 0.5, 2, 4 and 8 Gy. Both group of cells presented similar survival profiles with IC50 close to 4 Gy. When comparing the stemness of GSC and differentiated cells, we observed a significantly increase of MUSASHI-1 expression ( $p = 0.005$ ) on GSC (34%). After irradiation with 24 mGy/h for 1 week, the GSC showed 30% lower expression of MUSASHI-1, indicating a possible effect of chronic irradiation on differentiation of GSC. Considering the expression of hMTH1, we observed similar expression between the GSC and the differentiated ones and no significant differences after the irradiation in GSC. Possibly the overexpression of this protein even in the control made it hard to detect its modulation after radiation. Thus, the chronic irradiation induced differentiation and decreases neurospheres and proliferation in GSC, but it did not affect the hMTH1 expression. We expect that the present study will provide interesting results regarding glioma radiosensitization, and they may be used for discussing alternative therapies for patients with GBM, with a new approach based on decreased anti-oxidant defense combined to radiotherapy. Sensitizing tumor response to radiation and focusing radiation beams in the tumor (it is possible with modern techniques) may lead to decreasing radiation dose to healthy tissues avoiding acute as well as late effects of radiotherapy.

[paulo.godoy@su.se](mailto:paulo.godoy@su.se)



## Identification of novel tumour specific determinants of radiosensitivity using a CRISPR Whole Genome screen

**Giovanna Granata**, D. Jones, R. Prevo, D. Ebner, G. McKenna and G. Higgins

*Oxford Institute for Radiation Oncology, Oxford, UK*

Radiotherapy plays an important role in the curative management of cancer. Despite technical developments in treatment delivery, it is still associated with potentially severe side effects due to damage caused to normal tissues surrounding the tumour. One approach to reduce side effects is to increase tumour radiosensitivity without affecting the sensitivity of normal tissue to radiation. The aim of this work is to identify genes whose loss causes tumour specific radiosensitivity of lung cancer, which represents one of the most common and serious types of cancer. We therefore designed a genome wide screen in both a non-small-cell lung cancer cell line (A549) as well as a normal fibroblast cell line (MRC-5) aimed at identifying new clinically exploitable targets. To achieve genome-wide knockout of individual genes, we employed the CRISPR-Cas9 system, which represents a convenient and flexible tool for genome editing. Due to its versatility, this system can be exploited to perform high-throughput screens, allowing us to investigate thousands of genes in the context of the radiation response. The poster summarises the strategy adopted to screen both cell lines. Using two single guide RNA (sgRNA) libraries, we individually depleted a total of 19,050 genes and 1,864 genes encoding miRNAs.

## Cancer radiosensitivity under metabolic control

**Debora Grasso**, P. Danhier, V. Bol, V. Gragoire and P. Sonveaux

*Pole of Pharmacology, Institute of Experimental and Clinical Research (IREC), University of Louvain (UCL) Medical School, Brussels, Belgium*

A growing body of evidence indicates that mitochondria within cancer cells actively participate in tumor progression and treatment resistance. Ionizing radiation primarily induces ROS-dependent DNA damage as a consequence of water radiolysis. Mitochondria are well-known to play a central role in cellular responses to redox stress through critical functions that include ATP production fueling DNA repair pathways, apoptosis regulation and tunable mtROS production in dose ranges that can confer cytoprotection (low doses) or cytotoxicity (higher doses). Moreover, alterations of mitochondrial metabolism that decrease the mitochondrial membrane potential may lead to a redox imbalance capable of enhancing irradiation-induced DNA damage, chromosomal instability, and apoptosis. Our aim in this study was to investigate whether mitochondrial metabolism could modulate the intrinsic radiosensitivity of human cancer cell. For the fundamental proof-of-principle demonstration, we used human head-and-neck SQD9 squamous cell carcinoma cells, a type of cancer for which radiotherapy is a main treatment modality. We first generated radioresistant SQD9 cells by treating them chronically for up to 2 weeks with daily 2 Gy doses of  $\gamma$ -rays. Radioresistance was confirmed with clonogenic assays. Comparative metabolic characterization revealed that radioresistant SQD9 cells (SQD9-res) were more oxidative than wild-type cells (SQD9-wt), as SQD9-res had increased basal respiration, increased maximal respiration and an increased dependency on mitochondrial metabolism to generate ATP. There was no difference in glucose consumption and lactate production. Thus, radioresistance was associated to a shift to a more oxidative metabolism in the SQD9 model. We next aimed to identify a causal link between elevated oxidative activities and the intrinsic radioresistance. SQD9 cells were FACS-sorted for glucose uptake using fluorescent glucose analogue tracer 2-NBDG, and metabolically profiled. From the total SQD9-wt population, we isolated the 2% most glycolytic (SQD9-glyc) and 2% most oxidative (SQD9-ox) cells that were then irradiated and tested for clonogenicity. In vitro comparison provided early evidence that SQD9-ox are more radioresistant than SQD9-glyc cells. Conclusively, our experimental data indicate that switching from basal to a more oxidative metabolism can cause intrinsic radioresistance in human head-and-neck SQD9 cancer cells.

## A new efficient cancer treatment by combining ablation techniques and Auger electron therapy

**Torsten Groesser**, A. Jensen, F. Zhuravlev, J. Fonslet and M. Jensen

*Technical University of Denmark (DTU), Nutech, Hevesy Laboratory, Roskilde, Denmark*

Current ablation techniques are often inefficient, leaving behind viable micro tumors that can cause cancer recurrence and death of the patient. While Auger electron therapy is a highly promising new radiotherapy, it has currently no clinical use, since it needs to directly target the DNA to be effective. We aim to improve the efficiency of cell ablation by combining it with Auger electron therapy, whereby holes in the cells' membranes induced by the ablation will allow the Auger emitting radioisotopes access to the DNA, thus preventing tumor recurrence. We will label DNA-dyes with Auger-electron emitters to use in combination with ablation techniques such as cryosurgery (CS) or irreversible electroporation (IRE). The combination of such an invasive technique with Auger therapy allows easy injection of our radiolabeled compound right into the tumor volume shortly before the ablation insult occurs. This will permit free diffusion of our compound into the cancer cells. After binding to the DNA, the radioactive decay will occur in close proximity to the DNA. The Auger-cascade provides many short-range electrons which will deposit their energy near the decay side. This will cause a localized high ionizing density resulting in complex DNA damage. Our first in-vitro experiments were performed using a Chinese hamster cell line (V79) with a freezing protocol similar to the one used in cryotherapy. We are in the process of developing various compounds (DNA stains) labeled with a radioisotope emitting an Auger electron cascade. Preliminary data show a dramatic reduction in clonogenic cell survival after freezing cells. We suggest by adding our radio-labeled compound, we can induce DNA damage in addition to the cell membrane damage. This should reduce the survival fraction even further. To investigate the possibility of our compound to enter the tumor cells, we first measured the plasma membrane permeability during the freeze/thaw cycle. Our results indicate that plasma membrane permeability is highly increased especially when multiple freeze/thaw cycles are performed. Therefore, an Auger emitter bound to DNA dyes would have easy access to the DNA of cells damaged by the ablation treatment. The idea of combining radiation exposure from internalized Auger emitters with ablation techniques is quite unique but there are still several hurdles to overcome. Nevertheless, a successful outcome will be of huge benefit for future cancer treatments. In addition, it will open up a sophisticated way to study fundamental radiation effects of the Auger cascade in a more precise way than previously possible. For the reason that the method we are proposing will give us knowledge of the exact location of the decays due to the specific binding sites of our DNA dyes.

# Targeting improved antioxidant defense and dNTP sanitization is effective in eradicating cancer cells adapted to acute or chronic anoxia/re-oxygenation stress

**Christine Hansel**, J. Hlouschek, T. Helleday, V. Jendrossek and J. Matschke

*Institute of Cell Biology (Cancer Research), University Hospital Essen, Essen, Germany*

Introduction: Tumor hypoxia remains a major obstacle to successful radiotherapy. We recently demonstrated that exposure to repeated cycles of chronic severe hypoxia and intermittent reoxygenation drives the selection of cancer cells with increased tolerance to anoxia/reoxygenation stress and radioresistance. Cellular adaptation involved changes in the transcriptome as well as complex reprogramming of cell metabolism leading amongst others to improved antioxidant capacity and contributed to resistance to ionizing radiation in vitro and in vivo (Matschke et al., ARS 2016). We hypothesized, that the improved tolerance to oxidative stress will increase the ability of the cancer cells to cope with ROS-induced damage to free deoxy-nucleotides (dNTPs) required for DNA replication and may thus contribute to acquired resistance of cancer cells in advanced tumors to antineoplastic agents inhibiting the nucleotide-sanitizing enzyme MTH1 (7,8-dihydro-8-oxoguanine triphosphatase), ionizing radiation or both. Therefore, we aimed to explore potential differences in the sensitivity of cancer cells exposed to acute and chronic intermittent anoxia/reoxygenation stress to the clinically relevant MTH1-inhibitor TH1579 and to test whether a multi-targeting approach combining the glutathione withdrawer Piperlongumine and TH1579 may be suited to increase cancer cell sensitivity to TH1579 alone and in combination with ionizing radiation. Methods: We determined mRNA of MTH1 and other proteins of base excision repair pathway as well as protein levels of MTH1 in hypoxia/reoxygenation tolerant human NCI-H460 lung cancer, Du145 prostate cancer or T98G glioblastoma cells under normoxic (20% O<sub>2</sub>) and severely hypoxic (0.2% O<sub>2</sub>) conditions by using qRT-PCR and Western Blot analysis, respectively. Effects of treatment with TH1579 (0-1 µM) alone or in combination with the glutathione-antagonist Piperlongumine (10 µM) and/or ionizing radiation (0 - 10 Gy) was determined in short-term proliferation, apoptosis and cell death assays as well as long-term colony formation assays. Oxidative DNA damage was determined by measuring 8-oxo-Guanine (8-oxo-G) induction by using immunofluorescence. Results: Exposure to severe hypoxia or ionizing radiation altered expression of MTH1. Inhibition of MTH1 enhanced accumulation of ROS and increased 8-oxo-G formation and cell death under normoxic and severely hypoxic conditions. Importantly, though TH1579 alone was not able to radiosensitize ART and oxic control NCI-H460 cells, combined treatment with TH1579 and Piperlongumine increased ROS and cell death levels especially in ART NCI-H460 cells. Conclusion: Inhibition of MTH1 in combination with cellular antioxidant defense is suited for targeting phenotypic heterogeneity of cancer cells caused by adaptation to chronic cycling hypoxia in hypoxic tumor fractions.

## Influence of posttranslational modifications on radiation-induced DNA damage repair processes

**Anja Heselich**, L. Pack, G. Taucher-Scholz and B. Jakob

*GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany*

DNA damage induced by ion irradiation occurs very localized whereas damage induced by photon irradiation is homogeneously distributed over the cell nucleus. To allow recruitment of repair factors and efficient DNA double-strand break (DSB) rejoining DNA DSBs need to be accessible to a multitude of repair factors. It could be expected that especially clustered DSBs might demand extended alterations of the chromatin around the damage. Therefore we are especially interested in the impact various damage response factors have on the chromatin structure in processes like DNA repair and chromatin decondensation after densely ionizing irradiation. We started analyzing HP1 (heterochromatin protein 1) as a factor involved in heterochromatin (HC) compaction. CRISPR/cas9-mediated knock-out of HP1alpha w/wo combined siRNA-mediated knock-down of HP1beta or vice versa did not show any influence on DNA DSB rejoining or chromatin decondensation after targeted ion irradiation in HC compartments of NIH/3T3 mouse fibroblasts. In addition, we focused on proteins with relevant post translational modification activities, like SIRT6, a member of the sirtuin family, which shows strong mono(ADP)ribosylation and weak deacetylation activity. Repair studies in NIH/3T3 fibroblasts using  $\gamma$ H2AX as a DSB marker revealed an influence of sirtuin activity on DNA DSB rejoining after alpha-, but not after photon irradiation. Sirtuin inhibition resulted in impaired DNA DSB repair, becoming evident after longer incubation times (>8 h) after  $\alpha$ -irradiation (241Am, LET  $\sim 150$  keV/ $\mu$ m), with still elevated levels of  $\gamma$ H2AX foci compared to control cells 24 hrs after irradiation. In addition we are analyzing PARP1, which has been shown to be fast activated by SIRT6 via mono-PARYlation upon DNA damage induction, and is known to have important functions in DNA repair pathways. We focused on its possible influence on chromatin decondensation and used NIH/3T3 fibroblasts, in which the HC chromocenters can easily be distinguished from euchromatic areas based on DNA staining, for ion irradiation. Other than expected, after irradiation with charged particles (He, LET 76 keV/ $\mu$ m) only small differences in radiation induced heterochromatin decompaction could be observed in PARPi treated cells. As a next step we will address the question if the observed impaired DNA DSB rejoining after SIRTi is connected to chromatin alterations or rather based on a direct influence on repair factors. Funding: BMBF grant 02NUK037A

# Radiation and checkpoint blockade in the treatment of metastases

**Eva Hirschmann**, U. Gaipf, R. Fietkau, L. Distel and M. Hecht

*Strahlenklinik Erlangen, Erlangen, Germany*

Introduction: PD-1 and CTLA-4-Inhibitors have recently been approved for the treatment different metastatic tumors. Radiation is the common treatment for brain metastases, symptomatic bone metastases as well as palliation for other metastases. Preclinical studies showed synergetic effects when radiotherapy was combined with PD-1 inhibitors. However there exist only few clinical data from patients treated with this combination so far. Methods: Altogether 29 consecutive patients who received radiotherapy with concomitant PD-1-inhibitors were studied. 10 patients received additional CTLA-4 inhibitors. 13 patients started radiation therapy and PD-1-therapy at the same time, 16 patients received irradiation because of oligoprogression during PD-1 inhibitor therapy. The first group consists of 11 patients with metastatic melanoma and 2 patients with metastatic lung cancer. In 9 of them brain metastases were irradiated. The second group consists of 9 patients with metastatic melanoma, 3 patients with metastatic lung cancer and 4 patients with other metastatic tumors. In 8 of them brain metastases were irradiated. In each group a subgroup of 5 patients was treated with the triple combination of radiotherapy PD-1 and CTLA-4 inhibitors. Treatment related toxicity as well as the best response in field and out of the radiotherapy field and survival was analyzed. Results: The median follow up period was 6.5 months. In the group that started PD-1-therapy and irradiation simultaneously local tumor control was 67% (1 complete response (CR), 2 partial responses (PR), 3 stable diseases (SD)). Systemic tumor control was 44% (1 CR, 2 PR, 1 SD). Median duration of PD-1 inhibitor treatment was 4.6 months and overall survival was 5,2 months. In the group with radiotherapy after oligoprogression during PD-1 inhibitor therapy local tumor control was 92% (7 CR, 2 PR, 3 SD). Systemic tumor control was 57% (4 PR, 4 SD). Median duration of PD-1 inhibitor treatment was 1.7 months and overall survival was 3,8 months. Consequently an abscopal effect appeared in 4 patients (29%) after 3 months. There was one case of an asymptomatic intracerebral metastatic bleeding and one case of exfoliative dermatitis 1 week after irradiation. We observed one epileptic seizure, benign cardiac arrhythmia, moderate headache, pain in the limbs, dizziness, nausea, loss of hair, anemia and fatigue each in one patient. Conclusion: Toxicity of the combination of single or dual checkpoint blockade with radiation in metastatic tumors is acceptable. In this small patient collective a good local in field tumor control was observed. Abscopal effects were detected in single patients.

## Trichostatin A mitigates radiation induced gastrointestinal syndrome and bacterial translocation in gut

**Anup Kainthola**, N. Gupta and P. Agrawala

*Institute of Nuclear Medicine and Allied Sciences, Defence Research and Development Organization, New Delhi, India*

Radiation-induced gastrointestinal syndrome (RIGS) leads to disruption of mucosal barrier, bacterial invasion/ translocation, inflammation and sepsis. We aimed (a) to correlate specifically the incidence of gastrointestinal (GI) endogenous bacterial translocation to other organs and time dependent alteration in physiological status of the GI tract following exposure to 7Gy whole body  $\gamma$ -irradiation (Co60) and (b) to appraise the efficacy of HDAC inhibitor (Trichostatin A) in countering bacterial translocation and mitigating consequent infection. 4 different groups: a) Drug alone, b) Radiation alone, c) Radiation plus drug and d) Control with at least 5 mice in each were assigned for every assay. Xylose absorption and gastric emptying assays were done to assess the integrity of mucosal layer and permeability of GI tract. Bacterial translocation by endogenous bacterial gut flora (aerobic & anaerobic) was investigated using standard microbiological protocols. Translocated bacterial species into adjacent organs were identified by 16s rDNA sequencing and phylogenetic tree was constructed. Rate of infection in liver and mesenteric lymph nodes was calculated using published formula. 7 Gy irradiation caused endogenous gut flora to translocate and infect mesenteric lymph nodes, spleen and liver by day 8 (66.66% & 33.33% rate of infection by aerobic and anaerobic bacteria respectively) and gradually increased till 14th day. Liver was observed to have highest rate of infection. 16s rDNA sequencing revealed that on 7th day after exposure, *Escherichia coli* O104 showed translocation from GI to mesenteric lymph nodes in radiation group only. *Pseudomonas mendocina* was found in the liver of radiation group on 8th day. *Lysinibacillus sphaericus* was the only anaerobic bacteria to show radiation induced translocation through gut to the liver. A reduction in total number of bacteria was observed after irradiation in duodenum, jejunum and ileum, which soon approached to normalcy. Functionality of the intestine was found compromised which suggests no significant effect of TSA on gastric emptying of mice. However, spectrophotometric analysis for xylose absorption between radiation and radiation with drug group showed a significant difference on 5th and 9th day post irradiation (0.160, 0.224 and 0.165, 0.260 respectively) which shows recovery of integrity of intestinal mucosal layer and improved permeability in treated group. We conclude that Trichostatin A has mitigating properties towards radiation induced damage in GI. Apparently, TSA restored local intestinal homeostasis in terms of commensal bacterial flora and permeability. Integrity of luminal tight junctions was restored to an extent that functionality of gut returned close to normalcy in challenged mice.



# Challenges and problems while using data from NCBI Gene Expression Omnibus (GEO) to detect gene expression profiles – a case study regarding NSCLC cell lines treated with IR"

**Anna Katsiki**, C. S. Yeles, I. Liampa, E.-I. Vlahavas, A. A. Chatziioannou, A. G. Georgakilas and C. E. Vorgias

*Department of Biology, National and Kapodistrian University of Athens, Athens, Greece*

Despite the common usage of radiotherapy for the treatment of non-small cell lung cancer (NSCLC), outcomes for these cancers, upon treatment with ionizing radiation (IR), are still unsatisfactory. A deeper understanding of the mechanisms underlying resistance to IR is needed in order to design novel approaches to eliminate the radioresistant cells and prevent tumor recurrence and metastases. During the last decade, high-throughput methodologies such as mRNA microarray experiments have generated a vast amount of data that can be used towards unraveling the molecular pathways after treatment with IR. Analysis of mRNA microarray experiments for detecting gene expression profiles is a common methodology for Bioinformaticians. Therefore, a growing number of computational tools are currently available. Among them most powerful and widely used, are R programming language and Bioconductor, which are open source and provide the capability of many modifications depending on the user's requirements. Although they have been used for such analyses the last 15 years, there are many ambiguous points that each Bioinformatician handles arbitrarily. Like: (a) normalization and non-specific filtering methods; (b) cutoffs used to detect differentially expressed (DE) genes among various experimental conditions; (c) adjustment method for multiple comparisons; etc. The main steps for analyzing Affymetrix microarrays are: (a) reading from CEL files (raw data); (b) pre-processing (background adjustment, normalization and summarization); (c) non-specific filtering to remove probes that appear not to be expressed in any of the experimental conditions; (d) data quality assessment; (e) linear modelling and (f) moderated t-test to detect DE genes. Finally, annotation is running to match the probe names of the Affymetrix platform used in the experiment with the established gene names. Subsequently, functional enrichment analysis with the web tool "BioInfoMiner" (developed by e-NIOS Applications PC) may be performed to achieve an integrative interpretation and visualization of the aforementioned analysis. Here we suggest a bioinformatics based methodology for detecting DE genes using data from NCBI GEO. NCBI GEO is an international public repository that archives and freely distributes high-throughput functional genomics data submitted by the research community. In the current study we used the raw data with accession number GSE20549. GSE20549 contains time kinetics gene expression profiles of radioresistant H1299 and radiosensitive H460 NSCLC in response to IR. These cell lines have different p53 status, since H460 has a wild-type p53 while H1299 is p53-null. Both cell lines were irradiated with IR (2Gy) and total RNAs are collected at 0, 2, 4, 8, 12, and 24 h after radiation in triplicates. The samples were processed and hybridized to Affymetrix Human Gene 1.0 ST Arrays [transcript version]. Each cell line was analyzed separately so as to identify the DE genes using R statistical software/Bioconductor (R version 3.4.1). Preprocessing was performed using Robust Multichip Analysis (RMA) algorithm (oligo R package). Non-specific filtering was performed using function pOverA (genefilter R package). All comparisons among time points were made and after adjusting the p-value with fdr method, genes that have a log-fold change greater than  $\pm 0.5$  and a p-value lower than 0.01 were considered as DE (limma R package). After annotation, DE genes were used as input for BioInfoMiner which lead to 49 genes, common in both cell lines. The majority of the 49 genes is involved in the regulation of the cell cycle and in molecular pathways activated as a response to DNA damage stimuli. In conclusion, we propose a powerful methodology that can be used to eliminate the complexity of high-throughput data and help the molecular biologists to focus on a very small number of biologically relevant genes to the performed experiment.



## Identification of biologically active factors in ionizing radiation regulated secretome

**Tamara Kazimova**

*University Hospital Zurich, Zurich, Switzerland*

Ionizing radiation (IR) leads to DNA damage and genome instability. In addition, IR also leads to stress responses in tumor cells by activating signal transduction pathways and inducing secretion of numerous auto- and paracrine factors. As part of an exhaustive IR-dependent secretome analysis which was previously performed in our laboratory, placental growth factor (PIGF) was identified to be secreted in response to IR. It is a N-glycosylated, homodimeric protein and belongs to vascular endothelial growth factor (VEGF)-family. There are four splice isoforms of the protein and they bind to VEGFR1, the soluble version of the receptor and NRP1. PIGF expression is low to undetectable in most tissues in healthy subjects, but becomes significantly up regulated in disease. In this project, two different lung adenocarcinoma cell lines (Kras-mutated A549 and Kras-wild-type NCI-H125) were irradiated with increasing doses of IR (0, 5 and 10 Gy). Conditioned media (CM) from the cells were analyzed by ELISA. Only in CM derived from A549 cells increased PIGF levels could be detected in a time and dose-dependent way within the first 24 h after irradiation. To further analyze PIGF-expression, qRT-PCR was performed. PIGF expression was already upregulated after 4 h of irradiation with 5 and 10 Gy in A549 cells and remained upregulated up to 24 h. Interestingly, in NCI-H125 lung adenocarcinoma cells increased expression regulation could only be observed after 24 h. Next, PIGF was genetically targeted using PIGF-directed siRNA. Downregulation of PIGF was observed 48 and up to 96 hours after siRNA-treatment. CM was collected from unirradiated and irradiated, PIGF depleted cells (siPIGF) and control cells (siLuc). HUVECs were incubated with these differential conditioned media for 20 minutes and cell lysates were analyzed by western blotting. Interestingly, CM derived from irradiated control cells (siLuc) increased the pERK-level in the endothelial cells which was suppressed when incubated with CM derived from PIGF-depleted cells. Control ELISA was performed on these conditioned media and demonstrated lower PIGF level in CM derived from PIGF depleted cells. In conclusion, PIGF which has so far not been investigated in response to IR, might play a relevant role for the radiation response on the level of tumor angiogenesis.

# Mechanism of action of Dbait, an original class of DNA repair inhibitor

**Maria Koźlak**, W. Jdey, N. Berthault, PM. Girard and M. Dutreix

*Institut Curie, CNRS UMR3347, INSERM U1021, Orsay, France; Université Paris-Sud, Université Paris-Saclay, Orsay, France*

Defects of DNA repair pathways in tumor cells affect the response to ionizing radiation (IR) and can be exploited for targeted radio-sensitization strategies. Indeed, inhibitors of double strand breaks (DSBs) repair pathways have been shown to sensitize cancer cells to radiotherapy (RT). We have developed an original class of DNA repair inhibitor, DNA repair bait (Dbait in short) that inhibits three central repair pathways: Homologous Recombination (HR), Non Homologous End Joining (NHEJ) and Single Strand Break Repair (SSBR). These synthetic molecules are short double stranded oligonucleotides in hairpin-like structure mimicking double strand breaks. In cells, these molecules hijack many of the proteins involved in the repair of genomic double and single strand breaks. AsiDNA belongs to this class of Dbait molecules. They are short 32bp base pairs oligonucleotides coupled with cholesterol to facilitate cellular entry. Unlike non-tumor cells, some tumor cells are sensitive to AsiDNA treatment alone. Moreover, AsiDNA has been shown to sensitize tumors to radiation in vitro, in vivo and in clinic, while it has no effect on irradiated non-tumor cells and healthy tissues. Based on these observations, our purpose is to investigate the molecular mechanisms in normal and breast cancer cells involved in the response to AsiDNA and/or irradiation treatment, in order to understand the specificity of the radiosensitising effect of AsiDNA to cancer cells. We confirmed that AsiDNA sensitized breast cancer cells to irradiation without having effect on non-tumor cell lines. The selective sensitivity is not due to a difference in the uptake of the molecule between tumor and non-tumor cell lines. After several attempts in order to obtain resistant clones to AsiDNA treatment, we observed very unusual behaviour of various tumor cells treated with cycles of AsiDNA. Not only no resistance was recovered but tumor cells became more sensitive to AsiDNA after each cycle. Using the triple negative breast cancer (TNBC) MDA-MB-231 cells, we showed by transcriptome analysis that in AsiDNA "conditioned" cells, more than 2000 genes are down-regulated, encompassing 19 pathways, and 1190 are up-regulated. In a marked contrast, only 250 and 150 genes are up regulated and down regulated, respectively, in the non-tumor cells MCF-10A after the same number of treatments. In the next step, we will try to decipher how long this deregulation stand after "conditioning" treatment, what are the molecular mechanism of it and how these deregulations contribute to the acquired hyper-sensitivity of AsiDNA "conditioned" MDA-MB-231 cells.

## The membrane-targeted antineoplastic alkylphosphocholine erufosine modulates the cellular radiation response by interfering with the lipid compartment

**Adam Kryztofiak**, S. Oeck, R. Handrick, J. Rudner and V. Jendrossek

*Institute of Cell Biology (Cancer Research), University Hospital Essen, Essen, Germany*

Introduction: Cellular membranes are important integrators of growth, survival and death signals originating from the tumor microenvironment. Moreover, cellular membranes provide platforms for the activation of intracellular signaling cascades and are a source for lipid second messenger molecules that participate in the regulation of cell survival, death and metabolism. Therefore, they are attractive targets for the modulation of radiotherapy response. Erufosine (erucylphosphohomocholine, ErPC3) is a membrane-targeted synthetic phospholipid analogue with antineoplastic properties. In contrast to classical anticancer drugs and ionizing radiation (IR) which exert cytotoxicity mainly by inducing DNA damage, erufosine primarily interferes with cellular membranes. Erufosine affects cellular signaling response by influencing the membrane lipids which results in alterations of lipid second messengers and other signaling molecules. Moreover, erufosine changes lipid metabolism. Previous reports, including data from our group, revealed that erufosine and related drugs (alkylphosphocholines - APC) interfere with PI3K/Akt-dependent survival signals and trigger cell death in various cancer cell lines. Furthermore, these drugs increase the cytotoxic efficacy of ionizing radiation in vitro and in vivo. However, the molecular mechanisms of their radiosensitizing action are insufficiently understood. In the work presented here we aimed to identify the assumed unique molecular changes initiated by short and long term drug exposure that unexpectedly increased tumor cells sensitivity to ionizing radiation with a focus on membrane-related changes and lipid metabolism alterations. Methods: As experimental models we used human A549 lung carcinoma and T98G glioblastoma cell lines, which have been selected to be more resistant to erufosine and erucylphosphocholine, respectively, than non-selected control cells. Cells were treated with increasing concentrations of erufosine (0-50  $\mu$ M), IR (0-10 Gy) or the combination under normoxic (20% O<sub>2</sub>) and hypoxic (1% O<sub>2</sub>) conditions. Cytotoxicity of the treatment was determined in short-term assays (crystal violet staining of cell) and long-term assays (colony formation assays). The erufosine-related alteration in lipid trafficking and utilization was analyzed by flow cytometry and fluorescence microscopy using distinctive molecular probes for lipids, labeled with BODIPY fluorescent analogs. The effects of erufosine on cellular signaling related to survival (Akt), cell cycle regulation (MDM2, p53) and metabolism (AMPK) were assessed by western blot using specific antibodies. Results: Erufosine exerted potent cytotoxic effects in short-term and in long-term assays and increased the cytotoxic activity of IR. Cytotoxic activity was associated with inhibition of Akt and specific changes in the lipid compartment when cells were exposed to acute or chronic erufosine treatment. Additionally, acute and chronic erufosine treatment altered IR-induced G2 arrest. Interestingly, erufosine efficacy was not reduced under hypoxic conditions. Conclusion: Our data

# Identification of beta 8 integrin as novel determinant of pancreatic cancer cell radioresistance

Wei-Chun Lee, S. Jin and N. Cordes

*OncoRay – National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany and Helmholtz-Zentrum Dresden - Rossendorf, Germany*

Pancreatic ductal adenocarcinoma (PDAC) is one of the five most lethal malignancies in the world and has a 5-year relative overall survival rate of less than 5%. Thus, there is a great need for molecular-targeting strategies. As cell-matrix adhesion is essential for the survival, invasion and therapy resistance, we sought to identify the function of 117 focal adhesion proteins (FAP) in PDAC cell radioresistance. Intriguingly beta 8 integrin turned out to be one of the most potential novel targets in PDAC. Material and methods: For FAP detection, we performed a 3D endoribonuclease-prepared siRNA (esiRNA)-based screening (3DHTesiS) in PDAC cell culture (established and primary) grown in laminin-rich extracellular matrix (IrECM). After esiRNA-mediated knockdown and X-ray irradiation (2-6 Gy single dose), clonogenic survival assay and sphere formation were determined. Beta 8 integrin expression level and distribution were detected by using Western blot and immunofluorescence staining. Beta 8 integrin staining was also combined with vesicle trafficking proteins (Caveolin-1, APPL2) and the cis-Golgi matrix protein GM130. Fiji software was used to analyze vesicle distribution after irradiation and Pearson's correlation coefficients were calculated. Results: We identified a series of novel targets with radiosensitizing potential including beta 8 integrin. Without cytotoxicity, beta 8 integrin knockdown conferred a significant radiosensitizing effect in established patient-derived PDAC cell cultures. Moreover, beta 8 integrin depletion reduced invasion and sphere forming ability. Intriguingly, we found beta 8 integrin located in the perinuclear area colocalized with GM130 but neither in the cell membrane nor colocalized with Caveolin-1 and APPL2. Further, we observed an increased beta 8 integrin expression after irradiation associated with enhanced beta 8 integrin-positive vesicle formation in both cytoplasm and nucleus. This suggests that beta 8 integrin may contribute to intracellular vesicle trafficking under stress conditions. Summary: We successfully designed a high-throughput radiosensitivity screening method for cell growing in a physiological 3D matrix-based environment. Interestingly, beta 8 integrin has, although not found in the cell membrane to facilitate cell adhesion, a critical role in the radiation response of pancreatic cancer cell. Ongoing work will unravel the underlying mechanisms how beta 8 integrin is controlling cytoplasmic and nuclear survival pathways.

## c-MET as potential target for radio-sensitization in pancreatic cancer

**Ahmed Allam Mohamed,** A. R. Thomsen, M. Gothwal, C. Aldrian and T. B. Brunner

*Department of Radiation Oncology, Faculty of Medicine, University of Freiburg, Freiburg, Germany*

**Background and Purpose:** C-MET is a proto-oncogenic tyrosine kinase, which is activated by hepatocyte growth factor (HGF), a glycoprotein secreted by mesenchymal cells. C-MET overexpression and has been linked to poor prognosis, increasing proliferation, invasiveness, migration, and inhibition of apoptosis in many cancers. In pancreatic cancer, HGF acts in a paracrine fashion; it is secreted by stromal cells and acts on the c-MET receptors on cancer cells. **Aim of the work** to check if HGF – c-MET axis in has a role in cellular mechanisms of radiation-induced damage repair in pancreatic cancer. **Materials and methods:** Levels of c-MET and pc-MET were checked in pancreatic adenocarcinoma cell line “Panc-1” at 0Gy and 6 Gy on different time intervals after irradiation (0, 6, 24 hours after irradiation) in MDEM medium with 2.5% or 10% fetal calf serum (FCS) As a next step, we tested the effect of knockdown of c-MET on clonogenic assay, in which Panc-1 cells were cultured alone or with pancreatic cancer-associated fibroblasts (CAF) at 0,2,4,6 Gy. **Results:** The level of c-MET expression in panc-1 was not affected by irradiation, however, the level of phosphorylation of c-MET significantly increases at 6 and 24 hours after the radiation only in 10% FCS not in 2.5%. In our hands, c-MET knockdown showed no effect on radio-sensitivity of Panc-1 cell line in monoculture clonogenic survival assay and insignificantly increased the radiation sensitivity of Panc-1 co-cultured with CAF. **Conclusions:** C-MET phosphorylation takes place in Panc-1 cell line in response to radiation, with higher concentration of FCS (sufficient level of HGF), which indicates an active involvement of c-MET pathway in active cellular processes post-irradiation. However, we could not establish a real significance of this process on Panc-1 mechanisms of radiation-induced damage cell repair in 2D monoculture and co-culture.

# A model analysis to estimate the number of DNA double-strand breaks in cells exposed to X-rays

**Ryosuke Mori**, Y. Matsuya, Y. Yoshii and H. Date

*Graduate School of Health Sciences, Hokkaido University, Japan*

Background: When ionizing radiation such as X-rays is incident on cultured cells (mainly composed of water), secondary electrons are produced and their energies are deposited along the track. The energy deposition can induce some kinds of DNA damage, such as base damage, DNA single-strand breaks (DNA-SSBs) and DNA double-strand breaks (DNA-DSBs). Most of the damage can be repaired by DNA repair functions. However, due to their difficulty to repair, DNA-DSBs are interpreted as the main cause leading to cell killing after X-ray exposure. Purpose: To predict damage effects on healthy cells following X-ray exposure as the endpoint of DNA-DSBs, taking into account of cell nucleus dose and cell cycle distribution. Methods: We first measured the DNA amount in a nucleus in CHO-K1 cells via flow cytometric analysis with propidium iodide (PI) staining, and the number of DNA-DSBs per nucleus after irradiation (200kVp X-ray with 0.5mm Cu + 0.5mm Al filtration) by  $\gamma$ -H2AX foci formation assay. Next, we calculated the distribution of absorbed dose per cell nucleus using WLTrack (in-house Monte Carlo code). We then estimated the number of X-ray-induced DNA-DSBs by taking account of two factors: the dose and the DNA amount per nucleus. It has been reported that the number of background foci changes depending on the cell cycle of the cell nucleus (e.g., the number in S phase is higher than that in G1 phase). In this study, this number of background foci corresponding to percentage of the cells in S phase was also incorporated. Finally, we compared the estimated number of DNA-DSBs with the observed foci distribution under two phases: plateau and logarithmic growth. Results: To evaluate the influence of cell-cycle dependent DNA amount on DNA-DSB induction, we prepared beforehand two cell conditions: one in the logarithmic phase and the other in the plateau phase of the growth curve. The cell population under the plateau phase was composed of 78.9% in G0/G1, 12.6% in S and 8.48% in G2/M, and that under the logarithmic phase 35.4% in G0/G1, 41.2% in S and 23.4% in G2/M. The numbers of DNA-DSBs deduced considering both the variation of absorbed dose and the DNA amount per nucleus were: (1) for logarithmic growth phase, the average number of the foci was 55.97 (standard deviation 20.76) and the estimated value was 53.27 (15.98); (2) for plateau phase, the average number of the foci was 40.68 (standard deviation 14.59) and the estimated value is 42.20 (13.48). Conclusion: Although this study was performed with only one cell line (CHO-K1) under two typical conditions, our results suggest that the DNA amount depending on cell cycle and the statistical variation of local energy deposition by secondary electrons are both crucial to assay the probability of DNA-DSB induction in cells following X-rays exposure.

## Exploiting novel combined-modality approaches for the treatment of highly aggressive, invasive pancreatic ductal adenocarcinomas

**Michael Orth**, L. Posselt, S. Kirchleitner, J. Schuster, B. Stegen, C. Belka, M. Schnurr and K. Lauber

*Department of Radiation Oncology, Ludwig-Maximilians-University Munich, Germany*

Marked by an overall survival of less than 5% at 5 years past diagnosis, pancreatic ductal adenocarcinoma (PDAC) is one of the most devastating cancer entities among all. Even worse, its prevalence is assumed to considerably increase in near future. Its standard treatment encompasses surgical resection (if possible), frequently followed by radiochemotherapy settings implementing gemcitabine, capecitabine or 5'-FU. Nevertheless, treatment failure is frequent and inherent resistance towards irradiation and DNA-damaging chemotherapy is considered as a major reason. Therefore, novel molecularly targeted treatment approaches are needed, that can address this kind of resistance and that are capable of creating synergisms with classic treatment modalities. We screened a panel of ten human PDAC cell lines on their respective intrinsic radiosensitivity/-resistance and also examined the expression levels of multiple genes that are functionally connected to the DNA damage response (DDR) in these cell lines by qRT-PCR. By correlating the values we obtained for intrinsic radiosensitivity/-resistance of each cell line with the expression levels of DDR-related genes, we identified several genes of the DDR pathway to be specifically upregulated in radioresistant PDAC cells, particularly if compared to cells derived from non-transformed pancreatic tissue. One prominent candidate we found was the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), the kinase considered of being the master regulator of the non-homologous end joining (NHEJ) pathway. To examine the potential impact of DNA-PKcs upregulation on clonogenic survival of radioresistant PDAC cells after irradiation we employed pharmacological inhibition as well as RNA interference-mediated knockdown of gene expression. Strikingly, each of these approaches resulted in a decrease of the inherent capacity of radioresistant PDAC cells to deal with irradiation-induced DNA damages and, in consequence, in the decrease of clonogenic survival displayed by these cells. To confirm these results we analyzed the PDAC patient collective of the TCGA and found that DNA-PKcs expression levels are indeed upregulated in about 11% of all PDAC cases and that this upregulation is associated with impaired overall survival to significant extends. Currently, we test the in vivo efficacy of DNA-PKcs inhibition and fractionated, CT-based radiotherapy using an orthotopic PDAC mouse model to further explore whether the combination of DNA-PKcs inhibition and radio (chemo)therapy indeed represents a novel promising approach for the treatment of PDACs.



## HSP90 inhibitors as radiosensitizers: Lessons from different tumor models

**Michael Orth**, K. Seidl, J. Schuster, B. Stegen, A. Ernst, L. Kinzel, N. Brix, N. Winssinger, C. Belka and K. Lauber

*Department of Radiation Oncology, Ludwig-Maximilians-University Munich, Germany*

The chaperoning heat shock protein 90 (HSP90) is a factor of pivotal importance for both survival and progression of cancer cells as it supports the accurate folding and the stability of multitudes of client proteins. Small molecule inhibitors of HSP90 have been extensively tested for clinical purposes but (so far) failed to provide significant therapeutic benefit, at least when being applied as monotherapy. However, inhibition of HSP90 is still an attractive approach, particularly when being combined with other treatment modalities such as radiotherapy. Multiple studies on various tumor models have proven the radiosensitizing potential of various different HSP90 inhibitors, yet the mechanism(s) underlying the observed synergisms remained mostly unknown, thus precluding the identification of biomarkers for such treatment approaches. By using the pochoxime-derived HSP90 inhibitor NW457 (Barluenga et al. (2008), *Angewandte Chemie* 48, 4432-5) we confirmed the radiosensitizing potential of this class of drugs in several tumor entities including glioma, sarcoma and colorectal cancer (Ernst et al. (2015), *Cancer Lett* 365(2), 211-22; Kinzel et al. (2016), *Oncotarget* 7(28), 43199-219). As one major mechanism underlying the radiosensitizing potential of NW457 we identified the loss in protein stability of certain proteins related to the DNA damage response (DDR) such as ATM, ATR, CHK1 and NBS1. We now show that inhibition of HSP90 by NW457 radiosensitizes p53-deficient tumor cells to significant greater extents than tumor cells that are proficient for p53. Mechanistically, p53-proficient tumor cells arrest in G2-phase upon treatment with NW457 while p53-deficient cells exhibit a considerably higher tendency of progressing into the next M-phase resulting in enforced mitotic catastrophes upon concomitant exposure to ionizing irradiation. This difference between p53-proficient and p53-deficient cells did not depend on the canonical p53 signaling involving the major downstream effector p21CIP/WAF1 since p53-proficient cells actively repress the expression of several cell cycle regulating genes (among them cyclins and cdks) that are crucial for mitotic entry upon treatment with NW457. In contrast, p53-deficient cells continuously express these genes to high extends explaining their increased tendency to enter M-phases and subsequent mitotic catastrophes. Using a heterotopic colorectal mouse model we observed that mice bearing p53-deficient tumors benefit significantly more from HSP90 inhibition when combined with concomitant irradiation than mice that bear p53-proficient tumors. Thus, combinatorial treatment approaches encompassing HSP90 inhibition and radiotherapy are particularly attractive for the treatment of tumors that are deficient in p53 function

[michael.orth@med.uni-muenchen.de](mailto:michael.orth@med.uni-muenchen.de)



## Toward the pharmacological augmentation of radiotherapy

**Norman Reppingen**, M. Durante and C. Fournier

*GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany*

Combined pharmacological treatments offer new opportunities, which are also expected to increase the versatility of radiotherapy. It is also important to consider possible limits of each approach in the individual patient to enable appropriate patient stratification. Here we present a combined approach consisting of an anti malarial drug, a triterpenoid and a tyrosine kinase inhibitor (TKI). Each of these components was separately reported to be immune modulating in different models, and was shown to reduce tumor growth alone or combined with irradiation. This effects were shown by others to be also immune mediated. We found the combination of the three drugs to induce apoptosis in murine cancer cell lines CT26.WT and B16-F10 but not in the p53 negative 4T1 cells of murine metastatic breast cancer. The immune competent 4T1 model is highly resistant to radiation as well as chemotherapy and inducing aggressive metastasis to the lung, killing the animals in week 4-5. 4T1 tumors have relatively few mutations and low antigenicity, and are thus also a very challenging model for immunotherapy - and therefore a candidate test system for combined approaches. In the 4T1 model, our approach was put to vivo evaluation with and without irradiation. A single 16 Gy dose of x-rays to the tumor did lead to a small intermediate reduction of the pace of tumor growth. The treatment with our drug cocktail did show a more pronounced tumor growth retardation in comparison to irradiation. Contrariwise to the in vitro situation, the combination of drugs and irradiation was not additive / synergistic. Analysis of the possible effects behind this occurrence is ongoing, including immunity and the tumor microenvironment

# Combined effects of metformin and cisplatin on radiation sensitivity in non-small cell lung cancer

**Muhammad Assad Riaz**, A. Sak, YB. Erol , M. Groneberg, M. Melnikova, J. Thomale and M. Stuschke

*Strahlenklinik, Universitätsklinikum Essen, Germany*

**Purpose:** Cisplatin (CP) is most extensively used chemotherapeutic drug for lung cancer, but the development of resistance decreases its effectiveness in the treatments of non-small cell lung cancer cell lines (NSCLC). In this study, we examined the effects of metformin (MET), a widely used antidiabetic drug, on the radiosensitization of CP in NSCLC cells. **Methods:** Human NSCLC cell lines, A549 (cisplatin resistant) and H460 (cisplatin sensitive), were treated with MET, CP and/or combination of both drugs before ionization radiation (IR). Cell proliferation, clonogenic assays, Western blotting, cisplatin-GpG DNA adduct formation and immunocytochemistry were used to characterize the effects of treatments. **Results:** MET significantly inhibited NSCLC cell proliferation in a dose and time-dependent manner. The combination treatment of MET/CP and IR exhibited highest antiproliferative effect compared to IR alone, CP/IR or MET/IR. The results of clonogenic survival assay showed that radiosensitizing effect of CP was enhanced by MET on NSCLC when applied before CP/IR. At molecular level, MET increased cisplatin-DNA adducts level after CP treatment by about 10% and 30% in A549 and H460 cells, respectively. The combination of MET/CP with IR resulted a further increase in cisplatin-DNA adduct formation in A549 cell that was accompanied by reduced MEK1/2 phosphorylation and ERCC1 protein expression, a key enzyme in nucleotide excision repair pathway for the repair of cisplatin–DNA adducts. Furthermore, the combination of MET/CP and IR yielded by about 60% greater number of  $\gamma$ -H2AX foci at 1 h after IR compared to IR alone, suggesting increased DNA DSB damage signalling in NSCLC. Foci were reversed after 24 h of IR, showing DNA repair, whereas combined MET/CP and IR treatment showed relatively higher number of residual foci in both cell lines at 24 h suggesting increased residual DNA damage signaling. **Conclusion:** Our results suggest that MET can enhance the effect of combined radiotherapy and CP-treatment in NSCLC.

## Cellular differentiation exacerbates UV-radiation sensitivity in vitro in a human dopaminergic neuronal model

M. Temelie, C. Mustaciosu and Diana Savu

*The Department of Life and Environmental Physics, Horia Hulubei National Institute of Physics and Nuclear Engineering, Bucharest-Magurele, Romania*

UV radiations are well known cellular stressors. Following exposure to genotoxic factors the cells respond by molecular mechanisms aimed to repair the damages, block the proliferation or trigger cell death. Our study investigates how neuronal differentiation interferes with repair processes following UV-induced genotoxic stress, in a model of dopaminergic neurons (SHSY5Y). The present work proved a higher level of DNA damage induced by UVC radiation in the dose range of 0.358 - 2.150 mJ/cm<sup>2</sup> in differentiated cells compared to the proliferative variants. Cellular viability decreases proportionally with the irradiation dose and post-exposure time and correlates with an increased apoptosis through caspase 3/7 activation. These effects were enhanced in differentiated cells in which we have seen increased DNA damage, incomplete repair, exacerbated viability loss and apoptosis induction.

## AKLIDES® cell damage: development of an automated cell-based system for the quantitation of 8-OHdG

B. Debelec-Butuner, A. Bostancı, L. Heiserich, C. Eberle, F. Ozcan, M. Aslan,  
**Johannes Schulte-Pelkum**, D. Roggenbuck and K. S. Korkmaz

*Department of Pharmacology, Faculty of Pharmacy, Ege University, Bornova, Izmir, Turkey*

**Objective:** Detection of 8-OHdG-base damage has been a challenge for decades, though different analytical methods have been developed. Up to now no automated approach for quantitating either the total amount of base damage or the amount of base damage per cell from different sample-sources has been reported. We developed AKLIDES® Cell damage a novel method for automated damage detection on a single cell level based on fluorescence microscopy with LED light source and intelligent image processing and applied it to 8-OHdG base damage quantitation. The biomarker 8-OHdG: Inflammation induced reactive oxygen species are linked to mutations in protooncogenes and tumor suppressors resulting in genomic heterogeneity during cancer progression. Although continuously repaired by base excision repair (BER), 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a single nucleotide base lesion and recognized as a biomarker of oxidative DNA damage. The analysis of 8-OHdG is performed with a wide range of methods including immunofluorescence labeling with subsequent fluorescence intensity interpretation. The readout is usually given as average intensity. This approach is hindered by a dense background due to cytoplasmic RNA staining which interferes with DNA-related 8-OHdG quantitation. Other methods like IHC and ICC or LC-MS/MS quantitation produce either semi-quantitative data or carry the common disadvantages of quantitating the cell-based damage in oxidative conditions due to the sampling process, respectively. In contrast, immunofluorescence-based methods analyze 8-OHdG damage by different methods/algorithms involving simple image analysis. The AKLIDES® Technology: In this study we combined the conventional immunostaining of 8-OHdG-base damage with an automated immunofluorescence-based interpretation system, AKLIDES® Cell Damage. The platform enables automated fluorescence microscopy employing a LED light source with intelligent image processing software. Automatic focusing and cell identification are carried out using DAPI (blue) staining. For the determination of specific fluorescence signals in several z-levels, FITC (green) and APC (red) fluorescence dyes and respective detection channels are used. The implemented algorithms generate a 3-D matrix from the x-y-z coordinates of the detected foci and describe the foci using detailed parameters (nuclei amount, number of foci per cell, their sizes, volumes and the number of nuclei having foci). The system was initially designed for the reliable and efficient analysis of double-strand DNA breaks ( $\gamma$ -H2AX (S139) foci) in cells. It has been consistently used for clinical purposes such as to measure drug resistance and radiotherapy sensitivity. Results: Treatment of cells with 2M HCl before labeling 8-OHdG damage sites with specific antibodies facilitated antibody binding and resulted in a lower RNA labeling. Consequently, a lower background staining in the cytoplasm was detected supporting efficient 8-OHdG analysis. The number of foci was automatically counted on fluorescence-labeled cells at 5 confocal planes for the analysis of DNA damage by the AKLIDES® Cell Damage system (MEDIPAN GMBH, Germany). Amount of oxidative damage was defined as number of foci per cell. For comparison, samples were validated by LC-MS/MS analysis. The data are presented as average damage/cell; the number of cells having more than three foci; and total damage/100 cells in comparison to the 8-OHdG/dG ratio obtained by LC-MS/MS analysis. 8-OHdG/dG levels obtained by LC-MS/MS and AKLIDES® Cell Damage methods showed comparable results associated with the amount of base damage before and after oxidant (menadione) treatments. Conclusion: We developed an automated cell-based method for the quantitation of oxidative menadione-mediated 8-OHdG foci in the prostate cancer cell line LNCaP. Efficient 8-OHdG damage analysis can be performed with automated image interpretation by the AKLIDES® Cell Damage system using indirect immunofluorescence.

## Exposure to low-doses of ionizing radiation and mercury during neonatal development modifies the liver and kidney function in mice

**Noemí Serra**, R. Esplugas, M. Bellés, JC. Vallvé and V. Linares

*Laboratory of Toxicology and Environmental Health, Rovira i Virgili University, Reus, Spain*

In the 30 years since the Chernobyl nuclear power plant disaster, there is evidence of persistent levels of incorporated ionizing radiation (IR) in adults and children in the surrounding area. Cesium-137 ( $^{137}\text{Cs}$ ) produced by nuclear reactions is released into the air during and after the accident. On the other hand, people that lives in industrialized countries are exposed daily to a high number of environmental pollutants. The fetus and infant are especially vulnerable to toxicants, such as methylmercury (MeHg) that disrupt developmental processes during relatively narrow time windows. The objective of the present study was to evaluate renal and hepatic effects of low doses IR and MeHg when mice were exposed on postnatal day 10 (PND10). Male (C57BL/6J) mice were randomly assigned to six experimental groups: control (0.9% saline solution used as vehicle), Hg (20  $\mu\text{g/kgbw}$  of MeHg), Cs 4000 group ( $^{137}\text{Cs}$  with activity of 4000 Bq/kgbw), Cs 8000 ( $^{137}\text{Cs}$  with activity of 8000 Bq/kgbw), Hg/Cs 4000 group (20  $\mu\text{g/kgbw}$  of MeHg and  $^{137}\text{Cs}$  with activity of 4000 Bq/ kgbw) and Hg/Cs 8000 groups (20  $\mu\text{g/kgbw}$  of MeHg and  $^{137}\text{Cs}$  with activity of 8000 Bq/kgbw). To evaluate renal and hepatic effects, at the age of two months, urines were collected from animals of each group to determine LDH, GGT, NAG, and 8-isoPGF2 $\alpha$  levels. Blood samples were collected from the vena cava to determine LDH, GGT, NAG, ALP, ChE, GOT and GPT. Kidneys and liver were removed to quantify DNA damage (8-OH-dG) as well as to determine CYP1a2 mRNA expression in hepatic tissue. The results showed that GGT, GOT, GPT, ChE, NAG and Uric were altered when mice were co-exposed to  $^{137}\text{Cs}$  and MeHg. In addition, 8-isoPGF2 $\alpha$  levels were increased when mice were exposed to Hg/Cs8000. Hepatic CYP1a2 gene expression decreased significantly in mice co-exposed to  $^{137}\text{Cs}$  and MeHg. In conclusion, the results observed in this study show that  $^{137}\text{Cs}$  and MeHg co-exposure cause renal and liver damage.

# Antagonizing the CXCR4 chemokine receptor by AMD070 radiosensitizes HPV-negative human head and neck squamous cell carcinoma (HNSCC) lines

**Efe Cumhuri Sezgin**, A. Menegakis, D. Zips and S. Huber

*Department of Radiation Oncology, University of Tübingen, Tübingen, Germany*

Signaling by the chemokine stromal-cell-derived-factor-1 (SDF1, CXCL12) and its receptors CXCR4 and CXCR7 has been suggested to contribute to adaptation to hypoxia, malignant progression, tissue infiltration, distant metastasis, and therapy resistance of various tumor entities. In particular, SDF-1/CXCR4 signaling by HNSCC has been demonstrated to promote lymph node and distant metastasis, recruitment of CD11b-positive myeloid cells and subsequent vasculogenesis, as well as resistance of bone marrow-disseminated HNSCC cells to chemotherapy. Moreover, ionizing radiation at doses used for fractionated radiotherapy has been demonstrated to induce SDF1 signaling in several tumor entities. Here, we analyzed the impact of CXCR4 on DNA repair and radiosensitivity of human HNSCC cell lines with high (XF354), moderate (FaDu), and low (HSC4) CXCR4 expression. To this end, the effect of the CXCR4 antagonist AMD070 (1  $\mu$ M) on residual  $\gamma$ H2AX foci as a measure of residual DNA double strand breaks and clonogenic survival was determined in irradiated (0, 2, 4, and 6 Gy 6 MV photons) HNSCC cells by immunohistochemistry and delayed plating colony formation assay, respectively. As a result, AMD070 impaired DNA repair in FaDu cells as deduced from an increased number of residual  $\gamma$ H2AX foci 24 h after irradiation. In addition, AMD070 decreased the survival fraction of irradiated FaDu and XF354 but not HSC4 cells while having no effect on the plating efficiency of unirradiated cells. In conclusion, the data suggest that CXCR4 targeting by AMD070 radiosensitizes CXCR4-expressing human HPV-negative HNSCC cells in vitro. Since AMD070 is well tolerated in clinical trials on other diseases, the anti-tumor effect of combining fractionated radiotherapy with AMD070 chemotherapy should be analyzed in preclinical HNSCC in vivo models. Acknowledgments. ECS. is supported by a DAAD scholarship.

## Applying Broadband Dielectric Relaxation Spectroscopy (DRS) for the biophysical analysis of mammalian tissues under a variety of environmental stresses

**Maria Souli**, P. Klonos, AF. Fragopoulou, IS. Pateras, LH. Margaritis, D. Kletsas, VG. Gorgoulis, A. Kyritsis, L. Sihver and AG. Georgakilas

*Physics Department, School of Applied Mathematical and Physical Sciences, National Technical University of Athens, Zografou Campus, Athens, Greece; Atominstitut, Technische Universität Wien, Vienna, Austria*

**Purpose:** This study constitutes a synopsis presentation of our data for the detection of environmental stress-induced damage in mammalian tissues by the means of dielectric spectroscopy. The type of stress was different in each case. Firstly, we used non-ionizing and ionizing radiation as a typical environmental stress. Specifically, rat animals were exposed either to Digital Enhanced Cordless Telecommunication (DECT) radiofrequency electromagnetic radiation (RF-EMR) at 1880-1900 MHz or to  $\gamma$ -radiation (a dose of 5Gy) and the effects on cerebellum and skin were studied respectively. The other type of 'stress' (a type of pathophysiological state) was cancer and specifically human cancer lung tissues. The analysis was performed by means of Dielectric Relaxation Spectroscopy (DRS) and at the frequencies up to 1 MHz. The aim was to examine as to whether the method employed is sufficient enough for use in diagnostics. **Materials and Methods:** This preliminary study includes various types of tissue: human lung and prostate cancerous tissues, rat cerebellum isolated from young adult animals that have been whole-body exposed during prenatal only or pre- and post-natal life for 12 h/day to DECT base RF-EMR (at 3.7 V/m average electric field intensity), rat skin exposed to 5Gy  $\gamma$ -radiation and a set of different parts of a control rat to establish the experimental procedure. Each sample was inserted between two finely polished brass plates of a capacitor for dielectric measurements. An alternate voltage was applied and the conductivity,  $\sigma_{AC}$ , and the complex dielectric permittivity,  $\epsilon^*$ , were recorded isothermally (in nitrogen atmosphere) as a function of frequency in the range from 10-1 to 106 Hz at temperatures from 20 to -150°C on cooling and subsequently, from -150 to 25 °C on heating in steps of 5 and 10 °C using a Novocontrol Alpha Analyzer. **Results:** DRS allowed the detection of  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -dispersions, which are associated with dynamics of the various molecular groups in the tissues (membranes, proteins, water), as well as the recording of ionic conductivity related phenomena (dc-conductivity). Results were found consistent with previous work. Concerning the type of stress and tissue, rat skin exposed to a dose of 5Gy  $\gamma$ -radiation, was found to be less conductive than normal tissue at ambient temperature, probably due to dehydration. According to results with cancerous human lung tissues a strong connection to histopathologic characteristics, such as to those of tissue necrosis, is obtained. The same was found true for human prostate cancer tissues. The information regarding rat cerebellum exposed to DECT does not preclude, at this stage, univocal conclusions. However, this could be due to the mild effects imposed by DECT on tissues, as compare to those caused by  $\gamma$ -radiation. **Conclusion:** The contrast in electrical conductivity and other biophysical parameters of the different tissues, depending on their architecture and morphology, shows promise for distinguishing pathogenic and damage compared to healthy/control counterparts. These findings suggest that further studies should be applied with the aim to establish a standard protocol, exploit the sensitivity of the method and determine specific quantified thresholds for each pathogenic situation.



# Comet Assay analysis of DNA strand breaks in human cells after exposure to the DNA-incorporated Auger Electron Emitter Iodine-125

**Marcus Unverricht-Yeboah**, K. Holtmann and R.Kriehuber

*Department of Safety and Radiation Protection, Research Center Jülich, Germany*

Ionizing radiation (IR) causes various types of DNA damages e.g. single strand breaks (SSB) and double strand breaks (DSB), whereby the SSB/DSB ratio is shifted towards the DSB with increasing LET. For the DNA-incorporated Auger electron emitter Iodine-125 a SSB/DSB ratio of 5.4:1 is calculated based on computer simulations (Pomplun et al. 1996). In the presented work the SSB/DSB ratio induced by Iodine-125 was experimentally determined and compared to external homogenous  $\gamma$ -irradiation. SCL-II cells were labeled with I-125-iododeoxyuridine (I-125-UdR) and subsequently frozen for decay accumulation. Accordingly, external  $\gamma$ -irradiation (Cs-137 source) experiments were also performed in frozen cells. After exposure cells were thawed and used for the neutral or alkaline Comet Assay to quantify DSB or DSB & SSB, respectively. To avoid dose calculation for DNA-associated Iodine-125 the  $\gamma$ -H2AX assay was used in order to allow the comparison of the Comet Assay data between both investigated radiation qualities. Preliminary results show a lower SSB/DSB ratio induced by I-125-UdR when compared to external low-LET  $\gamma$  radiation comparable to the calculated values of Pomplun et al. (1996). This indicates that DNA-incorporated Iodine-125 induces a high-LET type DNA damage pattern.

Funded by Bundesministerium für Bildung und Forschung (BMBF), Grant 02NUK043A

**Reference:** Pomplun, E.; Terrissol, M.; Demonchy, M. (1996), Acta Oncologica 35(7): 857–862



## Reverse engineering the historical trend in risky UV-exposure from trends in cancer

**Arjan van Dijk**, A. Kloosterman, A. van der Reijden, F. Dekkers and H. Slaper

*National institute for public health and the environment (RIVM), Bilthoven, The Netherlands*

Lung cancer is mainly caused by smoking and skin cancer is mainly caused by UV exposure. Both causes of cancer undergo strong trends in how fashionable they are. For smoking, we have found historical prevalence records. For UV-exposure, such records could not be found. Of all types of cancer, skin cancer appears to have the strongest upward trend. This trend in incidence makes skin cancer worth studying, e.g. via cancer registry data. The unknown trend in the causative exposure behaviour makes it difficult to interpret the registry data. We have analysed multi-decade historical incidence records from Western European countries and Australia for several types of cancer. The age-specific incidence rates were parameterized with a multi-stage carcinogenesis model. Per type of cancer, all cancer-specific parameters were assumed to have one, unique value. Trends in time were parameterized by the introduction of a bimodal frailty, describing the “fraction of the population at risk”, as a function of the birth year. This parameter may reflect the propensity to engage in risky behaviour or conditions. For lung cancer, this interpretation was confirmed by comparison with the prevalence of smoking. For skin cancer, the same interpretation demonstrates that in many countries the fraction at risk has been increasing during most of the 20-th century by more than an order of magnitude. Only for Australia, this trend was broken starting with people born in the mid 1960’s, suggesting that the Australian awareness campaigns like “Slip! Slop! Slap!” and “SunSmart” are increasingly effective. At present, the fraction at risk is smaller for young Australians than for some European countries. Western European people thus face an attitude problem, and Australia shows that it can be done.

# Methadone interferes with the stress response of irradiated glioblastoma cells

**Tatjana Vatter**, L. Klumpp, D. Zips and S. Huber

*Department of Radiation Oncology, University of Tübingen, Germany*

Methadone which is used as maintenance medication for outpatient treatment of opioid dependence has been demonstrated in vitro to induce cell death and sensitivity to chemotherapy in leukemia and glioblastoma cells. On a compassionate use basis, glioblastoma patients are treated with methadone as a potential anti-cancer drug. Methadone reportedly signals via the  $\mu$ -opioid receptor and the coupled inhibitory G protein to voltage-gated  $\text{Ca}^{2+}$  channels. In addition, methadone exerts off-label effects and directly modulates cardiac voltage-gated Nav1.5  $\text{Na}^{+}$  channels, cardiac voltage-gated hERG  $\text{K}^{+}$  channels, and neuronal ionotropic NMDA glutamate receptors suggesting that methadone interferes with  $\text{Ca}^{2+}$ - and electrosignaling. Since potential methadone targets are expressed in glioblastoma cells and since  $\text{Ca}^{2+}$ - and electrosignaling has been demonstrated to promote survival of irradiated glioblastoma cells, we tested here in vitro for potential additive or synergistic anti-tumor effects of methadone treatment when combined with ionizing radiation. Expression of molecular methadone targets was determined on mRNA and protein levels in the human T98G, U251, and A172 glioblastoma cell lines by real-time RT-PCR and immunoblotting. In addition, the effect of methadone (20  $\mu\text{M}$ ) on clonogenic survival and cell cycle progression was assessed in irradiated T98G, U251 and A172 (0, 2, 4, 6 or 8 Gy 6 MV photons) 24 h and 48 h after irradiation by delayed plating colony formation assay and flow cytometry applying the Nicoletti propidium iodide DNA staining protocol, respectively. As a result, the human glioblastoma cell lines T98G, U251 and A172 expressed OPRM1  $\mu$ -opioid receptor, GRIN1 NMDA receptors, as well as CACNA1x voltage-gated  $\text{Ca}^{2+}$  (Cav) channel types at various extents. In flow cytometry experiments on cell cycle distribution, methadone significantly extended radiogenic G1 arrest in U251 and A172 cells and delayed radiogenic G2/M arrest in T98G cells. Moreover, methadone radiosensitized T98G and to some extent also A172 cells and conferred radioresistance to U251 cells. In addition, methadone increased plating efficacy of T98G cells. Combined, these data do not confirm previous reports on a cytotoxic activity of methadone in glioblastoma. Methadone-induced radiosensitization was only observed in one out of three glioblastoma lines which does not support the use of methadone in combination with radiotherapy in glioblastoma patients.

## Study of DNA damage signaling and its fate induced by low and high energy X-rays

A. Freneau, Aurelie Vaurijoux, P. Voisin, L. Roy and Gaetan Gruel

*Institute of Radioprotection and Nuclear Safety, Fontenay-aux-Roses, Île-de-France, France*

During a radiological examination, dose delivered to the patient's organs varies from a few tenths to a few tens of mGy. These low doses accumulate throughout the life of an individual, some patients being subjected to dozens of examinations in the same year. The risks associated with such exposures remain to be identified, understood and evaluated. Depending on the purpose of the examination, the type of radiation used is not the same. In a radiological examination (interventional radiology or mammography), low-energy X-radiation is used ( $< 100$  keV). For other radiodiagnosis procedures, the energy used is several MeV. It is currently considered that photons irrespective of their energy have the same radiation weighting factor. But, several studies have shown an increase in the relative biological effect of photons when their energy decreases, in particular an increase in the frequency of chromosomal aberrations and a decrease in clonogenic survival. In theory, these differences in biological efficacy could have their origin in the topological differences at the nanoscale of X-ray energy deposition as a function of their energy spectrum. Indeed, as the photon energy decreases, the nature of their interactions with living matter changes. To study this difference, we evaluated signaling of DNA damage by monitoring  $\gamma$ H2A.X and 53BP1 foci after exposure of G0/G1-phase synchronized human primary endothelial cells to 5 Gy of 40 kV, 220 kV and 4 MV X-rays. A large number of  $\gamma$ H2A.X and 53BP1 foci were analyzed from 30 min to 72 h using immunofluorescence labels and an automated detection of nuclei and foci was conducted. Different characteristics, such as size, intensity, spatial distribution of foci among others were explored. For the same dose of X-rays, no difference in the kinetics of the number of  $\gamma$ H2A.X foci per nucleus was shown among the three radiation qualities studied. The spatial distribution of foci among others seems similar. However, there would appear to be a difference in the size of the early and persistent foci, following exposure to X-rays of 4 MV compared to 40 kV and 220 kV. To go further, we will investigate the colocalization of other proteins which could reflect differences in nature and DNA damage processing.

# Prompt DNA double strand breaks (prDSBs) yield and acquired epithelial to mesenchymal transition (EMT): A potential association in determining DNA damage response

**Mohd Yasser**, Y. Cheng, F. Li, MSM. Hasan, E. Mladenov and G. Iliakis

*Institute of Medical Radiation Biology, University Hospital Essen, Germany*

DNA double-strand breaks (DSBs) are potentially lethal lesions and their misrepair can cause chromosomal translocations that feed carcinogenesis. Ionizing radiation (IR) induced DSBs include promptly induced DSB (prDSBs) that form immediately after irradiation, as well as DSBs that form minutes to hours after IR by the chemical evolution of thermally labile IR-sugar-lesions from strand-preserving to strand-breaking lesions (tDSBs). We find that tumor cells of different origin have significantly different prDSBs yields, hinting to cell-intrinsic factors modulating this parameter. Notably, we find a striking correlation between prDSBs induction and cell radiosensitivity to killing among tumor cell lines of epithelial origin. A surprising but intriguing observation we also made is that the induction of prDSB remains extremely low in all human fibroblasts examined thus far. Since fibroblasts are of mesenchymal cells, whereas tumor cell lines examined hitherto of epithelial origin, we hypothesized that prDSB yields dependent on unknown aspects of chromatin structure. We further hypothesized that this aspect of chromatin structure differs between epithelial and mesenchymal cells and underpins the dramatic differences in prDSB yields detected. To test this hypothesis, we examined systems in which epithelial to mesenchymal transition (EMT) was experimentally induced. Epithelial to mesenchymal transition (EMT) influences many processes associated with cancer development including malignant transformation and resistance to therapeutics. Despite this, little is known about the relationship between EMT and DNA damage response (DDR) in general, or the induction of prDSB in particular. Therefore, here we investigate correlations between prDSBs yield and EMT. We started with an induced mesenchymal system generated in human colorectal cancer cells of epithelial origin, HCT116 and HT29, by stably overexpressing Snail. Snail is a prominent inducer of EMT and causes a strong repression in the expression of the cell-cell adhesion molecule E-cadherin. Induction of prDSBs was measured by pulsed-field gel electrophoresis (PFGE) and cell survival after IR exposure using the clonogenic assay. The results show that EMT transition fails to alter prDSB yield. On the other hand, snail overexpressing HT29, but not HCT116 cells became more resistant to IR after EMT as compared to their parental cells. We conclude that experimentally mediated EMT fails to recapitulate important aspects of chromatin organization in epithelial versus mesenchymal cells that determine prDSBs yields in irradiated cells. Further work is required to uncover the molecular underpinnings of this important cellular response.

Supported by a grant from the Dean's office and by grants from the "Bundesministerium für Wirtschaft und Technologie"(BMWi: ESA-AO-08-IBER, 50WB1229).

[mohd.yasser@uk-essen.de](mailto:mohd.yasser@uk-essen.de)



A decorative graphic on the left side of the page, featuring a light blue, semi-transparent 3D model of a protein structure. The model shows a complex arrangement of alpha-helices and beta-sheets, with several subunits visible. The graphic is positioned vertically along the left edge, partially overlapping the dark blue banner.

# Poster Session 2



<b>P1</b>	<b>Ghassan Al-Massarani</b> Damascus, Syria	<i>"Long-term radiation exposure from interventional cardiology procedures induces endothelial damage"</i>
<b>P2</b>	<b>Katharina Batzke</b> Essen, Germany	<i>"Modulation of immune responses and radioresistance by neuroblastoma-derived and host-derived TrkB-target Galectin-1"</i>
<b>P3</b>	<b>Lindsay A. Beaton-Green</b> Ontario, Canada	<i>"Advancements in the Cytokinesis-Block Micronucleus assay as a biodosimetry tool using imaging flow cytometry"</i>
<b>P4</b>	<b>Deepti Bijlani</b> Canada	<i>"Radon mitigation actions follow-up study"</i>
<b>P5</b>	<b>Pierluigi Casolaro</b> Napoli, Italy	<i>"Absolute dose calibration of EBT3 Gafchromic films"</i>
<b>P6</b>	<b>Noemi Castelletti</b> Munich, Germany	<i>"Two distinct molecular pathways for the risk of lung adenocarcinoma from smoking and radiation"</i>
<b>P7</b>	<b>Lourdes Cruz-Garcia</b> Oxfordshire, United Kingdom	<i>"Inflammation-associated genes as biomarkers for monitoring radiation exposure during radiotherapy"</i>
<b>P8</b>	<b>Simon Deycmar</b> Zurich, Switzerland	<i>"Differential Response to Proton versus Photon Radiotherapy - Biological implications for new indications and combined treatment concepts"</i>
<b>P9</b>	<b>Daniel Fleischmann</b> Munich, Germany	<i>"Dynamic alterations in a miRNA signature of known prognostic value upon irradiation of glioblastoma cell lines"</i>
<b>P10</b>	<b>Noopur Gupta</b> New Delhi, India	<i>"Initial stage of Radiation Induced Gastrointestinal Syndrome and its modification by HDAC inhibitor (Diallyl sulphide)"</i>
<b>P11</b>	<b>Teena Haritwal</b> New Delhi, India	<i>"Mitigating effects of Trichostatin A against radiation induced damage to male reproductive system and progeny in C57Bl/6 mice"</i>
<b>P12</b>	<b>Carola Hartel</b> Darmstadt, Germany	<i>"Semi-automatic analysis of dicentric chromosomes for low-dose biodosimetry"</i>
<b>P13</b>	<b>Alexander Helm</b> Trento, Italy	<i>"Charged particles for a combination of radio- and immunotherapy"</i>



<b>P14</b>	<b>Julian Hlouschek</b> Essen, Germany	<i>"Importance of altered antioxidant defense for radioresistance of hypoxic cancer cells"</i>
<b>P15</b>	<b>Tímea Hülber</b> Budapest, Hungary	<i>"Robustness analysis of the Radosys Radometer-MN Series automated microscope"</i>
<b>P16</b>	<b>Annique Hunger</b> Neuherberg, Germany	<i>"Setup for tumor growth delay studies in small animals for low energy X-rays and small irradiation fields"</i>
<b>P17</b>	<b>Sophie Kalmbach</b> Essen, Germany	<i>"Identification of novel targets for rational chemoradiotherapy strategies in non-small-cell lung cancer"</i>
<b>P18</b>	<b>Justina Kazokaite</b> Vilnius, Lithuania	<i>"The carbonic anhydrase IX-selective inhibitor VD11-4-2 exhibits low toxicity in zebrafish and diminishes the acidification in Xenopus oocytes and cancer cell cultures"</i>
<b>P19</b>	<b>Julia Ketteler</b> Essen, Germany	<i>"Caveolin 1-deficiency in stromal fibroblasts mediates radiation resistance in human PC3 xenografts"</i>
<b>P20</b>	<b>Lukas Klumpp</b> Tübingen, Germany	<i>"KCa3.1 channels confer radioresistance to mesenchymal glioblastoma stem cells"</i>
<b>P21</b>	<b>Olena Klymenko</b> Munich, Germany	<i>"Patient-derived HNSCC cell lines and subclones as model systems to analyze tumor heterogeneity and radiosensitivity"</i>
<b>P22</b>	<b>Clelia Le Gallic</b> Bretigny-sur-Orge cedex, France	<i>"New strategies to mitigate accidental radiation-induced hematopoietic syndrome : reprogramming as a new frontier?"</i>
<b>P23</b>	<b>Andreas Maier</b> Darmstadt, Germany	<i>"Radon diffusion and solubility"</i>
<b>P24</b>	<b>Martina Matjanovski</b> Neuherberg, Germany	<i>"The CircRNA Interactome – Innovative Hallmarks of the Intra- and Extracellular Radiation Response"</i>
<b>P25</b>	<b>Johann Matschke</b> Essen, Germany	<i>"Adaptation to chronic cycling severe hypoxia involves reprogramming of cancer cell metabolism and increases radiation resistance of cancer cells"</i>
<b>P26</b>	<b>Eric Neuhaus</b> Tübingen, Germany	<i>"Tumor treating fields (TTF) perturb the calcium signaling of glioblastoma cells"</i>

<b>P27</b>	<b>Lily Nguyen</b> Munich, Germany, Institute of	<i>"Generation of radioresistant pancreatic cancer cell sub-lines by fractionated radiation"</i>
<b>P28</b>	<b>Zacharenia Nikitaki</b> Wien, Vienna, Austria	<i>"Primary DNA damage induced by high energetic protons"</i>
<b>P29</b>	<b>Antonio Pantelias</b> Athens, Greece	<i>"Development of a micro-PCC assay as a rapid and minimally invasive automatable approach for early triage biodosimetry"</i>
<b>P30</b>	<b>Veronika Paštyková</b> Prague, Czech Republic	<i>"In vitro study of dose rate effect on Leksell Gamma Knife Perfexion"</i>
<b>P31</b>	<b>Eszter Persa</b> Budapest, Hungary	<i>"Ionizing radiation effects on activation of dendritic cells in mice"</i>
<b>P32</b>	<b>Daniel Piehlmaier</b> Neuherberg, Germany	<i>"In-vitro and in-vivo characterization of a glioblastoma cell line panel"</i>
<b>P33</b>	<b>Felicitas Rapp</b> Darmstadt, Germany	<i>"Leukocyte adhesion to (primary) endothelial cells after pro-inflammatory stimulation and can be reduced by photon and charged particle exposure"</i>
<b>P34</b>	<b>Treewut Rassamegevanon</b> Dresden, Germany	<i>"Heterogeneous radiation response determined with a <math>\gamma</math>H2AX foci assay in human head and neck squamous cell carcinoma (hHNSCC) tumor models"</i>
<b>P35</b>	<b>Oliver Reiners</b> Düsseldorf, Germany	<i>"Non-cell-autonomous contribution to cancer cell radio susceptibility"</i>
<b>P36</b>	<b>Kai Rothkamm</b> Hamburg, Germany	<i>"Molecular targeting of the DNA damage response as a novel approach to deintensify the therapy of HPV-positive HNSCC"</i>
<b>P37</b>	<b>Michael Rückert</b> Erlangen-Nürnberg, Germany	<i>"Immunological basis of abscopal antitumor responses induced with radio-immunotherapy"</i>
<b>P38</b>	<b>Ludmila Schneider</b> Neuherberg, Germany	<i>"A prognostic signature of four 16q24.3 genes in radiotherapy-treated head and neck squamous cell carcinoma (HNSCC)"</i>
<b>P39</b>	<b>Tatsuya Shimasaki</b> Kumamoto, Japan	<i>"Radiation dose estimation by ESR dosimetry with tooth enamel for residents of Nagasaki and Fukushima in Japan"</i>

<b>P40</b>	<b>Alizee Steer</b> Essen, Germany	<i>"Divergent roles of fibroblasts on the radiation-response of cancer cells"</i>
<b>P41</b>	<b>Marin Terashima</b> Hokkaido, Japan	<i>"Verification of size-specific dose estimate (SSDE) calculation method for truncated X-ray CT images"</i>
<b>P42</b>	<b>Andreas Thomsen</b> Freiburg, Germany	<i>"Modelling pancreatic cancer cell interaction with tumor stroma in 2D vs. 3D culture - relevant implications for choice of model"</i>
<b>P43</b>	<b>Charlot Vandevoorde</b> Somerset West, South Africa	<i>"RBE variation as a function of depth in proton therapy: How to correlate microdosimetry with biological observations?"</i>
<b>P44</b>	<b>Christina Vasileiou</b> Athens, Greece	<i>"Detection of biomarkers after exposure to low doses of ionizing radiation through microarray data analysis and meta-analysis"</i>
<b>P45</b>	<b>Julia Wiedemann</b> Darmstadt, Germany	<i>"Establishment of a psoriatic skin model for radon treatment"</i>
<b>P46</b>	<b>Ryota Yamada</b> Hokkaido, Japan	<i>"Modelling of oxygen enhancement ratio in consideration of LET and oxygen partial pressure by using a microdosimetric-kinetic model"</i>

# Long-term radiation exposure from interventional cardiology procedures induces endothelial damage

**Ghassan Al-Massarani**, F. Najjar and M. Othman

*Atomic Energy Commission of Syria, Damascus, Syria*

Background: There has been a growing body of evidence on the adverse health effects of occupational exposures to ionizing radiation from interventional cardiology procedures. However, the association between occupational dose levels (<500 mSv) and late cardiovascular risks is still controversial. There are several mechanisms by which ionizing radiation may affect cardiovascular function. A plausible hypothesis is that endothelial damage caused by long-term radiation exposure may accelerate cardiovascular diseases. We therefore investigated if chronic occupational exposure in interventional cardiologists (ICs) could induce endothelial damage by measuring circulating endothelial cells (CECs) as a non-invasive biomarker. Methods: The study population comprised 45 ICs (72% male; age,  $36 \pm 8$  years) and 20 age- and sex-matched unexposed subjects (75% male; age,  $33 \pm 10$  years) considered as a control group. All participants received a complete assessment of health status by structured medical questionnaire including health history, lifestyle, habits, and medications used. Peripheral blood samples have been withdrawn from all participants to quantify circulating endothelial cells (CECs) using immunomagnetic separation (IMS) technique. Results: The amount of CECs in ICs were significantly higher than in control samples ( $105 \pm 84$  vs  $42 \pm 22$  cells/ml,  $p = 0.004$ ). However, there was no significant difference in the proportion of other blood elements (erythrocytes, leukocytes, granulocytes and platelets) in ICs compared to those of the healthy controls. Data analysis revealed that there was no significant correlation between CEC values and the other variables studied in ICs (age, sex, body mass index and smoking degree). Conclusion: This study shows that long-term radiation exposure in a cardiac catheterization laboratory is associated with increased CECs levels. However, long-term follow-up of these workers must be conducted to investigate whether CEC could be an early indicator of cardiovascular disease caused by long-term occupational exposure to ionizing radiation. Key words: Radiation exposure, circulating endothelial cells, interventional cardiologists.

## Modulation of immune responses and radioresistance by neuroblastoma-derived and host-derived TrkB-target Galectin-1

**Katharina Batzke**, G. Büchel, J. Schulte, L. Harrison, W. Hansen and A. Schramm

*Molecular Oncology, University Hospital Essen, Germany*

Neuroblastoma is the most common solid body tumor in childhood and is derived from primitive cells of the sympathetic nervous system. High expression of the neurotrophin receptor TrkB together with amplification of the MYCN oncogene defines the most aggressive form of neuroblastoma. Chemotherapy and external beam radiation constitute the backbone therapy of high-risk neuroblastoma, while TrkB expression has been linked to chemoresistance before. We previously identified the multifunctional protein Galectin-1 (Gal-1) as a target of TrkB to promote angiogenesis and invasiveness. In a murine neuroblastoma model, TH-MYCN, Gal-1 gene dosage did not affect tumour incidence. However, targeted Gal-1 gene disruption (Gal-1 ko/ko) correlated with both, impaired tumour angiogenesis as well as impaired T cell tumour infiltration, respectively. Furthermore, Gal-1 deficient CD4<sup>+</sup> T cells presented with reduced migratory activity, consistent with splenomegalies observed in double transgenic, TH-MYCN; Gal-1 ko/ko mice. In neuroblastoma cell lines, upregulation of TrkB and Gal-1 was induced by ionizing radiation as revealed by qPCR and Western Blot analyses. However, downregulation of Gal-1 using Gal-1 specific shRNA in murine neuroblastoma cell lines did not affect clonogenic cell survival upon ionizing radiation. These results are in line with a paracrine rather than an autocrine role of Gal-1 in modulating responses to radiotherapy. Interfering with Gal-1 functions in vivo will inform about the TrkB/Gal-1 axis in response to radiation and to contribute to a better understanding of the complex tumour-host interaction during chemo- and radiotherapy of neuroblastoma.

# Advancements in the Cytokinesis-Block Micronucleus assay as a biodosimetry tool using imaging flow cytometry

**Lindsay A. Beaton-Green**, M. A. Rodrigues, S. Lachapelle and R. C. Wilkins

*Environmental and Radiation Health Sciences Directorate, Ontario, Canada*

**Introduction:** In the case of a large-scale RN emergency, biodosimetry is an important tool for the triage of potentially exposed individuals. The biodosimetry toolbox consists of numerous assays, each with corresponding sensitivities and applications [1]. Of these, the Cytokinesis-Block Micronucleus (CBMN) assay is a well-established cytogenetic technique for estimating whole body doses of radiation in peripheral blood lymphocytes based on the frequency of micronuclei (MN) in binucleated cells [2]. The assay is traditionally scored by manual microscopy, but this process is time-consuming, tedious and can be error-prone due to scorer fatigue and bias. These problems are exacerbated when very large numbers of individuals must be screened for exposure. Imaging flow cytometry (IFC) is a rapidly developing technology in which cellular images are captured for each event that passes through the point of interrogation. Our group has successfully modified and adapted the microscope-based CBMN assay to the ImageStreamX® (ISX) (CBMN-ISX). Improvements in IFC software as well as sample processing methods have allowed for further optimization of both the sample-throughput and assay specificity. **Materials and Methods:** Whole blood collected from healthy, volunteers was irradiated with 250 kVp X-rays between 0-4 Gy, at a dose rate of 1.3 Gy/min. Whole blood (20 µL to 2 mL) was cultured in RPMI culture medium containing 10% fetal bovine serum, 2 mMol L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin and 1% PHA to stimulate cells to cycle. All cultures were incubated (37° C, 5% CO<sub>2</sub>) for 24 h before the addition of Cytochalasin-B to block cytokinesis. Cultures were then incubated for an additional 48 h before processing for analysis. To prepare the samples for analysis on the ISX, samples were treated using a combination of red blood cell lysing along with lymphocyte swelling, fixing and permeabilization for optimal sample stability and imaging. Intracellular anti-centromere staining was also tested. Samples were stained with a fluorescent DNA stain and images were acquired on the ISX® or the ISX®-Mark II (MilliporeSigma, Etobicoke, ON). **Results and Discussions:** Preliminary results have indicated the feasibility of this technique to provide dose estimates acceptable for triage ( $\pm 0.5$  Gy) in the range 0-4 Gy. Optimization of the assay has included determining the effects of hypotonic treatment to swell the cells for improved imaging, particularly for MN, reducing cytoplasmic staining to eliminate image artifacts and investigating alternative DNA dyes. Furthermore, work has been performed to incorporate the use of additional image information to generate a multi-parameter analysis that incorporates automated scoring of mono- and poly-nucleated lymphocytes. **Conclusions:** Details describing the recent modifications of the CBMN-ISX assay will be presented. This will include improvements in data analysis as well as enhancement of sample processing for improved imaging. The adaptation of the CBMN assay to IFC improves throughput due to rapid sample processing and

## Radon mitigation actions follow-up study

J. Whyte, **Deepti Bijlani**, R. Falcomer, I. Mohammad, J. Kisch, S. Reid, L. Close and R. Rogojan

*Health Canada, Canada*

**OBJECTIVES/BACKGROUND/ISSUE(S):** Radon is a naturally occurring radioactive gas which originates in rock and soil, and is found at varying concentrations in indoor environments. Exposure to high levels of radon over long periods leads to a higher risk of developing lung cancer. The National Radon Program at Health Canada conducted a large national residential survey to estimate the scope of the indoor radon problem in Canadian homes as one of the indicators of the effectiveness of the program. From these long-term (3-month) indoor measurements it was estimated that roughly 7% of Canadians are living in homes that are above the 200 Bq/m<sup>3</sup> radon guideline value. However defining the scope of the problem is only the first step in a national program. It is also important to understand how Canadians act to reduce high radon levels in their homes. Hence, we undertook the first ever national study in Canada to obtain information from Canadians regarding their behaviors towards reducing radon levels within their homes. **DESIGN/METHOD/DESCRIPTION:** Health Canada's National Radon Laboratory collaborated with Prairie Research Associates (PRA) under a Research Ethics Board (REB) submission to attempt to contact nearly 2000 previous subjects whose homes had tested above the Canadian radon guideline to ask them whether or not they had mitigated their high radon levels and to gather data from their responses. Participants who had mitigated were asked a series of questions regarding what steps were taken. They were also offered a free long-term post-mitigation radon test to gather some statistics on the radon reductions achieved since we already had their pre-mitigation radon result from their participation in the initial residential survey. Those who did not mitigate were asked a series of questions regarding why they had not taken action to reduce their indoor radon levels. **OUTPUTS/RESULTS:** Approximately 77% of eligible participants agreed to conduct the telephone survey on their mitigation behaviors. 29% of those who tested above the 200 Bq/m<sup>3</sup> reported having taken some action to reduce their radon levels. A smaller subset of participants who had tested in the 150-200 Bq/m<sup>3</sup> range was also surveyed and 5% of this group reported having mitigated their radon levels. Approximately 90% of participants, who reported having taken actions to reduce their radon levels, opted for the free follow-up long-term indoor radon test. Roughly 70% of participants experienced a reduction in their radon levels. Those who undertook sealing of entry points as their sole method of radon reduction experienced the smallest average radon reductions, roughly 13%. Conversely, those who reported installing an active soil depressurization system experienced the greatest average radon reductions, around 80%. The top two reasons reported for not mitigating were not thinking that their radon levels were a concern followed by the cost of mitigating. These two reasons for not mitigating have also been reported in other mitigation action surveys conducted in other countries. **IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS :** The mitigation rate of 29% reported by survey participants is encouraging for the National Radon Program and in line with international data, however much work remains to be done to educate Canadians to both test and to mitigate their homes.

[deepti.s.bijlani@hc-sc.gc.ca](mailto:deepti.s.bijlani@hc-sc.gc.ca)

# Absolute dose calibration of EBT<sub>3</sub> Gafchromic films

**Pierluigi Casolaro**, L. Campajola, F. Di Capua and L. Manti

*University of Napoli "Federico II" and National Institute for Nuclear Physics, Napoli, Italy*

Radiochromic dosimeter films are a commercial product available in a large number of different types. They can be used in a wide range of dose, fluence and incident radiation for different application fields. They are massively used in medical physics for therapy with particle beams and X and gamma radiations. In this work a full characterization of the performances of EBT<sub>3</sub> Gafchromic films has been accomplished: sensitivity, accuracy, precision, spatial resolution, dose-rate dependence and definition of dynamic range have been studied in depth and results are shown. In particular, the effect of more radiation sources on EBT<sub>3</sub> Gafchromic films has been investigated by means of the following sources: -250 kV X-ray from SIEMENS 250 kV X-ray tube of Physics Department from University "Federico II", Napoli (Italy). -Gamma from <sup>60</sup>Co-source of ISOF-CNR at Bologna (Italy). -Beta from 38 MBq <sup>90</sup>Sr/<sup>90</sup>Y radioactive source. -1 MeV electron beam from ILU-6 LINAC accelerator of Institute of Nuclear Chemistry Technology at Warsaw (Poland). -23 MeV proton beam from Tandem accelerator of LNS-INFN at Catania (Italy), proton beams of 50 MeV and 200 MeV from the cyclotron of Paul Sherrer Institute (PSI) at Villigen (Switzerland). The dose calibration was found to be absolute, that is independent of incident radiation type, energy and dose rate. This important and innovative feature adds up to others already known, making this kind of film a considerable tool for accurate dose assessment in radiotherapy and hadrontherapy applications. The dynamic range of the films was seen to be compatible with the typically demanded doses in medical physics application.



## "Two distinct molecular pathways for the risk of lung adenocarcinoma from smoking and radiation"

**Noemi Castelletti**, JC. Kaiser, GT. Stathopoulos, K. Furukawa and H. Küchenhoff

*Helmholtz Center Munich, Germany*

In addition to smoking ionising radiation is a known carcinogen for lung cancer. Evidence for the combined effect of both agents has been produced for the Life Span Study (LSS) cohort of atomic bomb survivors of Hiroshima and Nagasaki by statistical association using standard models of radioepidemiology [1,2]. However, little is known on the impact of detrimental molecular effects on lung adenocarcinoma incidence. We integrate molecular biology and radioepidemiology by developing mechanistic multi-scale models for the pathogenesis of lung adenocarcinoma. Since molecular measurements of cancer tissue are not available for the LSS we have analyzed biological data on different cohorts: North American patients from The Cancer Genome Atlas [2], Chinese patients [3] and the BATTLE trial [4]. The conceptual model design is motivated by the observation that genetic damage of people with adenocarcinoma can be grouped into two pathways: the EGFRMUT pathway and the KRASMUT-EGFR/KRASWT pathway. Smoking and radiation effects show markedly different patterns: both act on the apoptosisdeath rate of intermediate cells but on different pathways without any interaction. A linear radiation effect has been found in the EGFRMUT pathway and a linear-exponential smoking effect in the KRASMUT-EGFR/KRASWT pathway. Targets of radiation and smoking in different stages of the diseases process are identified based on goodness-of-fit criteria and biological plausibility. From the two pathways mechanistic model risk estimates for lung adenocarcinoma caused by both smoking and radiation are derived.

### References:

- [1] Furukawa et al., Radiation Research, 2010
- [2] Egawa et al., Radiation Research, 2012
- [3] The Cancer Genome Atlas, <http://cancergenome.nih.gov>
- [4] Wu et al., Nature Communications, 2015
- [5] Kabbout et al., Clinical Cancer Research, 2013

# Inflammation-associated genes as biomarkers for monitoring radiation exposure during radiotherapy

**Lourdes Cruz-Garcia**, L. Ponge, P. Widlak and C. Badie

*Radiation Effects Department, Centre for Radiation, Chemical and Environmental Hazards  
Public Health England Chilton, Didcot, Oxfordshire, United Kingdom*

Radiation therapy for cancer treatment has improved in precision and accuracy in the last few decades to reduce unnecessary irradiation of healthy tissue. However, healthy tissue exposure is not completely avoidable. Ionising radiation induces cell damage directly to the cells exposed but also may impact on cells which have not been hit. Non-targeted effects are induced through bystander signals produced by directly irradiated tissue which activate local inflammatory reactions. Hence, radiation-regulated genes involved in inflammatory pathways could be potentially used as bioindicators detecting exposure, assessing the radiation dose received and predict risks of developing radiation toxicity. Therefore, the present study has focused on the analysis of genes involved in inflammatory pathways and their response to radiation exposure in cancer patients treated with radiotherapy for two types of solid tumors (head and neck cancers and prostate), to discover inflammation-related biomarkers associated with radiation exposure. For this aim, cancer patients treated either with IMRT or Cyberknife were recruited. Blood samples from these patients were collected at 4 time points (before/ during/at end of treatment and a month afterwards). Gene expression analyses of already validated biomarkers involved in cell stress were performed in those patients to monitor transcriptional radiation response. Genes involved in inflammatory pathways were analysed using the nCounter Analysis System (Nanostring technologies) to screen hundreds of unique transcripts simultaneously. Our results confirmed the upregulation of expression in vivo of previously validated biomarkers ex vivo. Moreover we identified common inflammatory markers regulated by radiation exposure for the IMRT treated groups and interestingly also specific markers for each group. At this stage we hypothesize that different tumour locations and the type of treatment could be triggering different types of inflammatory responses, providing specific radiation-related inflammatory markers for each group. In conclusion, radiation exposure in vivo modulates inflammation-related genes which can be detected in blood samples at short and long-term post-exposure.

***Lourdes.CruzGarcia@phe.gov.uk***

# Differential Response to Proton versus Photon Radiotherapy - Biological implications for new indications and combined treatment concepts

**Simon Deycmar** and M. Pruschy

*Laboratory for Applied Radiobiology, Department of Radiation Oncology, University Hospital Zurich, Switzerland*

More than 50% of all patients diagnosed with solid tumors currently receive radiotherapy alone or in combination with surgery and/or chemotherapy. The most common form of radiotherapy used in clinics employs high energy linear accelerators (LINACs) to generate and precisely target a photon beam to the tumor mass. On the other hand, particle beam therapy, especially proton based radiotherapy, as used at the Paul Scherrer Institute, has evolved in the past decades from a niche treatment to a valid option. A 10% higher efficacy of proton versus photon irradiation is already implemented in the clinics even though little is known about the radiobiology of the relative biological effectiveness (RBE). Since this RBE can significantly vary in different tissues, cells or end points, a deeper look into the underlying mechanism for proton induced cell killing is inevitable. Previous research identified a differential requirement for the two major DNA double strand break (DSB) repair machineries in response to the two mentioned types of ionizing radiation. Thus, genetically defined cells with altered backgrounds of non-homologous end joining (NHEJ) or homologous recombination (HR) will be investigated on their response to the respective irradiation. Results gained from in vitro experiments will then be converted to an in vivo setting of inducible repair deficient xenografts to draw a correlation between genetic background and the preferable irradiation or combined chemoradiotherapy approach.

# Dynamic alterations in a miRNA signature of known prognostic value upon irradiation of glioblastoma cell lines

**Daniel Fleischmann**, A. Nieto, D. Piehlmaier, C. Belka, H. Zitzelsberger, K. Lauber, M. Niyazi and K. Unger

*Department of Radiation Oncology, LMU Munich, Munich, Germany*

Background: Previously, we have identified and validated a prognostic signature of four miRNAs (hsa-let-7a-5p, hsa-let-7b-5p, hsa-miR-125a-5p and hsa-miR-615-5p) in formalin-fixed paraffin-embedded (FFPE) tissue of a retrospective glioblastoma (GBM) patient cohort. In order to understand if and to which extent the signature miRNAs are involved in regulating the response to ionizing irradiation, the current project aims at assessing alterations in the miRNA signature during fractionated and single dose radiotherapy (RT) in seven established human GBM cell lines. Methods: Seven GBM cell lines (A172, LN18, LN229, T98G, U87, U138, U251) were irradiated with five daily fractions of 2 Gy or with a single dose of 10 Gy, and cell pellets were collected 1-8 days after irradiation. Total RNA extraction including small and large RNAs was performed for all samples, and quantitative real-time PCR (qRT-PCR) analyses are currently being performed for the four signature miRNAs and the small nucleolar RNA SNORD 61 as endogenous reference. Results: A total of 672 cell pellets were collected for the seven GBM cell lines. Base line miRNA expression levels were assessed in non-irradiated cells, and qRT-PCR analyses of the irradiated samples are still ongoing. Conclusion: The prognostic miRNA signature found in FFPE tissue of GBM patients was technically validated in GBM cell lines and is currently being assessed for alterations upon single dose and fractionated irradiation. Subsequently, gene expression profiling will be performed in order to identify potential targets of the signature miRNAs and to gain mechanistic insights if and to which extent the miRNAs regulate the radiation response.

## Initial stage of Radiation Induced Gastrointestinal Syndrome and its modification by HDAC inhibitor (Diallyl sulphide)

**Noopur Gupta,** A. Kainthola, A. Singhal, M. Tiwari and PK. Agrawala

*Institute of Medicine and Allied Sciences, New Delhi, India*

Background: Ionizing radiation exposure to GI system causes severe damage to the GI tissues. GI claims to be richest niche of microbes and hence this damage results in development of inflammation & possibilities of bacterial migration to adjacent organs and blood. Objectives: We focused on (a) Studying radiation mediated GI tissue damage (villi and crypts damage). (b) Establishing correlation between change in physiology of GI tract & microbiota alteration. (c) Estimating the efficacy of dietary HDAC inhibitor (Diallyl sulphide) in mitigating radiation effects and thereby checking microbial translocation. Methodology C57BL/6 male mice were used in 4 different groups (a) Radiation alone (7 Gy whole body gamma irradiation (Co60)) (b) Drug alone (c) Radiation plus drug (DAS) and (d) control. Bacterial isolation was done from duodenum, jejunum and ileum part of small intestine & adjacent organs (liver, spleen and mesenteric lymph nodes) to check the translocations at different time points. Alterations in physiology of GI tract were assessed by xylose absorption assay and FITC dextran assay for permeability test. Metabolism activity of the intestine was assessed by small intestine transit (SIT) assay and correlated with histological analysis (villi and crypts damage). Results: A significant reduction in microflora count in GI tract after 24 hrs of radiation exposure was observed which later recovered back to normal count. The damage in villi and crypts were seen. The translocation of bacterial strain was found on 8th day after radiation exposure on liver (33%), spleen (33%) and mesenteric lymphatic system (66.66%). *Lysinibacillus sphaericus*, and *Pseudomonas mendocina* (aerobic) showed the capability to translocate in the liver and *Escherichia coli* O104 translocated to mesenteric lymphatic system from gut. By mitigating the tissue injury, DAS was observed to control the translocation of bacteria from GI tract to other organs. The normal control value of small intestine transit was  $56.61 \pm 2$  % of the total length of the small intestine. The induction of radiation significantly increased SIT to  $66.6 \pm 2$  % while drug treated group SIT was  $60.8 \pm 2$  % on 3rd day of radiation exposure and became normal till 12th day. Conclusion: HDAC inhibitor DAS has potential capacity to control GI damage caused by radiation, able to maintain the normal microflora count in small intestine. Potentiality to prevent translocation of bacteria to other organs was observed in preliminary results. Further, the role of toll like receptors in the small intestine and their interaction with the microbes needs to be understood to correlate the action of immunity which subside the disease condition as is apparent from our study that even in translocated bacteria, the disease condition was not observed for at least 14 days post radiation.

# Mitigating effects of Trichostatin A against radiation induced damage to male reproductive system and progeny in C57Bl/6 mice

**Teena Haritwal**, M. Jain, P. Bhagwat, S. Parvez and PK. Agrawala

*Institute of Medicine and Allied Sciences, New Delhi, India*

Male reproductive system is highly sensitive to radiation, probably owing to the presence of rapidly proliferating cells besides other factors. The modulating action of Trichostatin A (TSA, a histone deacetylase inhibitor) against radiation injuries has been studied in various systems by our laboratory, but no information regarding male reproductive system is available so far. Objective - The aim of this study is to investigate the radiation induced alteration in testis and epididymal spermatozoa cell, its congenital effects and modification of those by TSA. Material and methods - We hypothesized that Histone Deacetylase inhibitors can minimize the germ cells depletion caused due to irradiation as well as other genotoxic effects of irradiation. Sperm head abnormality assay, cell viability assay of spermatozoa by 0.4% trypan blue, ROS level by flow cytometry, testicular weight, lipid peroxidase, catalase, and glutathione reductase activities were studied at different time points as 1, 3, 5 and 7 weeks of treatment in C57BL/6 mice model. For the same male mice were divided into four groups (i) control (ii) Irradiation only (iii) Drug i.e TSA only (iv) Irradiation + drug. Mice were exposed to a dose of 2 Gy  $\gamma$ -radiation and drug was administered 2 h post-irradiation. Breeding was done at 1, 3, 5 or 7 week post-irradiation time by housing male mice each from all four groups with two untreated virgin female mice for 2 days in individual cages. The litter size, birth weight and sex ratio of pups were also recorded. Results – It was observed that TSA could reduce mortality, motility and injuries of the spermatozoa cells and mitigated radiation-induced abnormalities in the sperms. Lipid peroxidase, catalase, and glutathione reductase activities studied in testes were comparable in control and drug + radiation group while radiation only group was found to have altered values for those parameters. Radiation was found to induce marked damage to germinal cells and disrupt cells in seminiferous tubules that were countered effectively by TSA administration. Reduction in the number of pups was observed in the irradiation only group. There were no pups at the 5 weeks treatment group and the percentage of chromosomal aberration in the bone marrow cells of the pups also remained high. Whereas in drug treated group the pup number increased and became comparable to control. Conclusion - In conclusion, post-treatment with HDAC inhibitors (TSA in this study) countered the germinal cells depletion and spermatozoa injuries caused by irradiation which could have contributed to the increased pup number as well as reduced the genotoxic effects in progeny.

## Semi-automatic analysis of dicentric chromosomes for low-dose biodosimetry

**Carola Hartel**, E. Nasonova, C. Fournier, B. Frey, U. Gaipf and S. Ritter

*GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany*

We have aimed to determine the cytogenetic risk associated with the therapeutic exposure to low doses of (densely or sparsely) ionizing radiation, which are applied during low-dose radiotherapy (LDRT) or radon therapy. The scoring of dicentric chromosomes (DCs) is a standard technique for biological dosimetry and the assessment of genotoxic risk. To determine the cytogenetically equivalent dose, the frequency of DCs in a sample of peripheral blood lymphocytes from an exposed individual is compared to a reference dose-effect curve. DCs can be scored manually; yet, to obtain meaningful results in the low-dose range, analysing a high number of metaphases is essential. Therefore, we use a commercially available software-based image acquisition and semi-automatic analysis system. To establish the reference dose-effect curve, peripheral blood lymphocytes from a healthy donor were exposed in vitro to X-rays (dose range 0-6 Gy). Metaphase spreads were prepared and were stained with Giemsa dye. DC scoring was performed using an Axio Imager Z2 microscope (Zeiss, Germany) and the DCscore/Metafer software (Metasystems, Germany). The automatically detected DCs were reviewed by an experienced scientist and false positives were removed. Only DCs which clearly have the typical shape and are accompanied by an acentric fragment are accepted as true DCs. A dose effect curve for the in vitro induction of DCs in peripheral blood lymphocytes was established from more than 100 000 analysed metaphases. We observed  $1.03 \pm 0.17$  DCs per 1000 analysed metaphases in the unexposed control and  $1.56 \pm 0.20$  in the sample exposed to a dose of 25 mGy (each based on 35 000 analysed metaphases), demonstrating that the exposure to a dose of 25 mGy can be distinguished from the background level. The generated dose-effect curve is now used as a reference for samples from patients undergoing low dose radiotherapy (LDRT). As inter-individual variations in the background level of aberrations may influence the results, especially when low doses are investigated, samples before as well as after therapy are analysed in a longitudinal manner from each patient. First results on the biodosimetry of LDRT will be presented. To determine the cytogenetic risk associated with therapeutic exposure to radon (in the framework of the RAD-ON02 study that is planned to be started in spring 2018) samples from patients undergoing radon treatment will be analysed. As radon gas is an emitter of densely ionizing alpha particles, in vitro reference data from exposure to alpha particles from radon gas and other alpha particle emitters, are required. Alpha-particle irradiated samples are currently under analysis and the results will be presented and compared to the X-ray calibration curve.

Supported by the Federal Ministry of Education and Research (BMBF), GREWIS under contract Nr. 02NUK017A.

[c.hartel@gsi.de](mailto:c.hartel@gsi.de)

# Charged particles for a combination of radio- and immunotherapy

**Alexander Helm**, W. Tinganelli, P. Simoniello, D. K. Ebner, F. Natale, A. Bisio, S. Yamada, T. Kamada, T. Shimokawa and M. Durante

*Trento Institute for Fundamental Physics and Applications, Trento, Italy*

Combined treatment of radiotherapy and immunotherapy is a topic of growing interest due to highly promising clinical results. Ionizing radiation has been found to be immunogenic, i.e. it may induce an immunogenic cell death in tumor cells during treatment which leads to an immune activation that in turn may result in the shrinkage of metastases out of the radiation field; a phenomenon termed abscopal effect. Several clinical cases of such abscopal effect have been reported following radiotherapy including particle therapy. Charged particles are assumed to further increase the immunogenic effect. The ultimate aim is the search for a combined treatment regimen applying charged particles (protons or carbon ions) and a concomitant administration of immunotherapeutics, which boost the immune response and mitigate immune suppressive signaling of radiation. In the framework of a collaboration between NIRS/QST (Japan) and TIFPA (Italy), we seek to set up such combination of particle radiation, namely carbon ions and protons and immunotherapeutic drugs to enhance the abscopal effect in a mouse model. The abscopal tumor model is based on immune competent mice, such as C3H/He mice, which are injected with tumor cells on both hind limbs prior to radiation treatment to allow for tumor growth. While only one tumor is exposed, the other resembles the abscopal tumor. The concomitant application of immunotherapeutics is tested and shrinkage of the abscopal tumor is the central endpoint, along with various quantitative and qualitative investigations of the systemic immune response. We will present preliminary results derived from this model.



## Importance of altered antioxidant defense for radioresistance of hypoxic cancer cells

**Julian Hlouschek**, C. Hansel, H. Riffkin\*, V. Jendrossek and J. Matschke

*Institute of Cell Biology (Cancer Research), University Hospital Essen, Germany*

Introduction: Hypoxia drives resistance to radio- and chemotherapy of solid tumours and goes along with a poor clinical outcome and malignant progression. We recently demonstrated that adaptation to chronic cycling hypoxia involves complex changes in cellular antioxidant defense, mitochondrial function and cell metabolism. Metabolic adaptation to severe hypoxia/reoxygenation stress involved amongst others glutamine-dependent up-regulation of cellular glutathione (GSH) levels leading to reduced basal and oxidative stress-induced cellular reactive oxygen species (ROS) levels. We speculate that targeting increased antioxidant defense of cancer cells will allow to reduce acquired resistance to ionizing radiation (IR) in areas of chronically hypoxic tumours with cycling hypoxia. Aim of the present study is to explore the mechanisms underlying the improved antioxidant defense of anoxia/reoxygenation tolerant (ART) cancer cells and the consequences of therapeutic targeting the mitochondrial antioxidant system for radiosensitivity of cancer cells exposed to acute or chronic cycling hypoxia. Methods: We used qRT-PCR and western blot analysis to validate the expression of the postulated mitochondrial (m) GSH carrier proteins (SLC25A1, SLC25A10, SLC25A11) in ART and control cells under normoxic (20 % O<sub>2</sub>) and severely hypoxic conditions (0.2 % O<sub>2</sub>). Consequences of pharmacologic inhibition of postulated mGSH carrier proteins by treatment with butylmalonate (BMA), benzene-1,2,3-tricarboxylate (BTA) and phenylsuccinate (PSA) (1-20 mM) alone or in combination with IR (0 – 10 Gy) was determined by short-term proliferation, apoptosis and cell death assays as well as long-term colony formation assays. Furthermore, GSH levels in different cell fractions were quantified upon exposure to the above-mentioned conditions. Results: We demonstrate that exposure to severe hypoxia or ionizing radiation leads to altered expression of the postulated mGSH carrier proteins. Additionally, inhibition of potential mGSH carrier proteins led to improved accumulation of ROS that was associated with a decrease in the number of viable cells and increased cell death under normoxic and severely hypoxic conditions. Importantly, combined treatment with inhibitors of mGSH uptake and IR sensitized ART cells and control cells to the cytotoxic action of IR. Conclusion: We conclude that mGSH levels contribute to the altered antioxidant defense of cancer cells exposed to chronic cycling hypoxia and impact the cellular radiation response. Our data suggest that pharmacologic inhibition of mGSH transporters may be suited for radiosensitization of cancer cells.

Supported by a grant of the DFG (GRK1739/2)

[julian.hlouschek@uk-essen.de](mailto:julian.hlouschek@uk-essen.de)

# Robustness analysis of the Radosys Radometer-MN Series automated microscope

**Tímea Hülber**, E. Kis, ZS. Kocsis, G. Sáfrány and C. Pesznyák

*Radosys Ltd, Budapest University of Technology and Economics - Department of Nuclear Techniques, Hungary*

Cytokinesis-blocked micronucleus assay (CBMN) is a well-accepted, widely used biodosimetric assay. Although the sample preparation protocol of the method is thoroughly described by international standards, there are uncontrollable factors that influence the visual features of the cells from slide to slide: The lymphocytes taken from various persons can respond slightly differently due to their varying levels of biological stress. These differences may be insignificant during visual scoring of the micronucleus frequency but can strongly influence the performance of an automatic scoring system which heavily depends on the geometrical and staining characteristics of the cells. In our study we identified the possible sample quality features that can influence the accuracy of the dedicated automatic micronucleus (MN) scorer microscope of Radosys Ltd. Additionally, we assessed the range of possible uncertainty of the automatically scored MN frequency caused by the alteration of these sample quality variations. The CBMN was performed on peripheral lymphocytes according to the IAEA protocol. The slides were stained by GIEMSA, scanned and analysed by Radosys Radometer-MN Series. The used blood samples were irradiated in vitro by 2 Gy X- and gamma- rays. We divided the same blood sample into smaller portions and processed them in slightly modified ways (for example: two groups were given to two different laboratories, few different slide pre-cleaning techniques were used, etc.) These alternations did not change the MN frequency but resulted in a set of samples that covers a wider range of sample quality. The effect of the proposed influencing factors (size of cells, cytoplasm and nucleus contrast, blurriness, staining intensity and inhomogeneity, number of artefacts) are determined with the help of the in-built revision function (so-called semi-automatic mode) of the automatic system: The found objects are listed and offered to be accepted or rejected by user. The option to add the missed objects is also possible. The quantity of MN-artefacts (stain residues and cell debris that can be confused with MN) and the size of the cells are found to be the most significant indicators of sample quality. In case of typical samples the detection rate of MN in binucleated cells is 74% $\pm$ 6%. The decrease in cell size results in the non-uniformity of the stain intensity of cytoplasm which reduces the MN segmentation capability (approximately 8% drop in cell size leads to 10% less MN detection rate). The effect of the presence of MN-like artefacts varies on a larger scale. They can change the MN detection rate with 10-40%. The contribution to the uncertainty of the other analysed factors is less than 5%. However the effects of all the examined factors can be compensated using the semi-automatic method. With the help of the interactive revision the semi-automatic MN frequencies for the subsamples of the same blood samples are found to be equal within the statistical uncertainty. In order to help to ensure the ideal work point for the automatic system we specified a set of criteria for sample quality. We also proposed and tested possible techniques (the temperature and surface pretreatment of the slide, type of staining, additional fixing/washing step) that can be used in order to improve sample quality even post-fixation state. This workflow leads to a more uniformed sample quality and a more robust automatic analysis.

## Setup for tumor growth delay studies in small animals for low energy X-rays and small irradiation fields

**Annique Hunger**, K. Burger, A.-K. Porth, K. Achterhold, B. Gleich, E. Beyreuther, F. Pfeiffer, S. Combs, J. J. Wilkens and T. E. Schmid

*Institute of Innovative Radiotherapy, Helmholtz Zentrum München, Neuherberg, Germany;  
Department of Radiation Oncology, Technical University of Munich, Munich, Germany*

**Introduction:** Tumor growth delay is a widely accepted method in experimental animal tumor models for assessment of treatment efficiencies. Most commonly, the tumor growth delay assay measures the growth of subcutaneous xenograft tumors in the hind leg of small animals. However, low energy photon beams and/or very small irradiation fields only allow for short penetration depths. Therefore, a dedicated irradiation setup was tested at the Small Animal Radiation Research Platform (SARRP, Xtrahl Ltd.) which is particularly well-suited for irradiating very small tumors with low energy X-rays. **Methods:** This study was performed with a human head and neck cancer cell line (FaDu). 2,000 FaDu cells per  $\mu\text{L}$  Matrigel® were subcutaneously injected into the right ear of immunocompromised NMRI (nu/nu) mice [1]. Tumors with a size of  $2 \times 2 \text{ mm}^2$  were irradiated with 3 or 6 Gy at the SARRP with 70 kVp X-rays and field size of  $4 \times 4 \text{ mm}^2$ . Tumor growth was determined over a follow-up of 20 days with a caliper. The tumor growth delay was compared between five homogeneously and three non-irradiated mice. 20 days after irradiation a single tumor cell suspension was prepared from xenograft tumors for in-vitro studies. **Results:** The tumor growth delay of homogeneously irradiated mice at the SARRP was determined with respect to a control group, which showed a tumor volume doubling time of  $2.75 \pm 0.4$  days. One mouse out of three showed a clear tumor growth delay at 3 Gy. In contrast, all tumors which were irradiated with 6 Gy were controlled. Tumor cells which were isolated from 3 Gy irradiated FaDu tumors showed normal growth characteristics and morphology after transfer into cell culture medium as compared to the established FaDu cell line. **Conclusion and Outlook:** We successfully implemented a xenograft tumor system in mouse ears and irradiation of FaDu xenograft tumors at the SARRP. This mouse model represents an accurate and simple method to determine the 3D tumor volume. In future, this developed model will be used to study the treatment efficiency of small irradiation fields and/or low X-ray energies. We envisage to use the developed method to investigate microbeam radiation therapy at the first commercially available inverse Compton scattering source in Munich, the Munich Compact Light Source [2].

**References:** [1] Oppelt et al., Radiat Environ Biophys (2015) 54:155-166, [2] Eggli et al., J. Synchrotron Rad. (2016) 23: 1137, Acknowledgements: This work was supported by the DFG-Cluster of Excellence “Munich-Centre for Advanced Photonics”.

# Identification of novel targets for rational chemoradiotherapy strategies in non-small-cell lung cancer

**Sophie Kalmbach**, F. Breitenbücher, S. Nothdurft, A. Schramm and M. Schuler

*Laboratory for Molecular Oncology, Department of Medical Oncology, University Hospital Essen, Germany*

Lung cancer is among the deadliest forms of cancer worldwide, with non-small cell lung cancers (NSCLC) accounting for approximately 85% of all cases. Radiotherapy is an important modality in the curative and palliative treatment of patients with NSCLC. Although the current development of innovative pharmacotherapies for NSCLC is tailored to tumor specific molecular aberrations, clinical radiotherapy still neglects the heterogeneous biology of this disease. We hypothesize that aberrant signal transduction pathways modulate the outcome of radiotherapy for NSCLC and set out to identify and validate novel targets for personalized chemoradiotherapy strategies in NSCLC. Conducting an unbiased functional genomic screen using a lentivirally encoded shRNA library in a human NSCLC model we have nominated several putative modulators of the radiation response in NSCLC. We have generated cell lines with stable shRNA expression for target validation in vivo. Mechanistic studies deciphering the modes of radioresistance and potentially defining additional targets are ongoing. Prevalence of expression and prognostic/predictive value of preclinically validated resistance factors will be studied in our institutional biobank including a large and well-characterized cohort of NSCLC patients treated with chemoradiotherapy. Our studies provide a framework for preclinical development of novel personalized chemoradiotherapy strategies in lung cancer.

## The carbonic anhydrase IX-selective inhibitor VD11-4-2 exhibits low toxicity in zebrafish and diminishes the acidification in *Xenopus* oocytes and cancer cell cultures

**Justina Kazokaite**, R. Niemans, A. Yaromina, A. Aspatwar, S. Parkkila, J. Deitmer, H. Becker, P. Lambin, L. J. Dubois and D. Matulis

*Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Vilnius University, Lithuania; Department of Radiation Oncology (MAASTRO Lab), Maastricht University Medical Centre, Netherlands*

Human carbonic anhydrase (CA) IX is one of the major players in the regulation of acid/base balance during the tumor progression in hypoxic milieu. Thus, CA IX has emerged as a promising anticancer drug target and an important biomarker for a broad range of hypoxic tumors. The recently developed compound VD11-4-2 was selected from our library comprised of over 700 CA IX-targeting compounds because VD11-4-2 showed high selectivity and affinity for CA IX in the picomolar range as confirmed by biophysical binding assays (fluorescent thermal shift assay and isothermal titration calorimetry) and the inhibition of CO<sub>2</sub> hydration. The inhibitor was investigated in 3 different model systems: zebrafish, *Xenopus* oocytes, and 2D monolayer human cancer cultures. VD11-4-2 did not lead to morphological changes on the zebrafish embryonic development and reached the IC<sub>50</sub> of 10 nM for heterologous CA IX expressed in *Xenopus* oocytes. In addition, the efficacy of the inhibitor was assessed in cervical (HeLa), breast (MDA-MB-231), and lung (A549, H460) carcinoma lines. The impact of VD11-4-2 on the hypoxia-induced intracellular and extracellular acidosis was evaluated. Western blot analysis and immunofluorescence microscopy demonstrated higher CA IX protein expression levels in response to hypoxia in HeLa, H460 and MDA-MB-231 cells as compared to A549 cells. Therefore, cell density and cell culture media for pH measurements was optimized for each cell line. A 4-fold lower concentration of sodium bicarbonate in the media for A549 was used to determine functional effects of the compound. VD11-4-2 (1  $\mu$ M) significantly reduced the rate of change in intracellular pH of MDA-MB-231 cells to around 60%. Moreover, VD11-4-2 significantly reduced hypoxia-induced extracellular acidification of HeLa, H460, MDA-MB-231 and A549 cells in a dose-dependent manner. This functional effect was the most pronounced in HeLa cells in which 50  $\mu$ M VD11-4-2 showed more than 50% efficiency. Thus, VD11-4-2 appears to be a promising agent for CA IX-specific therapy. Studies of the localization of compound in the cell as well as the effects of compound on H460 spheroid growth are currently under investigation. In addition, VD11-4-2 has been conjugated with metronidazole to determine if VD11-4-2 could increase the impact of irradiation on cancer cell proliferation. The effects of this derivative in combination with 0-4 Gy radiation doses on the clonogenicity of HeLa cells are currently studied.

# Caveolin 1-deficiency in stromal fibroblasts mediates radiation resistance in human PC3 xenografts

**Julia Ketteler**, A. Panic, H. Reis, A. Sak, C. Herskind, P. Maier, H. Rübben, V. Jendrossek and D. Klein,

*Institute of Cell Biology, University Hospital Essen, Germany*

Although there are good treatment options for benign prostate carcinoma, advanced stages still display high therapy resistance to radio- and chemotherapy. The membrane protein caveolin-1 (Cav1) functions here as an important oncogene. A characteristic switch in Cav1 expression occurs where prostate epithelial cells gain Cav1 expression in advanced and metastatic cancer stages whereas a decrease of stromal Cav1 can be observed in fibroblasts of the tumor microenvironment at advanced stages. Here we investigated the influence of differential Cav1 expression levels on the radiosensitivity of prostate carcinoma cells as well as the influence of stromal Cav1 expression on the radiation response of prostate carcinoma cells in vitro and in vivo. Cav1-silenced PC3 [PC3 Cav1(-)] showed an increased sensitivity to IR cells in vitro, while Cav1(-) fibroblasts showed increased radioresistance. In order to study the influence of stromal Cav1 for the radiation response of prostate carcinoma cells we started with an indirect approach using cell culture supernatants (SN) derived from the differentially Cav1 expressing HS5 fibroblasts. Here, epithelial PC3 cells cultured with HS5 Cav1(-) SN resulted in an increase in radioresistance. This results could be confirmed in vivo by implanting PC3 Cav1(-) cells with differential Cav1-expressing fibroblasts. Tumors derived from PC3(-)HS5(-) cells showed significantly increased growth after IR and thus an increase in radioresistance as compared to tumors derived from PC3(-)HS5(+) cells. Indication of radioresistance in advanced tumor stages could also be demonstrated by analyzing human prostate tissue for Cav1 and reactive stroma markers. Conclusively, the radiation response of human prostate tumors is critically regulated by Cav1 expression in stromal fibroblasts. Loss of stromal Cav1 expression in advanced tumor stages may thus contribute to resistance of these tumors to radiotherapy.

Supported by a grant of the DFG (GRK1739/2)

# KCa3.1 channels confer radioresistance to mesenchymal glioblastoma stem cells

**Lukas Klumpp**, EC. Sezgin, B. Stegen, D. Zips and S. Huber

*Department of Radiation Oncology, University of Tübingen; Dr. Margarete Fischer-Bosch-Institut of Clinical Pharmacology, Stuttgart, Germany*

Several tumor entities including brain tumors aberrantly overexpress intermediate conductance  $\text{Ca}^{2+}$  activated KCa3.1  $\text{K}^{+}$  channels. These channels contribute significantly to the transformed phenotype of the tumor cells. Moreover, KCa3.1 channels have been shown to confer resistance against radiotherapy suggesting KCa3.1 channels as promising new targets of future anti-cancer therapies. Here, we analyzed in glioblastoma cell lines and primary glioblastoma cells cultivated under stem cell growth promoting conditions KCa3.1 expression in subpopulations of glioblastoma stem cells, the radiation-induced upstream- and downstream signaling of these channels and their functional significance for DNA repair and clonogenic survival by real-time RT-PCR, immunoblotting, fura-2  $\text{Ca}^{2+}$ -imaging, patch-clamp recording, flow cytometry, immunofluorescence microscopy, and colony formation assay, respectively. As a result, KCa3.1 channel mRNA abundance correlated positively with the expression of the mesenchymal stem cell marker ALDH1A3 (pearson correlation coefficient = 0.82) and negatively with that of the proneural stem cell marker nestin (PCC = -0.63). Radiation (2 Gy with 6 MV photons) induced an increase in cytosolic free  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) that was paralleled by elevated KCa3.1 channel activity. Inhibition of KCa3.1 modulated radiation-induced cell cycle arrest and radiosensitized the glioblastoma cells in dependence on KCa3.1 expression. In particular, DNA double strand break repair of irradiated mesenchymal stem cell-enriched primary cell culture was impaired by KCa3.1 inhibition while that of low ALDH1A3-expressing cells was not. Combined, these data suggest that radiation stimulates KCa3.1  $\text{K}^{+}$  channel activity in particular in the mesenchymal subgroup of glioblastoma stem cells. Electrosignaling by these channels controls cell cycle, DNA repair and confer radioresistance. Since mesenchymal stem cells have been suggested to play an important role in therapy resistance of glioblastoma, KCa3.1 channels may be a promising target for eradication of these cells. Acknowledgement: LK is supported by the ICEPHA program of the University of Tübingen and the Robert Bosch Foundation for Medical Research, Stuttgart, and ECS by a DAAD scholarship.



# Patient-derived HNSCC cell lines and subclones as model systems to analyze tumor heterogeneity and radiosensitivity

**Olena Klymenko**, P. Baumeister, H. Zitzelsberger, U. Schötz, J. Heß, C. Belka and K. Lauber

*Department of Radiation Oncology, Ludwig-Maximilians-University Munich, Germany*

Head and neck squamous cell carcinomas (HNSCCs) account for approximately 90% of all head and neck neoplasms. Risk factors include tobacco use and alcohol consumption, and a novel subgroup associated with high-risk human papilloma virus infection is constantly on the rise. Current standard therapy of HNSCC involves radio(chemo)therapy in definitive settings as well as adjuvant to surgery. However, 5-year survival rates remain limited to 45-50% due to therapeutic failure. In this regard, the issue of intratumoral diversity and heterogeneity is receiving increasingly more interest, as sub-populations of tumor cells with distinct characteristics are considered to be responsible for therapy resistance. The present study was designed to establish a panel of patient-derived HNSCC cell lines with individual subclones and to subject them to molecular characterization and functional analyses of radiosensitivity. Several epithelial markers, including pan-Cytokeratin, EpCAM, EGFR, and CD44 were examined by immunofluorescence and flow cytometry. Expression of fibroblast-specific protein was measured in order to exclude contamination with tumor-associated fibroblasts. Moreover, clonogenic survival upon irradiation with single doses of 0-8Gy and a fractionated regimen of 4x 2Gy was analyzed. The established primary cell cultures and their sub-clones exhibit long-term proliferation capacity, and thus can be considered as immortalized. We observed clear inter-and intra-individual differences in epithelial marker expression and in clonogenic survival upon different irradiation regimens, suggesting that intratumoral heterogeneity can be reflected by these cell culture models – at least to a certain extent. We plan to further continue the establishment of new patient-derived HNSCC cell lines with individual subclones, and we want to characterize them by unbiased screening approaches, in order to find potential predictive and/or prognostic markers for individual radiosensitivity.



## New strategies to mitigate accidental radiation-induced hematopoietic syndrome : Reprogramming as a new frontier

**Clelia Le Gallic**, D. Riccobono, M. Valente, N. Jullien, N. Guitard, G. Cosler, C. Chargari, M. Drouet and S. Francois

*French Armed Forces Biomedical Research Institute (IRBA), Bretigny-sur-Orge cedex, France*

Following exposure to high doses of ionizing radiation (accident or nuclear attack), hematopoietic syndrome (HS) represents the first therapeutic challenge. Medical management would consist in early G-CSF treatment to stimulate residual hematopoiesis or allogeneic stem cell grafting if bone marrow has been severely depleted. However, regarding mortality and morbidity, unsatisfactory concern remains. Thus, identifying new drugs and developing new strategies remain priorities, especially in order to significantly raise up the hematopoietic stem and progenitors cells transplantation threshold and to cope with mass casualties scenario. Synthetic small interfering ribonucleic acids (siRNAs) may represent a new approach complementary to Emergency anti-apoptotic CytoKinothrapy. In order to in vivo mitigate RI-apoptosis, we set up a mouse model in which B6D2F1 mice were lethally irradiated (9 Gy gamma, LD90% 30 days) and then injected at 2 hours following irradiation with siRNA directed towards p53 gene. Preliminary results confirm that the efficacy of such strategy will depend on optimized cell penetration and rigorous gene(s) target(s) selection. As a second approach of our group has proposed the transient gene therapy based on short term secretion of morphogene(s) to stimulate residual stem cells and favor microenvironment repair. We investigated the hematopoietic response in rhesus monkey (n=4) to a single intra-osseous injection of xenogenic multipotent mesenchymal stem cells (ASCs) transduced with a Sonic-hedgehog (Shh) pIRES2 plasmid and grafted 2 days after a 8-Gy gamma irradiation. Thrombocytopenia and neutropenia duration were reduced in Shh-ASCs grafted animals when compared with mock-ASCs grafted controls (n=4). Areas under the curve (AUC) of platelets and polymorphonuclear cells between days 0 - day 30 and of hemoglobin between D0-D90 were higher. Finally, direct reprogramming of adult fibroblasts extrapolated from the Oct 4 Bahtia technology is under evaluation. The goal is to accelerate and amplify the production of a cell drug with a high hematopoietic potential in an autologous approach. To conclude, optimizing the accidental HS treatment in the future could be achieved by applying multiple complementary approaches.

## Radon diffusion and solubility

**Andreas Maier**, U. Weber, A. Hinrichs, G. Kraft and C. Fournier

*GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany*

In order to understand the interaction of radon with human organs, especially its anti-inflammatory effects, the knowledge of the permeation i.e. the diffusion and solubility of radon gas in different biological material is essential. For determining these parameters, a special setup was constructed and installed inside our radon exposure chamber where an  $\alpha$ -particle detector in a metal box is separated from the surrounding radon containing atmosphere. In this set-up, radon can diffuse only through a window containing the test sample and will reach the  $\alpha$ -particle detector, where the 5.49 MeV  $\alpha$ -decay of the penetrating radon is monitored as function of the diffusion time. In contrast to normal  $\alpha$  spectroscopy, the spectrum is broadened due to the different energy losses for the various path length in air of the isotropically emitted alphas and decreased solid angles for the larger penetration depth. In the experiments, the increase in time of the integral radon signal is measured after injection of a known radon activity concentration in the radon exposure chamber. By analyzing the time behavior of these values, the diffusion coefficient and the solubility of the sample can be obtained. First measurements with thin polyethylene foils (LDPE) were intended as proof of concept and showed agreement with data from literature. As a next step we will focus on biological samples like different fatty acids or saline solution and will continue to biological material like bone or fat. In our presentation we want to introduce the method, show our current results and give an outlook for future work. Work is supported by the German Federal Ministry of Education and Research (BMBF), project funding reference number 02NUK017A.

## The CircRNA Interactome – Innovative Hallmarks of the Intra- and Extracellular Radiation Response

**Martina Matjanovski**, J. Smida, S. Moertl, C. Brockhaus, K. Winkler, SV. Ovsepiyan, MJ. Atkinson and V. Bríd O'Leary

*Institute of Radiation Biology, Helmholtz Zentrum Munich - German Research Center for Environmental Health, Neuherberg, Germany*

CircRNA are a recently discovered novel type of non-coding RNAs (ncRNAs) that may act as potential post-transcriptional regulators. Generated by backsplicing of pre-mRNA in a process involving Quaking (QKI), circular RNAs (circRNAs) are characterized by tissue specificity, increased stability and enrichment within exosomes. Studies have shown that ionizing radiation (IR) can influence ncRNA transcription. However, it is unknown whether circRNAs or indeed QKI are regulated by IR. We have identified a number of circRNA species regulated by radiation response. Microarray circRNA profiling and next generation sequencing revealed that circRNA expression was altered by low and medium dose exposure. CircRNAs hsa\_circ\_0040573 and hsa\_circ\_0040571 transcribed from the WWOX (WW Domain Containing Oxidoreductase) tumor suppressor responded within hours to IR and were present in exosomes. Dualquasar labelled probes and in-situ hybridization demonstrated the intracellular distribution of hsa\_circ\_0040573 and hsa\_circ\_0040571 predominantly within the perinucleus. QKI knockdown removed nuclear expression of these circRNAs with no significant effect on cytosolic hsa\_circ\_0040573 and hsa\_circ\_0040571. Distinct QKI transcription between cell lines and its augmented interaction with circRNA was noted post IR. This foremost study provides evidence that QKI and circRNAs partake in the cellular irradiation response. Hsa\_circ\_0040573 and hsa\_circ\_0040571 as stable secreted circRNAs may afford vital characteristics worth syphoning as promising diagnostic radiotherapy biomarkers.

# Adaptation to chronic cycling severe hypoxia involves reprogramming of cancer cell metabolism and increases radiation resistance of cancer cells

**Johann Matschke**, T. Shlomi, L. Klein-Hitpass, H. Riffkin\*, J. Rudner and V. Jendrossek

*University Hospital Essen, Institute of Cell Biology (Cancer Research), Essen, Germany*

Introduction: Hypoxia-mediated resistance of solid tumors to ionizing radiation is a major obstacle to successful radiotherapy. We showed previously that chronic cycling hypoxia drives the evolution of anoxia/reoxygenation-tolerant (ART) cancer cells with increased resistance to ionizing radiation. Radiation resistance of ART cancer cells was associated with complex metabolic reprogramming (Matschke et al., Antioxid Redox Signal 2016,25:89-107; Matschke et al., Radiat Oncol 2016,11(1):75). Aim of the present study was to gain a more comprehensive understanding of the metabolic adaptation of cancer cells exposed to acute or chronic severe hypoxia/reoxygenation and to systematically explore opportunities for targeted pharmacologic intervention based on their suspected specific metabolic needs upon irradiation. Methods: We compared gene expression profiles of anoxia/reoxygenation-tolerant (ART) cancer cells and control cells by microarray analysis under normoxic or severely hypoxic conditions and validated genes of interest by qRT-PCR. We used LC-MS based high-throughput metabolomics, nutrient deprivation and drugs interfering with glycolysis or oxidative phosphorylation to characterize the cellular metabolic state. Cell function was determined by measuring cell viability, apoptosis and cell death. Furthermore, we investigated the mitochondrial phenotype of control cells and ART NCI-H460 cells under the above-mentioned conditions. Results: Our microarray data revealed that adaptation of NCH-H460 lung adenocarcinoma cells to acute or chronic cycling hypoxia involved complex changes in the expression of genes associated with glycolysis and oxidative phosphorylation. Nutrient deprivation studies showed a pronounced dependency of NCI-H460 cells of glucose for their short-term survival in acute or chronic severe hypoxia after IR. Furthermore, tolerance to severe hypoxia/reoxygenation stress was associated with the formation of enlarged mitochondria in ART NCI-H460 cells exposed to severe hypoxia. In line with these findings, functional analyses revealed that targeting glycolysis or oxidative phosphorylation with inhibitors of glucose uptake (Phloretin), hexokinase (2-Deoxy-glucose; Bromopyruvate) and Complex-I (Rotenone) sensitized particularly ART NCI-H460 cells to apoptotic cell death in severe hypoxia. The analysis of metabolic alterations in irradiated cancer cells by LC-MS based high-throughput metabolome analysis demonstrated a high and time-dependent need of irradiated cancer cells in glutamine and glucose. Conclusion: Metabolic reprogramming allows cancer cells to adapt to acute and chronic severe hypoxia/reoxygenation stress and to meet metabolic needs for survival upon exposure to ionizing radiation. Specific metabolic requirements under stress conditions such as severe hypoxia or irradiation render cancer cells vulnerable to metabolic inhibitors alone and in combination with ionizing radiation in a context and cell type-dependent manner.

Supported by grants of the DFG (GRK1739/1) and the Deutsche Krebshilfe (110355).

[johann.matschke@uk-essen.de](mailto:johann.matschke@uk-essen.de)

## Tumor treating fields (TTF) perturb the calcium signaling of glioblastoma cells

**Eric Neuhaus**, L. Zirjacks, U. Schöler, D. Zips and S. Huber

*Department of Radiation Oncology , University of Tübingen, Tübingen, Germany*

Tumor treating fields (TTF) represent a novel FDA-approved treatment modality for patients with newly diagnosed or recurrent glioblastoma multiforme. This therapy applies intermediate frequency alternating electric fields with low-intensity to the tumor volume by the use of non-invasive electrodes. A prospective clinical trial suggests a significant TTF-associated increase in median survival of glioblastoma patients. Despite these clinical data, the benefit of the TTF therapy is highly controversially debated probably since the molecular mechanism of a potential TTF interference with the glioblastoma biology is not well understood. TTF has been proposed to impair the formation of the mitotic spindle or to interfere with cytokinesis eventually causing cell death of fast proliferating tumor cells. In order to identify further potential molecular targets, here the effects of TTF on calcium-signaling and conductance of the plasma membrane were tested in human glioblastoma cells in vitro. To this end, a TTF-in-vitro-applicator was developed and connected to a function generator. Attached to the stage of an inverted microscope, the TTF-in-vitro-applicator allowed application of electromagnetic sine waves of variable amplitude and frequency to individual cells. Intracellular free calcium concentration (free [calcium]<sub>i</sub>) and membrane currents were recorded by Fura-2 calcium imaging and patch-clamp on-cell measurements in U251 and T98G glioblastoma cells, respectively. The expression of voltage-gated calcium (Cav) channels was determined by real-time RT-PCR and the effect of knock-down of the most abundant of these channels analyzed. As a result, acute application of TTF (200 kHz, 0.25-2.5 V/cm, 1-3 min) to U251 and T98G glioblastoma cells induced a long-lasting increase in free[calcium]<sub>i</sub> in an electric field intensity-dependent manner. This TTF-stimulated rise in free [calcium]<sub>i</sub> was observed in calcium-containing but not in calcium-free extracellular solution suggestive of a TTF-induced calcium-influx through generated by a calcium permeability in the plasma membrane. In accordance to this assumption, TTF (200 kHz, 2.5 V/cm, 1-2 min)-induced an increase in outward currents in patch-clamp on-cell recordings which were generated by channels with typical characteristics of calcium-activated BK K<sup>+</sup> channels. Furthermore, benidipine (1 μM), a non-specific inhibitor of voltage gated calcium-channels, reversibly prevented the TTF-induced calcium-influx suggesting the involvement of Cav channels. The RT-PCR data confirmed a high abundance of CACNA1C mRNA in both, T98G and U251 glioblastoma cells which was 5-10 times higher than the other Cav channels analyzed (CACNA1A, -1B, -1D, -1C, -1H, -1I, -1S). Knock-down of CACNA1C by RNA interference resulted in both, decreased steady state free cytosolic calcium concentration and lowering of the TTF-induced calcium-influx. In summary, this preliminary data suggest that TTF therapy targets calcium- and electrosignaling of glioblastoma cells. CACNA1C which encodes for the benidipine-sensitive cardiac and smooth muscle L-type Cav1.2 channel is one molecular TTF target in the plasma membrane. As to which extent a TTF-induced perturbation of the calcium- and electrosignaling might impact on glioblastoma cell survival has to await further studies.

# Generation of radioresistant pancreatic cancer cell sublines by fractionated radiation

**Lily Nguyen**, S. Dobiasch, D. Schilling, T. E. Schmid and S. E. Combs

*Department of Radiation Oncology, Klinikum rechts der Isar, Technische Universität München (TUM), Munich, Germany, Institute of Innovative Radiotherapy (iRT), Helmholtz Zentrum München, Neuherberg, Germany*

**Introduction:** Pancreatic cancer is known to be one of the most lethal cancers in humans, the 5-year survival rate is less than 5 %. Radiotherapy alone or in combination with chemotherapy has been used as a major therapeutic method in pancreatic cancer patients who are not eligible for surgery. However, only 12-40 % show response to radiation treatment. Recently, it was shown that specific microRNAs (miRNAs) are associated with the response to radiation treatment. The aim of our study is to generate in vitro isogenic models of radioresistant (RR) pancreatic cancer cell lines through exposure to fractionated radiation in order to analyse their miRNA profiles as new prognostic biomarkers. **Methods:** The radiosensitivity of six different pancreatic cancer cell lines (AsPc-1, BxPC-3, MiaPaCa-2, Panc-1, Su.86.86 and T3M4) was investigated by colony formation assay (CFA). For the generation of RR cell lines, parental cell lines were treated with 10 fractions of 2 Gy x-ray radiation until a total dose of 20 Gy was reached (Gulmay XStrahl irradiation device; 200 kV). At the end of this process, surviving cells were expanded and radiosensitivity was measured using CFA. **Results:** Our data revealed that the established pancreatic cancer cell lines are heterogenic concerning their radioresponse. Out of the six investigated cell lines, MiaPaCa-2 showed the highest radiosensitivity. Therefore, this cell line was chosen to generate a new radioresistant (RR) cell line by x-ray irradiation with 10 fractions of 2 Gy. The surviving MiaPaCa-2 cells (RR) demonstrated significantly higher radioresistance compared to the parental cell line ( $p=0.0321$  at 2 Gy;  $p=0.0004$  at 4 Gy;  $p=0.0057$  at 6 Gy;  $p=0.0023$  at 8 Gy). This is also reflected in a higher D50 and D10 value in the RR MiaPaCa-2 cells (RR: D50 =  $1.82 \pm 0.11$  Gy; Parental: D50 =  $1.59 \pm 0.19$  Gy; RR: D10 =  $4.80 \pm 0.11$  Gy; Parental: D10 =  $4.20 \pm 0.27$  Gy). **Conclusion and Outlook:** The exposure of pancreatic cancer cells to fractionated radiation schedules can select a subpopulation with modified radioresistance. To investigate the molecular response of cancer cells and develop novel biomarkers for response to radiotherapy, the miRNA profile of the isogenic (parental and RR) cell lines will be investigated by miRNA sequencing and miRNA expression analysis. The analysis of radioresistant sublines may provide new insights into the mechanisms underlying clinical radioresistance.

## Primary DNA damage induced by high energetic protons

**Zacharenia Nikitaki**, K. P. Brabcova, M. P. Souli, M. Foster, M. Puchalska, P. Pospisil and L. Sihver

*Atominstytut, Technische Universität Wien, Vienna, Austria // DNA Damage Laboratory, Physics Department, School of Applied Mathematical and Physical Sciences, National Technical University of Athens, Zografou Campus, 15780 Athens, Greece*

Understanding the biological impact of high energy protons is of great importance, especially for two reasons. First, protons are the dominant ionizing particles in galactic cosmic rays and solar particle events and therefore a health risk for future long-distance space travelers. Second, proton radiation is used in cancer therapy for its usually more selective dose delivery to the tumour, while decreasing unwanted dose in out-of-target healthy tissue volumes. Complex or clustered DNA damage, the most severe biological effect of ionizing radiation, can be studied using several different experimental approaches. We present here a simple but informative approach focused on primary DNA radiation damage induced by 198 MeV proton beam. Plasmid DNA in solution with a scavenger is chosen as a simple model system of a cell, omitting damages to membranes, and other cellular components, as well as cellular repair effects. The presence of the scavenger enables to separate and control the direct radiation effects from the indirect ones. We have used two different scavengers, coumarin-3-carboxylic acid (C3CA) and 2-Amino-2-hydroxymethyl-propane-1,3-diol (Tris) in three concentrations each one, selected to give –in pairs- equivalent values of scavenging capacity. After the irradiation, the samples of the plasmid DNA were treated (or not) with two of the E. coli base excision repair enzymes, namely, the formamidopyrimidine DNA glycosylase (Fpg) and the Endonuclease III (Nth). These proteins mostly catalyze cleavage of oxidized purines (Fpg) or pyrimidines (Nth) and, due to their associated lyase activity, convert the resulting abasic sites to strand breaks. The forms of plasmid DNA, damaged to a different grade, were separated with agarose gel electrophoresis and the damage in terms of strand breaks was calculated. The results are discussed comparatively for the two different scavengers used (C3CA and Tris) as well as for the two DNA repair enzymes (Fpg and Nth).



# Development of a micro-PCC assay as a rapid and minimally invasive automatable approach for early triage biodosimetry

**Antonio Pantelias**, G. Terzoudi and G. Pantelias

*Laboratory of Health Physics, Radiobiology and Cytogenetics, National Centre for Scientific Research "Demokritos", Athens, Greece*

In the present work we explore the applicability of cell fusion mediated premature chromosome condensation (PCC) methodology in peripheral blood lymphocytes for the development of a rapid, minimally invasive approach for early triage biodosimetry. Specifically, the main objective was to design a micro-PCC assay that could be applied to very small heparinized blood sample volumes of 50-150 $\mu$ l, using multi-tube racks or 96-deepwell plates, in order to obtain sufficient lymphocyte prematurely condensed chromosomes (PCCs) for biological dosimetry purposes. The development of such a micro-PCC assay for rapid dose estimation is at present a high priority for early triage in radiation emergencies in cases of large scale exposures. Towards this goal, the various steps of the standard PCC procedure were adapted, and lymphocytes corresponding to blood volumes of 50-150 $\mu$ l were successfully fused with CHO mitotic cells in 2ml round bottom safe-lock tubes or 96-well Deepwell plates of 2ml. The Deepwell plates are more advantageous since the various steps required by the protocol could be applied to all 96 wells simultaneously. The morphology of the lymphocyte PCCs obtained was practically identical to that obtained using the standard PCC assay and it allows, therefore, a simultaneous dose-estimation for at least 2x96 blood samples. In addition, the analysis of radiation-induced excess PCC fragments using Giemsa stain is simple and cost-effective. Interestingly, the use of only 1.5ml of hypotonic solution and the fixation of cells twice with 1.5ml of Carnoy's fixative in the 2ml tubes offers high quality PCC images. In cases of overexposed individuals whose blood samples arrive in the lab at least 10h after exposure, the micro-PCC assay was also successfully combined with fluorescence in situ hybridization (FISH) using simultaneously centromeric/telomeric (C/T) peptide nucleic acid (PNA) probes, for the accurate scoring of dicentric and centric ring chromosomes in lymphocyte PCCs. Absorbed dose estimation, by the analysis of Giemsa stained excess PCC fragments or C/T FISH stained dicentrics in lymphocyte PCCs, was facilitated using appropriate calibration curves constructed in our laboratory. The results obtained and the advantages of using an automatable micro-PCC assay, which will pave the way to the subsequent automation of the assay's workflow for early triage biodosimetry, will be presented.



## In vitro study of dose rate effect on Leksell Gamma Knife Perfexion

**Veronika Paštyková**, J. Vachelová, M. David, M. Hurychová, M. Davidková, J. Novotný Jr. and R. Liščák

*Na Homolce Hospital, Dep. of Medical Physics, Prague, Czech Republic*

**Objectives:** The main purpose of the study is to evaluate the radiobiological effect of the dose rate changes in Leksell Gamma Knife (LGK) clinical conditions. In principle there are two reasons why dose rate on LGK is reduced during patient irradiation: 1) Co-60 sources decay with half-life of 5.26 years and 2) using multiple isocenters and conformal treatment plans (e.g. with blocked beams). This pilot study is an experimental work performed in vitro with meduloblastoma DAOY cells. **Methods and materials:** A number of repeated experiments were performed with meduloblastoma DAOY cells irradiated on LGK Perfexion by various dose rates (0.35 – 3.31 Gy/min). The irradiation was performed in a spherical Elekta ABS plastic phantom with the special insert for the Eppendorf tube containing cells. Leksell GammaPlan treatment planning software was used to plan cell irradiation. The control of the different dose rates of LGK was achieved by two ways: 1) reloading of the Co-60 sources in our hospital after performing the first experimental campaigns and 2) sector blocking of the LGK collimator (0, 4 or 6 of 8 sectors of LGK were blocked to achieve full, half or quarter dose rate). To ensure homogenous irradiation of the cells 16 mm collimator was used. Plating efficiency and surviving fraction was determined for each experimental cell sample. Nine different doses in the range 0 – 6 Gy were used to have enough experimental points to obtain surviving curve. Linear quadratic model was used to fit experimental data. Surviving curves for different dose rates were plotted and compared, as well as the  $\alpha/\beta$  parameters. **Results:** This is an initial pilot study with very preliminary data. However, based on so far obtained data it could be observed higher cell survival for dose rates lower than 0.40 Gy/min compared to higher dose rates over 0.75 Gy/min. Currently, experiments continue with dose rates between 1.60 – 3.31 Gy/min taking into account more biological endpoints of the irradiation. **Conclusion:** Preliminary data from this study do show different cell survival for studied meduloblastoma DAOY cells based on a dose rate that was used for an irradiation. Higher survival is observed for a lower dose rate. However, more experimental work is needed to provide data beneficial for clinical practices of gamma knife.

This study was supported by the Ministry of Health, Czech Republic - conceptual development of research organization (Nemocnice Na Homolce - NNH, project n. 00024883).

# Ionizing radiation effects on activation of dendritic cells in mice

**Eszter Persa**, G. Safrany and K. Lumniczky

*National Public Health Institute, Budapest, Hungary*

Dendritic cells (DCs) are antigen presenting cells of the immune system. They have active role in initiating and promoting cellular immune response via T cell - DC interaction and cytokine secretion. DCs can also stimulate anti-tumor immunity by capturing and presenting tumor antigens. Danger signals (e.g. HMGB1) released by dying tumor cells after radiotherapy may facilitate DC activation. The aim of our studies was to determine the effect of ionizing radiation on phenotypical and functional parameters of DCs relevant in stimulating anti-tumor immune response. Mice were total-body irradiated with 0 (control), 0.1, 0.25 and 2 Gy X-rays. Spleen cells were isolated 24h after irradiation and DC cell surface markers were analysed by flow cytometry. Expression of costimulatory (CD40, CD80, and CD86), coinhibitory (B7-H1 (PD-L1)), danger responding (TLR-4) and antigen capturing (DEC205) molecules was determined. For testing antigen capture and antigen presentation isolated DCs and FITC labeled OVA peptide were used. The amount of OVA captured by DCs was followed by flow cytometry, as well. DC cytokine expression was measured by real-time qPCR. Our results showed a slight raise in the level of costimulatory molecules CD80 and CD86 after low dose irradiation and significant increase after 2 Gy. Irradiation seemed to have no effect on the expression of CD40, DEC205 and B7-H1 molecules. DCs irradiated with 2 Gy had increased ability to capture and present OVA peptides compared to non-irradiated DCs. Evaluation of cytokine gene expression is in progress. We showed that 2 Gy irradiation had a massive effect on activation status of DCs appeared in higher expression of costimulatory molecules and increase in their functional abilities. Pronounced activation status can reflect in more effective role of DCs in anti-tumor immune response. In addition, our findings point to a synergistic effect between radio- and immunotherapy.

## In-vitro and in-vivo characterization of a glioblastoma cell line panel

**Daniel Piehlmaier**, B. Stegen, A. Nieto, M. Niyazi, V. Ruf, J. Heß, H. Zitzelsberger, K. Lauber and K. Unger

*Abteilung Strahlenzytogenetik, Helmholtz Zentrum München, Neuherberg, Germany*

Introduction: Glioblastoma (GBM) is the most frequent and aggressive type of primary brain tumors which is characterized by a high degree of therapy resistance and invasiveness rendering it poorly accessible to medical treatment. Currently, the standard therapy includes a combination of surgery and radiochemotherapy followed by maintenance chemotherapy with temozolomide. Despite these aggressive treatment regimens, median overall survival times are limited to 15-18 months with large inter-individual differences. Recent advances in GBM research were focusing on the biological background of the disease and are usually acquired in cell culture experiments. However, experiments have shown that established GBM cell lines resemble the original tumor poorly. Therefore, in-vitro model systems have to be evaluated for their suitability to develop novel GBM treatment options. In this study, a preclinical toolbox for in-vitro and in-vivo experiments was established. Cytogenetic, phenotypic and transcriptomic properties of GBM cell lines were characterized that allow researchers to find the optimal model system for their experiments. Methods: Seven widely used GBM cell lines were analyzed in the present study (A172, LN18, LN229, T98G, U87, U138, and U251). Cell line identity was confirmed by STR typing, and the ploidy status was determined by SKY karyotyping. Additionally, the IDH1/2 status and MGMT promotor methylation status of the cell lines was assessed by Sanger and pyro-sequencing. Clonogenic survival assays were performed to characterize the cellular response towards irradiation or temozolomide treatment, respectively. Tumor growth and response to radiotherapy were analyzed in orthotopic xenograft models. Finally, global gene expression was determined by microarray analyses. Results: We found that the karyotype among the cell line panel varied from near diploid to pentaploid. The sensitivity towards ionizing irradiation and temozolomide treatment was highly variable and was not associated with ploidy status. All cell lines were negative for IDH1/2 mutations. Resistance towards temozolomide significantly correlated with MGMT mRNA expression. Differentially expressed genes were identified in MGMT expressing and non-expressing cell lines and high- and low-radioresistant groups. Tumor growth and radiosensitivity in-vivo was highly variable among the cell line panel. Conclusion: The present study provides comprehensive characterization of a widely used panel of GBM cell culture models that allows researchers to choose the optimal cell line for their individual experiments.

# Leukocyte adhesion to (primary) endothelial cells after pro-inflammatory stimulation and can be reduced by photon and charged particle exposure

**Felicitas Rapp**, P. Wendel, S. Ktitareva, N. Erbelinger and C. Fournier

*GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany*

For patients suffering from chronic inflammatory musculoskeletal diseases like rheumatoid arthritis (RA), low doses of ionizing radiation (low dose radiotherapy, LDRT) can be used for therapy. Photons are clinically used for RT, but the application of radon in spas or galleries is traditionally used for a long time. Positive effects like pain relief are reported by patients; however, the underlying mechanisms are yet unclear. Therefore, in the frame of the GREWIS consortium, the effect of radon- emitted  $\alpha$ - particles on cells located in the affected joints and a potential influence on the immune system is investigated. One hallmark of inflammation is the recruitment of leukocytes from the bloodstream into tissue via the endothelium. In this project, human primary endothelial cells (EC) were cultivated under pro-inflammatory conditions by stimulation with TNF- $\alpha$ . After irradiation with photons or helium ions, we used an adhesion assay with primary human peripheral blood lymphocytes (PBL) to quantify inflammation intensity. In addition, cultivation under static conditions was compared to physiological laminar flow in a specially designed Flow Chamber. Helium ions were used in this study because of their properties similar to  $\alpha$ - particles. Under pro-inflammatory conditions, low-dose irradiation results in a decrease of subsequent PBL adhesion. For helium ions, the effect is comparable to photons, indicating an effect of low doses of high LET-irradiation similar to radon- emitted  $\alpha$ - particles. The decrease of PBL adhesion is more pronounced under laminar flow, highlighting the influence of physiological culture conditions for primary cells. In line with this, EC morphology was found to be adapted under laminar flow, potentially linked to changed expression of surface molecules involved in adhesion. In the knee, blood vessels are in close vicinity to bone and adipose tissue (infrapatellar fat pad, IPFP). Adipocytes are known to secrete pro-inflammatory factors (adipokines, e.g. visfatin) which can contribute to the progression of RA. To this end, the effect on adipokines on EC is currently under investigation. Since radiation and inflammation-related effects were mostly investigated in single cell types so far, we also plan to assess interactions between different cell types located in the knee joint. First results will be presented.

Funding: BMBF grant 02NUK017A (GREWIS)

## Heterogeneous radiation response determined with a $\gamma$ H2AX foci assay in human head and neck squamous cell carcinoma (hHNSCC) tumor models

**Treewut Rassamegevanon**, S. Löck, U. Range, M. Krause, M. Baumann and C. von Neubeck

*OncoRay – National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Helmholtz-Zentrum Dresden - Rossendorf, Dresden, Germany*

**Introduction:** The  $\gamma$ H2AX foci assay holds the potential for treatment individualization and improvement of the treatment outcome. Based on ex vivo irradiated tumor specimens, the slope of dose response curves of residual  $\gamma$ H2AX foci could be used as a biomarker to determine the radiosensitivity of experimental tumors and patient derived tumor specimens (Menegakis et al. 2015). This study is focusing on the translation of the  $\gamma$ H2AX foci assay to clinical application and particularly on the representativeness of tumor biopsies for the whole tumor. The inter tumor model (SKX, FaDu, UT-SCC 5) as well as the intra-tumoral heterogeneities of  $\gamma$ H2AX foci were examined. **Methods and Results:** Excised tumors were cut in 8 pieces and reoxygenated in culture medium for a) 10 h or b) 24 h. Tumor specimens were irradiated ex vivo (0 Gy or 4 Gy); fixed and paraffin embedded 24 h post irradiation. The  $\gamma$ H2AX foci were detected by immunostaining. Foci counting was conducted exclusively within oxic regions determined by pimonidazole (hypoxic marker) staining and bromodeoxyuridine (BrdU, proliferation marker). The foci number was normalized (nfoci) to the unirradiated control and fitted by a linear mixed-effects model. The heterogeneities of nfoci were evaluated on each level of the analysis cascade i.e., a) all tumors per model (intra model), b) all tumor specimens of a particular tumor (intra-tumoral inter-specimen) and c) the microscopic region of interest within one tumor specimen (intra-tumoral intra-specimen) relative to the reoxygenation times. The results indicated no statistically significant effect of the reoxygenation times on the ex vivo set-up of  $\gamma$ H2AX foci assay. A significant intra model heterogeneity could be observed exclusively for FaDu ( $p=0.033$ ) but not for SKX ( $p=0.17$ ) and UT-SCC-5 ( $p=0.082$ ) tumors. All tumor models showed a significant intra-tumoral inter-specimen ( $p<0.01$ ) and intra-specimen ( $p<0.001$ ) heterogeneity. **Conclusion:** Comparable results for the ex vivo  $\gamma$ H2AX foci assay between 10 h and 24 h reoxygenation time improved the clinical practicability of the assay by providing a time flexibility in sample processing. A high degree of intra-tumoral heterogeneity emphasizes the importance of multiple biopsies per patient foci analysis and the necessity of an automated foci counting platform.

**Acknowledgement:** This work was supported by a grant of the Federal Ministry of Education and Research (BMBF 02NUK035C).

**Reference:** Menegakis et al., Radiother. Oncol. 116:473–79 (2015)

[Treewut.Rassamegevanon@uniklinikum-dresden.de](mailto:Treewut.Rassamegevanon@uniklinikum-dresden.de)

# Non-cell-autonomous contribution to cancer cell radio susceptibility

**Oliver Reiners**, A. Schütze, J. Morawitz, M. Sack, P. Brenneisen, V. Jendrossek, JW. Fischer and K. Röck

*Institute for Pharmacology and Clinical Pharmacology, University Hospital of Heinrich Heine University Düsseldorf, Germany*

Recently the role of the tumor microenvironment has been recognized as an important determinant of radiation responses. Here we investigate the influence of human stromal fibroblasts (hF) on radiation induced tumor cell death. To evaluate functional consequences of ionizing radiation on hF and tumor cells (esophageal squamous carcinoma cells, eSCC), cells were either cultured separately and irradiated (2Gy) or co-cultured after irradiation to investigate radiation dependent tumor-stroma interactions. Subsequently cells were monitored by time-lapse microscopy and cell death was assessed visually as well as by PARP cleavage, live cell staining with Hoechst33342/propidium iodide and quantification of sub-G1 cell cycle. The rate of cell death was barely altered in monocultures. Of note significantly increased cell death of eSCC was observed in co-culture with irradiated hF compared to co-cultures with mock-irradiated fibroblasts (16.10±4.25 fold of control). Microscopic evaluation of co-cultures revealed that hF and cancer cells are connected via tunneling nanotubes (TNT). Furthermore, TNT formation was found to be dependent on the extracellular matrix component hyaluronan. eSCC cell death was prevented by pharmacological inhibition of TNT formation by cytochalasin B as well as inhibition of hyaluronan synthesis by 4-MU. As TNTs are known to mediate intercellular protein or mitochondrial transfer we investigated which stromal proteins are shuttled into eSCC cells by trans-SILAC (stable isotope labeling by amino acids) experiments. For this purpose the proteome of fibroblasts was labelled with heavy amino acids. After co-culturing the cells were separated by FACS-sorting and labelled stromal proteins were identified in the cancer cells by mass spectrometry. Four consistently shuttled proteins were identified in the cancer cells, which were exclusively transferred after irradiation of both cell lines. Knock-down experiments of these proteins in hF were performed and SHANK1, which is not expressed in eSCC cells, was found to be responsible for the hF induced cell death of eSCC cells in response to radiation. In conclusion, by usage of a novel trans-SILAC approach, we provide evidence that in response to ionizing radiation stromal hF transfer SHANK1 into eSCC cells, thereby inducing cellular death. SHANK1 might therefore serve as potential new pharmacological target to sensitize cancer cells to radiation therapy.

## Molecular targeting of the DNA damage response as a novel approach to deintensify the therapy of HPV-positive HNSCC

C.-J. Busch , M. S. Kröger, M. Kriegs, J. D. Güster, S. Weissleder , **Kai Rothkamm**, A. Münscher and T. Rieckmann

*Department of Otolaryngology and Head and Neck Surgery, University Medical Center Hamburg Eppendorf, Hamburg, Germany*

**Background / Objectives:** Clinical data demonstrate an enhanced radiation sensitivity of HPV+ HNSCC, a feature also observed on the cellular level in HPV+ HNSCC cell lines. For the latter we could show that the underlying mechanism is a defect in DNA double-strand break repair associated with a profound and sustained arrest in G2 (Rieckmann 2013). Specific inhibitors of central components of the DNA damage response (DDR), such as PARP1, Wee1 and Chk1 are being tested in clinical trials in HNSCC and the intrinsic DNA repair defect of HPV+ HNSCC cells may render these tumors especially susceptible for further radiosensitization. **Methods:** Mechanistic proof of efficacy of the various inhibitors was performed using Western blot, immunofluorescence microscopy and assessment of cell cycle distribution. DDR-Inhibitors: PARP – Olaparib; Chk1 – PF00477736, LY2603618, Prexasertib; Wee1 – AZD-1775. Standard therapeutics: Cetuximab, cisplatin. Radiosensitization was assessed using colony formation assay. HPV+ HNSCC cells: UT-SCC-45, 93-VU-147T, UD-SCC-2, UM-SCC-47, UPCI-SCC-154. **Results:** While the inhibition of EGFR by cetuximab is being extensively tested for HPV+ HNSCC in phase 3 clinical trials, on the cellular level cetuximab completely failed to exert a meaningful cytotoxic effect or radiosensitization of any of the 5 HPV+ HNSCC strains tested (Güster 2014). In contrast, the inhibition of Chk1 interfered with the radiation-induced G2-arrest and resulted in radiosensitization in all HPV+ HNSCC cell lines, as well as the targeting of DNA repair processes through the inhibition of PARP1 (Busch 2013, Güster 2014, Busch 2017). Targeting Wee1 resulted in an accumulation of the HPV+ cells in the S-phase rather than in the intended release from the radiation-induced G2-arrest and it induced a compensatory activation of Chk1. The combined inhibition of Wee1 and Chk1, however, was already effective using massively reduced doses of both inhibitors and resulted in efficient radiosensitization (Busch 2017). In all cases the radiosensitizing effect was far stronger in the HPV+ HNSCC strains than in normal human fibroblasts used as a surrogate of p53-proficient normal tissue cells. **Conclusion:** While the inhibition of EGFR fails to confer radiosensitization of HPV+ HNSCC on the cellular level, the inhibition of the DNA damage response was found to be effective. Our data strongly suggest that these targeting approaches further interfere with the ability of HPV+ HNSCC cells to cope with radiation-induced DNA damage and may represent viable options for the deintensification of therapy. The verification of these results in independent patient-derived xenograft models as a next step towards a clinical use is currently being planned. **References:** Rieckmann et al. 2013. HNSCC cell lines positive for HPV and p16 possess higher cellular radiosensitivity due to an impaired DSB repair capacity. *Radiother Oncol* 107:242-6. Güster et al. 2014. The inhibition of PARP but not EGFR results in the radiosensitization of HPV/p16-positive HNSCC cell lines. *Radiother Oncol* 113:345-51. Busch et al. 2013. HPV-positive HNSCC cell lines but not primary human fibroblasts are radiosensitized by the inhibition of Chk1. *Radiother Oncol* 108:495-9. Busch et al. 2017. G2-checkpoint targeting and radiosensitization of HPV/p16-positive HNSCC cells through the inhibition of Chk1 and Wee1. *Radiother Oncol* 122:260-266.

[k.rothkamm@uke.de](mailto:k.rothkamm@uke.de)



# Immunological basis of abscopal antitumor responses induced with radio-immunotherapy

**Michael Rückert**, B. Frey, R. Fietkau and U.S. Gaipl

*Department of Radiation Oncology, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany*

**INTRODUCTION:** Radiotherapy (RT) is a common treatment for cancer patients to primarily achieve local tumor control. Through the induction of tumor cell death and the modulation of the tumor microenvironment ionizing irradiation can also stimulate the immune system. RT can therefore act as an in situ cancer vaccine, but most likely only in combination with further immune stimulation. Immunotherapies, such as killed autologous tumor cells applied as a therapeutic tumor vaccination and checkpoint inhibitors blocking the suppression of tumor-specific T cells represent an option to further elicit and boost antitumor immune responses, respectively. In some patients who have clinically been followed up by now, RT in combination with checkpoint inhibition led to the shrinkage of metastases outside of the radiation field – the so called “abscopal effect”. However, little is known about the underlying mechanisms. **METHODS:** In pre-clinical in vivo model systems we aim to examine how RT in combination with vaccination and immune checkpoint blockade induces strong systemic immune responses. Ectopic B16-F10 (melanoma) and CT26 (colorectal carcinoma) tumors on both flanks of syngeneic mouse strains serve as tumor model. One tumor will be irradiated while the other one (untreated) will be regarded as abscopal site. The efficiency of different RT fractionations in combination with anti-PD-1 checkpoint inhibition and a whole tumor cell vaccine generated with high hydrostatic pressure (HHP) will be compared. **RESULTS:** The local irradiation of mice in the abscopal-setting with tumors on both flanks and the generation of HHP-vaccines have been established. Identification of several immune cell types making up >80 % of all tumor infiltrating leukocytes (CD45+) was achieved with an approach using isolation and enrichment of CD45+ cells followed by staining with three different antibody panels and analysis with multicolor flow cytometry. First data demonstrate that already with RT alone the composition of tumor infiltrating immune cell types changes. Both tumors of untreated mice show a similar composition whereas in treated mice the composition of immune cells in the irradiated tumor and the abscopal tumor not only differs from untreated mice, but also from each other. **OUTLOOK:** After the establishment of an abscopal effect inducing treatment schedule by combining RT with both immunotherapies, the mechanistic basis of the systemic immune response will be investigated on cellular and molecular level. Tumor infiltrating immune cells will be closely monitored and compared between treated tumors, abscopal tumors and tumors of untreated mice.

**ACKNOWLEDGEMENT:** This work was supported by the doctoral training program GK1660 from the German Research Foundation (DFG).

[michael.rueckert@uk-erlangen.de](mailto:michael.rueckert@uk-erlangen.de)



## A prognostic signature of four 16q24.3 genes in radiotherapy-treated head and neck squamous cell carcinoma (HNSCC)

**Ludmila Schneider**, M. Selmansberger, L. Schüttrumpf, C. Maihöfer, U. Pflugradt, T. Kirchner, C. Woischke, A. Walch, C. Belka, H. Zitzelsberger, U. Ganswindt, K. Unger and J. Hess

*Research Unit Radiation Cytogenetics, Helmholtz Zentrum München, German Research Center for Environmental Health GmbH, Neuherberg, Germany; Clinical Cooperation Group 'Personalized Radiotherapy in Head and Neck Cancer' Helmholtz Zentrum München,*

The average 5-year survival rate of patients with advanced head and neck squamous cell carcinoma (HNSCC) is only about 40-50% which is mainly attributed to radio(chemo)resistance of treated tumors. For improved personalized treatment approaches prognostic markers for the identification of resistant patients are required. Previously, we have shown that increased copy number of the chromosomal band 16q24.3 is associated with impaired clinical outcome of HNSCC patients after radiotherapy (RTx, Bauer et al., 2008; Hess et al., 2017). In the current study, we aimed to identify a signature of genes located on 16q24.3 predicting overall survival in RTx treated patients. We applied a forward-selection approach on the expression data of 41 genes localized on the chromosomal band 16q24.3 of a RTx treated subgroup (n=99) of the The Cancer Genome Atlas (TCGA) head and neck cancer cohort. The data set was randomly split into a training (n=40) and a validation set (n=59). The resulting optimal cox model included the genes APRT, CENPBD1, CHMP1A and GALNS and calculated an individual risk score for each patient based on the expression of the signature genes weighted by the Cox-proportional hazard coefficients. A threshold that optimally allowed to assort patients into high- and low-risk in the training set was applied to the expression data of the validation set along with the initially generated cox model. Further, a gene-association network (GAN) was reconstructed from transcriptome data of the RTx treated subset of the TCGA HNSCC cohort using a partial correlation approach. The 4-gene-risk factor could be confirmed in the TCGA HNSCC validation set (p=0.02911; HR=3.85, 95% CI 1.05-13.89). Moreover, we were able to validate the signature in an independent cohort of HNSCC patients treated with adjuvant radiotherapy at the Department of Radiation Oncology, Ludwig-Maximilians-University Munich (LMU-KKG cohort; n=108). High-risk patients showed significantly impaired overall survival (p=0.02125; HR=2.01, 95% CI 1.10-3.70), recurrence-free survival (p=0.04206; HR=1.84, 95% CI 1.01-3.34) and locoregional recurrence-free survival (p=0.03641; HR=1.87, 95% CI 1.03-3.40). After adjustment for HPV-status in multivariate Cox-proportional hazard analysis, the 4-gene-classifier maintained its independent prognostic value. Furthermore, genes interacting with genes of the signature were identified after reconstruction of a gene association network. Subsequent pathway enrichment analysis of the first and second neighborhoods of the signature genes suggested involvement of tumorigenesis-associated processes such as cell cycle (e.g. M/G1 Transition, Cell cycle Checkpoints, Mitotic G1-G1/S phases), apoptosis and cell junctions. This provides first insights into the functional role of the prognostic 4-gene-signature. In order to strengthen validity, the signature needs to be applied on a further independent cohort of RTx treated HNSCC patients.

[ludmila.schneider@helmholtz-muenchen.de](mailto:ludmila.schneider@helmholtz-muenchen.de)

# Radiation dose estimation by ESR dosimetry with tooth enamel for residents of Nagasaki and Fukushima in Japan

**Tatsuya Shimasaki**, K. Yokota, M. Mine, Y. Shiraishi, K. Gotoh, N. Matsuda and S. Okada

*Institute of Resource Development and Analysis, Kumamoto University, Japan*

Tooth enamel is the only tissue in the human body that retains indefinitely the history of radiation exposure of Nagasaki Atomic bomb. Tooth enamel has not become widely used for determining radiation exposure of atomic bomb survivors, because any medical or dental diagnostic x rays of the teeth will confuse the measurement of their radiation exposure for atomic bomb. We present that the diagnostic x rays dose can be determined separately from radiation exposure of Nagasaki atomic bomb survivors. We began to request donations of extracted teeth from Adult Health Study participants of the Nagasaki ESR project in 1986. At present, 343 teeth have been collected, but only about 30% was found to be suitable for enamel separation and subsequent ESR measurement. To assess dental x rays dose, the enamel of each tooth was removed as two sides, buccal and lingual sides, and crushed to grain sizes 0.50-1.0 mm without any chemical treatment. We turned our attention to the problem of separating the contribution due to dental diagnostic x rays. Several tooth showed considerably larger ESR estimated doses in their buccal parts than in their lingual parts, which first seemed to indicate a considerably large contribution from any medical or dental diagnostic x rays exposure. We assessed that the dose ranging 0.04-0.7 Gy would be due to dental diagnostic x rays and natural radiation. The ESR dose of lingual parts of molars is least likely affected by factors other than atomic bomb gamma rays. Using ESR dosimetry of tooth enamel to estimate atomic bomb gamma ray dose received, we have recently examined teeth donated from Nagasaki atomic bomb survivors. Several teeth exposed in no shielding showed considerably lower ESR estimated doses than DS02 doses, but the other tooth enamel doses correlated quite well with DS02 doses. We found that the diagnostic x rays dose can be determined separately from radiation exposure of Nagasaki atomic bomb survivors. It is concluded that ESR dosimetry with division into two of tooth enamel is useful measures for retrospective dose estimation. Furthermore, we are going to report the radiation dose using tooth enamel which we obtained in Fukushima.

## Divergent roles of fibroblasts on the radiation-response of cancer cells

**Alizee Steer**, D. Klein, M. Stuschke and Verena Jendrossek

*Institute of Cell Biology (Cancer Research), University Hospital Essen, Essen, Germany*

Background: Increasing evidence indicates that the heterogeneous tumor stroma supports therapy resistance at multiple levels. Critical components of the stroma are fibroblasts and in particular cancer-associated fibroblasts (CAFs). CAFs secrete several signaling molecules to promote cancer cell growth, angiogenesis and metastasis and thus malignant progression. However, the impact of CAFs on the outcome of radiotherapy (RT) is poorly understood. Here, we aim to unravel how CAFs modulate the radiation response of tumors by supporting a radiation resistant cancer cell phenotype. Methods and Results: The impact of stromal fibroblasts and in particular CAFs for tumor growth and sensitivity to ionizing radiation (IR) was analyzed using murine MPR31.4 prostate carcinoma cells implanted subcutaneously either alone or in combination with NIH-3T3 stromal cells into male C57BL/6 mice. Co-implantation of cells resulted in increased tumor growth and reduced tumor growth delay after IR when compared to tumors generated by MPR31.4 carcinoma cells alone. In vitro, a potential effect of paracrine fibroblast signals on the cancer cells proliferation and cell death induction after RT was investigated by using an indirect co-culture (transwell) system. Different sets of fibroblasts (NIH-3T3, L929) and cancer cells (MPR, TrampC1, B16F10) were analyzed and revealed either a pro-tumorigenic or anti-tumorigenic effect. Conclusions: Our co-culture experiments reveal beneficial or adverse paracrine effects of fibroblasts on the survival of irradiated cancer cells in vitro, suggesting cell-type specific effects. A first set of in vivo investigations revealed that NIH-3T3 stromal fibroblasts increase the radiation resistance of MPR31.4 cancer cells in vivo. Further fibroblasts/ cancer cell sets are under current investigation. Future analyses will also explore if the RT responses of tumors corresponds with the activation state of fibroblasts at the time of irradiation.

# Verification of size-specific dose estimate (SSDE) calculation method for truncated X-ray CT images

**Marin Terashima**, K. Mizonobe and H. Date

*Graduate School of Health Sciences, Hokkaido University, Japan*

It is well known that exposed dose in X-ray CT examinations is relatively high compared to other diagnostic modalities. It is therefore necessary to evaluate the dose to patients with care. Although CTDI(vol) is commonly used as an index for the dose exposed in X-ray CT, this index itself does not take the body thickness into account. To solve this problem, the American Association of Physicists in Medicine (AAPM) Report 204 recommends the size-specific dose estimate (SSDE) as an index of radiation dose, which is obtained by multiplying CTDI(vol) by a conversion factor (CF). However, in CT truncated images, it is difficult to obtain the precise CF value and the overestimation of SSDE is of concern. In this study, we aim to propose a more accurate calculation method of SSDE by developing a program code to compensate for the area of the truncated section in CT images of thoracoabdominal and pelvic regions. We used artificially created truncated images for the thoracoabdominal and pelvic areas of a human body phantom (PBU-50) with an Aquilion ONE X-ray CT system. Using MATLAB software, the outline of the images excluding the bed was extracted, and the outline of the subjects was drawn by removing the contour from the field of view (FOV). Two approximate curves were created at the left and right defective portions of the contour, in which the area was corrected by adding the compensating area to the truncated area. Then, from the average CT value in the contour area, the conversion coefficient (CF) proposed by the AAPM report was determined to obtain three SSDEs for the original image, the truncated image, and the corrected image with comparison. It was assumed that the tissue of the corrected portion area was water. For the thoracoabdominal region (FOV 500 mm), the true area was 496.4 cm<sup>2</sup>, the CTDI(vol) value was 22.2 mGy, and the SSDE was 31.71 mGy. For the pelvic region (FOV 500 mm), the true area was 520.2 cm<sup>2</sup>, the CTDI(vol) value was 66.7 mGy and the SSDE was 97.25 mGy. In the thoracoabdominal region, for FOV 270 mm, the truncated area was 485.6 cm<sup>2</sup> and the differences of SSDE between the original image and the truncated image was 4.03%. After the correction, the truncated area was 530.4 cm<sup>2</sup> and the difference of SSDE was -0.18%. For FOV 280 mm, the truncated area was 502.6 cm<sup>2</sup> and the difference of SSDE was 2.39%. After the correction, the truncated area was 514.6 cm<sup>2</sup> and the difference of SSDE was 1.27%. In the pelvic region, for FOV 270 mm, the truncated area was 469.5 cm<sup>2</sup> and the difference of SSDE was 2.36%, and after the correction, the truncated area was 505.1 cm<sup>2</sup> and the difference of SSDE was -0.96%. For FOV 280 mm, the truncated area was 484.1 cm<sup>2</sup> and the difference of SSDE was 1.02%, and after the correction, the truncated area was 504.9 cm<sup>2</sup> and the difference of SSDE was -0.91%. By summarizing the results, it was found that the correction of the area of the truncated image enables us to obtain a more accurate dose value. However, corrections

## Modelling pancreatic cancer cell interaction with tumor stroma in 2D vs. 3D culture - relevant implications for choice of model

**Andreas Thomsen**, C. Aldrian, M. Gothwal, P. Lund, T. B. Brunner and A. Allam M.

*Department of Radiation Oncology, Faculty of Medicine, University of Freiburg, Freiburg, Germany*

**Background and Purpose:** The ability to overcome anoikis, anchorage-independent growth, is a hallmark of cancer cells, and an essential step in carcinogenesis and metastasis formation. In addition, the tumor microenvironment plays a crucial role in supporting cancer cells and resistance to therapy. For this reason, it can be argued that the 2D culture cancer-models are poorly representing the in vivo conditions. Recent studies showed that mesenchymal stem cells (MSCs) are recruited into pancreatic cancer xenografts and allocate themselves in the tumor stroma. The interaction between cancer cells and MSCs are still to be studied. **Aim of the work:** To investigate the difference between 3D and 2D culture conditions and to quantify the effect of tumor stroma in overcoming anoikis. **Materials and methods:** Human pancreatic cancer cell lines MIA PaCa-2, PSN-1 and Panc-1 were seeded in 2D direct co-culture with MSCs for clonal growth. In a 3D co-culture model, we first seeded a monolayer of MSCs into 6 well plates. Cancer cells lines were then loaded into agarose hydrogel arrays with conical microwells and placed on top of the MSC layer. Furthermore, we tested the effect of MSCs in altering radiation sensitivity of MIA PaCa-2 cells in both models in 0, 2, 4 and 6 Gy single doses. Readout was done on day 10 - 12 by selective staining of viable colonies and by image analysis of high resolution scans. **Results:** In 2D direct co-culture model, MSCs has no effect on the clonogenic survival rather the colonies were small in size (proliferation) in the presence of the MSCs. In contrast, all the 3 cancer cell lines showed significant enhanced colonies formation in the presence of MSCs (MIA PaCa-2: 3.8-fold increase). In the classical clonogenic survival assay for adherent growth (2D), co-culture with MSCs has no effect on altering the radiation sensitivity of MIA PaCa-2 cells. In contrast, when using the 3D agarose hydrogel array for anchorage-independent growth, MSCs have a radio-protective effect on MIA PaCa-2 cells, increasing both the colony forming rates and colony sizes. **Conclusions:** We demonstrate a relevant discrepancy in the results of co-culturing pancreatic cancer and mesenchymal stem cells in 2D vs. 3D conditions, with otherwise the same components and conditions. We argue that 3D colony formation mimics to a greater extent the in vivo conditions. Also, we present an agarose hydrogel array as a standardized and practical technique to test the 3D spheroid formation and cancer-stroma interactions.

# RBE variation as a function of depth in proton therapy: How to correlate microdosimetry with biological observations?

**Charlot Vandevoorde**, E. de Kock, J. Symons, S. Chiriotti, A. Parisi, M. De Saint-Hubert, L. Tran, L. Chartier, A. Rosenfeld and J. Slabbert

*NRF iThemba LABS, Somerset West, South Africa*

The potential clinical advantage of protons lies in their favorable depth dose curve compared to photons, characterized by a low dose in the entrance channel followed by a steep increase and sharp dose fall-off towards the end of their range in the so-called Bragg peak. This allows the positioning of the region of maximal energy within the treatment target, while damage to surrounding healthy organs and tissues is limited. Growing evidence suggests an increase in relative biological effectiveness (RBE) for protons in the distal part of the Bragg curve. This presents currently a key issue in proton therapy (PT), since any uncertainty in RBE results directly in an uncertainty in the biologically effective dose delivered to the patient. However, a generic fixed RBE of 1.1 is currently still used in clinical practice and the debate on variable versus constant RBE in PT treatment planning is still ongoing. Different studies demonstrated a strong correlation between RBE and dose-averaged linear energy transfer (LET<sub>d</sub>) and different RBE models have been proposed for a fast calculation of proton RBE from LET<sub>d</sub> values in treatment planning. However, there still exists a lack of biological input parameters and validation experiments are urgently needed. In this study, we evaluated the increase in RBE and LET<sub>d</sub> along the spread-out Bragg peak (SOBP) with special emphasis on the distal fall-off region, by performing both radiobiological and microdosimetric measurements within the same experiment. Chinese hamster ovary (CHO-K1) cells were grown in culture flasks and seeded in exact numbers in petri-dishes according to a dilution scheme that was previously established. A Perspex phantom was designed in house which allows a positioning accuracy of 0.1mm. The phantom consists of two Perspex plates that were specifically designed to hold a petri-dish or the silicon-on-insulator microdosimeter (MicroPlus™ Probe). Additional plates of various thicknesses were interposed in front of the petri-dish or the microdosimeter in order to obtain different water equivalent depths. Reference dosimetry was carried out with a Markus™ chamber and irradiations were performed with a 200 MeV proton beam generated at NRF iThemba LABS that was degraded to a R50 range of 120mm. A large field size of 100mm was applied and a 50mm SOBP was used. Cells were exposed to 2, 3, 4, 5, 6 and 8 Gy for colony survival analysis (CSA), in triplicate per dose point. A first set of irradiations was performed at the following positions for both CSA and microdosimetry, expressed as a percentage of the depth dose curve which was normalized to a reference position in the SOBP: 74,88% (entrance plateau), 100% (SOBP), 83.44%, 57.18%, 39.76% and 18.98%. The preliminary colony survival curves obtained from this first run, were used to calculate RBE values based on mean inaction doses. A clear increase in RBE with depth was observed, ranging from 1.10 for the 100% position in the SOBP to 1.44 for the 18.98% position in the distal fall-off. However, the cell survival curves of the positions in the distal fall-off indicate a saturation in RBE from 57.18% onwards (RBE of 1.41). Additional measurements and analysis will be undertaken to clarify this observation. The correlation between CSA results and microdosimetry readings will be presented and the results will be critically evaluated against existing RBE models. Acknowledgement: We wish to thank the Physics Advisory Committee (PAC) of NRF iThemba LABS for support and beam time allocation.

[cvandevoorde@tlabs.ac.za](mailto:cvandevoorde@tlabs.ac.za)



## Detection of biomarkers after exposure to low doses of ionizing radiation through microarray data analysis and meta-analysis

**Christina Vasileiou**, D.D. Michalettou, P. Kontou, P. Bagos, I. Michalopoulos and A. G. Georgakilas

*DNA Damage laboratory, Physics Department, National Technical University of Athens, Athens, Greece*

The use of Ionizing Radiation (IR) is currently one of the most common approaches in the diagnosis and treatment of many kinds of disease, including most types of cancer. Evidence from multiple sources suggest the involvement of inflammation and oxidative stress to be the main contributors of high dose radiation effects. Our interest was to identify the effects of low dose radiation in human tissues. To this end, we compare up- or down-regulated genes in response to low and high dose and the biological networks they participate in. Human samples categorized as irradiated with low or high dose of radiation and non-irradiated (control samples) were selected and gene expression was determined using microarray data analysis: Raw data from various studies were downloaded from GEO public microarray database (GEO series GSE8917; GSE12435; GSE16935; GSE23901; GSE29344; GSE52918; GSE59861). GEO data were background-corrected, quantile normalized and log2 transformed, using the Limma package from Bioconductor suite which is based on the R programming language. We also downloaded various genomic data from Biomart. We designed a MySQL database in order to store our data and conduct a meta-analysis to select statistically significant differentially expressed genes. Using STRINGdb and WebGestalt, we were able to construct gene networks and identify biological processes, which are related to IR response. Different cell-response effects were observed between high and low doses. Response in high dose irradiation was clearly distinguishable including genes associated with the onset of cancer, activation of p21 inhibitor, activation of tumor suppressor proteins such as p53 and several DNA repair mechanisms ensuring the cell's response to radiation. However the correlation of the low dose response is not clear. MiRNA activation appears to be involved in gene silencing, regulation of cell cycle phase transitions and pathways for pyrimidine metabolism. Furthermore, we observed the activation of the GAB1 Signalosome pathway, leading to AKT signaling that has been associated with cancer and the distinct presence of CDKN1A gene in the majority of results for exposure to low dose irradiation, the expression of which leads to the production of p53-dependent p21 protein, gives evidence of activation of the p53 tumor suppressor protein and all the corresponding repair mechanisms. Keywords: ionizing radiation, low dose, high dose, gene differential expression, bioinformatics, microarray analysis, database, meta-analysis, biomarkers, biological networks.

# Establishment of a psoriatic skin model for radon treatment

**Julia Wiedemann**, C. L. Witzler and C. Fournier

*Biophysics, GSI, Helmholtz Centre for Heavy Ion Research Darmstadt, Germany*

Psoriasis is a chronic inflammatory skin disease which affects more than 25 million of people in North America and Europe. Clinically it is characterized by red plaques with white scales which are typically located at elbows, knees and scalp and can be clearly distinguished from non-lesional skin. The disease can be triggered by many factors like injury and trauma but in general it is due to an overshooting and persisting activation of the immune system. For the manifestation, interleukines and cytokines (IL-17, IL-22, TNF-alpha and IFN-gamma) released by T-lymphocytes (Th17, Th1 and Th22 cells) and keratinocytes are essential to contribute to the inflammatory cytokine milieu and to trigger an amplifying feedback loop, which is in turn responsible for the pathogenesis. Psoriasis is on the indication list for the therapy in radon galleries. However, the mechanisms underlying the clinical benefit are unknown. Due to ethical concerns, skin from psoriasis patients is not easily available. Therefore we established a model system for psoriasis on the level of keratinocytes to investigate the potential effects of alpha irradiation on cells that exhibit a key role in the progression of the malign state in psoriasis. Keratinocytes are therefore treated with cytokines relevant in the IL17/IL23 axis. A geneexpression study of psoriatic markers was conducted to show induction of a psoriaforme phenotype. A method to irradiate this model system with alpha irradiation from an <sup>241</sup>Am-source is under development. Results of the induction of a psoriatic phenotype, the effects of UV-B irradiation, used as a reference irradiation type, and technical aspects of irradiation with an alpha-source will be presented.

This work is supported by the Forschungsinstitut Bad Gastein (FOI-15/08-031WIE), EURADON, the Radon Gallery of Bad Gastein and GREWIS (02NUK017A)



## Modelling of oxygen enhancement ratio in consideration of LET and oxygen partial pressure by using a microdosimetric-kinetic model

Ryota Yamada, Y. Matsuya and H. Date

*Graduate School of Health Sciences, Hokkaido University, Japan*

A reduction of the radiation effects on tumor under lack of oxygen is attributed to low radiosensitivities of hypoxic tumor cells. Radioresistance of the hypoxic cells arises from a diminished DNA damage in which the initial lesions can be repaired under hypoxic conditions. This property is expressed by the oxygen enhancement ratio (OER). The OER value is defined as the ratio of the dose in hypoxic to that in oxic condition at the same biologic endpoint. Oxygen enhancement depends on several factors such as the linear energy transfer (LET), oxygen partial pressure of the tumor during irradiation and the biological endpoint. In recent decades, there are a number of reports concerning the OER value from a view point of cell-killing model parameters. The aim of this study was to develop a model for deriving OER as a function of both LET and oxygen partial pressure of tumor. Conventionally, the linear-quadratic model (LQM) has been used to describe the differences in radiosensitivity and cell survival fraction between hypoxic and oxic conditions. While the LQM is very simple by using two coefficients of dose and dose squared ( $\alpha$ ,  $\beta$ ), the MKM used in this study enables us to consider microscopic dose depositions and repairs of potentially lethal damage after irradiation by using two cell-specific parameters ( $\alpha_0$ ,  $\beta_0$ ) and one microdosimetric parameter ( $\gamma$ ). We further incorporated the Alper-Howard-Francis model into the MKM parameters ( $\alpha_0$ ,  $\beta_0$ ) that vary depending on oxygen partial pressures, respectively. We assumed that the  $\alpha_0$  is simply proportional to LET in the clinical LET range while the  $\beta_0$  is constant. These cell-specific parameters were determined by fitting the MKM formula to experimental cell survival data reported by Hirayama et al. (for CHO cell line), and the OER was calculated value as the ratio of doses under hypoxia and aerobic conditions at 10% survival level. Thus, without major changes of cell survival formula, this model enable us to evaluate OER depending on LET an oxygen partial pressure. By fitting the SF formula of MKM to the experimental cell survival data, we could determine a proper curve for the data. The MKM analysis shows that the OER values are 2.80 for LET = 1.7 keV/ $\mu$ m (X-rays, 200kVp) and 1.83 for LET = 80 keV/ $\mu$ m (nucleon beam, 290MeV) at the 10% survival level, in good agreement with experimental cell survival in the literature. We found that the OER modelling used here can reproduce not only the experimental OER value but also its dependence of LET and oxygen partial pressure on cell survival. The OER value can be predicted by determined parameters without change of the formula in the MKM, and we hope to describe the biological processes of tumor regrowth in the future.

A decorative graphic on the left side of the page, featuring a light blue, semi-transparent 3D model of a protein structure. The model shows a complex arrangement of alpha-helices and beta-sheets, with several subunits visible. It is positioned vertically along the left edge, partially overlapping the dark blue banner.

# Poster Session 3



<b>P1</b>	<b>Rozina Aktar</b> Dresden ,Germany	<i>"Orthotopic Transplantation of Cancer Cells into Mice Lung Using a Stereotactic Technique"</i>
<b>P2</b>	<b>Nicole Averbeck</b> Darmstadt, Germany	<i>"Proliferating versus quiescent cells: Repair and survival of low- or high-LET radiation induced DNA double-strand breaks "</i>
<b>P3</b>	<b>Sofia Barbieri</b> Pavia, Italy	<i>"Track-structure simulation of <math>\gamma</math>-H2AX foci and comparison with experimental results: unravelling the role of radiation quality"</i>
<b>P4</b>	<b>Carina Barent</b> Darmstadt, Germany	<i>"RNF138 stimulates DNA-end resection upon heavy-ion-irradiation in human G1-phase cells"</i>
<b>P5</b>	<b>Jacqueline Bernardino-Sgherri</b> Paris, France	<i>"In vivo radioprotective effect of the cellular prion protein"</i>
<b>P6</b>	<b>Anna Broich</b> Essen, Germany	<i>"Molecular mechanisms linking repair of DNA double strand breaks by alternative end joining to cell growth-state"</i>
<b>P7</b>	<b>Shipra Chaudhary</b> Essen, Germany	<i>"DSB clusters as chromatin destabilizers and source of DSB processing errors"</i>
<b>P8</b>	<b>Volker Dahmen</b> Jülich, Germany	<i>"Processing of Complex DNA Lesions in Mammalian Cells Induced by I-125 labeled Triplex-Forming Oligonucleotides"</i>
<b>P9</b>	<b>Marie Davidková</b> Prague, Czech Republic	<i>"Biological efficiency of double scattering and pencil beam scanning modes in proton therapy"</i>
<b>P10</b>	<b>Flavia Zita Francies</b> Johannesburg, South Africa	<i>"Micronucleus assay for Fanconi Anaemia diagnosis with ionising radiation and mitomycin C"</i>
<b>P11</b>	<b>Anastasios Georgoulis</b> Athens, Greece	<i>"Biological assessment of the safe use of Dental Cone Beam Computed Tomography, at a molecular level"</i>
<b>P12</b>	<b>Christin Glowa</b> Heidelberg, Germany	<i>"Carbon ions are more effective than photons after 1, 2 and 6 fractions in three sublines of syngeneic rat prostate tumors"</i>
<b>P13</b>	<b>Ielizaveta Gorodetska</b> Dresden, Germany	<i>"The role of BRCA1 in regulation of prostate cancer cell radioresistance and tumorigenicity"</i>

<b>P14</b>	<b>Mohammad S. Mortoga Hasan</b> Essen, Germany	<i>"Regulated systems of I-SceI expression for in-depth studies of the biological effects of DSBs and DSB-clusters"</i>
<b>P15</b>	<b>Michael Hausmann</b> Heidelberg, Germany	<i>"Radiation-induced re-arrangements of molecular complexes studied in 3D-conserved cells and cell nuclei by super-resolution localization microscopy"</i>
<b>P16</b>	<b>Katarina Ilicic</b> Munich, Germany	<i>"Interaction of DNA damage on micrometer and nanometer scale results in reduced cell survival"</i>
<b>P17</b>	<b>Tatsuhiko Imaoka</b> Japan	<i>"Dose Rate Dependence of Rat Mammary Carcinogenesis Following Protracted <math>\gamma</math> Ray Exposure"</i>
<b>P18</b>	<b>Shizuko Kakinuma</b> Japan	<i>"Genomic alterations associated with radiation exposure in mice model"</i>
<b>P19</b>	<b>Dmitry Klovov</b> Canada	<i>"Effects of low-dose gamma-radiation on myogenic properties of muscle stem cells in the context of aging"</i>
<b>P20</b>	<b>Maria Koshanskaya</b> Vienna, Austria	<i>"Modification of radiation-induced oral mucositis by heparin – preclinical studies"</i>
<b>P21</b>	<b>Jakob Kowaliuk</b> Vienna, Austria	<i>"Thalidomide ameliorates early radiation-induced urinary bladder impairment"</i>
<b>P22</b>	<b>Lisa Marie Krieger</b> Essen, Germany	<i>"The influence of chromatin structure on DNA double strand break repair pathway choice"</i>
<b>P23</b>	<b>Pelin Kucuk</b> Essen, Germany	<i>"Growth state dependence of alt-EJ and the role of the chromatin structure in this response"</i>
<b>P24</b>	<b>Sarah Kunze</b> Neuherberg, Germany	<i>"Low-dose ionizing radiation did not lead to cataract formation in mice"</i>
<b>P25</b>	<b>Charlotte Lepleux</b> Caen, France	<i>"Study of bystander signals emitted by chondrosarcoma cells irradiated in vitro with X-rays and carbon ions"</i>
<b>P26</b>	<b>Grainne Manning</b> Oxfordshire, United Kingdom	<i>"Tracking Kras mutations in human and murine acute myeloid leukaemia: role in radiation leukaemogenesis"</i>

<b>P27</b>	<b>Yusuke Matsuya</b> Hokkaido, Japan	<i>"Application of the IMK model to cell survival curves following the exposure to intensity modulated radiation fields"</i>
<b>P28</b>	<b>Veronika Mladenova</b> Essen, Germany	<i>"Generation of human cell lines to explore the biological consequences of double-strand break clustering"</i>
<b>P29</b>	<b>Elizaveta Moskaleva</b> Moscow, Russia	<i>"DNA double-strand breaks measured by the level of histone <math>\gamma</math>H2AX in mouse neural stem cells after <math>\gamma</math>-irradiation at low and sublethal doses"</i>
<b>P30</b>	<b>Elizaveta Moskaleva</b> Moscow, Russia	<i>"The different sensitivity of mesenchymal stem cells from different mouse tissues to malignant transformation under the action of mixed gamma-neutron radiation"</i>
<b>P31</b>	<b>Mayumi Nishimura</b> Japan	<i>"Refractoriness to neutron-induced mammary carcinogenesis in parous rats"</i>
<b>P32</b>	<b>Andreas Ntargaras</b> Athens, Greece	<i>"Study of the systemic nature of ionizing radiation through the detection of complex DNA lesions on mice tissues"</i>
<b>P33</b>	<b>Franziska Papenfuß</b> Heidelberg, Germany	<i>"Super Resolution Localization Microscopy visualizes endothelial agonists and membrane protein turnover under anticancer therapy conditions"</i>
<b>P34</b>	<b>Annika Reddig</b> Magdeburg, Germany	<i>"Application of automated <math>\gamma</math>H2AX foci assessment to examine the impact of 7 Tesla magnetic resonance imaging on DNA damage formation in human blood lymphocytes"</i>
<b>P35</b>	<b>Alexander Rühle</b> Heidelberg, Germany	<i>"Radiation resistance of mesenchymal stem cells is independent of their tissue of origin"</i>
<b>P36</b>	<b>Traimate Sangsuwan</b> Sweden	<i>"Gamma radiation effects on Drosophila melanogaster development of embryo"</i>
<b>P37</b>	<b>Zoé Schmal</b> Homburg/Saar, Germany	<i>"Impact of repetitive low-dose irradiation on neurogenesis in adult and juvenile hippocampus"</i>
<b>P38</b>	<b>Yoshiya Shimada</b> Japan	<i>"Environmental enrichment modifies radiation response of intestinal cells in B6C3F1 mice"</i>
<b>P39</b>	<b>Mattia Siragusa</b> Denmark	<i>"Tritiated water effects at high dose rates on V79 cell survival "</i>

<b>P40</b>	<b>Tünde Szatmári</b> Budapest, Hungary	<i>"The effects of whole body low dose irradiation on the composition of extracellular vesicles and cytokine levels in murine blood plasma"</i>
<b>P41</b>	<b>Ikuno Takahashi</b> Hiroshima, Japan	<i>"Peripheral artery disease prevalence in the Japanese atomic bomb survivors, Hiroshima and Nagasaki"</i>
<b>P42</b>	<b>Feng Ru Tang</b> Singapore, Singapore	<i>"Neuropsychological changes and relevant neurocytoarchitectonic abnormality of the dentate gyrus after early life acute radiation exposure to mice"</i>
<b>P43</b>	<b>Vasiliki Tasiou</b> Essen, Germany	<i>"Effects of changes in chromatin structure on alt-EJ"</i>
<b>P44</b>	<b>Susanne Tonnemacher</b> Frankfurt am Main, Germany	<i>"Use of Tetracysteine and ReAsH as a Marker for 53BP1 in Fluorescence and Electron Microscopy"</i>
<b>P45</b>	<b>Ioanna Tremi</b> Athens, Greece	<i>"Radiosensitizing effects of the novel PARP inhibitor BMN673 on CHO c-NHEJ-Mutants"</i>
<b>P46</b>	<b>Bram Verstraete</b> Ghent, Belgium	<i>"Radiation-induced DNA damage response of hematopoietic stem cells after proton irradiation"</i>
<b>P47</b>	<b>Ermenegilda Vitale</b> Naples, Italy	<i>"Can be Solanum lycopersicum L. cv 'Micro-Tom' a good candidate for growth in Space? Testing the effects of High-LET ionizing radiation on plant growth, photosynthesis and"</i>
<b>P48</b>	<b>Jennifer Wadsworth</b> Edinburgh, United Kingdom	<i>"SELLR (Subsurface Experiment of Life in Low Radiation): Effects of ultra-low radiation environments on bacterial growth"</i>
<b>P49</b>	<b>Alexandra Wolf</b> Essen, Germany	<i>"Effect of HIF-1<math>\alpha</math> on radiation sensitivity and DNA repair of tumor cells"</i>
<b>P50</b>	<b>Constantinos Yeles</b> Athens, Greece	<i>"A unifying bioinformatic analysis of transcriptomic profiling experiments of radiation-induced, bystander effects uncovers distinct cellular mechanisms of response"</i>
<b>P51</b>	<b>Brian Yudhistiara</b> Heidelberg, Germany	<i>"The influence of a magnetic field on photon and particle therapy in normal human cells"</i>
<b>P52</b>	<b>Sebastian Zahnreich</b> Mainz, Germany	<i>"Repair deficiency of radiation-induced DNA double strand breaks in Fanconi's anemia fibroblasts"</i>

# Orthotopic Transplantation of Cancer Cells into Mice Lung Using a Stereotactic Technique

**Rozina Aktar**, R. Bütof , F. Tillner , M. Baumann and M. Krause

*OncoRay - National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technical University Dresden ,German Cancer Consortium (DKTK) Dresden, Germany*

Theoretical considerations, Monte-Carlo simulations and experimental findings suggest that DNA-incorporated Auger electron emitters (AEE) cause primarily complex and clustered DNA lesions. It was previously shown that the shape of AEE-induced cell survival curves resembles that of High-LET irradiation and, therefore, poses the question of an increased biological effectiveness and a separate quality factor for Auger electrons. During electron capture or internal conversion an electron vacancy in an inner atomic shell is created. Filling the electron vacancy by a higher shell electron can initiate a process of non-radiative energy transmission, commonly termed as “Auger effect”. During the process numerous low-energy Auger electrons (up to 27 in the case of Iodine-125) with a short range are emitted leading to energy densities and free radical production in the close vicinity of the emitter exceeding that of a 5 MeV alpha-particle traversing the DNA double-helix. Experimental data demonstrates, that the cyto- and genotoxicity of AEE is comparable to low-LET radiation per unit dose when the AEE is exclusively located in the cytoplasm. However, in case of DNA- incorporation RBEs ranging from 5 – 9 are frequently reported. Employing the alkaline and neutral comet assay, the high DSB/SSB ratio of I-125-iododeoxyuridine derived from Monte-Carlo simulations could be experimentally confirmed. The unique properties of AEE and the possibility to target DNA in a sequence-specific manner using AEE-labeled Triplex-forming oligonucleotides (TFOs) enable to study the repair of complex DNA lesions at defined sites in more detail. A transgenic SCL-II p2RT strain carrying the stably integrated recoverable p2RT vector system harboring a specific triplex target sequence for TFO-p2RT will help to analyze the repair efficiency of complex DNA lesions regarding mutation frequency, mutation type and mutation localization.



## Proliferating versus quiescent cells: Repair and survival of low- or high-LET radiation induced DNA double-strand breaks

**Nicole Averbeck**, T. Syzonenko, E. Abdollahi, B. Jakob and G. Taucher-Scholz

*GSI Helmholtzzentrum für Schwerionenforschung, Darmstadt, Germany*

DNA-damage complexity and the cell-cycle stage influence the repair processes of DNA double-strand breaks (DSBs). The different processing of DSBs in G2- versus G1-phase cells in dependence of damage complexity is well characterized. Processes in quiescent G0 cells, however, are scarcely studied and hence we started to characterize DSB repair and survival of DNA damage of different complexity in normal human fibroblasts that were either in G1 phase or in G0 phase (quiescent). X-ray induced rather simple DSBs are repaired by G0 cells, yet, far slower than in G1 cells. Inhibiting alternative (alt) NHEJ, which is characterized by slow repair kinetics, with PARP1 inhibitor caused no repair deficiency suggesting that the slower repair kinetics in G0 cells is not based on the usage of the alt-NHEJ pathway. Impairing classical NHEJ with a DNA-PKcs inhibitor caused a severe repair defect in G1 and G0 cells. This suggests that c-NHEJ is the main DSB repair pathway not only in G1 cells but also in G0 cells. The slower kinetics in G0 cells may be based on a more compact chromatin structure as FLIM data and Western analyses on heterochromatic histone markers suggest. Interestingly, the repair kinetics of complex heavy-ion induced DSBs is hardly different in G1 and G0 cells. While in G1 cells complex heavy-ion induced DSBs are repaired slower than less complex X-ray induced DSB, show G0 cells similar repair kinetics for both damage types. The obtained repair data are mirrored in survival data.

This work is part of the DFG funded graduate school GRK1657 and funded by the BMBF grant 02NUK037A.

# Track-structure simulation of $\gamma$ -H2AX foci and comparison with experimental results: unravelling the role of radiation quality

**Sofia Barbieri**, G. Babini, G. Baiocco, J. Morini, W. Friedland, M. Buonanno and A. Ottolenghi

*Physics Department, University of Pavia, Italy*

The pattern of discrete energy depositions along a radiation track depends on radiation quality and determines the spatial distribution of DNA damage. High LET radiation causes hardly repairable cluster damages, whose deleterious effects are exploited with beneficial consequences in hadrontherapy. On the other hand, the production of secondary radiation, such as neutrons, during particle therapy might contribute to the risk of radiation-induced secondary cancer. Different experimental assays help to detect radiation-induced DNA lesions and the dynamics of their induction and repair and to unravel the underlying mechanisms, however, track-structure codes have become a unique mean to characterize the damage complexity, eventually correlated to the late biological outcome, with respect to the initial physical events. In this work, the strategy for benchmarking the results of track-structure simulations against experimental results will be discussed: the Monte Carlo biophysical code PARTRAC will be used to quantify complex DNA damages and to predict the induction of  $\gamma$ -H2AX foci after irradiation with different radiation qualities, namely low-LET X-rays, high-LET alpha particles and neutron broad beams. In particular, the LET/dose dependence has been investigated. From the experimental point of view, irradiation of both normal and cancer cells has been performed at the Radiological Research Accelerator Facility, Columbia University, New York, USA with the chosen radiation qualities. For neutrons, the available broad beam simulates the neutron spectrum from an improvised nuclear device. The induction of  $\gamma$ -H2AX foci has been chosen as endpoint for the comparison with PARTRAC simulations, and precisely we characterized their spatial distribution and the temporal kinetics. Furthermore, different LETs (65-80-110-160 keV/ $\mu$ m) for alpha particles have also been tested on cancer cells.

## RNF138 stimulates DNA-end resection upon heavy-ion-irradiation in human G1-phase cells

**Carina Barent**, L. Niederreiter, A. Heselich, B. Jakob, G. Taucher-Scholz and N. B. Aeverbeck

*GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany*

Radiation represents a widely used clinical cancer treatment. In comparison to photon therapy radiotherapy with carbon-ions offers several advantages; among them a higher relative biological effectiveness (RBE). A crucial factor of the biological effects of radiation is the induction of DNA damage; primarily DNA double-strand breaks (DSBs). The DSB's reparability and also the quality of their repair determine the cell survival after irradiation. Photon-induced DSBs are repaired resection dependent only in the S/G2 cell-cycle phase, whereas at complex, ion-induced DSBs resection dependent repair occurs also in G1-phase cells. Because all resection dependent repair pathways known so far in G1 phase are error-prone, this most likely contributes to the effectiveness of high-LET radiation. To reveal the processes at heavy-ion induced DSBs in G1 phase, we study the regulation of resection upon heavy-ion irradiation in this cell-cycle phase. The ubiquitin ligase RNF138 stimulates DNA-end resection in S/G2 cells by RNF138-dependent Ku removal from DNA breaks. Furthermore, the ubiquitination of the resection factor CtIP by RNF138 is essential for CTIP's recruitment to DSB sites. Hence, we investigate whether RNF138 is required for resection in G1-phase cells upon induction of complex DSBs as well. We found that RNF138 is similarly expressed in S/G2 and G1 cells. Its knockdown decreases the fraction of RPA- and CtIP-positive cells upon irradiation with carbon ions not only in G2- but also in G1-phase cells. This suggests that after induction of complex DNA lesions RNF138 is required for resection-dependent repair in G1 cells.

This work is part of the DFG funded graduate school GRK1657 and funded by the BMBF grant 02NUK037A.

## In vivo radioprotective effect of the cellular prion protein

**Jacqueline Bernardino-Sgherri**, C. Siberchicot, N. Gault, V. Barocca, P.-H. Roméo, J. P. Radicella and A. Bravard

*CEA, Paris, France*

The cellular prion protein (PrP<sup>c</sup>) is expressed in almost all tissues and at particularly high levels in the brain and the immune system. Its physiological role is still unclear although its conformational variant called scrapie-PrP<sup>c</sup> (PrP<sup>Sc</sup>) is responsible for the transmissible spongiform encephalopathies. We have recently showed (Bravard et al 2015 NAR 43 :904-16), both in vitro and in vivo, that PrP<sup>c</sup> protects neuronal cells from death after treatment with the alkylating agent methylmethane sulfonate. This protective effect was due to a more efficient repair of the MMS-induced DNA lesions in the presence of PrP<sup>c</sup>. On the contrary, cells that express less amounts or no PrP<sup>c</sup> do not efficiently repair these DNA lesions, leading to increased death rates. Indeed, we showed that PrP<sup>c</sup> directly interacts with Ape1, a key endonuclease of the main cellular DNA repair pathway, the base excision repair. By interacting with Ape1, PrP<sup>c</sup> stimulates specifically its DNA repair activity and therefore better cellular survival. Here we show that PrP<sup>c</sup> has also an in vivo radioprotective effect : 1) Prnp knockout mice (KO) exhibit a slightly higher sensitivity to Total Body Radiation exposure ; 2) We found that within the first 18 hours after irradiation at 7 Gy, the myeloid lineage cells in the bone marrow of KO mice are significantly decreased compared to Wild type mice (WT), whereas the lymphoid lineage show no change between both genotypes; 3) More specifically, within the first hours after irradiation, both the KO common and granulocyte myeloid progenitors show higher apoptosis rates compared to WT. We are now characterizing these bone marrow myeloid progenitors for their PrP<sup>c</sup> expression and Ape1 activity to test whether the radioprotective effect of PrP<sup>c</sup> in the myeloid lineage is linked to its property to positively modulate the DNA repair activity of APE1.

## Molecular mechanisms linking repair of DNA double strand breaks by alternative end joining to cell growth-state

Anna Broich, S.K. Singh and G. Iliakis

*Institute of Medical Radiation Biology, University of Duisburg-Essen, Medical School,  
Essen, Germany*

The human body is comprised of somatic cycling and non-cycling cells, whereby non-cycling cells vastly dominate proportionally. Progression through the cell cycle can be directly linked to the presence of growth factors. In contrast, in the absence of growth factors cells exit the cell cycle and enter G0 phase. The cell cycle status is a major determinant of the ionizing radiation response. Many adverse effects of ionizing radiation have their origins in the induction of DNA double strand breaks (DSBs). In higher eukaryotes, DSBs are predominantly removed by classical non-homologous end joining (c-NHEJ), which is highly dependent on DNA-PKcs. C-NHEJ operates with similar efficiency throughout the cell cycle and fails to show marked efficiency fluctuations with changing growth conditions. Cells with defects in c-NHEJ repair the majority of ionizing radiation induced DSBs using a second form of NHEJ operating with a high propensity for misrepair, thus leading to the formation of chromosomal translocations. This alternative form of NHEJ (alt-EJ) constitutes a potential backup mechanism for c-NHEJ. Unlike c-NHEJ, alt-EJ shows pronounced efficiency fluctuations throughout the cell cycle being most efficient in G2 phase. It is recognized that complete understanding of the response of tumor and healthy tissue to radiation therapy will require elucidation of the mechanisms underpinning responses of both cycling and non-cycling cells. While the response of cycling cells to radiation has been studied extensively, work on non-cycling cells is lagging behind. Here, address the role of cell cycle phase and growth state in the regulation of DSB processing. We describe biological systems allowing experimentation in defined growth stages and cell cycle phases. Alt-EJ, again in contrast to c-NHEJ, becomes severely compromised as cells cease cycling and enter G0 phase. The release of G0 phase cells into the cell cycle leads to recovery of DSB-repair by alt-EJ. Deficiency of DSB repair by alt-EJ during G0 phase correlates with a reduction in the abundance of CtIP protein. The reduction in CtIP protein levels is not caused by a reduction in its mRNA and points to regulatory mechanisms of protein stability. Acknowledgment: Work supported by DFG Research Training Group 1739, as well as by grants from the BMBF.

# DSB clusters as chromatin destabilizers and source of DSB processing errors

**Shipra Chaudhary**, E. Mladenov and G. Iliakis

*Institute of Medical Radiation Biology, University of Duisburg-Essen, Essen, Germany*

DSB are the most deleterious lesions for the integrity of the cellular genomes. Often their repair can lead to the formation of chromosomal translocations. This form of misrepair underlies cell death or transformation to a carcinogenic state. To counteract the detrimental effects of DSBs, cells have evolved multiple DSB repair pathways; error-free homologous recombination repair (HRR), and potentially error-prone, classical, non-homologous end-joining (c-NHEJ), which is orchestrated by the activity of DNA-PKcs. It is now widely accepted that when c-NHEJ and /or HRR are globally or locally inactivated, an alternative form of end-joining (alt-EJ) is engaged in the repair of DSBs. Alt-EJ operates with speed and fidelity markedly lower than c-NHEJ, which increase the probability for chromosome translocations, and generate more extensive sequence alterations at the junction. In this framework of thinking, chromosome translocations can be considered as accidents that compromise NHEJ or HRR allowing the operation of alt-EJ. While the potential sources of such accidents are multiple and diverse, DSB complexity is considered as one of them. DSB complexity is typically defined by the nature and the number of chemical alterations that accompany a DSB in close vicinity to the break site. The present project focuses on the characterization of the role of DSB complexity in chromosome translocation formation with particular emphasis on DSB clusters and the role of cell cycle phase in the final outcome. It is proposed to test the hypothesis that DSB clusters represent a highly dangerous form of DNA damage with a particularly high risk for misrepair as a result of the ensuing destabilization of chromatin. DSB clustering as a cause of irreversible radiation effects has been suggested in the past but mainly tested after IR using mathematical modeling and fitting to cell survival and DSB-repair results. Also the comparison between cell killing due to exposure to high LET radiation or low LET radiation is done on the basis of mathematical models. To overcome the limitations of the stochastic nature of IR-mediated induction of DSBs, we introduced a restriction endonuclease (RE)-based system in order to provide conclusive answers to this important question and to generate a basis for a comparison of the effects between these two DSB inducing modalities. The approach taken involves the generation of DSBs by I-SceI. Previously published work shows significant correlation between cell killing and chromosomal aberrations formation with increasing DSB clustering. Translocation-formation from DSB-clusters utilizes alt-EJ in their formation that showed a strong PARP1 dependence. Immunofluorescence experiments show formation of single γH2AX foci by both single and double strand breaks that suggest similar activation of early DNA damage response (DDR)(1). There was also an enhanced recruitment of the mediator protein 53BP1 at DSB-clusters in comparison to single-DSBs. These results suggest a strong dependence of 53BP1 foci formation on the complexity of the DSB. The above results were obtained from previously generated system in Chinese Hamster Ovarian cell lines harboring different levels of complexities. In order to investigate the possible consequences of DSB-clustering on DSB repair efficiency and to translate the previously obtained results to the human systems, constructs harboring various I-SceI sites engineered at different distances and orientations have already been established and validated at our lab.

**References:** [1] A. Schipler, et al.,(2016); Nucleic Acid Research; 44(16).

**Funding:** BMBF [02NUK043B-COLLAR] 2. DFG [GRK1739]

[shipra.chaudhary@uk-essen.de](mailto:shipra.chaudhary@uk-essen.de)

# Processing of Complex DNA Lesions in Mammalian Cells Induced by I-125 labeled Triplex-Forming Oligonucleotides

Volker Dahmen and R. Kriehuber

*Department of Safety and Radiation Protection, Forschungszentrum Jülich, Germany*

Introduction: Triplex-forming oligonucleotides (TFOs) are known for their ability to bind DNA in a sequence specific manner and are therefore a promising tool to manipulate genes or gene regulatory units. TFOs labeled with the Auger-Electron-Emitter Iodine-125 can induce complex but localized damage to the DNA. Using radionuclide-labeled TFO the subsequent cellular damage was analyzed regarding mutation frequency, mutation type and mutation localization. Methods: The human squamous cell carcinoma cell line SCL-II was used as the wildtype strain SCL-II WT and the transgenic strain SCL-II p2RT. In the conducted experiments the SCL-II WT strain was transiently transfected with an in vitro pre-formed DNA-triplex of the p2RT vector containing the target sequence and its specific TFO, the I-125-TFO p2RT. The transgenic SCL-II p2RT strain carry the stably integrated p2RT vector system harboring the specific triplex target sequence for TFO-p2RT and was, therefore, transfected with the I-125-TFO p2RT only. Efficient delivery of vector + TFO or TFO only was ensured by electroporation with the Nucleofector I system (Lonza GmbH, Basel). After storage at -150°C for decay accumulation the samples were analyzed for mutation frequency, type and localization using blue/white screening and sequencing. Results: In the SCL-II WT cell line an almost four fold increased mutation frequency at the target region on the p2RT vector was found when compared to the negative controls. In contrast, the SCL-II p2RT transgenic strain did not show a significant increase of the mutation frequency in comparison to the negative controls. Sequencing revealed that most mutants displayed large deletions of more than 100 bp located at the TFO p2RT target site. Conclusions: The local complex DNA damage induced by the decay of the TFO delivered Iodine-125 is likely to be accountable for the increased mutation rate in the SCL-II WT cells. Since this increase could not be detected in the SCL-II p2RT transgenic strain it can be hypothesized that the site-specific triplex formation between I-125-TFO p2RT and its target sequence is inhibited to some extent in the cellular environment.

# Biological efficiency of double scattering and pencil beam scanning modes in proton therapy

A. Michaelidesová, J. Konířová, J. Vachelová, M. Falk, I. Falková, K. Pachnerová Brabcová, M. Karabín, V. Štěpán, V. Vondráček and **Marie Davídková**

*Department of Radiation Dosimetry, Nuclear Physics Institute of the CAS, Prague, Czech Republic*

Until recently, double scattering (DS) has been the most common irradiation mode in proton cancer therapy. The latest technological advances brought into clinical practice scanning of target volume using proton pencil beam. However, biological consequences of the use of pencil beam scanning (PBS) instead of double scattering mode have not been described yet. The most of the biological studies of relative biological effectiveness of therapeutic proton beam were carried out using double scattering mode. The dose rates in pencil beam scanning are locally essentially higher than those in double scattering mode, which can affect the cellular response. DAOY medulloblastoma cells were irradiated by both irradiation modes using the same proton energies and doses at Proton Therapy Center Czech. The initial energy of protons used for the irradiations was selected according to the clinical options in double scattering. The same energy was then adjusted for the pencil beam scanning mode. The irradiation took place in two different positions, 2 and 23.6 cm in water, adjusted using RW3 and PMMA plates. The first position corresponds to the plateau region and the second one to the peak of Bragg curve. Cell samples were irradiated also by a Co-60 source for comparison. Cells were grown in T25 tissue culture flasks (TPP) in improved MEM (GIBCO) supplied by 10% FBS (BIOSERA) and 1% of standard antibiotics Pen/Strep (Sigma Aldrich). Several parameters of cell response (cell survival and apoptosis, gH2AX foci and micronuclei formation) were followed in various times post irradiation. The majority of radiation damage in living matter is caused by indirect effects of ionizing radiation by means of oxidative radical attack. Radiation induced hydroxyl radical yields were measured for both irradiation modes using solutions containing different concentrations of coumarin-3-carboxylic acid as a probe. This setup enables to determine hydroxyl radical kinetics. The experimental results were compared to values predicted by Geant4 track structure simulations. Based on our results, we conclude that pencil beam scanning mode is slightly more efficient than double scattering mode.



## Micronucleus assay for Fanconi Anaemia diagnosis with ionising radiation and mitomycin C

**Flavia Zita Francies**, R. Wainwright, J. Poole, J. P. Slabbert and A. Baeyens

*Radiobiology, Department of Radiation Sciences, University of the Witwatersrand, Johannesburg, South Africa; iThemba LABS - NRF, Somerset West, South Africa*

**Objectives:** Fanconi Anaemia (FA) is an autosomal recessive disorder that is clinically characterised by congenital and developmental abnormalities and haematological defects. FA cells are hypersensitive to DNA cross-linking agents such as mitomycin C (MMC). The association of FA genes in DNA repair is crucial in the maintenance of genomic stability. Deficiencies in DNA repair genes are linked to increased chromosomal radiosensitivity. FA patients undergoing radiotherapy have shown adverse normal tissue side-effects. Data on chromosomal radiosensitivity to ionising radiation in FA patients is, however, very limited. The main aim of this study was to determine the chromosomal radiosensitivity and genomic instability of 14 FA patients (homozygotes) and parents (heterozygotes) and compare it to controls using 3 different micronucleus (MN) assays. **Methods:** In the cytokinesis-block G0 MN assay, blood samples of FA patients and parents were exposed to ionising radiation of 2 and 4Gy. Cell division of the lymphocytes was stimulated by the addition of phytohaemagglutinin (PHA) and 23 hours later, cytokinesis was blocked by adding cytochalasin B (Cyto B). To detect damage induced in the G0 phase of the cell cycle, cells were harvested 70 hours post irradiation. In the cytokinesis-block S/G2 MN assay, lymphocytes were first stimulated with PHA. After 72hrs, Cyto B is added immediately following irradiation. The culture is stopped 8 hours post irradiation to detect DNA damage in the S/G2 phase of the cell cycle. In the third MN assay, a similar protocol was used as in the cytokinesis-block G0 MN assay but the chromosomal damage was induced by the addition of MMC instead of irradiation. Following harvest, slides for all assays were stained with acridine orange. Micronuclei were scored using a fluorescent microscope. **Results:** Spontaneous micronuclei values in FA patients are significantly higher when compared to parents and controls indicating genomic instability. FA patients also exhibited higher irradiated micronuclei frequencies. Chromosomal damage induced by MMC was also significantly higher in FA patients and parents compared to controls. **Conclusion:** Chromosomal radiosensitivity and genomic instability are demonstrated in FA patients. However, chromosomal radiosensitivity in FA patients is most prominent in the S/G2 phase. The MMC MN assay seemed to be the most sensitive assay to distinguish FA homozygotes from FA heterozygotes and controls.

# Biological assessment of the safe use of Dental Cone Beam Computed Tomography, at a molecular level

**Anastasios Georgoulis**, L. Berkas, V. Drakou, A. Tsoka, G. Terzoudi, U. Knippschild and C. Vorgias

*Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Athens, Athens, Greece*

The dental cone beam computed tomograph (DCBCT) is a new generation tomograph which has recently become an upcoming diagnostic tool in daily dental practice. The DCBCT provides a three-dimensional display of high diagnostic value of the maxillofacial complex. It has an advantage on other medical CT, as it exposes the patient to the less possible ionizing radiation, due to the conical beam irradiation. Nonetheless, ionizing radiation causes multiple damage to the DNA, depending on the time and dose of exposure to it. It is known that the maintenance of the genome's integrity is a prerequisite for a healthy organism, since it prevents the development of tumors. Until nowadays, the DNA damage caused by the DCBCT have been examined only on macromolecular level, studying the formation of micronuclei. Purpose of this study is to assess the impact of the ionizing radiation of DCBCT on epithelial cell line HEK 293 at a molecular level. Specifically, we examined the alterations of the factors that involve in various functions in the cells, as the DNA repair mechanisms, the cell cycle regulation, the apoptosis and the cell signaling. Hitherto, our results show alterations in expression of molecules involved in DNA repair but not cell cycle control pathways. Characteristic foci of phosphorylated  $\gamma$ H2AX, a marker of ds DNA damage, were clearly detected in HEK293 cell nuclei, in just half an hour after irradiation. In accordance, altered protein levels of key molecules involved in DNA repair such as BRCA1 and Rad51, were observed. More specifically, BRCA1 protein was significantly induced at least half an hour after irradiation, while Rad51 protein sustained quite higher than normal levels 48hours following irradiation. Our data clearly imply that DCBCT irradiation of HEK293 cells results in at least temporary modification of molecules involved in DNA damage detection and repair. BRCA1 is implicated in detection of DNA damage and further regulation of the consequent repair processes, while Rad51 is a key factor of homologous recombination, a high fidelity DNA repair mechanism.

## Carbon ions are more effective than photons after 1, 2 and 6 fractions in three sublines of syngeneic rat prostate tumors

**Christin Glowa**, P. Peschke, S. Brons, P. E. Huber, J. Debus and C. P. Karger

*Department of Radiotherapy and Radiooncology, University Hospital of Heidelberg, Heidelberg, Germany*

Background: Carbon ions (12C-ions) show an increased relative biological effectiveness (RBE) relative to photons. However, the underlying mechanisms in vivo are still unknown. Therefore, it is difficult to identify, which tumor characteristics (e.g. hypoxic status, differentiation) would be suited best for 12C-ion therapy. To investigate the impact of differentiation on RBE as well as the optimal fractionation schedule, dose-response curves for photons and 12C-ions were determined for three well characterized sublines of a rat prostate adenocarcinoma after irradiation with 1, 2 and 6 fractions (fx). Methods: Tumor fragments of three Dunning prostate tumor R3327sublines (AT1, HI and H) were transplanted s.c. into the distal thigh of male Copenhagen rats. Tumors were treated with single, 2 fx or 6 fx irradiations with increasing doses of either 12C-ions or 6 MeV photons. Primary endpoint was local tumor control within 300 days. RBEs were calculated based on TCD50-values (dose at 50% tumor control probability) of photons and 12C-ions, respectively. Results: Local tumor control was achieved with both, 12C-ions and photons, in all sublines and a higher effectiveness was found for 12C-ions. The RBE for local tumor control after single dose irradiation increased from  $1.62 \pm 0.11$  (H) to  $2.08 \pm 0.13$  (HI) to  $2.30 \pm 0.08$  (AT1). Variation of TCD50-values between tumor sublines was significantly smaller for 12C-ions than for photons and the dose-response curves for 12C-ions were steeper. With decreasing dose per fraction (increasing number of fractions), the RBE increased. Conclusions: The RBE of 12C-ions is highest for the anaplastic AT1-tumor and smallest for the well-differentiated H-tumor, which indicates a clear correlation between decreasing differentiation status and increasing RBE. 12C-ions may therefore be beneficial especially in undifferentiated tumors, which are highly resistant against photon irradiation. RBE changes were predominantly caused by changes in the photon response. This supports the assumption that the response to 12C-ions is less dependent on intra- and inter-tumor heterogeneity than for photons.

# The role of BRCA1 in regulation of prostate cancer cell radioresistance and tumorigenicity

**Ielizaveta Gorodetska**, C. Peitzsch, I. Kozeretska and A. Dubrovskaya

1) OncoRay-National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden and Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany; 2) Department of General and Medical Genetics, ESC "The Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

**Introduction:** BRCA1 (BRCA1 Cancer gene 1) is a tumor suppressor gene, and its mutant phenotype is associated with different types of cancers including prostate cancer. It was shown that BRCA1 protein is involved in DNA repair and regulation of transcription, cell cycle and apoptosis in response to DNA damage. Recent studies also showed a role of BRCA1 in chromatin remodeling, epigenetic regulation of gene expression and cell differentiation. The aim of this study is to understand the regulatory role of BRCA1-dependent epigenetic cell reprogramming for the radioresistant and tumor initiating prostate cancer cell populations. **Methods:** BRCA1 expression was knocked down in prostate cancer cell lines DU145 and LNCaP by small interfering (si) RNA. Aldehyde dehydrogenase (ALDH) activity was measured by ALDEFLUOR assay and flow cytometry. Clonogenic survival and radiosensitivity was analyzed for 12 primary prostate cancer cultures using 3D Matrigel colony-formation assay. Global comparative gene expression data for these primary cultures were analyzed by Ingenuity pathway (IPA) analysis. The Cayman Epigenetics Screening Library which contain more than 140 small molecules was applied to DU145 cell lines stably transfected with SORE6-mCMVp-dsmCherry-PURO or SORE6-mCMVp-dsGFP-PURO reporters which respond to the core stem cell transcription factors OCT4 and SOX2. DU145 cell lines stably transfected with mCMVp-dsCopGFP-PURO or mCMVp-dsmCherry-PURO were used as control. Analysis of the cancer stem cell (CSC)-enriched SOX2/OCT4 positive cell population by flow cytometry and analysis of  $\gamma$ H2A.X staining by Celigo cytometry were used as readout. **Results:** The results of radiobiological colony forming analysis demonstrated that siRNA - mediated knockdown of BRCA1 expression led to the radiosensitization of prostate cancer cells. We have demonstrated that BRCA1 knockdown increases prostate cancer cell population with a high ALDH activity which is a marker of tumor initiating and radioresistant prostate cancer cell populations. These results suggest that the loss of BRCA1 expression could lead to an increase in tumor stem/progenitor cell population. To identify the regulators of prostate cancer cell population positive for expression of SOX2 and OCT4 which are well characterized regulators of tumor initiating cells and putative regulators of tumor cell radioresistance, we performed screening of the library of 140 chemical epigenetic regulators using the established DU145 SORE6-mCherry reporter cell line and corresponding control cell line. By this screening we have identified 12 compounds which increased  $\gamma$ H2A.X signal in response to irradiation and 4 compounds which decreased SOX2/OCT4 positive population, and therefore can potentially be used for tumor cell radiosensitization. Ingenuity pathway (IPA) analysis of the gene expression data and clonogenic survival of 12 primary prostate cancer cell cultures identified BRCA1-dependent DNA damage response as a one of the key regulator of cancer radioresistance. The BRCA1-dependent gene expression was downregulated in the radiosensitive (n=6) as compared to radioresistant (n=6) primary prostate cancer tissue cultures. **Conclusions:** Our results suggest that BRCA1 play an important role for prostate cancer cell radioresistance as well that the loss of BRCA1 expression leads to an increase in the tumor stem/progenitor cell population. By the screening of 140 chemical epigenetic regulators we have identified 12 compounds which increased  $\gamma$ H2A.X signal in response to irradiation and 4 compounds which decreased SOX2/OCT4 positive population, and can potentially be used for prostate cancer cell radiosensitization. The potency of these compounds to regulate BRCA1-dependent cell reprogramming and DNA repair is currently under investigation.

[Liza.Gorodetska@uniklinikum-dresden.de](mailto:Liza.Gorodetska@uniklinikum-dresden.de)

## Regulated systems of I-SceI expression for in-depth studies of the biological effects of DSBs and DSB-clusters

**Mohammad Sharif Mortoga Hasan**, V. Mladenova, E. Mladenov and G. Iliakis

*Institute of Medical Radiation Biology, University of Duisburg- Essen, Essen, Germany*

Cells exposed to Ionization Radiation (IR) accumulate DNA double strand break (DSBs) which are the most critical DNA lesion generating adverse biological consequences and if left unrepaired, could results in chromosomal aberration and cell death. It has been suggested that DSBs could be classified in distinct types, from simple DSBs with ligatable DNA end to DSBs clusters, which could destabilize locally the chromatin structure. While the repair of simple DSBs is well characterized the responses initiated by the DSBs clusters remains obscure. To date models based on mathematical or computational approaches have been used to assess the effect of simple DSBs. However, a precise biological model system mimicking DSBs clustering is still missing. In order to study such DSB-cluster associated adverse biological consequences, a model system based on the generation of DSBs by restriction endonuclease (I-SceI) has been established. Rodent CHO cell line harboring I-SceI sites engineered at different configurations with increasing DSB-cluster complexity was characterized for DNA damage responses after induction of DSBs at I-SceI sites. Obtained results show significant correlation between cell killings together with chromosomal aberrations with formation of DSBs clusters. The formation of chromosomal translocation is PARP1 dependent, which suggests that processing of DSB clusters is achieved by alternative end joining (alt-EJ). Moreover, equal activation of DNA Damage Response (DDR) signaling measured by  $\gamma$ -H2AX foci formation was observed in single-DSBs and DSB- clusters of different complexity. However the enhanced recruitment of the mediator protein 53BP1 was observed at DSB-clusters in comparison to simple-DSBs, suggesting a strong dependence of 53BP1 foci formation on the complexity of the DSB. In order to investigate the possible consequences of DSB-clustering on DSB repair efficiency and to translate the previously obtained results to the human systems, constructs harboring various I-SceI sites engineered at different distances and orientations have already been established and validated at our lab. Furthermore, to obviate the transfection related issues with rodent model, we have stably integrated into the genome of our newly generated I-SceI homing human cell lines, a chimeric I-SceI expression cassette (ddSceGR) where destabilizing domain (dd) derived from the FKBP12 is fused to N-terminus and ligand binding domain of the rat GR (Glucocorticoid receptor) is added to the C-terminus. Here we investigate the biological effects of I-SceI mediated DSBs and DSB-clusters of different levels of complexity in cell survival, DNA repair pathway activation and abruption of genomic integrity and some preliminary results based on this inducible I-SceI system will be presented.

# Radiation-induced re-arrangements of molecular complexes studied in 3D-conserved cells and cell nuclei by super-resolution localization microscopy

**Michael Hausmann**, M. Krufczik, E. Wagner, R. Chojowski, F. Theda, Ma.Eryilmaz, JH. Lee, S. Schumann, G.Hildenbrand and H. Scherthan

*Kirchhoff-Institute for Physics, University of Heidelberg, Germany*

Novel light microscopic super-resolution techniques enable optical resolution down to about 10 nm even in 3D conserved cells or cell nuclei. Localization microscopy, as one of these techniques, is based on the concept of using fluorescent labels that can be switched between two different spectral states (e.g. off/on) to achieve temporal isolation and thus spatial separation of molecular signals leading to pointillist images and quantitative spatial and structural parameter. Multi-colour localization microscopy has been applied in multiple studies of molecular re-arrangements after exposure to ionizing radiation and during repair processes. These experiments include ErbB2-receptor arrangements in membranes, conformational changes of chromatin, and recruitment of repair proteins and repair foci formation under different radiation and repair conditions. After specific labelling by antibodies against heterochromatin or oligo-nucleotide nano-probing against ALU-repeats, network-like structures were detected and characteristic changes were elucidated after X-irradiation and during an up to 48 hrs time course of repair. The data indicate dose and repair process-dependent de-compaction and re-compaction of the different types of chromatin addressed. The recruitment to and loss of repair proteins and foci formation (e.g.  $\gamma$ -H2AX, MRE11) at DNA damage sites was analysed at the nanoscale together with local compaction changes of their chromatin surroundings. The data show a dose-dependent early increase of the  $\gamma$ -H2AX DSB marker. During the early repair, the formation of dense  $\gamma$ -H2AX signals at dsDNA damage chromatin regions was increasing followed by continuous foci relaxation in the later repair phase. In addition the spatial interaction between foci formation and local chromatin re-arrangements was shown. A dose response was also observed by the spatial arrangement of ErbB2 receptors and their internalisation into the cytoplasm after X-irradiation. Since these receptors are involved in repair pathways, our measurements revealed radiation induced spatio-temporal modifications of these pathway endpoints. In conclusion, the investigations shown here demonstrate the broad potential of localization microscopy in biological radiation research in order to better understand spatial re-arrangements of molecular complexes and mechanisms behind radiation and repair response of individual cells.



## Interaction of DNA damage on micrometer and nanometer scale results in reduced cell survival

**Katarina Ilicic**, C. Greubel, T. Friedrich, D. Walsh, J. Reindl, S. Girst, C., Siebenwirth, M. Scholz, G. Dollinger and T. Schmid

*Department of Radiation Oncology, Technical University Munich, Germany*

Radiation of high linear energy transfer (LET), such as heavy ions, are well known to have an increased relative biological effectiveness (RBE) compared to low LET radiation. The biological difference between high and low LET radiation is a consequence of different spatial dose and DNA damage distributions of the charged particles that pass through the biological material. The highly inhomogeneous dose deposition of high LET particles where the dose is concentrated around the ion trajectories is supposed to be the reason for the enhanced RBE due to formation of complex DNA damage which is more difficult to repair, contrary to quasi-homogeneous distribution in the case of low-LET particles. The biological optimization of radiotherapy as well as the improvement of risk assessment in radiation protection requires an accurate knowledge on how the RBE depends on the spatial dose distribution of energy deposition and thus the LET. The aim of the present study is to assess in detail the influence of different spatial distributions of DNA damage on the RBE with respect to clonogenic cell survival. Using the ion microbeam SNAKE at the Munich tandem accelerator, Chinese hamster ovary cells (CHO-K1) were irradiated with 1.7 Gy of 20 MeV (LET= 2.65 keV/ $\mu\text{m}$ ) protons. The cells were exposed either to a grid like irradiation where at each spot 117, 234, 468 protons were focused to a spot size of about  $0.6 \times 1.2 \mu\text{m}^2$  or homogeneous proton irradiation. The mean dose and the number of induced DNA DSB was the same in all irradiation cases, only the spatial distance of DSB was varied. The measured cell survival was compared with the prediction calculations obtained using the biophysical local effect model (LEM IV). Experimentally obtained cell survival results showed a significantly reduced cell survival in all grid irradiation cases ( $0.39 \pm 0.04$ ;  $0.41 \pm 0.05$ ;  $0.42 \pm 0.05$ ) compared to homogeneously distributed protons ( $0.75 \pm 0.09$ ) of the same mean dose. This clearly demonstrates the importance of the interaction of DSB on the micrometer scale. Although the total number of induced DSB was not altered, the DNA repair is meant to result in more lethal chromosome aberrations and thus less cell survival due to the increased local density of DSB after grid like irradiation compared to a homogeneous proton application. The spatial proximity of DNA DSB on the micrometer scale as well as their interaction was confirmed when comparing cell survival after grid-like high LET radiation using lithium or carbon ions. Additionally, the exposure of cells to grid-like irradiation with lithium and carbon ions resulted in a reduced cell survival compared to the proton grid irradiations. This clearly demonstrates the importance of the induced DNA damage distribution on nanometer scale. The experimental data are in excellent agreement with the LEM calculations, supporting the existence of different spatial scales of DNA damage.

# Dose Rate Dependence of Rat Mammary Carcinogenesis Following Protracted $\gamma$ Ray Exposure

**Tatsuhiko Imaoka**, M. Nishimura, K. Daino, A. Hosoki, M. Takabatake, Y. Nishimura, T. Kokubo, T. Morioka, Y. Shimada and S. Kakinuma

*National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology, Japan*

There is firm evidence on the risk of breast cancer after high dose rate radiation exposure. Only inconsistent evidence has been provided, however, about the risk at low dose rates. We aimed at providing experimental evidence with a longer observation period than previous studies. Female Sprague-Dawley rats were whole-body irradiated with  $\gamma$  rays, which started at i) 3 weeks of age (before puberty) at a dose rate of 6 mGy/h (total dose, 1–4 Gy), ii) 7 weeks of age (after puberty) at the same dose rate (total dose, 1–8 Gy) or iii) 7 weeks of age at 3–60 mGy/h (total, 4 Gy), and observed until 90 weeks. Results were compared with those of high dose rate irradiation (30 Gy/h; total, 0.2–4 Gy) at 3, 7 and 13 weeks of age. As a result, irradiation at 6 mGy/h did not alter Cox's hazard ratio at doses 1–8 Gy, whereas that at 30 Gy/h resulted in dose dependent increase, at any age examined. Irradiation of 4 Gy at 60 mGy/h significantly increased, whereas that at 3–24 mGy/h did not alter, the hazard ratio. Thus,  $\gamma$  ray induction of rat mammary carcinogenesis is considerably small below a certain dose rate.



## Genomic alterations associated with radiation exposure in mice model

**Shizuko Kakinuma**, C. Tsuruoka, M. Sunaoshi, Y. Amasaki and Y. Shimada

*National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology, Japan*

Radiation is one of the identified causes of cancer. Studies of atomic bomb survivors show the risk of solid cancer mortality is positively associated with radiation dose above 0.1 Gy, but is uncertain below 0.1 Gy. Since the Fukushima Daiichi Nuclear Power Plant accident, public concern over the cancer risk associated with low dose radiation has been heightened. Yet, the available epidemiological data on low dose/low dose rate radiation are controversial. Using animal models, we have been investigating the characteristics of genetic changes in radiation-induced tumors. T-cell lymphomas of B6C3F1 mice are frequently developed after weekly exposure for four consecutive weeks, and they harbor mutations and concomitant loss of heterozygosity of tumor suppressor genes such as Pten and Ikaros. Loss of the wild allele of Pten is mediated by mitotic recombination, but that of Ikaros is interstitial deletion. Ptch1 heterozygous mice are prone to spontaneous and radiation-induced medulloblastoma (MB) development. Genomic alteration analysis of MB induced after high dose radiation exposure revealed that one can distinguish spontaneous from radiation-induced tumors by the mode of loss of the wild allele of Ptch1. Loss is mediated by mitotic recombination in spontaneous tumors, while it occurs by interstitial deletion in radiation-induced ones. On the other hand, acute or chronic exposure at 0.1 Gy did not increase the incidence of MB. Interestingly, the interstitial deletions were observed in 10% of mice after acute exposure, but only in 1-2% of that after chronic exposure, comparable to those in non-exposed mice. The mechanisms of genomic alteration in radiation-induced tumors will be discussed.

# Effects of low-dose gamma-radiation on myogenic properties of muscle stem cells: the aging context

**Dmitry Klovov**, S. Sebastian, J. Kavumkal, L. Bertrand and M. Flegal

*Canadian Nuclear Laboratories, Chalk River, Canada*

Exposure to low-dose ionizing radiation (LDR) has been associated with aging related health effects. An aging related decline in functional/regenerative properties of muscle stem cells in elderly people, as well as other muscle dystrophy conditions, represent a major health issue. Effects of LDR exposures, such as those common for CT scans, on functional properties of muscle stem cells are not known. We studied how <sup>60</sup>Co gamma-irradiation (10 and 100 mGy) affected cell proliferation and differentiation into mature muscle fibers in cultures of C2C12 mouse myoblasts or primary normal human myoblasts. We observed a substantial decrease in differentiation capacity in control unirradiated myoblasts with age/time of culture. Thus the fusion index dropped from 45% to <5% over 90 days of culture in C2C12 cells. Surprisingly, we observed that this loss of differentiation potential was partially restored in cultures exposed to LDR at early passages. This was accompanied by higher protein levels of MyH3 and Myogenin in myotubes formed by LDR exposed myoblasts compared to ones formed by the unirradiated muscle stem cells. Irradiated myoblast cultures maintained higher levels of Pax7 gene expression associated with higher stemness potential. RNAseq analysis revealed that LDR induced changes in gene expression patterns are characterized by higher motility/migratory gene expression, providing a mechanistic link to enhanced differentiation of LDR exposed muscle stem cells. Lastly, cellular proliferation of myoblast cultures was not affected by LDR at any of the time points examined. In conclusion, our results indicate that exposure to low doses of gamma-radiation does not negatively affect functions of muscle stem cells and that such exposures may enhance retention of their functional/regenerative properties with age.

## Modification of radiation-induced oral mucositis by heparin – preclinical studies

**Maria Koshanskaya**, E.Bozsaky, S. Gruber, P.Kuess and W. Dörr

*Department of Radiotherapy- ATRAB - Applied and Translational Radiobiology and  
Christian Doppler Laboratory for Medical Radiation Research for Radiation Oncology,  
Medical University of Vienna, Austria*

**Purpose or Objective:** Severe oral mucositis is a frequent and often dose-limiting early side effect of radio(chemo)therapy for head and neck tumors. Although it has a major impact on the patient's quality of life and tumor control probability, no biology-based prophylactic or mitigative approach has been generally introduced into clinical practice. Unfractionated or low-molecular-weight heparin (UFH, LMWH) both modulate multiple biological processes, such as proliferation, inflammation, infection, immune response and others. The purpose of the present, preclinical study was to investigate the effect of systemic administration of UFH or LMWH, on radiation-induced oral mucositis. **Materials and Methods:** This preclinical study was performed in the well-established animal model of the lower tongue mucosa in the inbred C3H/Neu mouse strain. For functional studies, mice were irradiated using either single dose or fractionated irradiation protocols with 5x3 Gy/week, given over one (days 0-4) or two (days 0-4, 7-11) weeks. All fractionation protocols were concluded by local top-up irradiation using graded doses to generate complete dose-effect curves. Daily doses of UFH or LMWH (40 or 200 I.U./mouse, respectively) were applied subcutaneously over varying time intervals. Mucosal ulceration was analyzed as clinically relevant endpoint. For mechanistic studies, groups of 5 mice were sacrificed daily, the tongues excised and subjected to histological/immunohistochemical analyzes. **Results:** Preliminary data clearly show that systemic application of the heparins significantly increased isoeffective doses for the induction of mucosal ulceration in fractionated irradiation protocols. Moreover, a tentative reduction of ulcer duration and prolonged latency was observed. **Conclusion:** These data provide the first evidence of a protective and/or mitigative effect of heparins for radiation-induced oral mucositis. Further studies are ongoing in order to optimize the drug administration protocols and to characterize the underlying mechanisms of the mucoprotective effect. These data will also be presented.

# Thalidomide ameliorates early radiation-induced urinary bladder impairment

**Jakob Kowaliuk**, E. Bozsaky, S. Sarsarshahi, P. Kuess and W. Dörr

*Medical University of Vienna, Department of Radiotherapy- ATRAB - Applied and Translational Radiobiology, Vienna, Austria*

The urinary bladder represents an important organ at risk in the radiotherapy of pelvic tumors. Exposure to significant radiation doses results in a decrease in bladder capacity. Patients suffer from dysuria, urgency, incontinence, and increased micturition frequency, including nocturia. The radiation response occurs in three distinct phases: (1) a reversible, biphasic early response, (2) a symptom-free latent phase, which can - inversely depending on the radiation dose - last from months to many years and (3) an irreversible late phase eventually resulting in fibrosis. Local inflammatory processes are significantly involved in the pathogenesis, with a potential role of the transcription factor NF- $\kappa$ B. Therefore, thalidomide, a potent NF- $\kappa$ B inhibitor, is studied in a mouse model for its potential to prevent or alleviate bladder dysfunction. Thalidomide mediated NF- $\kappa$ B inhibition clearly reduces radiation-induced functional urinary bladder changes (response: >50 % reduction in bladder capacity at the intravesical pressure of 10 mm Hg). Daily administration from day 0 – day 15 or day 15 – day 30 significantly increased the radiation tolerance. This may influence consequential late changes in the urinary bladder.

# The influence of chromatin structure on DNA double strand break repair pathway choice

Lisa Marie Krieger and G. Iliakis

*Institute of Medical Radiation Biology, University of Duisburg-Essen, Essen, Germany*

Double strand breaks (DSBs) are a natural consequence of the cellular metabolism, but can also be produced by exogenous agents like ionizing radiation (IR). DSBs can lead to chromosomal aberrations that promote cell death, genomic instability and tumorigenesis. Cells of higher eukaryotes evolved highly conserved repair pathways to process this form of DNA damage. Beside the only error-free repair by homologous recombination (HR), there are two forms of end joining processes: classical non-homologous end joining (c-NHEJ) and alternative end joining (alt-EJ). Particularly alt-EJ is known to be mutagenic and implicated in formation of translocations. The work presented here focuses on chromatin structure as a parameter determining repair pathway choice. In G2-phase of the cell cycle, all three repair pathways can be active and we think that chromatin structure surrounding the DSB plays an important role in repair pathway choice. Here, we employ hypo- and hypertonic medium to relax or condense chromatin, respectively, and investigate effects on DSB repair and DNA damage response. We study DSB repair and DNA damage response using human wild type retinal pigmented epithelium (RPE) cells and immunofluorescence confocal microscopy, as well as flow cytometry as methods to detect ionizing radiation-induced foci (IRIF), or accretion of DNA damage repair proteins to chromatin, respectively. To achieve condensation/decondensation of chromatin, media with different molar salt concentrations (150 mM isotonic; 75 mM hypotonic; 300 mM hypertonic) are used. The results show a high sensitivity of HRR to condensation or decondensation of chromatin. Especially, hypotonic treatment abrogates ATM initiated DNA damage response signaling. Notably, the effects of chromatin alteration on DNA damage response appear to be reversible, suggesting build-in flexibility in such changes in cellular physiology. Experiments on the effects of postirradiation chromatin alterations on checkpoint response and cell survival are underway and will be presented. Finally, the effects of small molecule inhibitors such as Trichostatin A and Chaetocin that are known to alter chromatin structure are underway and will also be summarized. Ultimate goal of these studies is to define the relative requirements for and sensitivity to chromatin structure alterations of the three repair pathways processing DSBs.

**Acknowledgments:** Work supported by Grants from the BMBF (02NUK037B).

# Growth state dependence of alt-EJ and the role of the chromatin structure in this response

Pelin Kucuk and G. Iliakis

*Institute of Medical Radiation Biology, University of Duisburg-Essen, Essen, Germany*

DNA Double Strand Breaks (DSBs) are one of the most deleterious lesions induced by Ionizing Radiation (IR). Classical Non-Homologous End Joining (c-NHEJ), which is active throughout the cell cycle and Homologous Recombination Repair (HRR), which is only active in late S and G2 phases, are the major DSB repair pathways. Increasing evidence suggests that, when c-NHEJ is chemically or genetically compromised, the majority of DSBs are repaired by an alternative end joining pathway (alt-EJ). Contrary to c-NHEJ, alt-EJ functions with slow kinetics and utilizes PARP, MRN and DNA Ligases I and III, whereas c-NHEJ requires Ku, DNA-PKcs and DNA Ligase IV. To date, many efforts have been made to characterize the mechanistic underpinnings of alt-EJ. In the present study, we investigated the role of growth state and chromatin structure in alt-EJ. For this purpose, we used mouse embryonic fibroblasts (MEFs) with genetic defects in c-NHEJ, along with wild type (WT) MEFs as controls. Through passaging every 2 days, cells were kept in a phase of exponential growth; by removing serum from the medium, cells were arrested in G0/G1, referred as 'SD (Serum Deprived)'. In order to alter the chromatin structure, cells were treated with HDACis (Histone Deacetylase inhibitors), which are known to relax the chromatin through hyperacetylation of lysine residues on histone tails and thus reduction of the histone-DNA interaction. Cell cycle distribution and acetylation levels, which reflect growth state and chromatin structure, respectively, were monitored by flow cytometry. Repair of DSBs, induced by exposing cells to 20 Gy of X-rays, was measured by pulsed-field gel electrophoresis (PFGE). Our findings indicate that alt-EJ activity is growth state dependent and abrogated in plateau phase. However, alt-EJ activity remains unchanged following HDACi treatments, indicating that the dependence of alt-EJ on growth state may be mediated by mechanisms other than acetylation-dependent alterations in chromatin structure.

## Low-dose ionizing radiation did not lead to cataract formation in mice

**Sarah Kunze**, C. Dalke, F. Neff, SM. Hölter, S. Hornhardt, H. Zitzelsberger, S. Tapio, M. Atkinson, U. Kulka and J. Graw

*Helmholtz Center Munich, German Research Centre for Environmental Health, Institute of Developmental Genetics, Neuherberg, Germany*

Ionizing radiation is well known to cause cataracts, but the question of a corresponding threshold for the lens is still open. For a better understanding, wild-type mice and heterozygous *Ercc2* mutants were irradiated at the age of 10 weeks with doses between 0 Gy - 0.5 Gy (60Co-source, dose-rate 0.063 Gy/min). *ERCC2* (Excision Repair Cross-Complementation Group 2, also known as XPD, xeroderma pigmentosum group D; OMIM 278730) is a DNA repair helicase and transcription factor. Because of its recessive mode of inheritance, only homozygous mouse mutants develop cataracts 3 weeks after birth<sup>1</sup>. Heterozygous mutants showed an increased radiation-sensitivity in lymphocytes and were hypothesized to show an earlier onset of cataracts after irradiation. Irradiated mice were sacrificed at different time points (4/24 h, 12/18/24 months after irradiation) and various organs (brain, eyes, blood, liver, spleen and others) were collected for detailed analyses. 24 months post irradiation, both mutants and wild types did not develop cataracts. Sections of the eyes were examined immunohistochemically to analyse molecular changes and damages. Staining against 8-OHG (8-hydroxyguanosine; oxidation products of RNA and DNA) showed a higher basal oxidative stress level in mutant mice, but no increased ROS-level related to radiation were observed. Staining against DNaseIIb was performed to analyse variations in lens fibre cell differentiation. The distribution of DNaseIIb showed no differences between wild types and mutants at all time points and radiation doses. Histological analyses showed no major morphological changes in all animals. The absence of cataract formation or other molecular changes in the lens is in contrast to previous findings in mice<sup>2</sup>. As the study is still in progress, further analyses are planned to confirm these results in lens organ cultures and lens epithelial cell culture. Since radiation-induced tumours were observed in other organs of these irradiated mice, we conclude that the eye lens is not one of the most radiation-sensitive organs. This work is supported by the German Federal Ministry of Education and Research (FKZ 02NUK045A).

# Study of bystander signals emitted by chondrosarcoma cells irradiated in vitro with X-rays and carbon ions

**Charlotte Lepleux**, Y. Saintigny and F. Chevalier

*LARIA – Cimap / IRCM - Commissariat à l’Energie Atomique et aux Energies Alternatives,  
Caen, France*

For the treatment of cancer resistant to conventional radiotherapy, hadrontherapy with carbon ions seems to be a good alternative. Hadrontherapy uses accelerated ions; this technique is interesting when the tumor is deep and isolated or located close to sensible organs. Indeed, the dose deposit is maximal within the tumor volume (Bragg peak), and minimal before and after the tumor, preserving the surrounding healthy tissues. Chondrosarcoma is a good candidate for hadrontherapy because this cancer is radio and chemo resistant, and can be un-operable, when located in the skull base. However, it is necessary to evaluate the secondary effects of this irradiation type, especially the interaction between irradiated and non-irradiated cells. Among these effects, the radiation-induced bystander effect involves stress signals emitted by irradiated cells adjacent or very close to non-irradiated cells; bystander molecules can induce a biological response with damages usually observed with irradiated cells [1,2]. To study this phenomenon, we used a protocol of medium transfer. Cells are irradiated with X-rays or carbon ions and then the bystander supernatant, containing the signals emitted by irradiated cells, is transferred on non-irradiated cells. Chondrosarcoma cells and chondrocytes were analyzed as emitting and/or receptor cells of bystander signals. We use different technical strategies, such as clonogenic assay, to study the survival cells fraction after treatment; multiplex Elisa analysis of conditioned medium for the identification of bystander factors and flow cytometry for cell cycle analysis of irradiated and bystander cells. Our results showed a significant reduction of chondrocyte survival (until 65%) after transfer of conditioned medium from chondrosarcoma cells irradiated with low doses of X-Rays and C-ions. By diluting this medium, the phenomenon decreased proportionally, confirming the presence of bystander factors. Some of these factors were partially observed using multiplex analysis of cell cytokines. As observed by flow cytometry, the reduction of survival rate didn’t affect the repartition of bystander cells on each cycle phases. Taken together, these results showed the capacity of chondrosarcoma cells to secrete bystander signals, particularly at low irradiation dose, and the capacity of chondrocyte cells to receive these signals. Even if in vivo experiments are still required, these results open the discussion with the medical staff for protocol adaptations during radiotherapy, in order to limit the damaging impact of bystander effect within the healthy tissues.

[1] K.M. Prise, J.M. O’Sullivan, Nat. Rev. Cancer. 9 (2009) 351–360.

[2] F. Chevalier, D.H. Hamdi, Y. Saintigny, J.-L. Lefaix, Mutation Research/Reviews in Mutation Research. 763 (2014) 280–293.



## Tracking Kras mutations in human and murine acute myeloid leukaemia: role in radiation leukaemogenesis

**Grainne Manning**, L. Cruz Garcia, K. Manola, M. Pagoni, N. Brown, R. Finnon, J. Zyla, J. Polanska and C. Badie

*Public Health England, Centre for Radiation, Chemical and Environmental Hazards,  
Oxfordshire, UK*

Therapy-related acute myeloid leukaemia (t-AML) is a well-recognised complication of cytotoxic therapy for the treatment of a primary cancer. Radiation induces leukaemia in mice as well as in humans and the CBA mouse model is widely used in radiation leukemogenesis studies as at least some of the mechanisms of leukaemogenesis have been identified (Sfpi1/PU.1 interstitial chromosome 2 deletion and point mutation). It represents an ideal model system to improve understanding of the biological mechanisms underlying radiation-induced cancers. Kras is one of the most frequent AML mutations in human but its role in radiation-induced AML (rAML) is unclear. In this study we have screened 134 mouse rAML samples for Kras mutations specifically at the G12 codon mutation, (glycine to aspartic acid (G-->D) exon 1, chromosome 6) as it is the most commonly mutated codon, and has been shown to be an activating mutation. After PCR amplification, the samples were analysed by Sanger sequencing and mutations confirmed by pyrosequencing. We identified 3 leukaemic cases with a Kras mutation. The first resulted in a change of codon 12 (GGT-->GAT, present in two samples with an amino acid change of Gly-->Asp). The second mutation, present in the third sample is also affecting codon 12 (GGT-->CGT, resulting in an amino acid change of Gly-->Arg). Both PolyPhen-2 and PredictSNP algorithms predict a deleterious effect (87%). KRAS G12 mutations in human de novo and radiotherapy-related AML samples is also currently under analysis and data will be presented. In this study we have identified the presence of Kras mutations for the first time in the murine CBA mouse model of rAML in 3/134 (2.2% ) of cases, either a GGT-->GAT mutation together with a TGC Sfpi1/PU.1 hemizygous deletion and point mutation or a GGT-->CGT mutation with no Sfpi1 mutation (deletion under investigation). For one of these cases, the calculated percentage of leukaemic cells carrying the mutations allowed us to propose a model of clonal evolution in rAML, where we have reconstituted the sequence of mutational events in rAML, confirming the co-operative effect role of Kras mutation.

# "Application of the IMK model to cell survival curves following the exposure to intensity modulated radiation fields"

**Yusuke Matsuya**, S. J. McMahon, K. M. Prise and H. Date

*Graduate School of Health Sciences, Hokkaido University, Hokkaido, Japan*

Radiation-hit cells transmit cell-killing signals to (non-hit) bystander cells via intercellular signalling, an example of a "non-targeted effects" (NTEs). In radiotherapy using modulated fields, cells in the out-of-field region see greater cell killing than explained by the dose they receive alone. Here we evaluated in-field or out-of-field effects on surviving fraction of cells using a new mathematical model. We developed a new cell-killing model, the Integrated Microdosimetric-Kinetic (IMK) model incorporating potentially lethal damage repair (PLDR) and NTEs so as to consider the signal transmission from in-field cells to out-of-field cells and vice versa. To test the performance of the IMK model it was applied to measured cell survival data in AGO1522 cells exposed to X-rays with uniformed or modulated fields. The rate of PLDR in AGO1522 cell line (represented as  $(a+c)$  [h<sup>-1</sup>] in the model) was deduced from the cell survival recovery curve. Radiation quality of X-rays represented by dose-mean linear energy  $yD$  was calculated by using 2 types of Monte Carlo simulation codes, the Particle and Heavy Ion Transfer code System (PHITS) for photons' procedure and the an in-house code (WLTRACK) for electrons procedure. The model was then fitted to the data with a maximum likelihood method with Monte Carlo techniques in which 5 cell-specific parameters for DNA-targeted effects and NTEs were determined. The value of PLDR  $(a+c)$  for in-field and uniform-field cells were  $2.24 \times 10^{-2}$  and 0.312 [h<sup>-1</sup>], respectively. This reflects the reduction in split-dose recovery seen in modulated exposures. Based on the IMK model, it was found that in addition to causing out-of-field cell killing, in-field and uniform-field cell survival curves showed reductions in survival due to signalling effects from hit cells in the 0 to 6 Gy dose range. These predictions were found to be in agreement with experimental observations of in-field survival following DMSO treatment. In summary, the IMK model analysis shows that while signalling from in-field cells is the dominant contribution to out-of-field cell survival, it also contributes significantly to survival in irradiated populations.

## Generation of human cell lines to explore the biological consequences of double-strand break clustering

**Veronika Mladenova**, E. Mladenov and G. Iliakis

*Institute of Medical Radiation Biology, University Hospital Essen, Germany*

Ionizing radiation (IR) generates a broad spectrum of DNA lesions, encompassing single-strand breaks (SSBs), a variety of oxidative base modifications, DNA crosslinks as well as DNA double-strand breaks (DSBs). However, from the plethora of lesions induced by IR, DSBs elicit the most detrimental effects, including genomic rearrangements, chromosome aberrations, cell death, genetic mutations, and cancer. IR-induced DSBs are generated within clusters of ionizations and frequently comprise base damages and single-strand breaks in their vicinity generating unique DNA damage-clustering effect referred to as increased DSB “complexity”. Moreover, high-LET ionization modalities may also result in formation of DSB-clusters, comprising two or multiple DSBs that destabilize the chromatin structure, which may compromise the overall DSB processing. The increased biological effectiveness of high-LET radiation, such as alpha-particles emitted by radon gas or accelerated ion particles from the cosmic radiation, could be also attributed to the DSB-cluster formation. In order to confirm this long-known effect we utilize a recently introduced biological model, based on the generation of clonal human cell lines, allowing direct analysis of the assumptions regarding the biological effects of single DSBs and DSB-clusters. In the following study, derivatives of RPE-1 (retinal pigment epithelial), 82-6 hTert and lung carcinoma A549 cells, harboring multiple genomic integrations of engineered cassettes carrying I-SceI recognition sequence(s), were generated by “Sleeping beauty” transposon technology. We present the initial steps including generation, characterization and validation of the aforementioned human cell clones. The cleavage of I-SceI sites by transient expression of I-SceI endonuclease results in generation of single DSBs or DSB-clusters with increasing complexity as monitored by the accumulation of gamma-H2AX and 53BP1 at the sites of the damage. Such model system allows to assess the biological consequences of DSB-clustering at multiple genomic loci, its impact on chromatin integrity, its influence on the activation and efficiency of DSB signaling and DNA repair, as well as its effect on cell viability.

# DNA double-strand breaks measured by the level of histone $\gamma$ H2AX in mouse neural stem cells after $\gamma$ -irradiation at low and sublethal doses

G. Posypanova, M. Ratushnyak, A. Abisheva, Y. Semochkina and

**Elizaveta Moskaleva**

*National Research Centre "Kurchatov Institute", Moscow, Russia*

One of the late effects of ionizing radiation on the brain is the development of such impairments of cognitive functions as decreased ability to learn, impaired memory and attention. These complications are observed in 20-50% of patients after radiation therapy of brain and head and neck tumors. It is believed that the high sensitivity of cognitive functions to radiation is largely determined by a disturbance of neurogenesis in the subgranular region of the dentate gyrus within the hippocampus as a result of damage of the neural stem cells (NSC). The damaging effect of radiation is determined by the appearance of DNA DSB. Their level and the rate of repair determine both the survival of cells, and the level of mutations accumulation. The aim of this work was to study the sensitivity of cultured mouse NSC to  $\gamma$ -irradiation at low and sublethal doses by estimation of their clonogenic activity and survival in comparison with the level of DNA DSB accumulation and elimination measured as the level of histone  $\gamma$ H2AX in NSC nuclei.

The NSCs were isolated from the brain of newborn mice and cultured as neurospheres in DMEM/F12 culture medium containing the necessary additives in low-adhesive plates. NSCs were characterized by the level of nestin, markers of differentiation  $\beta$ -tubulin III, GFAP, O4 and NG2 and antigen CD133, using fluorescently-labeled antibodies to these proteins by flow cytometry. NSCs were exposed to 0.1 Gy, 1, 2 and 4 Gy at the facility "GUT-200M" ( $^{60}\text{Co}$ ) at room temperature in a culture medium. To measure clonogenic activity the neurospheres were fixed one week after irradiation, stained with hematoxylin and photographed for counting. The number of colonies was counted using the program Image-Pro Plus. The NSCs survival after irradiation was evaluated using the Mosmann MTT-test or sulforodamine B. The level of histone  $\gamma$ H2AX after irradiation at doses of 1 Gy and higher was determined using monoclonal antibodies to this protein by flow cytometry, and after irradiation at doses of 0.1 and 1 Gy using fluorescence microscopy. The statistical processing of the results was carried out using the Student's method using the computer program "Origin".

Cultured NSCs were nestin-positive, and only a small number of cells containing markers of astrocytes (GFAP), neurons ( $\beta$ -tubulin III), oligodendrocytes (O4), and oligodendrocyte precursors (NG2) were present in the general cell population. The clonogenic activity and survival of the NSC decreased in proportion to the dose of  $\gamma$ -radiation. In 7 days after irradiation at doses of 0.1; 1, 2 and 4 Gy the clonogenic activity of NSC was 100; 90; 83 and 20% of control, and survival rate 100; 74; 21 and 9%, respectively. Thus, clonogenic cells corresponding to the actual NSCs were more radioresistant than the general population of cells, which contains, in addition to stem cells some part of progenitor cells. One hour after NSC irradiation at doses of 1-4 Gy, the level of histone  $\gamma$ H2AX increased in proportion to the dose. In 24 hours after irradiation at these doses, the amount of histone  $\gamma$ H2AX in the NSC was returned to the control level. After NSCs irradiation at the doses of 0.1 and 1 Gy, an increasing of the number of histone  $\gamma$ H2AX foci in the cells nuclei was detected by fluorescence microscopy, which returned to the control level after 24 hours only after irradiation at a dose of 1 Gy. The decrease in the number of foci  $\gamma$ H2AX after irradiation at a dose of 0.1 Gy occurred more slowly. The clonogenic activity and survival of the NSC after irradiation at a dose of 0.1 Gy did not differ from the control. But after NSC irradiation at this dose, the stimulation of NSC differentiation into neurons was observed. Thus, complete DNA DSB repair was observed in the mouse NSCs in 24 hours after irradiation at doses of 1-4 Gy. The DNA DSB repair was slower after NSCs irradiation at a dose of 0.1 Gy. This work was partially supported by RFBR grant № 17-29-01033

## The different sensitivity of mesenchymal stem cells from different mouse tissues to malignant transformation under the action of mixed gamma-neutron radiation

**Elizaveta Moskaleva**, Y. Semochkina, A. Rodina, S. Arzumanov and G. Posypanova

*National Research Centre "Kurchatov Institute", Russia*

Mesenchymal stem cells (MSC) are the long-living cells that are present in almost all organs and tissues. Therefore one believes that in the organism certain MSCs could be transformed into cancer stem cells as a result of the spontaneous and induced mutations accumulation and trigger the growth of tumors. Earlier, we demonstrated the possibility of tumors development when the irradiated bone marrow (BM) MSC were administered subcutaneously into syngeneic mice and the absence of tumors after transplantation of control and irradiated MSCs from the brain (BR) after  $\gamma$ -radiation of MSCs (Moskaleva E. et al, 2017). The aim of this work was to study the possibility of malignant transformation of control and irradiated mouse MSC-BM, MSC-BR and MSC from adipose tissue (AT) after mixed  $\gamma$ -neutron ( $\gamma, n$ ) irradiation. The irradiation was carried out in a collimated beam of neutrons and gamma quanta at a special station of a nuclear reactor. MSCs at passage 29 were exposed to 0,05; 0,5 and 2 Gy, cultured for 10 passages and  $1 \times 10^6$  cells in 100  $\mu$ l of culture medium were transplanted subcutaneously into syngeneic mice line C57Black/6. The maximum RBE value for  $\gamma, n$ -radiation was 8 at a dose of 0.5 Gy. At higher doses in the range from 2 to 5 Gy, the RBE did not depend on the dose and was 2 - 2.5. Tumors were detected after 5 months only after transplantation into syngeneic mice of MSC-BM irradiated at doses of 0.05; 0.5 and 2 Gy. After transplantation of control MSC-BM and of control and irradiated MSC-BR and MSC-AT, no tumors were detected. After subcutaneous injection of  $\gamma$ -irradiated at doses of 0.1; 1 and 6 Gy MSC-AT, that is, at those doses at which tumors developed from irradiated MSC-BM under these conditions, no tumors from irradiated MSC-AT were detected. The dependence of the frequency of tumors development from irradiated MSC-BM on the dose of  $\gamma, n$ -irradiation was in the form of a bell. The level of histone  $\gamma$ H2AX increased after MSC irradiation at a dose of 0.5 Gy in 1.2 times, and at a dose of 1.0 Gy in 2 times and returned to the control level 24 hours after irradiation. An increase in the apoptosis level and the block of the cell cycle 24 hours after irradiation were detected starting at a dose of 0.5 Gy. It was found that the level of TGF $\beta$  secretion by all studied MSCs was practically the same. The level of cytokines VEGF and HGF were maximal in the culture medium of MSC-AT. The level of VEGF in MSC-AT was 10 times higher than in MSC-BM and 20 times higher than in MSC-BR. Secretion of HGF in MSC-AT was 8 times higher than in MSC-BM and 3.7 times higher than in MSC-BR. The maximal level of IL6 secretion was found in MSC-BR. After  $\gamma, n$ -irradiation an increase of VEGF secretion in MSC-BM, a decrease of IL6 secretion in MSC-BM and MSC-BR, and an increase in its secretion in MSC-AT were detected. Thus, under the action of  $\gamma, n$ -radiation, as well as after the action of  $\gamma$ -radiation, the secretion profile of the investigated cytokines is changed, depending both on the dose and on the type of radiation. Analyzing the possible association of tumors development from irradiated MSC-BM with the peculiarities of the cytokines secretion in these cells, we must again note a much lower level of the cytokines VEGF, HGF and IL6 secretion in MSC-BM in comparison with MSC-BR and, especially, MSC-AT. Apparently, the low level of these cytokines secretion in MSC-BM is more associated with malignant transformation of MSC-BM than changes in the level of their secretion after irradiation. The obtained results testify the high sensitivity of MSC-BM to malignant transformation after ionizing irradiation of different kinds and on the much higher resistance to transformation of mouse MSC-BR and MSC-AT. The mechanisms of these differences are not yet known.

This work was supported by RFBR grant №15-29-01234.

[moskalevaey@mail.ru](mailto:moskalevaey@mail.ru)

## Refractoriness to neutron-induced mammary carcinogenesis in parous rats

**Mayumi Nishimura**, T. Imaoka, K. Daino, M. Takabatake, T. Kokubo and Y. Shimada

*National Institute of Radiological Sciences National Institutes for Quantum and  
Radiological Science and Technology, Japan*

Breast cancer is one of the most common cancers in females throughout the world, and is well known to be modified by reproductive history. Women who carry full term pregnancy at early age of life are significantly less likely to develop breast cancer than nulliparous women. Similar to humans, parous rats have also reduced risk of chemically induced mammary carcinogenesis as compared to nulliparous rats. A-bomb survivor study reports that early-age at first full-term pregnancy and multiple birth are protective against radiation-related as well as baseline breast cancer. Quality of radiation, such as LET and energy of radiation, is a critical determinant for the risk of radiogenic cancer. Experimental studies have shown that neutrons exhibits an increased risk of cancer compared to gamma or X rays. Little data are available, however, about the effect of reproductive history on neutron carcinogenesis. In this study, we investigated the effects of pregnancy on neutron-induced mammary carcinogenesis. Female Sprague-Dawley nulliparous rats at 35 weeks after birth (sexually matured and middle aged) were whole-body irradiated with either gamma rays (Cs-137, 2 and 4 Gy) or fast neutrons of an average energy of 2 MeV (0.05, 0.2 and 0.5 Gy). Parous rats, which gave birth and breast fed twice prior to 30 weeks of age, were also irradiated at 35 weeks after birth. Rats were observed until 100 weeks of age and the incidence for mammary carcinoma, as compared to the non-irradiated group, was determined based on palpation records and pathological examination. The data to date indicate that the dose response of neutron induction of mammary carcinoma was concave downward while that of gamma-ray induced ones was concave upward, pointing in the direction of higher RBE at lower doses. Although parity in this experiment did not reduce the incidence of spontaneous mammary carcinoma, it, surprisingly, inhibited completely an induction of mammary carcinomas not only by gamma rays but also by neutrons. The data are now under analysis by Cox regression hazard ratio estimates. The biological mechanism(s) of the suppression of radiation-induced mammary carcinogenesis by full-term pregnancy will be discussed. Our findings offer information useful for risk assessment of breast cancer after female with cancer therapy with high energy particle radiations and for exposure of female astronauts to space radiation.



## Study of the systemic nature of ionizing radiation through the detection of complex DNA lesions on mice tissues

**Andreas Ntargaras**, S. I. Giannakandropoulou, V. Kotsaris, G. Terzoudi, O. A. Martin and A. G. Georgakilas

*National Technical University of Athens, Athens, Greece*

The ionizing radiation, through the direct cause of DNA lesions, is thought to be affecting not only the irradiated cells but also induce damages to areas distant from the irradiated area through systemic mechanisms. Clustered or complex DNA damages are the most harmful for the cell because they are repair resistant and highly mutagenic leading often to genomic instability and potentially carcinogenesis. For this reason, their proper repair is very important for the cell's normal function. In an effort to understand these phenomena, a study was conducted in two stages. In the initial, we studied isolated DNA from the tongue and the colon of a single group of mice (C57BL/6J (BJ6) from Ozgene Australia). The tissues were extracted 24 and 96 hours after the irradiation process, which used two different X-ray beam setups, micro-beam and broad-beam, each yielding two separate doses, 10 and 40 Gy. After confirming the systemic nature of ionizing radiation, a new cycle of experiments with a more specific set of parameters was uninitialized. In order to target the possible mechanisms implicated in the induction of DNA damage at distant sites i.e. the immune response, different sets of mice were used as positive controls: mice with deficient immune system, with antibody against the macrophages etc. These tissues were extracted at the 3rd and 7th day after initial irradiation. The method that we followed, during both experimental cycles, was an adaptation of agarose gel DNA electrophoresis and number average length analysis (NALA). For the detection of the DNA damages, we used two human DNA repair enzymes, APE1 (for abasic sites) and OGG1 (for oxidized purines). The number of the clusters which were detected with the APE1 enzyme were less than the OGG1 enzyme, and we also observed more efficient DNA repair mechanisms compared with the clusters of the OGG1 enzyme. The levels of DNA damages which were evaluated, were high enough compared to non-irradiated samples. Average values during the initial set of experiments for tongue tissues were 2.6 clusters/Mbp with OGG1 and 1.3 clusters/Mbp with APE1. For colon the same values were 3.1 clusters/Mbp and 1.3 clusters/Mbp respectively. In the second part of the experiments the average values for tongue tissues were 2.1 clusters/Mbp with OGG1 and 1.8 clusters/Mbp with APE1. The respective values for colon were 2.5 clusters/Mbp and 2.1 clusters/Mbp. It is becoming clear that cells exposed to ionizing radiation can communicate their DNA damage response status to cells that have not been directly irradiated. The results of this study indicate the induction of systemic responses and DNA lesions *in vivo* after local exposure to high levels of radiation and underline the major biological significance of this phenomenon.

# Super Resolution Localization Microscopy visualizes endothelial agonists and membrane protein turnover under anticancer therapy conditions

**Franziska Papenfuß**, G. Pilarczyk, F. Bestvater, M. Gunkel and M. Hausmann

*Kirchhoff-Institute for Physics, Heidelberg University, Heidelberg, Germany*

Introduction: In cancer therapy, ionizing radiation and antibody treatment are known for a successive tumour suppressive effect. In some cases, however, the treatment outcome seems to be behind the expectations. This has arisen the idea that a treatment induced release of neuregulin (NRG) in the tumour endothelium may lead to an adverse, i.e., tumour protecting effect. In epithelium NRG works in an antagonistic fashion to trastuzumab. Spectral Position Determination Microscopy (SPDM), an embodiment of Localization Microscopy, enables investigations of topological arrangements of for instance ErbB2 receptors, proNeuregulin (proNRG), an inactive precursor of NRG, and its sheddase BACE1. Methods: Human primary endothelial cells were treated with trastuzumab, neuregulin1 (NRG1) or irradiated by 6MeV-photons with a dose of 4Gy. ErbB2 receptors, proNRG and BACE1 were tagged with specific, fluorescently labelled antibodies. Their positions in the perinuclear cytosol and in the cell periphery at the membrane were determined using SPDM. SPDM facilitates quantitative distance distributions between single molecules for analyses of spatial arrangements on the nano-scale. Results: In endothelial cells, ErbB2 receptors, proNRG and BACE1 reveal a characteristic spatial organisation for each protein ensemble. For ErbB2 receptors, distance distributions indicate dimer formation in the cell periphery after irradiation as well as stimulation with NRG1. After irradiation compatible distance distributions were found in the perinuclear region whereas NRG1 treatment did not show this behaviour suggesting prevention of autocrine signalling in endothelium. Trastuzumab leads to reduced dimer formation in the cell periphery but shows no effect in the perinuclear cytosol. ProNRG and BACE1 distance distributions in cell periphery become broader after irradiation. In the perinuclear cytosol immobile BACE1 islets are entered by mobile proNRG indicating its cleavage. After NRG1 treatment, no significant change in the distance distributions is observed at the cell periphery. In the perinuclear cytosol, however, an adaption of proNRG to the characteristic organisation of BACE1 is measured indicating NRG release and thus suggesting autocrine signalling. Trastuzumab has two complementary effects on the BACE1/proNRG system: While proNRG is stimulated to enter BACE1 islets, the BACE1 islets themselves disaggregate. Conclusion: In endothelial cells, irradiation seems to trigger an adverse tumour protecting signal cascade via the release of NRG which ends in a positive feedback loop with additional NRG release due to autocrine signalling. Moreover, it can be shown that NRG and trastuzumab effect ErbB2 receptors in the cell periphery only; these conformation changes are not processed to the perinuclear cytosol. Consequently, the NRG-trastuzumab-antagonism is not applicable. In addition, trastuzumab acts on the BACE1/proNRG system.



## Application of automated $\gamma$ H2AX foci assessment to examine the impact of 7 Tesla magnetic resonance imaging on DNA damage formation in human blood lymphocytes

**Annika Reddig**, M. Fatahi, B. Friebe, D. Roggenbuck, O. Speck and D. Reinhold

*Institute of Molecular and Clinical Immunology, Otto von Guericke University Magdeburg, Magdeburg, Germany*

Phosphorylated histone protein H2AX ( $\gamma$ H2AX) represents a promising biomarker to assess radiation- and drug-induced DNA double-strand breaks and to analyze individual DNA repair capacity. To replace subjective and time-consuming manual  $\gamma$ H2AX focus quantification and to make this immunocytochemical assay available for standardized routine applications, the imaging system AKLIDES® was developed, combining microscope image acquisition, processing and analysis. In the present work, we compared the sensitivity of this fully automated  $\gamma$ H2AX analysis tool with  $\gamma$ H2AX determination by flow cytometry and western blotting. We confirmed that microscopic  $\gamma$ H2AX focus quantification represents the most sensitive approach and is most suitable when low  $\gamma$ H2AX levels are investigated. Further, we applied this automated  $\gamma$ H2AX assay as genotoxicity test to study radiation-induced DNA damage in human peripheral blood lymphocytes. Whereas former studies reported a dose-dependent induction of  $\gamma$ H2AX foci after x-ray-based medical imaging, such as computed tomography (CT), inconsistent results have been published regarding the genotoxic potential of non-ionizing radiation used during magnetic resonance imaging (MRI). Previous analyses were mainly performed after high-field MRI with magnetic field strength between 1–3 Tesla (T) but lack data about the genotoxic impact of ultra-high-field MRI ( $\geq 7$  T). For the first time, we investigated  $\gamma$ H2AX formation in lymphocytes after in vitro and in vivo 7 T MRI exposure. Lymphocytes and patients exposed to CT served as positive controls. Results revealed no significant alterations of  $\gamma$ H2AX foci in isolated lymphocytes before and after in vitro 7 T MRI ( $n = 16$ ). Additionally, our in vivo study also indicated no significant changes in  $\gamma$ H2AX focus levels in patients undergoing MRI ( $n = 43$ ), independent of applied MRI field strength (1–7 T) and administration of gadolinium-based contrast agent. In contrast, in vitro and in vivo CT exposure significantly increased the amount of  $\gamma$ H2AX foci per cell. Furthermore, we studied the genotoxic impact of in vivo 7 T MRI in repeatedly exposed subjects ( $n = 11$ ) compared to non-MR-exposed control group ( $n = 11$ ). Frequent 7 T MRI exposures did not result in detectable alterations in baseline or radiation-induced  $\gamma$ H2AX foci or micronuclei level and did not indicate changes in DSB repair kinetics. Thereby, these investigations support the acceptance of high-field and ultra-high-field MRI as a safe diagnostic imaging technique. Furthermore, our studies successfully demonstrated that the AKLIDES® system represents a fast and sensitive tool to analyze  $\gamma$ H2AX foci formation in peripheral blood lymphocytes. Thus, this will further facilitate the translation of  $\gamma$ H2AX as biomarker into genotoxicity assessment and clinical research.

# Radiation resistance of mesenchymal stem cells is independent of their tissue of origin

**Alexander Rühle**, R. L. Perez, C. Glowa, K.-J. Weber, A. D. Ho, R. Saffrich, P. E. Huber and N. H. Nicolay

*Department of Molecular and Radiation Oncology, German Cancer Research Center, Heidelberg, Germany*

**Background** Mesenchymal stem cells (MSCs) have been shown to attenuate radiation-induced tissue damage. MSCs were first isolated from bone marrow, but during the last years MSCs have been obtained from various tissues. While it could be shown that bone marrow-MSCs (bm-MSCs) are relatively radioresistant, the influence of ionizing radiation on MSCs of other human tissues is largely unknown. **Material and Methods** The survival of MSCs isolated from human bone marrow (bm-MSCs), adipose tissue (ad-MSCs) and Wharton's jelly (wj-MSCs) was measured by clonogenic assays, proliferation assays and metabolic assays. MSC morphology, adhesion ability, velocity, surface marker expression and the differentiation potential along the adipogenic, osteogenic and chondrogenic lineages was measured. Cell cycle distribution and apoptosis levels of MSCs were assessed using flow cytometry and DNA double strand break repair capacities were evaluated by immunofluorescence. The influence of irradiation on the expression of various cell cycle checkpoint and DNA repair proteins was investigated using Western Blots. **Results** The radiosensitivities of MSCs from different tissues of origin were comparable and similar to that of differentiated fibroblasts. However, there was a trend towards a slightly higher radiation resistance in ad-MSCs compared to bm-MSCs and wj-MSCs. Irradiation of ad-MSCs and bm-MSCs led to a strong accumulation of cells in the G2 phase of the cell cycle, whereas wj-MSCs showed no G2/M phase arrest after irradiation. Ad-MSCs and bm-MSCs showed relatively low levels of apoptosis after ionizing radiation, while there was a significant increase of apoptotic wj-MSCs after irradiation. Irradiation did not significantly influence cellular morphology, adhesion and migratory abilities irrespective of the tissue of origin; and ad-MSCs, bm-MSCs and wj-MSCs preserved their potential for adipogenic, osteogenic and chondrogenic differentiation. Immunofluorescence analyses of  $\gamma$ H2AX foci showed an efficient repair of radiation-induced DNA double strand breaks. DNA damage signaling proteins were found elevated after irradiation. **Conclusion** We could demonstrate for the first time that MSCs derived from different human tissues exhibit a comparable radioresistance, irrespective of the otherwise heterogeneous characteristics of these stem cells. MSCs completely maintained their defining stem cell characteristics independent of their tissue of origin. The observed radiation resistance of MSCs from various tissues may be useful when devising new studies for MSC-based treatments of radiation-induced side effects.

## Gamma radiation effects on *Drosophila melanogaster* development of embryo

**Traimate Sangsuwan**, M. Mannervik and S. Haghdooost

*Centre for Radiation Protection Research, Sweden*

Radiation protection research is challenged by the demands to provide a mechanistic understanding of effects of low doses and low dose rate on cells and organs to improve the risk estimates for cancerous and noncancerous effects such as vascular diseases, ageing process or reduced cognitive ability. It has been suggested these effects may be epigenetically mediated. The epigenetic event refers to changes in gene expression that do not involve changes in the underlying DNA sequence. Changes in patterns of histone acetylation, methylation and DNA base methylation are hallmarks of epigenetic events in several organisms. Most of the studies related to effect on epigenetic and differentiation effects of radiation have been done using high dose at high dose rate. It is still debating whether chronic low doses of gamma radiation influence the developmental processes or not. The developmental processes are controlled by epigenetic mechanisms. At the level of embryogenesis, epigenetic processes play an important role. In the present project we are studying the effect of dose and dose rates of gamma radiation on *Drosophila* embryos development as epigenetic model. In the first step of the project we established the dose response relation for embryonic development and further effects will be investigated. *Drosophila* embryos of different stage were collected and irradiated at the certain time point after egg laying to identify radiosensitivity of different embryonic stage. The very early stage about 20 minute after egg laying (AEL) is in the syncytium where it has only 1 to 8 nucleus. The middle embryonic stage 5 hours AEL and the nearly late embryonic stage about 10 hours AEL were irradiate chronically and acutely of gamma radiation. The radiation response of eggs survival will observe to determine radiosensitivity of different embryonic stages during development. This is also followed up the survival of larvae, pupae and adults from the irradiated eggs. Aim of the study is to investigate the effect of chronic low dose and different dose rate of gamma irradiation during *Drosophila* embryo development and try to answer if chronic low dose rate radiation could induced histone modification during *Drosophila melanogaster* embryogenesis.

# Impact of repetitive low-dose irradiation on neurogenesis in adult and juvenile hippocampus

Zoé Schmal and C. E. Rübe

*Departement of Radiation Oncology, Saarland University, Homburg/Saar, Germany*

**Introduction:** High-precision radiotherapy such as intensity-modulated radiotherapy (IMRT) enables precise sculpting of the dose distribution to the tumor, with deliberate avoidance of critical tissue structures. With IMRT, however, larger volumes of non-targeted tissue is repetitively exposed to low doses of ionizing irradiation, the consequences of which are unknown. Here, we examined brain tissue of adult and juvenile mice to provide a mechanistic understanding of how low-dose irradiation may adversely affect normal tissue in the short and long term. **Methods:** Following daily low-dose irradiation (with 5x, 10x, 15x, or 20x 0.1 Gy) of adult and juvenile C57BL6 mice, the DNA damage response was characterized by quantifying the induction and persistence of 53BP1 foci in mature Neurons (NeuN) by immunofluorescence microscopy. To examine the effect on the diverse cell populations, the number of neuronal stem (Sox2) and precursor (DCX) cells was quantified in the hippocampus by immunofluorescence analysis. Dynamics in axon and dendrite formation during fractionated low-dose irradiation were assessed in DCX-positive precursor cells. Moreover, radiation-induced changes in stem cell fate was analyzed using repeated BrdU cell-labeling during the first week of irradiation, after which the number of new neurons (NeuN), Astrocytes (GFAP) and Microglia (Iba1) were compared. **Results:** Even after very low doses of ionizing radiation the amount of DNA damages accumulated until 1 month post irradiation before declining. The number of proliferating precursor cells together with their outgrowth and dendritic branching decreased significantly and was most pronounced in the hippocampus of juvenile mice, both shortly after and up to 6 months post IR. In contrast, stem cells were initially unaffected however their numbers declined significantly between 1 month and 6 months after irradiation particularly in the juvenile hippocampus. In addition, cell fate shifted focus away from building new neurons and towards the formation of glia cells such as astrocytes and microglia. Altered synaptic plasticity may lead to functional changes in neuronal network activity, and consequently, to cognitive dysfunction with impairments in learning and memory performance. **Conclusion:** These preclinical studies provide valuable insights into the pathogenesis of brain injury in the non-targeted tissue exposed to low-dose irradiation. The resulting impaired neurogenesis may lead to serious side effects, such as severe cognitive impairment. A greater awareness of potential deleterious effects of fractionated low-dose irradiation may encourage the development of techniques to reduce the volume of low-dose irradiated brain tissue thereby potentially sparing the stem cell compartments within the brain. **Acknowledgement:** This research project is funded by the German Federal Ministry of Education and Research (02NUK035A).

## Environmental enrichment modifies radiation response of intestinal cells in B6C3F1 mice

S. Yokomizo , M. Nishimura, T. Morioka, Y. Nishimura, C. Tsuruoka, Y. Shang , S. Yamazaki, S. Kakinuma and **Yoshiya Shimada**

*National Institute of Radiological Sciences, QST, Japan*

Increasing evidence shows that exposure to an enriched environment (EE), a housing condition providing positive physical and mental stimulation, leads to improved metabolic health, resistance to diet-induced obesity and suppression of cancer growth. In this study, we established EE protocol in our laboratory and started to examine the effect of EE on deleterious effects of ionizing radiation. In a preliminary experiment, we found that EE to male B6C3F1 mice for eight weeks significantly decreased adipose tissue weight, but increased spleen weight. Serum total cholesterol, triglyceride, and glucose were also decreased by EE. Immunohistochemical staining study showed that EE had substantially higher levels of UCP1 in the brown fat, which contribute to dissipate energy directly as heat through uncoupling fatty acid oxidation and thus to promote energy expenditure. Furthermore, EE significantly decreased the serum levels of leptin, a major adipocyte hormone. These results indicate that our EE system works. Using this EE protocol, we first examined the effect of EE on radiation-induced apoptosis and cancer induction in small intestine and colon. Compared to standard environment (SE), EE appeared to induce apoptosis more efficiently in the crypt cells of colon, but not in those of small intestine, suggesting EE works to eliminate damaged cells from colonic crypts. For carcinogenesis study, we used ApcMin/+ mice with B6C3F1 background. Male ApcMin/+ mice were irradiated with X rays (2Gy) at 2 weeks after birth, when intestinal tumors are reported to efficiently induce by irradiation. After weaning at 3 weeks of age, they were housed in SE or EE cages for subsequent 17 weeks. Preliminary data indicate a reduction of polyps in colon in mice housed in EE compared to those in SE. But this beneficial effect was negligibly observed in small intestine, suggesting tissue dependence of EE on modification of risk of cancer.

# Tritiated water effects at high dose rates on V79 cell survival

Mattia Siragusa, PM. Fredericia, M.Jensen and T. Groesser

*Technical University of Denmark (DTU), Denmark*

Tritium ( $^3\text{H}$ ) is a radioactive isotope of hydrogen. It transforms into  $^3\text{He}$ , emitting a beta particle with a maximum energy of 18.6 keV and an average of 5.7 keV. Its physical half-life is 12.3 years. The maximum penetration depth of the beta electron is approximately 7  $\mu\text{m}$  in water, which is short if compared to the average size of mammalian cells. Tritium mainly exists in the environment as tritiated water (HTO) or in organic molecules as organically bound tritium. As HTO behaves just like water, it can freely diffuse between all cellular compartments, quickly providing a uniform distribution of this low-energy electron emitter. A critical step for estimating the Relative Biological Effectiveness (RBE) of HTO is related to the accuracy in calculating the absorbed dose. Calculations are made difficult mainly because of the dynamic exchange of tritium in biomolecules, especially during long exposures. In this work, RBE values were obtained using a high dose-rate of 3 Gy/h in order to reduce the exposure time. In addition, the dose-rate of the external gamma-ray source ( $^{60}\text{Co}$ ) was matched with the HTO dose-rate. Furthermore, V79 cells were grown either in suspension, or as attached to the flasks. The geometrical configuration was taken into account when calculating the absorbed dose through the use of the newly developed program COOLER. To calculate RBE values of HTO, clonogenic cell survivals was measured, and dose-effect curves were produced. The clonogenic cell survival was scored by eye. In addition, a newly developed method for an ImageJ based analysis, named CoCoNUT, was introduced and used to count the number of colonies after picture acquisition. The calculated RBE values for HTO at 10% survival fraction (D10) for adherent cells were 1.7 and 2.1 respectively when compared to external gamma-rays using acute exposures (90 Gy/h) or when using the matched dose rate. For cells in Suspension cultures revealed RBEs of 1.2 when compared to a similar dose-rate of external gamma-rays. These RBE values fit well with previously published RBE values, but indicate the importance of dose-rate and cell geometry when measuring RBE values in vitro. Results from a direct comparison of clonogenic cell survival curves for V79 cells growing under different geometrical conditions showed a similar (to each other) behavior.

## The effects of whole body low dose irradiation on the composition of extracellular vesicles and cytokine levels in murine blood plasma

**Tünde Szatmári**, E. Kis, A. Benedek, A. Balogh, G. Sáfrány and K. Lumniczky

*National Public Health Institute, Department of Radiobiology and Radiohygiene,  
Budapest, Hungary*

Background: Biological responses to low and high doses of ionizing radiation are different. Low dose radiation exposure might have important consequences in radiation protection, because of occupational exposures and its frequent usage during diagnostic imaging procedures. However, the effects and consequent health implications of exposures to low dose radiation are still controversial; the immunological responses elicited following low-dose irradiation are not elucidated. Soluble factors such as cytokines, ROS, microRNAs, as well as extracellular vesicles (EVs) play an important role in the development of a biological radiation response. The purpose of this study is to investigate effects of whole body low dose irradiation on blood plasma in vivo. Methods: C57BL/6 mice were irradiated with low dose X-rays (0.1 Gy), and as a high dose control with 2 Gy. Sham-irradiated animals were used as controls. Twenty four hours later mice were sacrificed, blood was collected in heparinized tubes and plasma was separated by centrifugation. To characterize the EV's cargo, EVs were isolated from the blood plasma of 5 animals and pooled. Total RNA was isolated from EVs and subjected to miRNA profiling (Exiqon, Denmark). The irradiation induced changes in miRNA cargo of EVs were assessed by bioinformatical tools. For the study of the cytokine expression, plasma samples from 4-5 animals were pooled and analysed using a proteome profiler antibody array (Mouse XL Cytokine Array Kit, ARY028, R&D Systems). Next, to investigate the possible role of EVs in mediating radiation induced bystander effects of low dose irradiation in vivo, we analysed the cytokine levels of plasma from non-irradiated mice receiving EVs from irradiated mice, through tail-vein injection. We used the same method as for directly irradiated mice. Results: In EVs isolated from plasma of irradiated mice 7 miRNAs were differentially expressed following low-dose irradiation and 11 after high dose, respectively, with a cut-off value of 1.5 fold change and p-value  $\leq 0.05$ . Although we did not find any overlap in these two miRNA sets, a target prediction and pathway analysis revealed that a set of signalling pathways, including several DNA damage repair related ones (TGF $\beta$ , FOXO, MAPK, ER $\beta$  signalling pathways) are altered by miRNAs irrespective of the irradiation dose. In contrast, a number of pathways, such as HTLV-I infection or leucocyte transendothelial migration were modified exclusively by miRNAs from mice irradiated with low dose. A number of cytokines had significantly altered levels following both 0.1 Gy and 2 Gy irradiation. The observed changes were small, but significant. We also identified several proteins which were affected only by low dose irradiation. The levels of a set of cytokines in the plasma of EV recipient animals also changed significantly. These changes were partially overlapping with the changes in directly irradiated mice: the levels of M-CSF, TSG-14, Angiopoietin-1 and MMP2 increased in directly irradiated and bystander animals. Conclusion: we show that low dose irradiation alters the microRNA composition of EVs and protein levels in murine blood plasma. We identified differentially expressed miRNAs and cytokines which are modified only by low-dose irradiation. In addition, we present evidences that EVs could mediate radiation induced bystander signals in vivo in blood plasma. This work was supported by DOREMI EU-FP7 project.



# Peripheral artery disease prevalence in the Japanese atomic bomb survivors, Hiroshima and Nagasaki

**Ikuno Takahashi**, J. Cologne, D. Haruta, M. Misumi, M. Yamada, A. Hida and W. Ohishi

*Department of Clinical Studies, Radiation Effects Research Foundation (RERF), Hiroshima, Japan*

[Background & purpose] Use of medical radiotherapy has increased markedly in recent decades, and past reports suggested that total body irradiation at dose levels of 0.5-1.0 Gy or higher could be responsible for atherosclerosis. Peripheral artery disease (PAD) is involved in a polyvascular disease and a manifestation of systematic atherosclerosis. Whether the consequence of low-to-moderate doses of radiation increases risk of PAD remains to be undetermined. The purpose of this study was to examine the association between radiation exposure and the prevalence of PAD/ upstroke time (UT) as a marker of early stage of atherosclerosis among Japanese atomic bomb survivors. [Methods] The association with radiation exposure from the atomic bombing was assessed in 3,476 participants (41.1% men, mean age ( $\pm$  standard deviation) 74.8 (6.4) years) with a cross-sectional survey in 2010-2014. Ankle-brachial indexes (ABI) and UT were obtained using oscillometric VP-2000. PAD was defined based on ABI of 1.0 or less or a prior history related to revascularization before the survey. Other potential risk variables were collected using data from RERF health examinations, including medical interview, blood tests, and physiological examinations: smoking history, high sensitivity C-reactive protein (hs-CRP), white blood cell (WBC) count, body mass index (BMI), systolic/diastolic blood pressure, total cholesterol, LDL/HDL cholesterol (LDL/HDL-C), triglycerides, glucose, HbA1c, estimated glomerular filtration rate (eGFR), hypertension (HT), diabetes (DM), and dyslipidemia (Dysl). Individual skin doses were based on the revised dose-reconstruction system (DS02R1). The relationship of PAD to potential risk variables was assessed with stepwise logistic regression with considering the effects of age, smoking, eGFR, hs-CRP, Dysl, and HT. That of UT was analyzed with a bivariate linear regression model fit with the generalized estimating equation (GEE) approach allowing for correlation between left and right UT in lower legs with considering the effects of age, sex, smoking, BMI, blood pressure, total cholesterol, LDL/HDL-C, WBC, hs-CRP, HbA1c and eGFR. [Results] Of 3,476 participants, 79 (2.3%) were identified as having prevalent PAD. Multivariate regression analysis indicated that dose was unrelated to PAD prevalence (odds ratio per Gy, 0.83, 95% confidence interval [0.57 to 1.21],  $p=0.347$ ). UT appeared to increase with radiation dose, but not significantly (change per Gy: +1.09 msec [-0.17 to 2.36],  $p=0.091$ ). The limited number of persons exposed to high doses of radiation (24 participants with 2 Gy+) may link with a lack of our finding a dose relation to PAD prevalence. [Conclusions] In the Japanese atomic bomb survivors (dose  $\leq 4$ Gy), UT may be associated with radiation dose, but we found no significant association of radiation dose with prevalent PAD.



## Neuropyschological changes and relevant neurocytoarchitectonic abnormality of the dentate gyrus after early life acute radiation exposure to mice

**Feng Ru Tang**, H. Wang, H. Y. Shen and G. Sethi

*Radiation Physiology Laboratory, Singapore Nuclear Research and Safety Initiative,  
National University of Singapore, Singapore*

Acute irradiation with 5Gy to postnatal day 3, 10 and 21 (P3, P10, P21) mice drastically reduced the number of Ki67 labelled cells in the hilus and subgranular zone (SGZ) of the dentate gyrus 1 day after the exposure. While there was no obvious change in the number of NeuN immunopositive neurons in the dentate gyrus 1 day after irradiation at P10 and P21, drastic loss of these neurons in the low blade of the stratum granulosum and in the hilus of the dentate gyrus was observed after irradiation at P3. Irradiation also reduced the number of newly generated neurons labelled by doublecortin (DCX) and NeuroD. At 7 days after irradiation, the reduction of Ki67 immunopositive cells in the dentate gyrus became less obvious after irradiation at the three postnatal days. Much less NeuN immunopositive granular cells in the low blade of the stratum granulosum was still observed in mice irradiated on P3 when compared to the control mice. There was also drastic reduction of DCX immunopositive dendrites and dendritic branches in the stratum moleculare, and reduction of NeuroD immunopositive neurons in SGZ of DG. By 4 months after irradiation at P3, P10 and P21, the reduction of the number of Ki67, DCX and NeuroD immunopositive neurons in SGZ still existed. Irradiation of P3 mice induced granule cell hypoplasia and aberrant cell genesis labelled by Ki67 or neurogenesis labelled by DCX in the stratum granulosum or stratum moleculare. Behaviour tests indicated that irradiation induced spatial memory impairment and depression when the exposure occurred at P3 or P3 and P10 respectively. At molecular level, up-regulation of miR34a-5p at 1 and 7 day(s) was observed after irradiation at postnatal day 3, but not at P10 and P21. It suggests that miR34a-5p may serve as an early biomarker for detection of the development of radiation-induced Alzheimer's disease (AD) and Depression.

# Effects of changes in chromatin structure on alt-EJ

Vasiliki Tasiou and G. Iliakis

*Institute of Medical Radiation Biology, University of Duisburg-Essen, Essen, Germany*

DNA of all eukaryotic cells is organized in dynamic chromatin compartments (eu- and heterochromatin), controlling the activity of many nuclear processes associated with DNA metabolism. It is well established that DNA replication and transcription are strongly affected by chromatin status. However, an increasing number of reports also suggests a role for chromatin structure in DNA double-strand break (DSB) repair. In eukaryotic cells DSBs are repaired mainly by classical, DNA-PKcs-dependent, Non-Homologous End-Joining (c-NHEJ), which operates throughout the cell cycle. Homologous Recombination Repair (HRR) is also processing a subset of DSBs and operates in S and G2 phase of the cell cycle. When the activity of c-NHEJ is genetically or chemically compromised a third, alternative pathway of end joining (alt-EJ), operating as a back-up, comes to the fore with activity that is substantially increased in G2 phase. However, there is evidence that the efficiency of alt-EJ is severely compromised in quiescent cells generated either by serum deprivation, or by allowing cell cultures to grow to a plateau-phase. Our hypothesis is that the expected higher level of chromatin structure compaction in quiescent cells contributes to the observed diminished efficiency of alt-EJ pathway. To test this postulate, we used a specific inhibitor of the methyltransferase enzyme SUV39 that is known to relax the chromatin. This inhibitor, Chaetocin, is already in use as an anticancer drug. Pre-irradiation treatment of serum-deprived HCT116 LIG4<sup>-/-</sup> (c-NHEJ mutant) with chaetocin improves repair of DSBs by alt-EJ. Indeed, under these conditions alt-EJ reaches efficiency levels similar to those normally seen in exponentially growing cells. To further elucidate the mechanism of alt-EJ suppression in quiescent cells, we used western blot analysis to compare the levels of CtIP in actively growing and serum deprived cells. We find a substantial decrease in the levels of CtIP in serum deprived cells. Notably, CtIP levels recover after treatment of cells with Chaetocin. Experiments are under way to further analyze the mechanistic underpinning of alt-EJ and its regulation in the different stages of cell growth. We particularly focus on the role of chromatin structure in the regulation of gene expression of key components of alt-EJ pathway and their role in DNA end-resection. Results along these lines will be presented.

## Use of Tetracysteine and ReAsH as a Marker for 53BP1 in Fluorescence and Electron Microscopy

**Susanne Tonnemacher**, G. Becker, A. Heselich, G. Taucher-Scholz and B. Jakob

*GSI Helmholtz Center for Heavy Ion Research, Goethe Universität Frankfurt am Main, Germany*

Correlative light and electron microscopy can combine the advantages of live imaging of dynamic processes by means of fluorescence microscopy (FM) with the high resolution of electron microscopy (EM). For this, markers are needed that can be detected in both microscope types connected to the different imaging modalities. In FM, typically GFP or related proteins are used to tag the protein of interest but in EM these cannot be detected and discriminated by the electron beam due to their protein nature. To overcome this limitation in the analysis and in the identification of double strand break (DSB) sites after irradiation, we are establishing the use of the repair protein 53BP1 tagged to Tetracysteine (TC). With a size of 4 kDa (including an additional V5-epitope), the proposed TC-tag is much smaller than GFP, lowering the probability of interference with the protein function. In FM, the TC-tag can be imaged when it is bound to Fluorescein Arsenical Hairpin (FIAsH, green fluorescence) or Resorufin Arsenical Hairpin Binder (ReAsH, red fluorescence). FIAsH-EDT2 and ReAsH-EDT2 are membrane permeable and non-fluorescent as long as they are not bound to the TC-tag [1]. The TC-tag bound to ReAsH can be used as a marker in EM as well. To detect ReAsH in EM one can make use of the photoconversion of diaminobenzidine (DAB) causing polymerization of DAB. The obtained product can be contrasted with uranyl acetate (UrA) leading to an electron dense material. Using this method, it should be possible to have live experiments and afterwards image the region of interest at high resolution in EM. First results establishing and x-ray irradiating a stable expressing NIH-53BP1-TC cell line will be shown.

**Acknowledgement::** This work was supported by BMBF Grant 02NUK037A and the graduate school HGS-HiRe

### References:

[1] Gaietta, Guido, et al. "Multicolor and electron microscopic imaging of connexin trafficking." *Science* 296.5567 (2002): 503-507.

# Radiosensitizing effects of the novel PARP inhibitor BMN673 on CHO c-NHEJ-Mutants

Ioanna Tremi, A. Soni, G. Iliakis, A. Georgakilas

*Institute of Medical Radiation Biology, University of Duisburg-Essen, Medical School, Essen, Germany; School of Applied Mathematics and Physical Sciences, Department of Physics, National Technical University of Athens, Greece*

Poly (ADP-ribose) polymerase-1 (Parp1) is the founding member of a family of enzymes consisting of 17 members that catalyze the addition of ADP-ribose units to a wide range of proteins, including proteins involved in DNA repair. Parp1 is implicated in double strand breaks (DSB) repair through the alternative end joining (alt-EJ) pathway. Alt-EJ is enhanced when DNA-PK dependent classical non-homologous end joining (c-NHEJ) is compromised. Parp inhibitors (Parpi) have shown great promise in cancer treatment through synthetic lethality when used as single agent but combination with ionizing radiation (IR) has not been widely considered. Talazoparib (BMN673) is one of the most recently developed and most potent Parpi. Extensive work in our laboratory shows strong radiosensitization by BMN673 in several tumor cells lines, as well as selected mutants of homologous recombination repair (HRR) and classical non-homologous end-joining (c-NHEJ). The observed effects strongly suggest the operation of mechanisms that go beyond those typically envisioned for classical Parpi. Indeed, we showed that even short treatments (1h) with BMN673 prior to IR, result in large radiosensitization and that BMN673 exerts a complex set of effects on DSB processing including inhibition of c-NHEJ and a pronounced increase in resection at DSBs that is associated with an increase in alt-EJ. As an extension to these studies, here we investigate a possible role of DNA-PK in the molecular mechanisms underpinning BMN673-induced radiosensitization. Specifically, we examine BMN673 radiosensitization in different DNA-PK mutants. The results obtained show significantly higher radiosensitization by BMN673 in several of these mutants as compared to the wild type cells. Furthermore, all mutants examined also show stronger IR-induced G2-checkpoint than wild type cells after BMN673 treatment. The significance of these observations will be analyzed and discussed.

Work supported by grants from BMBF and the DAAD.

[IOANNATREMI@hotmail.gr](mailto:IOANNATREMI@hotmail.gr)

## Radiation-induced DNA damage response of hematopoietic stem cells after proton irradiation

**Bram Verstraete**, C. Vandevoorde, J. Slabbert, A. Vral and A. Baeyens

*Department of Basic Medical Sciences, Ghent University, Ghent, Belgium*

The hematopoietic system is one of the most radiosensitive organs. Haematopoietic stem and progenitor cells (HSPCs) give rise to all types of mature blood cells and are an ideal model to study consequences of radiation exposure such as radiation-induced leukemia. In the field of radiotherapy, proton therapy has become a focus of attention the last few years. Protons are very useful to treat tumors located close to radiosensitive tissues and in anatomical sites where surgical access is limited, e.g. for brain tumors. The significant decrease of dose to non-target tissues compared to standard X-ray therapy is particularly important for pediatric patients. HSPCs may be exposed in vivo to radiation during the therapy either directly, when part of the bone marrow is irradiated, or by exposing HSPCs of the peripheral blood when they pass the radiation field. Since 17.5%-27.8% of the red bone marrow is located in the head of children in the first five years of age, it is important to take this into account in the clinical treatment of pediatric brain tumors. The high radiosensitivity of children with respect to radiation-induced leukemia warrants studies on the response of HSPCs to radiation-induced DNA damage as well as on the mutagenic consequences of DNA misrepair. The aim of this study was to investigate the differences in DNA damage and repair in HSPCs induced by photon and proton irradiations. HSPCs were isolated from umbilical cord blood by using density gradient centrifugation and CD34 immunomagnetic Microbead kit (Miltenyi Biotec). The CD34+ stem cells were in vitro irradiated with photons (Ugent) and protons (iThemba LABS) with ranges between 0Gy and 2Gy. The DNA double strand damage and repair was determined with  $\gamma$ H2AX immunostaining 0.5h, 4h, 12h and 24h after exposure. The micronucleus assay was used to assess the mutagenic effect of the radiation exposure. Dose response curves and time kinetics of DNA DSBs induction and repair of the HSPCs for photon and proton radiation were compared and will be presented.

# Can be *Solanum lycopersicum* L. cv 'Micro-Tom' a good candidate for growth in Space? Testing the effects of High-LET ionizing radiation on plant growth, photosynthesis and antioxidants

**Ermenegilda Vitale**, V. De Micco, C. Amitrano, M. Turano, B. Hay Mele, L. Manti and C. Arena

*Department of Biology, University of Naples Federico II, Naples, Italy*

The possibility to cultivate plants in Space represents an ongoing challenge because in extraterrestrial environments, plant growth is controlled by factors, some of which are also acting on Earth (e.g. temperature, relative humidity, light) while others are peculiar such as altered gravity in addition to a profoundly different ionizing radiation (IR) field [1]. In particular, exposure to Space IR may determine several outcomes depending on the dose, radiation quality (high vs. low LET), exposure rate (acute vs. chronic), but also by the intrinsic characteristics of the organism, such as species, cultivars, developmental stage, structure of organs and tissues and genetic traits [2]. Generally, plant response to IR is manifestly dose-dependent with irreparable damage at high doses, sublethal consequences at intermediate levels and stimulatory effects at low dose [3][4]. In this study we explore the possibility that low doses of heavy ions, namely C ions at dose of 25 Gy delivered at seed stage, may exert beneficial effects on *Solanum lycopersicum* L. cv 'Micro-Tom' on physiological, biochemical and anatomical traits, promoting the fruit ripening and quality. This would have important consequences in the view of its utilization on board of the Bioregenerative Life Support Systems (BLSSs) as food for crew. For this purpose, the plant life cycle from germination to fruit harvesting was monitored in terms of plant growth, photosynthetic efficiency, leaf anatomical traits and antioxidant production in leaf and fruits. The irradiation did not affect plant germination. Plants from irradiated seeds showed reduced height and a more compact size. The PSII quantum yield as well as the electron transport rate was promoted in irradiated compared to control plants. These data are consistent with a high level of D1 protein and photosynthetic pigment content in the leaves. As regards fruit, plants from irradiated seeds showed a significantly higher content of ascorbic acid, carotenoids and anthocyanins.

[1][2][3]De Micco et al., 2011. *Radiat. Environ. Biophys.* 50:1-19

[4]Arena et al., 2014. *Acta Astronaut.* 104:419-431

## SELLR (Subsurface Experiment of Life in Low Radiation): Effects of ultra-low radiation environments on bacterial growth

Jennifer Wadsworth, C. Cockell and S. Paling

*University of Edinburgh, Edinburgh, UK*

The effects of high radiation exposure on life have been extensively researched, however the effects of the lack of radiation have received limited attention from the scientific community, and are still largely unclear. The two main competing hypotheses are the linear no-threshold (LNT) model and hormesis model. The LNT model predicts a positive linear correlation between the radiation dose and damage caused to a life form, whilst the hormesis model predicts once radiation exposure is under a certain limit the damage induced by the lack of radiation is greater than at exposure to a small dose of radiation. Experiments to date have not yet been able to clearly dismiss either model. Bacterial growth assays were performed under ultra-low ionising radiation (100 mGy/y) in the Boulby International Subsurface Astrobiology Laboratory (BISAL) facilities of the Boulby mine (North East England). At ultra-low doses, a non-linear relationship was observed between bacterial growth and radiation, showing the lack of positive or negative growth responses compared to controls. This suggests a new model should be considered consisting of a linear dose-risk response with a threshold at low radiation.

# Effect of HIF-1 $\alpha$ on radiation sensitivity and DNA repair of tumor cells

**Alexandra Wolf**, H. Riffkin, U. Brockmeier, J. Baumann, K. Göpelt, G. Iliakis and E. Metzen

*Institute of Physiology, University Hospital Essen, Essen, Germany*

Poor prognosis of many solid tumors is often associated with hypoxic regions and an increased level of hypoxia-inducible-factor-1 $\alpha$  (HIF-1 $\alpha$ ). Previous findings indicate that HIF-1 $\alpha$  expression is relevant for radiation resistance. HIF-1 $\alpha$  has an enhanced activity in tumors after radiation treatment (RT) and also mediates low radiation sensitivity. We used cultured Lewis Lung Carcinoma (LLC) cells to investigate sensitivity to RT after knockdown of HIF-1 $\alpha$ . We observed delayed DNA repair in the knockdown cells after RT. Using  $\gamma$ H2AX, a sensitive marker for DNA double strand breaks (DSB), we demonstrated a prolonged expression of  $\gamma$ H2AX after hypoxic treatment and RT in the HIF-1 $\alpha$  deficient cells. Results of pulse field gel electrophoresis showed an impaired repair kinetic in cells with reduced HIF-1 $\alpha$ . Moreover, these cells displayed an increased rate of apoptosis after RT. Furthermore, observation of long term effects performed by delayed plating colony formation assay confirmed enhanced radiation sensitivity after knockdown of HIF-1 $\alpha$ . These results imply that inactivation of HIF-1 $\alpha$  leads to the enhanced radiation sensitivity of tumor cells which is potentially helpful for the development of novel tumor therapies.



## A unifying bioinformatic analysis of transcriptomic profiling experiments of radiation-induced, bystander effects uncovers distinct cellular mechanisms of response

**Constantinos Yeles,** E.-I. Vlachavas, O. Papadodima, E. Pilalis, C. E. Vorgias, A. G. Georgakilas and A. Chatziioannou

*Department of Biochemistry and Molecular Biology, National and Kapodistrian University of Athens and Metabolic Engineering and Bioinformatics Research Team, Institute of Biology Medicinal Chemistry and Biotechnology, National Hellenic, Athens, Greece*

Over the past years, novel approaches in radiation biology and therapy have introduced the existence of recently identified systemic phenomena, named as non-targeted effects, or more precisely, radiation-induced bystander effects (RIBE). In detail, ionizing radiation can damage the cellular genome directly or indirectly by the generation of direct damage (breaks etc.) and also reactive oxygen and nitrogen species. In this phenomenon, bystander cells exhibit stress effects, as a result of signals derived from the directly irradiated cells. In addition, it has been suggested that RIBE have been linked to specific molecular mechanisms, such as cell growth, inflammation, formation of micronuclei, delay in cell cycle along with transformation of non-irradiated cells and DNA damage response. In parallel, last decade various “omics”-technologies (microarrays, NGS) have generated available transcriptomic data, for the interrogation of the above phenomena. On this premise, we applied a computational pipeline for the integrative analysis of gene expression in several microarray datasets from GEO (Gene Expression Omnibus) (GSE55869, GSE32091, GSE21059, GSE25772, GSE18760, GSE12435, GSE8993) regarding radiation-induced, bystander effects in human cell lines. Complete microarray analysis (for each cohort) was performed with R software/Bioconductor (R version 3.3.2). Through Limma moderated t-test along with the BioInfoMiner gene analytics platform of e-NIOS Applications PC, we derived lists of differentially expressed (DE) genes, when contrasting bystander and irradiated samples versus sham-irradiated controls, and performed subsequent functional enrichment analysis. The aforementioned workflow, resulted in interesting findings concerning the interpretation of the total results. Firstly, from a simple overlap comparison of the DE gene lists of the 4 datasets (a-particles and carbon-ion bystander vs control samples) we identified a small subset of common genes, which include: IL1A, IL1B, NFKBIZ, SAT1 and TNFAIP3. In this point, it is worth noting that from partially overlapping comparisons, such as 2 out of 3 out of 4 datasets, other common DE genes are also identified: CXCL2, GOS2, MT1E, PTGS2, CXCL8 and FGF2. The biological interpretation of the DE gene lists, employing GO enrichment analysis results, revealed specific common biological processes, overrepresented in both “bystander” and “irradiated” comparisons: negative regulation of growth, cellular response to Zn<sup>2+</sup> and positive regulation of protein oligomerization. On the other hand, in bystander and irradiated samples irradiated with carbon-ion particles, different gene ontology terms were significantly enriched, related with response to mechanical stimulus, response to hypoxia and positive regulation of extrinsic apoptotic signaling pathway. In conclusion, these distinct biological processes indicate discrete response mechanisms, between different particle types of ionizing radiation and the importance of applying bioinformatics for the systematic analysis also of the non-targeted radiation effects.

# The influence of a magnetic field on photon and particle therapy in normal human cells

**Brian Yudhistiara**, K. Weber, J. Debus and H. Hauswald

*University of Heidelberg, Heidelberg, Germany*

**Background** The introduction of magnetic resonance imaging (MRI)-guided radiotherapy where the magnetic field and photon or particle beams are applied simultaneously demands knowledge about potential changes in the biological effects of the radiotherapy. The aim of this experimental study was to examine possible differences in the vitality of human normal cells when exposed to photon and particle radiation in the presence of a magnetic field (simulating the conditions of a MRI system). **Methods** For TK6 human lymphoblastoid cells, the clonogenicity was determined 12-14 days after exposure with 1 to 4 Gy photons (6 MV) with or without a magnetic field of 1 Tesla. Changes in cell cycle distributions and rates of radiation induced apoptosis (FACS analysis of cells with sub-G1 DNA content) were secondary end points and were analyzed following 4 Gy photons during 12 hours after exposure (+/- magnetic field). Furthermore, we compared the clonogenic survival of the cells exposed to a therapeutic charged particle beam (1 to 4 Gy protons or 0.5 to 2 Gy carbon ions) in the presence or absence of the magnetic field. **Results** Clonogenic survival of the TK6 cells after photon irradiation displayed a simple exponential dose-dependence, and the radiation sensitivity parameter ( $\alpha = 1.57/\text{Gy}$ ) was in accordance with earlier studies. Identical results (within error bars) were obtained in the presence of a magnetic field of 1.0 Tesla. The derived survival fractions at 2 Gy (SF2 values) were 9% (without magnetic field) and 8.5% (with magnetic field), respectively. The FACS analysis of cell cycle progression also showed identical results, both for the increase in G2/M and the decrease in S-phase fractions after 12 hours regardless of the exposure to a magnetic field. The treatment-specific apoptosis after irradiation also confirmed the absence of an altered DNA damage response as error bars overlap at all time points (ranging from 1% at 8 hours to 4% at 14 hours). Furthermore, no significant difference in the TK6 cells clonogenic survival was observed after irradiation with carbon ions or protons. **Conclusion** The application of a 1.0 Tesla magnetic field does not affect the clonogenicity of TK6 cells irradiated with photons, protons, or carbon ions. This supports combination of MRI and linear accelerators or particle beam lines for patients, but further research regarding the effects in living tissues is certainly warranted.

## Repair deficiency of radiation-induced DNA double strand breaks in Fanconi's anemia fibroblasts

**Sebastian Zahnreich**, B. Weber, D. Galetzka, T. Haaf, D. Schindler and H. Schmidberger

*Department of Radiation Oncology and Radiotherapy, University Medical Centre Mainz, Germany*

The Fanconi anaemia (FA)/BRCA pathway comprises 21 gene products (FANC A–V) with essential roles in DNA interstrand crosslink (ICL) repair and homologous recombination (HR). However, upon DNA damage induced by ionizing radiation in vitro, the radiation sensitivity of FA cells is widely considered as normal. This is in contrast to a frequently observed radiation hypersensitivity of FA patients during radiotherapy. To this end, we set out to investigate the repair of radiation-induced DNA double strand breaks (DSB) in G1- and G2-phase FA fibroblasts of the complementation groups A, B, C, D1, D2, E, F and G compared to a healthy donor. Hypersensitivity of FA cells towards ICL was confirmed after Mitomycin C treatment by a pronounced G2-arrest and an accumulation of DSB quantified as  $\gamma$ -H2AX-signals by flow cytometry. Using  $\gamma$ -H2AX analysis in combination with cell cycle markers by fluorescence microscopy, FA fibroblasts showed a decreased repair capacity of radiation-induced DSB during G2-phase 6h after the exposure to 2Gy X-rays compared to the healthy donor. G2 radiosensitivity of FA fibroblasts was confirmed using the premature chromosome condensation technique on G2-cells 8h after the exposure to 3 Gy X-rays by an elevated yield of chromatid breaks and exchanges. Our results demonstrate radiation sensitivity in a variety of FA complementation groups by an impaired and more erroneous repair of DSB in G2-cells compared to a healthy donor. These findings must be considered for planned medical exposures of FA patients to ionizing radiation and might reveal new functions of FA complementation group members in HR as well as potential modulators of the clinical radiation response during radiotherapy.

Funded by the German Ministry of Education and Research, Grant 02NUK042A

# ERRS-GBS 2017 Essen

How to get **there!**



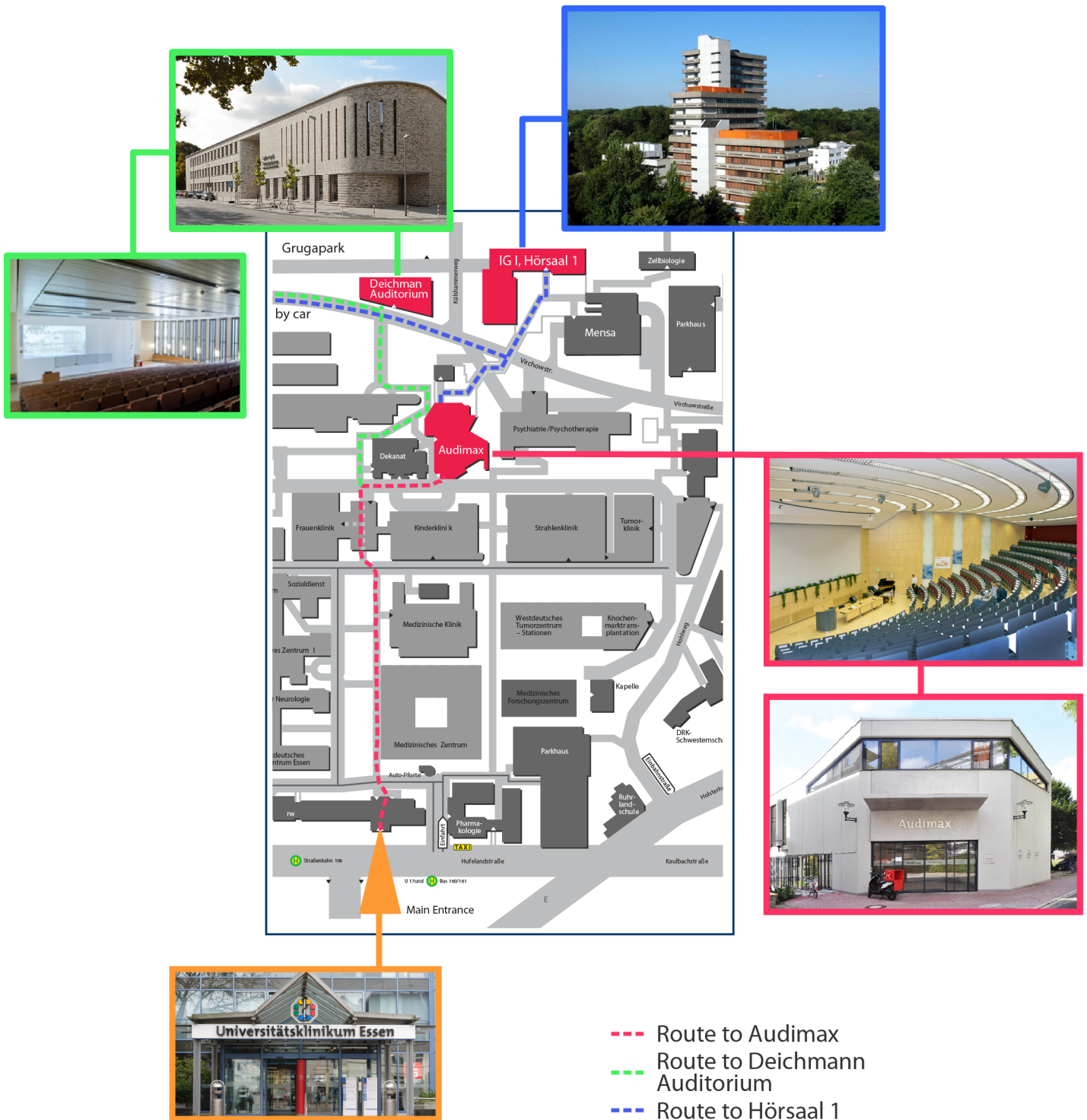
## Conference Venue & Registration Site

Universitätsklinikum Essen  
Lehr- und Lernzentrum (LLZ)  
Virchowstraße 163a,  
D-45147 Essen



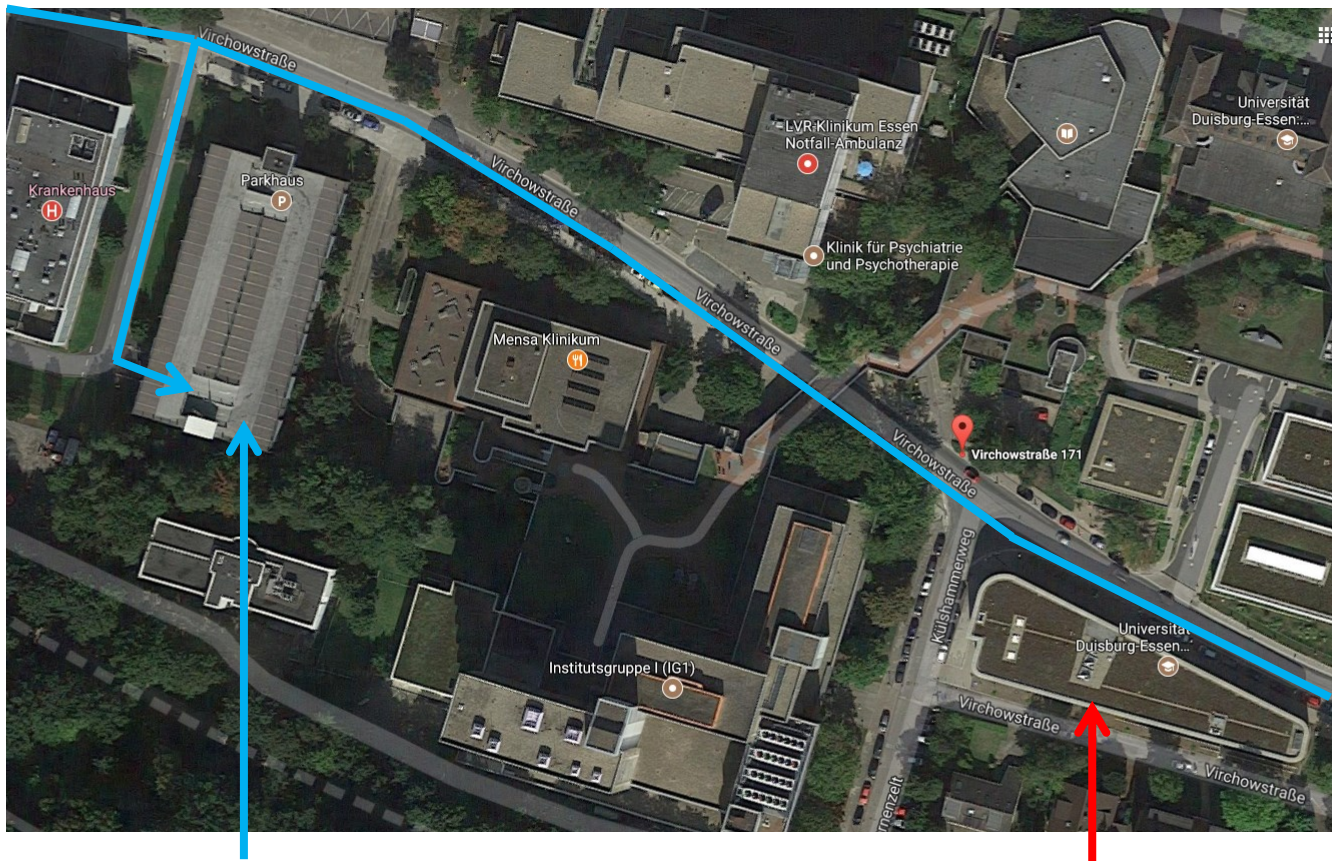


# Site map



43rd Annual Meeting of ERRS and 20th Annual Meeting of GBS, Essen, Germany, 2017

## Arriving by car



Parking

Lehr- und  
Lernzentrum

Enter the following address in your navigation system:

Virchowstraße 163a  
45147 Essen

A limited number of parking slots will be available free of charge\* at:

Parkhaus 2 (50 m from LLZ)  
Virchowstraße 175  
45147 Essen

*\*Details as to how to take advantage of this option will be provided at the meeting.*





# ✈ Arriving by plane

Departure/Arrival Area  
and access  
to SkyTrain

Düsseldorf  
Airport train  
station



## Düsseldorf Airport (DUS)

- From Arrivals follow signs to “SkyTrain”
- Take SkyTrain to Düsseldorf Airport train station
- Trains to Essen pass every few minutes and take 25-40 min to reach Essen Main Train Station. No ticket is required to enter departure platforms. Train options include:
  - S1 (S-Bahn), direction: Dortmund
  - RE1 (regional express), direction: Hamm (Westf.)
  - RE2, direction: Münster (Westf.)
  - RE6, direction: Minden (Westf.)
- The provided public transportation pass is valid in the above trains. Caution! Pass is not valid on IC or ICE trains!
- Follow instructions below from Essen Main Train Station to Venue venue site

*“Düsseldorf Airport has the Düsseldorf Airport terminal train station in the Arrivals hall. Do not use this station! It only services Düsseldorf city”*





# Arriving by train



## From Essen Main Train Station (Hauptbahnhof)

- Follow signs to subway (U-Bahn) U17 à Margarethenhöhe. No ticket is required to enter departure platforms. Use provided pass to travel.
- Disembark at „Holsterhauser Platz“
- Walk down Robert-Koch-Straße to Essen Medical School (Universitätsklinikum Essen).
- Follow ERRS-GBS 2017 signposting to Venue Site











# ERRS-GBS 2017 Public Transport Pass (the back side of your nametag)

It allows you to use the entire local public transport network in the greater area of Essen (EVAG). It includes Düsseldorf Airport and the Rhine-Ruhr-area (VRR, see map below for exact information on area of validity). It is valid from September 17<sup>th</sup> to September 22<sup>nd</sup>, 3 a.m. It includes busses, trams, subways (U-Bahn), urban trains (S-Bahn) and regional express trains (RE). It **does not** include Intercity (IC) and Intercity Express trains (ICE).

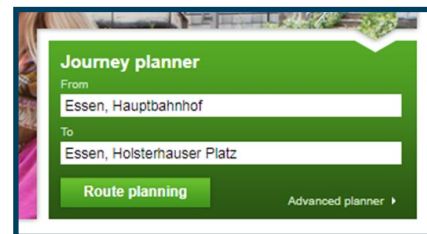
*"Please note, that the pass is non-transferrable and must not be given or sold to other persons!"*

To enable pass use **before registration**, a PDF of the pass is included in this mail. Please print both the pass in actual size and color, as well as the **e-mail it came with** and carry it with you as your pass. You do not need a further ticket to enter the departure platforms. There are occasional controls on the train! The pass is not valid without the e-mail. E-mail identifies you as the rightful pass-owner!

For train schedules go to VRR:

<http://www.vrr.de/en/index.html>.

You can choose stations, addresses or points of interest as starting and end points.



From

Transport updates available

Essen, Hauptbahnhof, Essen, Holsterhauser Platz, Essen, Werden S, Essen, Pastoratsberg 2, Düsseldorf, Flughafen Bahnhof, more...

To

Essen, Holsterhauser Platz, Essen, Hauptbahnhof, Essen, Klinikum, Essen, Gemarkenplatz, Essen, Berliner Platz, more...

New Journey

Update

I'd like to 

depart

 at 

08

 : 

00

 on 

18.09.2017

I want to plan a regular journey

Advanced settings

Journeys

Send Link

Earlier First journey

	Departure	Arrival	Duration	Interchanges	Price (Adult/Child)
1. Journey	07:56	08:07	00:11	1x	A3
2. Journey	08:04	08:10	00:06	0x	A3
08:04 from Essen Hauptbahnhof Bay 2					
U-Bahn U17					
Direction Essen Margarethenhöhe					
08:10 to Essen Holsterhauser Platz Bay 2					
3. Journey	08:06	08:17	00:11	1x	A3
4. Journey	08:14	08:20	00:06	0x	A3

Later Last journey

Map (2. Journey)

Print

1. 07:56 - 08:07

U18

2. 08:04 - 08:10

U17

3. 08:06 - 08:17

U18

4. 08:14 - 08:20

U17

Print journey selection

Downloads for origin

Stop timetable

A PDF file of the desired service's stop







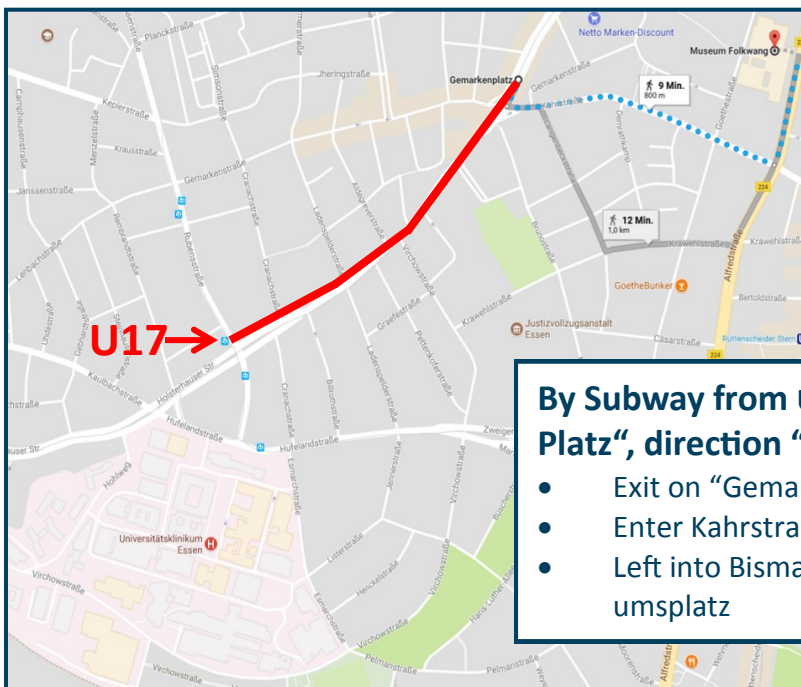
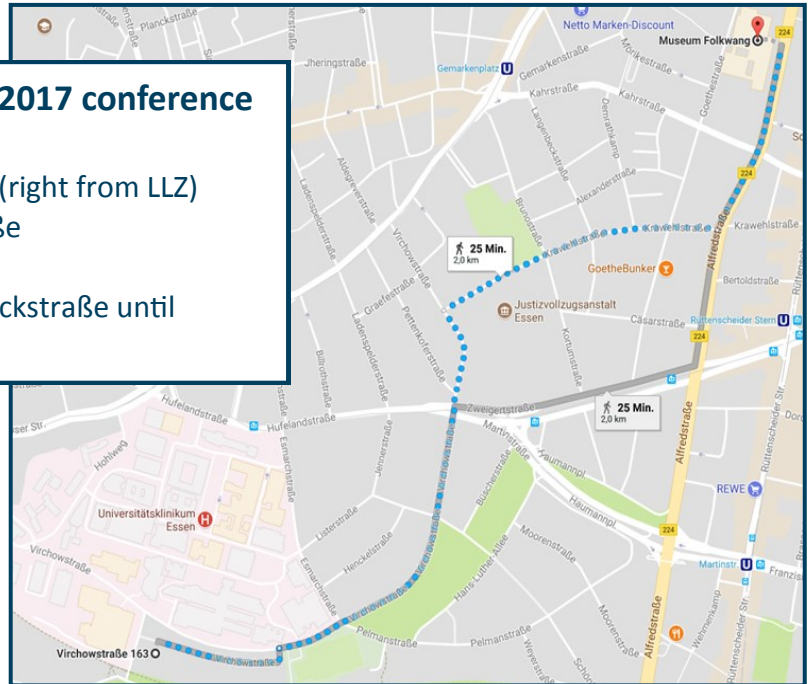


# Social Evening

## Optional Guided Tour: Art Museum Folkwang Museumsplatz 1, D-45128 Essen

### Walking from ERRS GBS 2017 conference venue

- East on Virchowstraße (right from LLZ)
- Right into Krawehlstraße
- Left into Alfredstraße
- Go straight into Bismarckstraße until Museumsplatz



### By Subway from U17 „Holsterhauser Platz“, direction “Karlsplatz”

- Exit on “Gemarkenplatz” (first stop)
- Enter Kahrstraße
- Left into Bismarckstraße until Museumsplatz





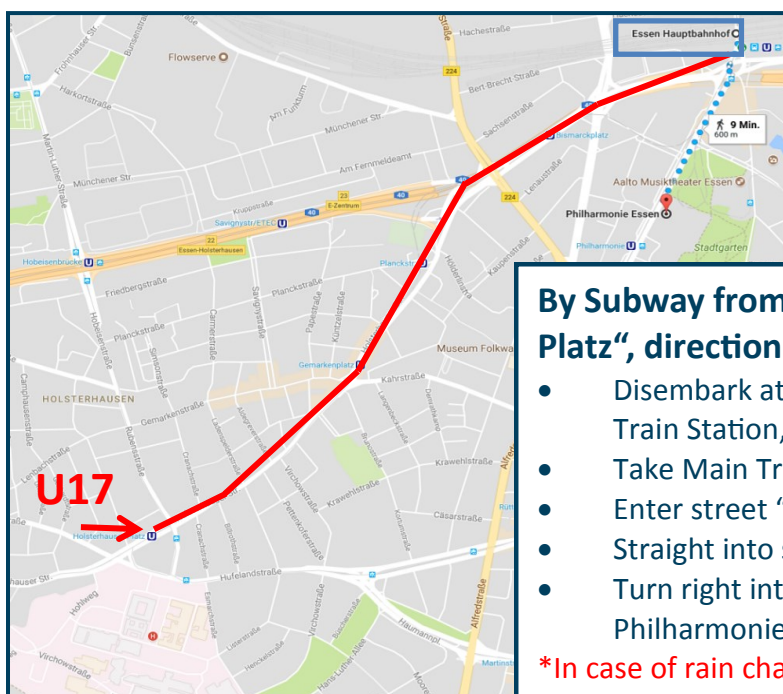
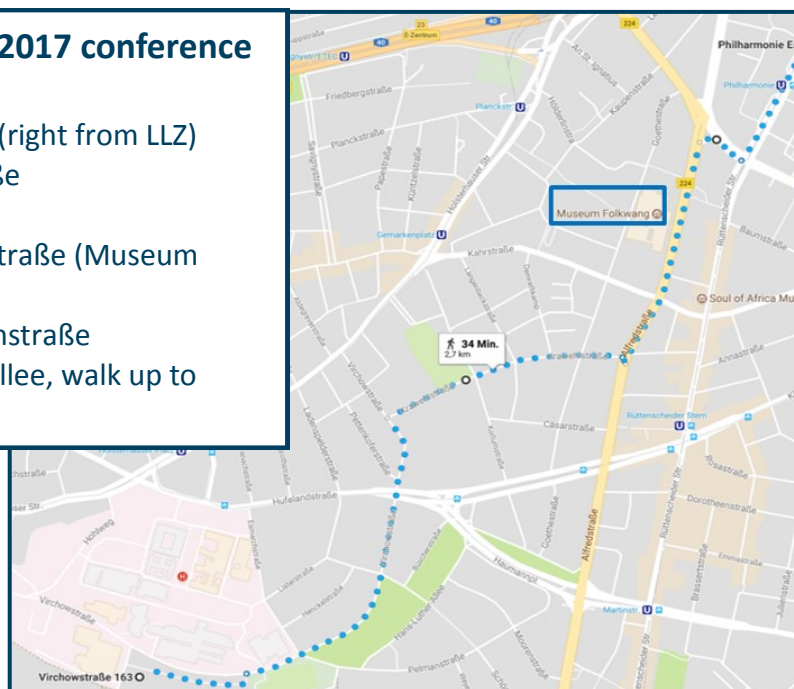
# Social Evening

Conference Dinner: Philharmonie Essen

Huyssenallee 53, D-45128 Essen

## Walking from ERRS GBS 2017 conference venue

- East on Virchowstraße (right from LLZ)
- Right into Krawehlstraße
- Left into Alfredstraße
- Straight into Bismarckstraße (Museum Folkwang)
- Turn right into Friedrichstraße
- Turn left into Huyssenallee, walk up to Philharmonie



## By Subway from U17 „Holsterhauser Platz“, direction “Karlsplatz”

- Disembark at “Hauptbahnhof” (Essen Main Train Station, 7min ride)\*
- Take Main Train Station exit “Freiheit”
- Enter street “Am Hauptbahnhof”
- Straight into street “Freiheit”
- Turn right into Huyssenallee, walk up to Philharmonie

\*In case of rain change into U11 to Essen Messe W.-Süd/Gruga, disembark at “Philharmonie”



## **Cab**

The Venue Site (LLZ) can be reached by cab. Please give Virchowstraße 163a as address to the cab driver.

It will cost about 10€ from Essen Main Train Station and 70€ from Düsseldorf Airport.

## **Cab Services in Essen**

### **Taxi Essen**

+49 201 77 77 77

### **Taxi Beige**

+49 201 70 70 60

### **Taxi 24Std**

+49 201 55 89 22 19



A decorative graphic of a virus particle, showing a spherical head and a textured, segmented tail, is positioned along the left edge of the page.

# List of Participants





1. **Amir Abdollahi**  
[a.amir@dkfz.de](mailto:a.amir@dkfz.de)  
Molecular and Translational Radiation Oncology  
Heidelberg Institute of Radiation Oncology (HIRO), NCRO
2. **Michael Abend**  
[michaelabend@bundeswehr.org](mailto:michaelabend@bundeswehr.org)  
Bundeswehr Institute of Radiobiology  
Bundeswehr
3. **Gabriel Adrian**  
[gabriel.adrian@med.lu.se](mailto:gabriel.adrian@med.lu.se)  
Department of Clinical Sciences Lund, Oncology and Pathology  
Lund University, Faculty of Medicine
4. **An Aerts**  
[an.aerts@sckcen.be](mailto:an.aerts@sckcen.be)  
Radiobiology Unit  
SCK•CEN The Belgian Nuclear Research Institute
5. **Vidhula Ahire**  
[vidhula4@gmail.com](mailto:vidhula4@gmail.com)  
Radiobiology  
Academic Medical Center
6. **Rozina Aktar**  
[Rozina.Aktar@uniklinikum-dresden.de](mailto:Rozina.Aktar@uniklinikum-dresden.de)  
Medical Faculty  
OncoRay, Medical Faculty and University Hospital Carl Gustav Carus
7. **Ghassan Al Massarani**  
[galmassarani@aec.org.sy](mailto:galmassarani@aec.org.sy)  
Radiation Medicine Department  
AECS
8. **Klaudia Al-Refae**  
[Klaudia.Al-Refae@uk-essen.de](mailto:Klaudia.Al-Refae@uk-essen.de)  
University Hospital Essen  
Institute of Cell Biology (Tumor Research)
9. **Nataša Anastasov**  
[natasa.anastasov@helmholtz-muenchen.de](mailto:natasa.anastasov@helmholtz-muenchen.de)  
Institute of Radiation Biology  
Helmholtz Zentrum München
10. **Heike Anders**  
[heike.anders@med.uni-muenchen.de](mailto:heike.anders@med.uni-muenchen.de)  
Clinic for Radiotherapy  
Ludwig-Maximilians-Universität
11. **Rhona Anderson**  
[rhona.anderson@brunel.ac.uk](mailto:rhona.anderson@brunel.ac.uk)  
Institute of Environment, Health and Societies  
Brunel University London
12. **Mike Atkinson**  
[atkinson@helmholtz-muenchen.de](mailto:atkinson@helmholtz-muenchen.de)  
Institute of Radiation Biology  
Helmholtz Zentrum München
13. **Nicole Aeverbeck**  
[n.aeverbeck@gsi.de](mailto:n.aeverbeck@gsi.de)  
Biophysics  
GSI Helmholtzzentrum für Schwerionenforschung
14. **Hosein Azimian**  
[hosein\\_azimian@yahoo.com](mailto:hosein_azimian@yahoo.com)  
Department of medical physics  
Mashhad university of medical sciences
15. **Sarah Baatout**  
[sbaatout@sckcen.be](mailto:sbaatout@sckcen.be)  
Radiobiology Unit  
Belgian Nuclear Research Centre
16. **Christophe Badie**  
[christophe.badie@phe.gov.uk](mailto:christophe.badie@phe.gov.uk)  
Radiation Effects Department  
Public Health England
17. **Ans Baeyens**  
[Ans.Baeyens@Ugent.be](mailto:Ans.Baeyens@Ugent.be)  
Basic Medical Sciences  
University Ghent
18. **Evangelos Balanikas**  
[v.balanikas@gmail.com](mailto:v.balanikas@gmail.com)  
Department of Physics  
National Technical University of Athens
19. **Lara Barazzuol**  
[l.barazzuol@umcg.nl](mailto:l.barazzuol@umcg.nl)  
Department of Radiotherapy  
University Medical Center Groningen
20. **Sofia Barbieri**  
[sofia.barbieri01@universitadipavia.it](mailto:sofia.barbieri01@universitadipavia.it)  
Department of Physics  
University of Pavia
21. **Mary Helen Barcellos-Hoff**  
[maryhelen.barcellos-hoff@ucsf.edu](mailto:maryhelen.barcellos-hoff@ucsf.edu)  
Radiation Oncology  
University of California, San Francisco
22. **Carina Barent**  
[c.barent@gsi.de](mailto:c.barent@gsi.de)  
Biophysics  
GSI Helmholtzzentrum für Schwerionenforschung GmbH
23. **Katharina Batzke**  
[katharina.batzke@uk-essen.de](mailto:katharina.batzke@uk-essen.de)  
Molecular Oncology  
University Hospital Essen
24. **Lindsay Beaton-Green**  
[lindsay.beaton@hc-sc.gc.ca](mailto:lindsay.beaton@hc-sc.gc.ca)  
Consumer and Clinical Radiation Protection Bureau  
Health Canada

25. Niels Belmans  
[nbelmans@sckcen.be](mailto:nbelmans@sckcen.be)  
Radiobiology Unit  
University of Hasselt/Belgian Nuclear Research Centre
26. Marc Benderitter  
[marc.benderitter@irsn.fr](mailto:marc.benderitter@irsn.fr)  
SERAMED  
IRSN
27. Jacqueline Bernardino-Sgherri  
[jacqueline.bernardino@cea.fr](mailto:jacqueline.bernardino@cea.fr)  
Institute of Cellular and Molecular Radiobiology  
CEA
28. Elke Beyreuther  
[E.Beyreuther@hzdr.de](mailto:E.Beyreuther@hzdr.de)  
Institute of Radiation Physics  
Helmholtz-Zentrum Dresden-Rossendorf
29. Deepti Bijlani  
[deepti.s.bijlani@hc-sc.gc.ca](mailto:deepti.s.bijlani@hc-sc.gc.ca)  
HEALTH CANADA  
FEDERAL GOVERNMENT CANADA
30. Kerstin Borgmann  
[borgmann@uke.de](mailto:borgmann@uke.de)  
Laboratory of Radiobiology & Experimental Radiooncology, Clinic for  
University Medical Center Hamburg-Eppendorf
31. Petra Boukamp  
[p.boukamp@dkfz-heidelberg.de](mailto:p.boukamp@dkfz-heidelberg.de)  
Genetics of skin Carcinogenesis  
DKFZ/ IUF
32. Simon Boulton  
[simon.boulton@crick.ac.uk](mailto:simon.boulton@crick.ac.uk)  
DSB Repair Metabolism  
The Francis Crick Institute
33. Herbert Braselmann  
[braselm@helmholtz-muenchen.de](mailto:braselm@helmholtz-muenchen.de)  
Research Unit Radiation Cytogenetics  
Helmholtz Zentrum München
34. Anne Bravard  
[anne.bravard@cea.fr](mailto:anne.bravard@cea.fr)  
DRF/Institut François Jacob/IRCM  
CEA
35. Nikko Brix  
[nikko.brix@med.uni-muenchen.de](mailto:nikko.brix@med.uni-muenchen.de)  
Department of Radiation Oncology  
Ludwig-Maximilians-University of Munich
36. Anna Broich  
[anna.broich@uk-essen.de](mailto:anna.broich@uk-essen.de)  
Institute of Medical Radiation Biology  
University Hospital Essen
37. Martin Bucher  
[mbucher@bfs.de](mailto:mbucher@bfs.de)  
Radiation Protection and Health  
Federal Office for Radiation Protection
38. Helmut Bühler  
[helmut.buehler@rub.de](mailto:helmut.buehler@rub.de)  
Klinik für Radiotherapie und Radio-Onkologie  
Ruhr-Universität Bochum
39. Serge Candéias  
[serge.candeias@cea.fr](mailto:serge.candeias@cea.fr)  
Laboratory of Chemistry and Biology of Metals, UMR5249 CEA-CNRS-UGA  
Commissariat à l'Energie Atomique
40. Mario Pietro Carante  
[mariopietro.carante01@ateneopv.it](mailto:mariopietro.carante01@ateneopv.it)  
Physics department  
University of Pavia
41. Pierluigi Casolaro  
[casolaro@na.infn.it](mailto:casolaro@na.infn.it)  
Department of Physics  
University of Napoli "Federico II"
42. Noemi Castelletti  
[noemi.castelletti@helmholtz-muenchen.de](mailto:noemi.castelletti@helmholtz-muenchen.de)  
Institute of Radiation Protection (ISS)  
Helmholtz Zentrum Munich, Deutsches Forschungszentrum für Gesundheit
43. Antonina Cebulska-Wasilewska  
[b7wasile@cyf-kr.edu.pl](mailto:b7wasile@cyf-kr.edu.pl)  
Laboratory of Individual and Environmental Dosimetry  
Central Laboratory for Radiological Protection (CLOR)
44. Aristotelis Chatziioannou  
[achatz@eie.gr](mailto:achatz@eie.gr)  
Biophysics/DNA Damage Lab, Department of Physics  
National Technical University of Athens
45. Shipra Chaudhary  
[shipra.chaudhary@uk-essen.de](mailto:shipra.chaudhary@uk-essen.de)  
Institute of Medical Radiation Biology  
University Duisburg-Essen
46. I-Peng Chen  
[ipengchen@gmail.com](mailto:ipengchen@gmail.com)  
Mol Cell Biology  
Dermatology, Hospital Buxtehude
47. Lei Cheng  
[lei.cheng@su.se](mailto:lei.cheng@su.se)  
Department of Molecular Biosciences, The Wenner-Gren Institute  
Stockholm University
48. Francois Chevalier  
[chevalier@ganil.fr](mailto:chevalier@ganil.fr)  
LARIA  
CEA

49. Emma Coninx  
[emma.coninx@sckcen.be](mailto:emma.coninx@sckcen.be)  
Radiobiology Unit  
Belgian Nuclear Research Center (SCK-CEN)
50. Rob Coppes  
[r.p.coppes@umcg.nl](mailto:r.p.coppes@umcg.nl)  
Departments of Radiation Oncology and Cell Biology  
University Medical Center Groningen
51. Nils Cordes  
[nils.cordes@oncoray.de](mailto:nils.cordes@oncoray.de)  
Molekulare und Zelluläre Strahlenbiologie  
OncoRay® - National Center for Radiation Research in Oncology
52. Lourdes Cruz-Garcia  
[lourdes.cruzgarcia@phe.gov.uk](mailto:lourdes.cruzgarcia@phe.gov.uk)  
Radiation Effects Department  
Public Health England
53. Daniel Cullen  
[d13123391@mydit.ie](mailto:d13123391@mydit.ie)  
DIT Centre for Radiation and Environmental Science  
Dublin Institute of Technology
54. Noami Daems  
[noami.daems@sckcen.be](mailto:noami.daems@sckcen.be)  
Radiobiology unit  
UNamur/SCK-CEN
55. Volker Dahmen  
[v.dahmen@fz-juelich.de](mailto:v.dahmen@fz-juelich.de)  
Institute of Safety and Radiation Protection  
Forschungszentrum Jülich GmbH
56. Claudia Dalke  
[dalke@helmholtz-muenchen.de](mailto:dalke@helmholtz-muenchen.de)  
Institute of Developmental Genetics  
Helmholtz Zentrum München
57. Marie Davidková  
[davidkova@ujf.cas.cz](mailto:davidkova@ujf.cas.cz)  
Dept. of Radiation Dosimetry  
Nuclear Physics Institute of the CAS
58. Mozghan Dehghan Harati  
[mozghan.dehghan-harati@klinikum.uni-tuebingen.de](mailto:mozghan.dehghan-harati@klinikum.uni-tuebingen.de)  
Radiation Oncology  
University of Tübingen
59. Fieke Dekkers  
[Fieke.dekkers@rivm.nl](mailto:Fieke.dekkers@rivm.nl)  
cVLH  
National Institute for Public Health and the Environment
60. Lisa Deloch  
[Lisa.Deloch@uk-erlangen.de](mailto:Lisa.Deloch@uk-erlangen.de)  
Radiation Oncology  
Universitätsklinikum Erlangen
61. Sara Sofia Deville  
[SaraSofia.Deville@uniklinikum-dresden.de](mailto:SaraSofia.Deville@uniklinikum-dresden.de)  
OncoRay - National Center for Radiation Research in Oncology  
Universitätsklinikum Carl Gustav Carus
62. Simon Deycmar  
[simon.deycmar@usz.ch](mailto:simon.deycmar@usz.ch)  
Dept. of Radiation Oncology  
Laboratory of Applied Radiobiology
63. Angela Diana  
[angela.diana@oncology.ox.ac.uk](mailto:angela.diana@oncology.ox.ac.uk)  
Oncology  
University of Oxford
64. Klaus Dittmann  
[klaus.dittmann@uni-tuebingen.de](mailto:klaus.dittmann@uni-tuebingen.de)  
Department of Radiation Oncology, Division of Radiobiology  
University of Tübingen
65. Bastien Doix  
[bastien.doix@uclouvain.be](mailto:bastien.doix@uclouvain.be)  
Institut de Recherche Expérimentale et Clinique (pole FATH)  
Université Catholique de Louvain
66. Wolfgang Dörr  
[wolfgang.doerr@meduniwien.ac.at](mailto:wolfgang.doerr@meduniwien.ac.at)  
ATRA - Applied and Translational Radiobiology, Dept. Radiation  
Medical University of Vienna
67. Varvara Drakou  
[vdrakou@biol.uoa.gr](mailto:vdrakou@biol.uoa.gr)  
Biology  
National and Kapodistrian University of Athens
68. Ludwig Dubois  
[Maastrlab@maastrichtuniversity.nl](mailto:Maastrlab@maastrichtuniversity.nl)  
Radiation Oncology  
Maastricht University Medical Centre
69. Marco Durante  
[Marco.Durante@tifpa.infn.it](mailto:Marco.Durante@tifpa.infn.it)  
TIFPA  
INFN
70. Marie Dutreix  
[marie.dutreix@curie.fr](mailto:marie.dutreix@curie.fr)  
AG Löbrich - Radiation Biology and DNA Repair  
TU Darmstadt
71. Michael Ensminger  
[ensminger@bio.tu-darmstadt.de](mailto:ensminger@bio.tu-darmstadt.de)  
University Hospital Essen  
Department of Radiotherapy
72. Yasin Bahadır Erol  
[yasinbahadirerol@gmail.com](mailto:yasinbahadirerol@gmail.com)

- 73. Roser Esplugas**  
[roser.esplugas@urv.cat](mailto:roser.esplugas@urv.cat)  
Physiology Unit, DCMB  
Universitat Rovira i Virgili
- 74. Sofia Ferreira**  
[sofia.morgadinho-ferreira@curie.fr](mailto:sofia.morgadinho-ferreira@curie.fr)  
U1021  
Institut Curie
- 75. Daniel F. Fleischmann**  
[daniel.fleischmann@med.uni-muenchen.de](mailto:daniel.fleischmann@med.uni-muenchen.de)  
Radiation Oncology  
LMU Munich
- 76. Marco Foiani**  
[marco.foiani@ifom.eu](mailto:marco.foiani@ifom.eu)  
Genome Integrity  
IFOM- the FIRC Institute of Molecular Oncology
- 77. Silvia Formenti**  
[FORMENTI@MED.CORNELL.EDU](mailto:FORMENTI@MED.CORNELL.EDU)  
RADIATION ONCOLOGY  
NYP/WEILL CORNELL MEDICINE
- 78. Claudia Fournier**  
[c.fournier@gsi.de](mailto:c.fournier@gsi.de)  
Biophysik  
GSI Helmholtzzentrum
- 79. Giorgos Fragoulis**  
[g.fragkoulis.92@gmail.com](mailto:g.fragkoulis.92@gmail.com)  
Physics Department  
National and Technical University of Athens
- 80. Flavia Zita Francies**  
[flavia.zita4@gmail.com](mailto:flavia.zita4@gmail.com)  
Radiation Sciences  
University of the Witwatersrand
- 81. Nicolaas Franken**  
[n.a.franken@amc.uva.nl](mailto:n.a.franken@amc.uva.nl)  
Laboratory for Experimental Oncology and Radiobiology (LEXOR), Center  
Academic Medical Center, University of Amsterdam
- 82. Benjamin Frey**  
[benjamin.frey@uk-erlangen.de](mailto:benjamin.frey@uk-erlangen.de)  
Department of Radiation Oncology  
Universitätsklinikum, Friedrich-Alexander-Universität erlangen-Nürnberg
- 83. Werner Friedland**  
[friedland@helmholtz-muenchen.de](mailto:friedland@helmholtz-muenchen.de)  
Department of Radiation Sciences  
Helmholtz Zentrum München
- 84. Thomas Friedrich**  
[t.friedrich@gsi.de](mailto:t.friedrich@gsi.de)  
Biophysics  
GSI Darmstadt
- 85. Moritz Frister**  
[m.frister@dkfz-heidelberg.de](mailto:m.frister@dkfz-heidelberg.de)  
Molecular Radiation Oncology E055  
Deutsches Krebsforschungszentrum Heidelberg
- 86. Florian Frohns**  
[Frohns@bio.tu-darmstadt.de](mailto:Frohns@bio.tu-darmstadt.de)  
Radiation Biology and DNA Repair  
TU Darmstadt
- 87. Udo Gaipf**  
[udo.gaipf@uk-erlangen.de](mailto:udo.gaipf@uk-erlangen.de)  
Department of Radiation Oncology  
Universitätsklinikum Erlangen
- 88. Danuta Galetzka**  
[Danuta.Galetzka@unimedizin-mainz.de](mailto:Danuta.Galetzka@unimedizin-mainz.de)  
Department of Radiation Oncology and Radiotherapy  
University Medical Centre Johannes Gutenberg University
- 89. George Garinis**  
[garinis@imbb.forth.gr](mailto:garinis@imbb.forth.gr)  
DNA damage and mammalian physiology  
Institute of Molecular Biology and Biotechnology-FORTH
- 90. Ana Gasol Garcia**  
[a.gasolgarcia@vumc.nl](mailto:a.gasolgarcia@vumc.nl)  
Radiobiology  
VU medisch centrum
- 91. Alexandros Georgakilas**  
[alexg@mail.ntua.gr](mailto:alexg@mail.ntua.gr)  
Applied Physics  
National Technical University of Athens
- 92. Anastasios Georgoulis**  
[tgeorgoulis@med.uoa.gr](mailto:tgeorgoulis@med.uoa.gr)  
Molecular Biology and Biochemistry  
National and Kapodistrian University of Athens
- 93. Stavroula Isidora**  
[linaki\\_1612@hotmail.com](mailto:linaki_1612@hotmail.com)  
Applied Physics  
national technical university of athens
- 94. Ulrich Giesen**  
[ulrich.giesen@ptb.de](mailto:ulrich.giesen@ptb.de)  
Radiation effects  
PTB
- 95. Lorena Giuranno**  
[l.giuranno@maastrichtuniversity.nl](mailto:l.giuranno@maastrichtuniversity.nl)  
Radiation Oncology  
Maastricht Univeristy
- 96. Christin Glowa**  
[c.glowa@dkfz.de](mailto:c.glowa@dkfz.de)  
Department of Radiotherapy and Radiation Oncology  
University Hospital Heidelberg

97. Paulo Godoy  
[paulo.godoy@su.se](mailto:paulo.godoy@su.se)  
The Wenner-Gren Institute  
University of Stockholm
98. Maria Gomolka  
[mgomolka@bfs.de](mailto:mgomolka@bfs.de)  
Department of Radiation Protection and Health  
Federal Office for Radiation Protection (BfS)
99. Ielizaveta Gorodetska  
[Liza.Gorodetska@uniklinikum-dresden.de](mailto:Liza.Gorodetska@uniklinikum-dresden.de)  
Biomarkers for the Individualized Radiotherapy, OncoRay - National  
Technische Universität Dresden
100. Sylvia Graeber  
[sylvia.graeber@kit.edu](mailto:sylvia.graeber@kit.edu)  
Wassertechnologie und Entsorgung  
Projekträger Karlsruhe
101. Giovanna Granata  
[giovanna.granata@oncology.ox.ac.uk](mailto:giovanna.granata@oncology.ox.ac.uk)  
Oxford Institute for Radiation Oncology  
University of Oxford
102. Debora Grasso  
[d.grasso@uclouvain.be](mailto:d.grasso@uclouvain.be)  
IREC - Institute of Experimental and Clinical Research (FATH)  
Université Catholique de Louvain
103. Torsten Groesser  
[togro@dtu.dk](mailto:togro@dtu.dk)  
Hevesy Laboratory  
Technical University Denmark
104. Gina Grünheid  
[g.gruenheid@medipan.de](mailto:g.gruenheid@medipan.de)  
Marketing  
MEDIPAN GMBH
105. Noopur Gupta  
[noopurgupta3105@gmail.com](mailto:noopurgupta3105@gmail.com)  
Radiation genetics and Epigenetics  
Institute of Nuclear Medicine and Allied Sciences
106. Siamak Haghdooost  
[Siamak.Haghdooost@su.se](mailto:Siamak.Haghdooost@su.se)  
Department of Molecular Bioscience, The Wenner-Gren Institute  
Stockholm University
107. Thanos Halazonetis  
[thanos.halazonetis@unige.ch](mailto:thanos.halazonetis@unige.ch)  
Department of Molecular Biology  
University of Geneva
108. Ester Hammond  
[sharon.draper@oncology.ox.ac.uk](mailto:sharon.draper@oncology.ox.ac.uk)  
Department of Oncology  
University of Oxford
109. Christine Hansel  
[christine.hansel@uk-essen.de](mailto:christine.hansel@uk-essen.de)  
Institut für Zellbiologie  
Uniklinikum Essen
110. Teena Haritwal  
[haritwalteena@gmail.com](mailto:haritwalteena@gmail.com)  
Radiation genetics and Epigenetics  
Institute of Nuclear Medicine and Allied Sciences
111. Carola Hartel  
[c.hartel@gsi.de](mailto:c.hartel@gsi.de)  
Biophysics Department  
GSI Helmholtz Center for Heavy Ion Research
112. Mohammad Sharif Mortoga Hasan  
[sharif.mortoga@uk-essen.de](mailto:sharif.mortoga@uk-essen.de)  
Institute for Medical Radiation Biology  
University of Duisburg-Essen
113. Robert Hase  
[rhase@faxitron.com](mailto:rhase@faxitron.com)  
Life Sciences & NDT  
Faxitron
114. Michael Hausmann  
[hausmann@kip.uni-heidelberg.de](mailto:hausmann@kip.uni-heidelberg.de)  
Kirchhoff-Institute for Physics  
University of Heidelberg
115. Stephanie Hehlhans  
[stephanie.hehlhans@kqu.de](mailto:stephanie.hehlhans@kqu.de)  
Klinik für Strahlentherapie und Onkologie  
Goethe-Universität Frankfurt
116. Thomas Helleday  
[thomas.helleday@scilifelab.se](mailto:thomas.helleday@scilifelab.se)  
Division of Translational Medicine and Chemical Biology, Department of  
Karolinska Institutet
117. Christine Hellweg  
[christine.hellweg@dlr.de](mailto:christine.hellweg@dlr.de)  
Radiation Biology  
German Aerospace Center (DLR), Institute of Aerospace Medicine
118. Alexander Helm  
[alexander.helm@tifpa.infn.it](mailto:alexander.helm@tifpa.infn.it)  
Trento Institute for Fundamental Physics and Applications (TIFPA)  
Trento Institute for Fundamental Physics and Applications (TIFPA)
119. Roman Hennel  
[roman.hennel@med.uni-muenchen.de](mailto:roman.hennel@med.uni-muenchen.de)  
Department of Radiation Oncology  
Ludwig-Maximilians-Universität München
120. Stefan Henning  
[stefan.henning@elbekliniken.de](mailto:stefan.henning@elbekliniken.de)  
Molekulare Zellbiologie  
Elbekliniken



- 121. Carsten Herskind**  
[carsten.herskind@medma.uni-heidelberg.de](mailto:carsten.herskind@medma.uni-heidelberg.de)  
Dept. of Radiation Oncology  
Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg
- 122. Anja Heselich**  
[a.heselich@gsi.de](mailto:a.heselich@gsi.de)  
Department for Biophysics  
GSI Helmholtz Center for Heavy Ion Research
- 123. Julia Hess**  
[julia.hess@helmholtz-muenchen.de](mailto:julia.hess@helmholtz-muenchen.de)  
Research Unit Radiation Cytogenetics  
Helmholtz Zentrum München
- 124. Eva Hirschmann**  
[Eva.Hirschmann@uk-erlangen.de](mailto:Eva.Hirschmann@uk-erlangen.de)  
Strahlenklinik Erlangen  
FAU Erlangen-Nürnberg
- 125. Daniela Hladik**  
[daniela.hladik@helmholtz-muenchen.de](mailto:daniela.hladik@helmholtz-muenchen.de)  
Institute of Radiation Biology  
Helmholtz Center Munich
- 126. Julian Hlouschek**  
[julian.hlouschek@uk-essen.de](mailto:julian.hlouschek@uk-essen.de)  
Institute of Cell Biology  
University Duisburg-Essen
- 127. Peter Huber**  
[p.huber@dkfz.de](mailto:p.huber@dkfz.de)  
Radiation Oncology  
dkfz
- 128. Stephan Huber**  
[stephan.huber@uni-tuebingen.de](mailto:stephan.huber@uni-tuebingen.de)  
Radiation Oncology  
University of Tübingen
- 129. Timea Hülber**  
[thulber@radosys.com](mailto:thulber@radosys.com)  
Department of Nuclear Techniques  
Budapest University of Technology
- 130. Timothy Humphrey**  
[timothy.humphrey@oncology.ox.ac.uk](mailto:timothy.humphrey@oncology.ox.ac.uk)  
CRUK-MRC Oxford Institute for Radiation Oncology  
University of Oxford
- 131. Annique Hunger**  
[annique.hunger@helmholtz-muenchen.de](mailto:annique.hunger@helmholtz-muenchen.de)  
Department of Radiation Sciences  
Institute of Innovative Radiotherapy
- 132. George Iliakis**  
[georg.iliakis@uk-essen.de](mailto:georg.iliakis@uk-essen.de)  
Institute of Medical Radiation Biology  
University of Duisburg-Essen, Medical School
- 133. Katharina Ilicic**  
[katarina.ilicic@tum.de](mailto:katarina.ilicic@tum.de)  
Department of Radiation Oncology  
Technical University Munich
- 134. Tatsuhiko Imaoka**  
[imaoka.tatsuhiko@qst.go.jp](mailto:imaoka.tatsuhiko@qst.go.jp)  
National Institute of Radiological Sciences  
National Institutes for Quantum and Radiological Science and Technology
- 135. Burkhard Jakob**  
[b.jakob@gsi.de](mailto:b.jakob@gsi.de)  
Biophysik  
GSI Helmholtzzentrum für Schwerionenforschung
- 136. Penny Jeggo**  
[P.A.Jeggo@sussex.ac.uk](mailto:P.A.Jeggo@sussex.ac.uk)  
Genome Damage and Stability Centre,  
University of Sussex
- 137. Verena Jendrossek**  
[verena.jendrossek@uni-due.de](mailto:verena.jendrossek@uni-due.de)  
Institute of Cell Biology (Cancer Research)  
University of Duisburg-Essen, University Hospital Essen
- 138. Hua Jing**  
[h.jing@dkfz-heidelberg.de](mailto:h.jing@dkfz-heidelberg.de)  
Deutsches Konsortium für Translationale Krebsforschung  
Deutsches Krebsforschungszentrum
- 139. Christian Johannes**  
[christian.johannes@uni-due.de](mailto:christian.johannes@uni-due.de)  
Fakultät Biologie, Molekulare Genetik I  
Universität Duisburg-Essen
- 140. George Don Jones**  
[gdj2@le.ac.uk](mailto:gdj2@le.ac.uk)  
Cancer Studies  
University of Leicester
- 141. Sylwia Kabacik**  
[sylwia.kabacik@phe.gov.uk](mailto:sylwia.kabacik@phe.gov.uk)  
Radiation Effects Department  
Public Health England
- 142. Anup Kainthola**  
[anupmicrobio@gmail.com](mailto:anupmicrobio@gmail.com)  
Division of Radiation Biosciences  
Institute of Nuclear Medicine & Allied Sciences, DRDO, New Delhi
- 143. Shizuko Kakinuma**  
[kakinuma.shizuko@qst.go.jp](mailto:kakinuma.shizuko@qst.go.jp)  
National Institute of Radiological Sciences  
National Institutes of Quantum and Radiological Sciences and Technology
- 144. Sophie Kalmbach**  
[sophie.ziegler@uk-essen.de](mailto:sophie.ziegler@uk-essen.de)  
Molecular Oncology  
University Hospital Essen

- 145. Maria Anna Kasma**  
[Marianna.kasma@gmail.com](mailto:Marianna.kasma@gmail.com)  
Applied Physics  
national technical university of athens
- 146. Anna Katsiki**  
[akatsik@biol.uoa.gr](mailto:akatsik@biol.uoa.gr)  
Faculty of Biology, Dept. of Biochemistry-Molecular Biology  
National and Kapodistrian University of Athens
- 147. Tamara Kazimova**  
[tamara.kazimova@usz.ch](mailto:tamara.kazimova@usz.ch)  
Department of Radiation Oncology  
University of Zurich
- 148. Justina Kazokaite**  
[kazokaite@ibt.lt](mailto:kazokaite@ibt.lt)  
Department of Biothermodynamics and Drug Design  
Institute of Biotechnology, Vilnius University
- 149. Julia Ketteler**  
[julia.ketteler@uk-essen.de](mailto:julia.ketteler@uk-essen.de)  
Institute of Cell Biology (Cancer Research)  
University Duisburg-Essen, University Hospital Essen
- 150. Nagma Khan**  
[nagma.khan@oncology.ox.ac.uk](mailto:nagma.khan@oncology.ox.ac.uk)  
Dept of Oncology  
University of Oxford
- 151. Diana Klein**  
[Diana.Klein@uk-essen.de](mailto:Diana.Klein@uk-essen.de)  
Univerity Medical Center, University of Duisburg-Essen  
Institute for Cell Biology (Cancer Research)
- 152. Dmitry Klovov**  
[dmitry.klovov@cnl.ca](mailto:dmitry.klovov@cnl.ca)  
Radiobiology and Health  
Canadian Nuclear Laboratories
- 153. Lukas Klumpp**  
[lukas.klumpp@med.uni-tuebingen.de](mailto:lukas.klumpp@med.uni-tuebingen.de)  
Department of Radiation Oncology  
University Hospital Tuebingen
- 154. Olena Klymenko**  
[olena.klymenko@med.uni-muenchen.de](mailto:olena.klymenko@med.uni-muenchen.de)  
Department of Radiation Oncology  
Ludwig-Maximilians-University Munich
- 155. Ioanna Kokkinopoulou**  
[iwanna-k@biol.uoa.gr](mailto:iwanna-k@biol.uoa.gr)  
Department of Biology  
National and Kapodistrian University of Athens
- 156. Katrien Konings**  
[kkonings@sckcen.be](mailto:kkonings@sckcen.be)  
Interdisciplinary Biosciences  
SCK-CEN
- 157. Maria Koshanskaya**  
[maria.koshanskaya@meduniwien.ac.at](mailto:maria.koshanskaya@meduniwien.ac.at)  
Radiotherapy  
Medical University of Vienna
- 158. Jakob Kowaliuk**  
[jakob.kowaliuk@meduniwien.ac.at](mailto:jakob.kowaliuk@meduniwien.ac.at)  
Department of Radiotherapy  
Medical University of Vienna
- 159. Maria Koźlak**  
[maria.kozlak@curie.fr](mailto:maria.kozlak@curie.fr)  
U1021  
Institut Curie
- 160. Eleftheria Kravvariti**  
[larakrav@biol.uoa.gr](mailto:larakrav@biol.uoa.gr)  
Department of Biochemistry and Molecular Biology  
National and Kapodistrian University of Athens
- 161. Lisa Marie Krieger**  
[lisamarie.krieger@uk-essen.de](mailto:lisamarie.krieger@uk-essen.de)  
Institute of Medical Radiation Biology  
University Hospital Essen
- 162. Malte Kriegs**  
[m.kriegs@uke.de](mailto:m.kriegs@uke.de)  
Lab of Radiobiology & Experimental Radiation Oncology  
University Medical Center Hamburg-Eppendorf
- 163. Ralf Kriehuber**  
[r.kriehuber@fz-juelich.de](mailto:r.kriehuber@fz-juelich.de)  
Safety and Radiation Protection  
Forschungszentrum Jülich
- 164. Adam Krysztofiak**  
[adam.krysztofiak@uk-essen.de](mailto:adam.krysztofiak@uk-essen.de)  
Institute of Cell Biology (Cancer Research)  
University Hospital Essen, University of Duisburg-Essen
- 165. Pelin Küçük**  
[pelin.kucuk@uk-essen.de](mailto:pelin.kucuk@uk-essen.de)  
Institute of Medical Radiation Biology  
University of Duisburg-Essen, Medical School
- 166. Ulrike Kulka**  
[ukulka@bfs.de](mailto:ukulka@bfs.de)  
Radiation Protection and Health  
Federal Office For Radiation Protection
- 167. Neeraj Kumari**  
[kmneeraj05@yahoo.com](mailto:kmneeraj05@yahoo.com)  
Capacity Enhancement and Product Implementation  
Institute of Nuclear Medicine and Allied Sciences
- 168. Sarah Kunze**  
[sarah.kunze@helmholtz-muenchen.de](mailto:sarah.kunze@helmholtz-muenchen.de)  
Institute of Developmental Diseases  
Helmholtz Center Munich



- 169. Bodo Laube**  
[laube@bio.tu-darmstadt.de](mailto:laube@bio.tu-darmstadt.de)  
Neurophysiology and Neurosensory Systems  
Technische Universität Darmstadt
- 170. Clelia Le Gallic**  
[clelia.legallic@gmail.com](mailto:clelia.legallic@gmail.com)  
Department of Radiation BioEffects (EBR)  
French Armed Forces Biomedical Research Institute (IRBA)
- 171. Wei-Chun Lee**  
[weichunlee1984@gmail.com](mailto:weichunlee1984@gmail.com)  
oncoray  
TU Dresden
- 172. Charlotte Lepleux**  
[lepleux@ganil.fr](mailto:lepleux@ganil.fr)  
LARIA  
CEA
- 173. Andrew Lessey**  
[alismith@xstrahl.com](mailto:alismith@xstrahl.com)  
Life Science  
Xstrahl Ltd
- 174. Fanghua Li**  
[fanghua.li@uk-essen.de](mailto:fanghua.li@uk-essen.de)  
Institute of medical radiation biology  
University Duisburg-Essen
- 175. Pavel Lobachevsky**  
[pavel.lobachevsky@petermac.org](mailto:pavel.lobachevsky@petermac.org)  
Cancer Research Division  
Peter MacCallum Cancer Centre
- 176. Markus Löbrich**  
[lobrich@bio.tu-darmstadt.de](mailto:lobrich@bio.tu-darmstadt.de)  
Darmstadt University of Technology  
Radiation Biology and DNA Repair
- 177. Daniel Lopez**  
[dflopezb@unal.edu.co](mailto:dflopezb@unal.edu.co)  
School of Medicine  
National University of Colombia
- 178. Ramon Lopez Perez**  
[r.lopez@dkfz.de](mailto:r.lopez@dkfz.de)  
Molecular and Radiation Oncology  
German Cancer Research Center (dkfz)
- 179. Katalin Lumniczky**  
[lumniczky.katalin@osski.hu](mailto:lumniczky.katalin@osski.hu)  
Radiobiology and Radiohygiene  
National Public Health Institute
- 180. Lovisa Lundholm**  
[lovisa.lundholm@su.se](mailto:lovisa.lundholm@su.se)  
Department of Molecular Biosciences, The Wenner-Gren Institute  
Stockholm University
- 181. Fiona Lyng**  
[Fiona.lyng@dit.ie](mailto:Fiona.lyng@dit.ie)  
RESC  
Dublin Institute of Technology
- 182. Heidi Lyng**  
[heidi.lyng@rr-research.no](mailto:heidi.lyng@rr-research.no)  
Department of Radiation Biology  
Oslo University Hospital
- 183. Simon Magin**  
[simon.magin@uk-essen.de](mailto:simon.magin@uk-essen.de)  
Institute of medical radiation biology  
University Hospital Essen
- 184. Andreas Maier**  
[a.maier@gsi.de](mailto:a.maier@gsi.de)  
Biophysic  
GSI Helmholtzzentrum für Schwerionenforschung GmbH
- 185. Grainne Manning**  
[grainne.manning@phe.gov.uk](mailto:grainne.manning@phe.gov.uk)  
Radiation Effects Department  
Public Health England
- 186. Wael Mansour**  
[wmansour@uke.de](mailto:wmansour@uke.de)  
Lab of Radiobiology and Experimental Radiation Oncology  
University Medical Cancer Center Hamburg Eppendorf
- 187. Lorenzo Manti**  
[lorenzo.manti@unina.it](mailto:lorenzo.manti@unina.it)  
Department of Physics "E. Pancini"  
University of Naples Federico II
- 188. Noëlle Mathieu**  
[noelle.mathieu@irsn.fr](mailto:noelle.mathieu@irsn.fr)  
PRP-HOM  
Institute of Radioprotection and Nuclear Safety
- 189. Martina Matjanovski**  
[martina.matjanovski@helmholtz-](mailto:martina.matjanovski@helmholtz-)  
Institute of radiation biology  
Helmholtz Zentrum München
- 190. Johann Matschke**  
[johann.matschke@uk-essen.de](mailto:johann.matschke@uk-essen.de)  
Institute of Cell Biology (Cancer Research)  
University Hospital Essen
- 191. Yusuke Matsuya**  
[y-matsuya1028@frontier.hokudai.ac.jp](mailto:y-matsuya1028@frontier.hokudai.ac.jp)  
Graduate School of Health Sciences  
Hokkaido University
- 192. Beatrice Mattheis-Koschel**  
[BeatriceMattheis-Koschel@xstrahl.com](mailto:BeatriceMattheis-Koschel@xstrahl.com)  
Radiotherapy  
Xstrahl Ltd

- 193. Ifigeneia Mavragani**  
[ifimav@mail.ntua.gr](mailto:ifimav@mail.ntua.gr)  
Dept. of Physics  
National Technical University of Athens (NTUA)
- 194. André Claude Mbouombouo Mfossa**  
[ammfossa@sckcen.be](mailto:ammfossa@sckcen.be)  
Radiobiology Unit  
Belgian Nuclear Research Centre (SCKCEN)
- 195. Gillies McKenna**  
[gillies.mckenna@oncology.ox.ac.uk](mailto:gillies.mckenna@oncology.ox.ac.uk)  
Oxford Institute for Radiation Oncology  
Oxford University
- 196. William McLaughlin**  
[wmclaughlin@pxinc.com](mailto:wmclaughlin@pxinc.com)  
Small Animal Image Guided Radiation Therapy Systems  
Precision X-Ray Inc.
- 197. Christina Anna Meidani**  
[sbi1200150@gmail.com](mailto:sbi1200150@gmail.com)  
Biology  
University of Athens
- 198. Sarah Meneceur**  
[sarah.meneceur@uniklinikum-dresden.de](mailto:sarah.meneceur@uniklinikum-dresden.de)  
Institute of translational radiooncology  
Universitätsklinikum Dresden - OncoRay
- 199. Malihe Mesbah**  
[malihe.mesbah@uk-essen.de](mailto:malihe.mesbah@uk-essen.de)  
Institut für Medizinische Strahlenbiologie  
Universitätsklinikum Essen
- 200. Felix Meyer**  
[fe.meyer@uke.de](mailto:fe.meyer@uke.de)  
Laboratory of Radiobiology & Experimental Radiooncology  
University Medical Center Hamburg-Eppendorf
- 201. Mouna Mhamdi-Ghodbani**  
[Mouna.Mhamdi-Ghodbani@elbekliniken.de](mailto:Mouna.Mhamdi-Ghodbani@elbekliniken.de)  
Dermatologie/Molekulare Zellbiologie  
Elbe Klinikum Buxtehude
- 202. Theodora-Dafni Michalettou**  
[daphne\\_lettos@yahoo.gr](mailto:daphne_lettos@yahoo.gr)  
Applied Mathematical and Physical Sciences  
National Technical University of Athens
- 203. Johanna Mirsch**  
[mirsch@bio.tu-darmstadt.de](mailto:mirsch@bio.tu-darmstadt.de)  
Radiation biology and DNA repair  
TU Darmstadt
- 204. Emil Mladenov**  
[emil.mladenov@uk-essen.de](mailto:emil.mladenov@uk-essen.de)  
Institute of Medical Radiation Biology  
University of Duisburg-Essen, Medical school
- 205. Veronika Mladenova**  
[Veronika.Mladenova@uk-essen.de](mailto:Veronika.Mladenova@uk-essen.de)  
Institute of Medical Radiation Biology  
University of Duisburg-Essen, Medical School
- 206. Simone Moertl**  
[moertl@helmholtz-muenchen.de](mailto:moertl@helmholtz-muenchen.de)  
Institute for Radiation Biology  
Helmholtz Zentrum München
- 207. Ahmed Allam Mohamed**  
[mohamed.ahmed.allam.abdelhamed@uniklinik-freiburg.de](mailto:mohamed.ahmed.allam.abdelhamed@uniklinik-freiburg.de)  
Radiation therapy  
Uniklinik Freiburg
- 208. Christian Möllers**  
[Christian.moellers@uk-essen.de](mailto:Christian.moellers@uk-essen.de)  
Institute of Medical Radiation Biology  
University of Duisburg-Essen, Medical School
- 209. Marjan Moreels**  
[marjan.moreels@sckcen.be](mailto:marjan.moreels@sckcen.be)  
Radiobiology Lab  
SCK-CEN
- 210. Ryosuke Mori**  
[rokomisyeru@eis.hokudai.ac.jp](mailto:rokomisyeru@eis.hokudai.ac.jp)  
Graduate School of Health Sciences  
Hokkaido University
- 211. Stefanie Mosel**  
[stefanie.schlesiger@uni-due.de](mailto:stefanie.schlesiger@uni-due.de)  
Molecular Biology II  
University of Duisburg-Essen
- 212. Elizaveta Moskaleva**  
[moskalevaey@mail.ru](mailto:moskalevaey@mail.ru)  
Department of Biology and Molecular Medicine  
National Research Centre "Kurchatov Institute"
- 213. Carmel Mothersill**  
[mothers@mcmaster.ca](mailto:mothers@mcmaster.ca)  
Dept Medical Physics and Applied Radiation Sciences  
McMaster University
- 214. Wolfgang-Ulrich Müller**  
[wolfgang-ulrich.mueller@uni-due.de](mailto:wolfgang-ulrich.mueller@uni-due.de)  
retired  
retired
- 215. Ruth Muschel**  
[ruth.muschel@oncology.ox.ac.uk](mailto:ruth.muschel@oncology.ox.ac.uk)  
Oxford Institute  
University of Oxford
- 216. Tamara Mußfeldt**  
[tamara.mussfeldt@uk-essen.de](mailto:tamara.mussfeldt@uk-essen.de)  
Institut für Medizinische Strahlenbiologie  
Universitätsklinikum Essen

- 217. Eric Neuhaus**  
[eric.neuhaus@me.com](mailto:eric.neuhaus@me.com)  
Department of Radiation Oncology  
University Hospital Tuebingen
- 218. Lily Nguyen**  
[lily.nguyen@helmholtz-muenchen.de](mailto:lily.nguyen@helmholtz-muenchen.de)  
Institute of Innovative Radiotherapy (iRT)  
Helmholtz Zentrum Muenchen
- 219. Gabriele Niedermann**  
[gabriele.niedermann@uniklinik-freiburg.de](mailto:gabriele.niedermann@uniklinik-freiburg.de)  
Dept. of Radiation Oncology  
University Freiburg
- 220. Zacharenia Nikitaki**  
[znikitaki@mail.ntua.gr](mailto:znikitaki@mail.ntua.gr)  
Physics Department  
National Technical University of Athens
- 221. Shwetajali Nimker**  
[shwet5859@gmail.com](mailto:shwet5859@gmail.com)  
Natural Radiation Response Mechanism division  
Institute of Nuclear Medicine and Allied Sciences
- 222. Mayumi Nishimura**  
[nishimura.mayumi@qst.go.jp](mailto:nishimura.mayumi@qst.go.jp)  
National Institute of Radiological Sciences  
National Institutes for Quantum and Radiological Science and Technology
- 223. Andreas Ntargaras**  
[a.ntargaras@gmail.com](mailto:a.ntargaras@gmail.com)  
DNA Damage Laboratory Physics Department  
National Technical University of Athens
- 224. Chioma Nwankwo**  
[rapuluchi@yahoo.com](mailto:rapuluchi@yahoo.com)  
National Institute of Radiation Protection and Research  
University of Ibadan
- 225. Veronica Olivo Pimentel**  
[v.olivopimentel@maastrichtuniversity.nl](mailto:v.olivopimentel@maastrichtuniversity.nl)  
Department of Radiation Oncology  
Maastricht University
- 226. Michael Orth**  
[michael.orth@med.uni-muenchen.de](mailto:michael.orth@med.uni-muenchen.de)  
Radiation Oncology  
University of Munich (LMU)
- 227. Andrea Ottolenghi**  
[Andrea.Ottolenghi@unipv.it](mailto:Andrea.Ottolenghi@unipv.it)  
Physics Department  
University of Pavia
- 228. Jagdish Paithankar**  
[jagdish.paithankar@gmail.com](mailto:jagdish.paithankar@gmail.com)  
Applied Zoology  
Mangalore University
- 229. Antonio Pantelias**  
[antonio.pantelias@gmail.com](mailto:antonio.pantelias@gmail.com)  
Laboratory of Health Physics, Radiobiology & Cytogenetics  
National Centre for Scientific Research "Demokritos"
- 230. Gabriel Pantelias**  
[gabriel@ipta.demokritos.gr](mailto:gabriel@ipta.demokritos.gr)  
Institute of Nuclear & Radiological Sciences & Technology, Energy &  
National Centre for Scientific Research "Demokritos"
- 231. Konstantinos Papadimitriou**  
[kpapadimitriou@aua.gr](mailto:kpapadimitriou@aua.gr)  
Biochemistry and Molecular Biology  
National and Kapodistrian University of Athens
- 232. Franziska Papenfuß**  
[franziska.papenfuss@kip.uni-heidelberg.de](mailto:franziska.papenfuss@kip.uni-heidelberg.de)  
Kirchhoff-Institut für Physik  
Ruprecht-Karls-Universität Heidelberg
- 233. Veronika Paštyková**  
[veronika.pastykova@gmail.com](mailto:veronika.pastykova@gmail.com)  
Dosimetry and Application of Ionizing Radiation  
Faculty of Nuclear Sciences and Physical Engineering, CTU in Prague
- 234. Apostolos Evgenios Pavlos**  
[evpavlos@biol.uoa.gr](mailto:evpavlos@biol.uoa.gr)  
Biochemistry and Molecular Biology  
National and Kapodistrian University of Athens
- 235. Eszter Persa**  
[persaeszter@yahoo.com](mailto:persaeszter@yahoo.com)  
Department of Radiobiology and Radiohygiene  
National Public Health Institute
- 236. Saskia Pfeiffer**  
[saskia.pfeiffer@kit.edu](mailto:saskia.pfeiffer@kit.edu)  
Wassertechnologie und Entsorgung  
Projekträger Karlsruhe
- 237. Daniel Piehlmaier**  
[daniel.piehlmaier@helmholtz-muenchen.de](mailto:daniel.piehlmaier@helmholtz-muenchen.de)  
Institut für Strahlenzytogenetik  
Helmholtz Zentrum München
- 238. Gerasimos Pollakis**  
[gerpolx@hotmail.com](mailto:gerpolx@hotmail.com)  
School of applied mathematical and physical science  
National technical University of Athens
- 239. Martin Pruschy**  
[martin.pruschy@usz.ch](mailto:martin.pruschy@usz.ch)  
Radiation Oncology  
University Hospital Zurich
- 240. Roel Quintens**  
[roel.quintens@sckcen.be](mailto:roel.quintens@sckcen.be)  
Radiobiology Unit  
Belgian Nuclear Research Centre

- 241. Ken Raj**  
[ken.raj@phe.gov.uk](mailto:ken.raj@phe.gov.uk)  
Radiation Effects Department  
Public Health England
- 242. Satish Rao Bola Sadashiva**  
[rao.satish@manipal.edu](mailto:rao.satish@manipal.edu)  
Department of Radiation Biology & Toxicology  
School of Life Sciences,
- 243. Alexander Rapp**  
[rapp@bio.tu-darmstadt.de](mailto:rapp@bio.tu-darmstadt.de)  
Cell Biology and Epigenetics  
Technische Universität Darmstadt
- 244. Felicitas Rapp**  
[f.merz@gsi.de](mailto:f.merz@gsi.de)  
Biophysics  
GSI Helmholtz center for heavy ion research
- 245. Treewut Rassamegevanon**  
[treewut.rassamegevanon@uniklinikum-dresden.de](mailto:treewut.rassamegevanon@uniklinikum-dresden.de)  
Faculty of Medicine  
OncoRay – National Center for Radiation Research in Oncology
- 246. Conrad Rauber**  
[conrad.rauber@gustaveroussy.fr](mailto:conrad.rauber@gustaveroussy.fr)
- 247. Annika Reddig**  
[annika.reddig@med.ovgu.de](mailto:annika.reddig@med.ovgu.de)  
Institute of Molecular and Clinical Immunology  
Otto von Guericke University Magdeburg
- 248. Judith Reindl**  
[judith.reindl@unibw.de](mailto:judith.reindl@unibw.de)  
Institut für angewandte Physik und Messtechnik  
Universität der Bundeswehr München
- 249. Oliver Reiners**  
[olrei101@hhu.de](mailto:olrei101@hhu.de)  
Institut für Pharmakologie und Klinische Pharmakologie  
Heinrich-Heine-Universität Düsseldorf
- 250. Christian Reinhardt**  
[christian.reinhardt@uk-koeln.de](mailto:christian.reinhardt@uk-koeln.de)
- 251. Günther Reitz**  
[guenther.reitz@dlr.de](mailto:guenther.reitz@dlr.de)  
Radiation Biology  
German Aerospace Center
- 252. Norman Reppingen**  
[N.Reppingen@gsi.de](mailto:N.Reppingen@gsi.de)  
Biophysics, GSI  
GSI Helmholtzzentrum für Schwerionenforschung
- 253. Muhammad Assad Riaz**  
[assad.muhammad@uni-due.de](mailto:assad.muhammad@uni-due.de)  
Klinik für Strahlentherapie, Universitätsklinikum Essen  
Universität Duisburg-Essen
- 254. Carmen Rios**  
[carmen.rios@nih.gov](mailto:carmen.rios@nih.gov)  
RADIATION AND NUCLEAR COUNTERMENT  
NIH/NIAID
- 255. Sylvia Ritter**  
[s.ritter@gsi.de](mailto:s.ritter@gsi.de)  
Biophysics  
GSI Helmholtzcenter for Heavy Ion Research
- 256. Franz Rödel**  
[franz.roedel@kgu.de](mailto:franz.roedel@kgu.de)  
Radiotherapy and Oncology  
Goethe-University Frankfurt am Main
- 257. Emmy Rogakou**  
[emrogakou@med.uoa.gr](mailto:emrogakou@med.uoa.gr)  
EpigenFocus  
Red Biotechnology
- 258. Kai Rothkamm**  
[k.rothkamm@uke.de](mailto:k.rothkamm@uke.de)  
Radiobiology & Experimental Radiation Oncology  
University Medical Center Hamburg-Eppendorf
- 259. Claudia E. Rübe**  
[claudia.ruebe@uks.eu](mailto:claudia.ruebe@uks.eu)  
Radiation Oncology  
Saarland University
- 260. Michael Rückert**  
[michael.rueckert@uk-erlangen.de](mailto:michael.rueckert@uk-erlangen.de)  
Radiation Oncology  
Universitätsklinikum Erlangen
- 261. Justine Rudner**  
[justine.rudner@uk-essen.de](mailto:justine.rudner@uk-essen.de)  
Insitute for Cell Biology (Tumor Research)  
University Hospital Essen
- 262. Alexander Rühle**  
[ruehle.alexander@gmx.de](mailto:ruehle.alexander@gmx.de)  
Molecular Radiooncology  
German Cancer Research Center
- 263. Werner Rühm**  
[werner.ruehm@helmholtz-muenchen.de](mailto:werner.ruehm@helmholtz-muenchen.de)  
Institute of Radiation Protection  
Helmholtz Zentrum München
- 264. Anderson Ryan**  
[sharon.draper@oncology.ox.ac.uk](mailto:sharon.draper@oncology.ox.ac.uk)  
Department of Oncology  
University of Oxford

- 265. Sunilgowda S N**  
[Sunilgowda.sn4492@gmail.com](mailto:Sunilgowda.sn4492@gmail.com)  
School of Chemical and biotechnology  
SASTRA University
- 266. Géza Sáfrány**  
[safrany.geza@osski.hu](mailto:safrany.geza@osski.hu)  
Radiobiology and Radiohygiene  
National Public Health Institute
- 267. Yannick Saintigny**  
[yannick.saintigny@cea.fr](mailto:yannick.saintigny@cea.fr)  
IRCM  
CEA
- 268. Traimate Sangsuwan**  
[traimate.sangsuwan@su.se](mailto:traimate.sangsuwan@su.se)  
Stockholm University  
Centre for Radiation Protection Research
- 269. Kohei Sasaki**  
[sasaki-k@hus.ac.jp](mailto:sasaki-k@hus.ac.jp)  
Faculty of Health Science  
Hokkaido University of Science
- 270. Diana Savu**  
[dsavu@nipne.ro](mailto:dsavu@nipne.ro)  
The Department of Life and Environmental Sciences  
Horia Hulubei National Institute for R&D in Physics and Nuclear
- 271. Harry Scherthan**  
[h.scherthan@tum.de](mailto:h.scherthan@tum.de)  
RadBio  
Bundeswehr Institute of Radiobiology affil. to the Univ. of Ulm
- 272. Zoé Schmal**  
[zoe.schmal@uks.eu](mailto:zoe.schmal@uks.eu)  
Labor für Strahlentherapie  
Universität des Saarlandes
- 273. Thomas Schmid**  
[thomas.schmid@helmholtz-muenchen.de](mailto:thomas.schmid@helmholtz-muenchen.de)  
Institute of Innovative Radiotherapy (iRT)  
Helmholtz Zentrum München
- 274. Sabine Schmitz**  
[sa.schmitz@fz-juelich.de](mailto:sa.schmitz@fz-juelich.de)  
Safety and Radiation Protection  
Forschungszentrum Juelich GmbH
- 275. Ludmila Schneider**  
[ludmila.schneider@helmholtz-muenchen.de](mailto:ludmila.schneider@helmholtz-muenchen.de)  
Department of Radiation Sciences  
Helmholtz Zentrum München
- 276. Paul Schofield**  
[pns12@cam.ac.uk](mailto:pns12@cam.ac.uk)  
Dept Physiology Development and Neuroscience  
University of Cambridge
- 277. Michael Scholz**  
[m.scholz@gsi.de](mailto:m.scholz@gsi.de)  
Biophysics  
GSI Helmholtzzentrum für Schwerionenforschung
- 278. Ulrike Schötz**  
[ulrike.schoetz@gmail.com](mailto:ulrike.schoetz@gmail.com)  
LMU München  
Strahlentherapie Großhadern
- 279. Alexander Schramm**  
[alexander.schramm@uk-essen.de](mailto:alexander.schramm@uk-essen.de)  
Medical Oncology  
University Hospital Essen
- 280. Insa Sigrid Schröder**  
[i.schroeder@gsi.de](mailto:i.schroeder@gsi.de)  
Biophysics Department  
Helmholtz Center for Heavy Ion Research
- 281. Johannes Schulte-Pelkum**  
[j.schulte-pelkum@medipan.de](mailto:j.schulte-pelkum@medipan.de)  
Sales Department  
MEDIPAN GMBH
- 282. Björn Schumacher**  
[bjoern.schumacher@uni-koeln.de](mailto:bjoern.schumacher@uni-koeln.de)  
Institute for Genome Stability in Aging and Disease, CECAD Research  
University of Cologne
- 283. Christian Schunck**  
[cschunck@metasystems.de](mailto:cschunck@metasystems.de)  
Marketing  
MetaSystems GmbH
- 284. Martin Selmansberger**  
[martin.selmansberger@helmholtz-](mailto:martin.selmansberger@helmholtz-)  
Department of Radiation Cytogenetics  
Helmholtz Zentrum München
- 285. Noemi Serra**  
[noemi.serra@urv.cat](mailto:noemi.serra@urv.cat)  
Physiology Unit, DCMB  
Universitat Rovira i Virgili
- 286. Lisa Sevenich**  
[sevenich@gsh.uni-frankfurt.de](mailto:sevenich@gsh.uni-frankfurt.de)  
Georg-Speyer-Haus  
Institute for Tumor Biology and Experimental Therapy
- 287. Colin Seymour**  
[seymouc@mcmaster.ca](mailto:seymouc@mcmaster.ca)  
Dept Medical Physics and Applied Radiation Sciences  
McMaster University
- 288. Efe Cumhur Sezgin**  
[efe.sezgin@uni-tuebingen.de](mailto:efe.sezgin@uni-tuebingen.de)  
Department of Radiation Oncology  
Universitätsklinikum Tübingen



- 289. Yoshiya Shimada**  
[shimada.yoshiya@gst.go.jp](mailto:shimada.yoshiya@gst.go.jp)  
National Institute of Radiological Sciences  
National Institutes for Quantum and Radiological Science and Technology
- 290. Tatsuya Shimasaki**  
[tshima@kumamoto-u.ac.jp](mailto:tshima@kumamoto-u.ac.jp)  
Institute of Resource Development and Analysis  
Kumamoto University
- 291. Vijay Singh**  
[vijay.singh@usuhs.edu](mailto:vijay.singh@usuhs.edu)  
Pharmacology and Experimental Therapeutics  
USUHS
- 292. Mattia Siragusa**  
[masir@dtu.dk](mailto:masir@dtu.dk)  
Nutech  
DTU
- 293. Peter Sminia**  
[p.sminia@vumc.nl](mailto:p.sminia@vumc.nl)  
Radiation Oncology / Laboratory for Radiobiology  
VU University medical center / Cancer Center Amsterdam
- 294. Aashish Soni**  
[aashish.soni@uk-essen.de](mailto:aashish.soni@uk-essen.de)  
Institute of Medical Radiation Biology  
University of Duisburg-Essen, Medical School
- 295. Claus Sørensen**  
[claus.storgaard@bric.ku.dk](mailto:claus.storgaard@bric.ku.dk)  
BRIC - Biotech Research and Innovation Center  
University of Copenhagen
- 296. Maria Souli**  
[maria\\_souli@yahoo.gr](mailto:maria_souli@yahoo.gr)  
Physics Department of NTUA and Atominstut of TU Wien  
National Technical University of Athens- Technische Universität Wien
- 297. Christin Starzonek**  
[Christin.Starzonek@elbekliniken.de](mailto:Christin.Starzonek@elbekliniken.de)  
Dermatologie/Molekulare Zellbiologie  
Elbe Klinikum Buxtehude
- 298. Alizée Steer**  
[alizee.steer@uk-essen.de](mailto:alizee.steer@uk-essen.de)  
Institute of Cell Biology (Cancer Research)  
Uniklinikum
- 299. Sjors Stouten**  
[sjors.stouten@rivm.nl](mailto:sjors.stouten@rivm.nl)  
Modelling and Scenario Research department  
Netherlands National Institute for Public Health and the Environment
- 300. Christian Streffer**  
[streffer.essen@t-online.de](mailto:streffer.essen@t-online.de)  
Medical Faculty  
University Clinics Essen
- 301. Tünde Szatmári**  
[szatmari.tunde@osski.hu](mailto:szatmari.tunde@osski.hu)  
Radiobiology and Radiohygiene  
National Public Health Institute
- 302. Ikuno Takahashi**  
[iktakaha@gmail.com](mailto:iktakaha@gmail.com)  
Department of Clinical Studies  
Radiation Effects Research Foundation
- 303. Feng Ru Tang**  
[tangfr@gmail.com](mailto:tangfr@gmail.com)  
Singapore Nuclear Research and Safety Initiative  
National University of Singapore
- 304. Vasiliki Tasiou**  
[vasiliki.tasiou@uk-essen.de](mailto:vasiliki.tasiou@uk-essen.de)  
Institute of Medical Radiation Biology  
University Duisburg-Essen
- 305. Konstantina Tatsi**  
[kontat@biol.uoa.gr](mailto:kontat@biol.uoa.gr)  
Biology  
National and Kapodistrian University of Athens
- 306. Marin Terashima**  
[marin0429@eis.hokudai.ac.jp](mailto:marin0429@eis.hokudai.ac.jp)  
Hokkaido University  
Graduate of Health Science
- 307. Georgia Terzoudi**  
[gterzoudi@rrp.demokritos.gr](mailto:gterzoudi@rrp.demokritos.gr)  
Institute of Nuclear & Radiological Sciences & Technology, Energy &  
National Centre for Scientific Research "Demokritos"
- 308. Marcel Thissen**  
[marcel.thissen@uk-essen.de](mailto:marcel.thissen@uk-essen.de)  
Institute of Medical Radiation Biology  
Uniklinik Essen
- 309. Andreas R. Thomsen**  
[andreas.thomsen@uniklinik-freiburg.de](mailto:andreas.thomsen@uniklinik-freiburg.de)  
Department of Radiation Oncology  
University of Freiburg, Faculty of Medicine
- 310. Sara Timm**  
[sara.timm@uks.eu](mailto:sara.timm@uks.eu)  
Labor für molekulare Radioonkologie  
Universität des Saarlandes
- 311. Beate Timmermann**  
[beate.timmermann@uk-essen.de](mailto:beate.timmermann@uk-essen.de)  
West German Proton Therapy Center  
University Hospital Essen
- 312. Aadhya Tiwari**  
[aadhya.tiwari@uni-tuebingen.de](mailto:aadhya.tiwari@uni-tuebingen.de)  
Department of Radiation Oncology  
University of Tuebingen

- 313. Susanne Tonnemacher**  
[S.Tonnemacher@gsi.de](mailto:S.Tonnemacher@gsi.de)  
Biophysics  
GSI Helmholtzzentrum für Schwerionenforschung
- 314. Mahmoud Toulany**  
[mahmoud.toulany@uni-tuebingen.de](mailto:mahmoud.toulany@uni-tuebingen.de)  
Division of Radiation Biology & Molecular Environmental Research,  
University of Tuebingen
- 315. Ioanna Tremi**  
[ioannatremi@hotmail.gr](mailto:ioannatremi@hotmail.gr)  
Institute of Medical Radiation Biology  
University of Duisburg-Essen
- 316. Angeliki Tsoka**  
[agg.tsoka@gmail.com](mailto:agg.tsoka@gmail.com)  
Molecular Biology and Biochemistry  
National and Kapodistrian University of Athens
- 317. Amanda Tulk**  
[alismith@xstrahl.com](mailto:alismith@xstrahl.com)  
Life Science  
Xstrahl Ltd
- 318. Kristian Unger**  
[unger@helmholtz-muenchen.de](mailto:unger@helmholtz-muenchen.de)  
Research Unit Radiation Cytogenetics  
Helmholtz Zentrum München
- 319. Marcus Unverricht-Yeboah**  
[m.unverricht@fz-juelich.de](mailto:m.unverricht@fz-juelich.de)  
Department of Safety and Radiation Protection  
Forschungszentrum Jülich
- 320. Haico van Attikum**  
[h.van.attikum@lumc.nl](mailto:h.van.attikum@lumc.nl)  
Department of Human Genetics  
Leiden University Medical Center
- 321. Anneke van der Reijden**  
[Anneke.van.der.Reijden@RIVM.nl](mailto:Anneke.van.der.Reijden@RIVM.nl)  
Centre for Environmental Safety and Security  
RIVM
- 322. Arjan van Dijk**  
[Arjan.van.Dijk@RIVM.nl](mailto:Arjan.van.Dijk@RIVM.nl)  
Centre for Environmental Safety and Security  
RIVM
- 323. Dik van Gent**  
[d.vangent@erasmusmc.nl](mailto:d.vangent@erasmusmc.nl)  
Molecular Genetics  
Erasmus MC
- 324. Charlot Vandevoorde**  
[cvandevoorde@tlabs.ac.za](mailto:cvandevoorde@tlabs.ac.za)  
Radiation Biophysics  
NRF iThemba LABS
- 325. Christina Vasileiou**  
[christinevasil@gmail.com](mailto:christinevasil@gmail.com)  
Applied Mathematics and Physical Sciences  
National and technical university of Athens
- 326. Tatjana Vatter**  
[TatjanaVatter@gmx.de](mailto:TatjanaVatter@gmx.de)  
Department of Radiation Oncology  
University Hospital Tuebingen
- 327. Aurelie Vaurijoux**  
[aurelie.vaurijoux@irsn.fr](mailto:aurelie.vaurijoux@irsn.fr)  
Radiobiology and Epidemiology Department  
Institute of Radioprotection and Nuclear Safety
- 328. Anne Vehlow**  
[anne.vehlow@nct-dresden.de](mailto:anne.vehlow@nct-dresden.de)  
Nationales Centrum für Tumorerkrankungen (NCT) Dresden  
Deutsches Krebsforschungszentrum (DKFZ)
- 329. Mieke Verslegers**  
[mversleg@sckcen.be](mailto:mversleg@sckcen.be)  
Radiobiology Unit  
Belgian Nuclear Research Centre SCK-CEN
- 330. Bram Verstraete**  
[Bram.Verstraete@UGent.be](mailto:Bram.Verstraete@UGent.be)  
Basic Medical Sciences, Radiobiology group  
Ghent University
- 331. Ermenegilda Vitale**  
[erm.vitale@studenti.unina.it](mailto:erm.vitale@studenti.unina.it)  
Department of Biology  
University of Naples Federico II
- 332. Beate Volkmer**  
[beate.volkmer@elbekliniken.de](mailto:beate.volkmer@elbekliniken.de)  
Dep. of Molecular Cellbiology  
Elbe Kliniken Stade-Buxtehude
- 333. Cläre von Neubeck**  
[claere.vonneubeck@uniklinikum-dresden.de](mailto:claere.vonneubeck@uniklinikum-dresden.de)  
Translational Radiooncology  
OncoRay, German Cancer Consortium, German Cancer Research Center
- 334. Marc Vooijs**  
[mvooyis@gmail.com](mailto:mvooyis@gmail.com)  
Radiation Oncology  
Maastricht University
- 335. Konstantinos Vorgias**  
[cvorgias@biol.uoa.gr](mailto:cvorgias@biol.uoa.gr)  
Department of Biology, Section of Biochemistry-Mol Biology  
National and Kapodistrian University of Athens
- 336. Jennifer Wadsworth**  
[j.l.wadsworth@sms.ed.ac.uk](mailto:j.l.wadsworth@sms.ed.ac.uk)  
School of Physics and Astronomy  
University of Edinburgh



- 337. Dietrich Walsh**  
[dietrich.walsh@unibw.de](mailto:dietrich.walsh@unibw.de)  
Institut für angewandte Physik und Messtechnik  
Universität der Bundeswehr München
- 338. Klaus-Josef Weber**  
[klaus-josef.weber@med.uni-heidelberg.de](mailto:klaus-josef.weber@med.uni-heidelberg.de)  
Radiation Oncology  
Heidelberg University Clinics
- 339. Julia Wiedemann**  
[j.wiedemann@gsi.de](mailto:j.wiedemann@gsi.de)  
Biophysics  
GSI Darmstadt
- 340. Lisa Wiesmüller**  
[lisa.wiesmueller@uni-ulm.de](mailto:lisa.wiesmueller@uni-ulm.de)  
Department of Obstetrics and Gynaecology of the University of Ulm  
Ulm University
- 341. Florian Wirsdörfer**  
[florian.wirsdoerfer@uk-essen.de](mailto:florian.wirsdoerfer@uk-essen.de)  
Institute of Cellbiology (Cancer Research)  
University Hospital Essen
- 342. Andrzej Wojcik**  
[andrzej.wojcik@su.se](mailto:andrzej.wojcik@su.se)  
Department of Molecular Biosciences - MBW  
Stockholm University
- 343. Alexandra Wolf**  
[alexandra.wolf@uk-essen.de](mailto:alexandra.wolf@uk-essen.de)  
Physiology  
UK Essen
- 344. Markus Wolf**  
[markus.wolf@uni-due.de](mailto:markus.wolf@uni-due.de)  
Molecular Biology I  
University of Duisburg-Essen
- 345. Ryota Yamada**  
[minato2525x@eis.hokudai.ac.jp](mailto:minato2525x@eis.hokudai.ac.jp)  
Graduate School of Health Sciences  
Hokkaido University
- 346. Mohd Yasser**  
[mohd.yasser@uk-essen.de](mailto:mohd.yasser@uk-essen.de)  
Institute of Medical Radiation Biology  
University of Duisburg
- 347. Konstantinos Yeles**  
[yeles.konstantinos@gmail.com](mailto:yeles.konstantinos@gmail.com)  
Biochemistry-Molecular Biology  
National and Kapodistrian University of Athens
- 348. Michio Yoshimura**  
[myossy@kuhp.kyoto-u.ac.jp](mailto:myossy@kuhp.kyoto-u.ac.jp)  
Department of Radiation Oncology & Image-applied therapy  
Kyoto University
- 349. Brian Yudhistiara**  
[yudhistiara@stud.uni-heidelberg.de](mailto:yudhistiara@stud.uni-heidelberg.de)  
Radiation Oncology  
University Hospital Heidelberg
- 350. Sebastian Zahnreich**  
[zahnreic@uni-mainz.de](mailto:zahnreic@uni-mainz.de)  
Radiation Oncology and Radiotherapy  
University Medical Centre
- 351. Athanassios Zees**  
[azees@biol.uoa.gr](mailto:azees@biol.uoa.gr)  
Faculty of Biology, Department of Biochemistry and Molecular Biology  
National and Kapodistrian University of Athens
- 352. Erika Zernickel**  
[erika.zernickel@uk-essen.de](mailto:erika.zernickel@uk-essen.de)  
Department of Radiotherapy  
Uniklinik
- 353. Xuanwei Zhang**  
[xuanwei.zhang@uniklinik-freiburg.de](mailto:xuanwei.zhang@uniklinik-freiburg.de)  
Klinik für Strahlenheilkunde  
University of Freiburg
- 354. Horst Zitzelsberger**  
[zitzelsberger@helmholtz-muenchen.de](mailto:zitzelsberger@helmholtz-muenchen.de)  
Research Unit Radiation Cytogenetics  
Helmholtz Zentrum München
- 355. Friedo Zölzer**  
[zoelzer@zsf.jcu.cz](mailto:zoelzer@zsf.jcu.cz)  
Institute of Radiology, Toxicology and Civil Protection  
University of South Bohemia



# Wireless-LAN access



## Guest Account Details

### Credentials for Guest User: ERRS2017

<b>Guest User Name:</b>	<b>ERRS2017</b>
<b>Password:</b>	<b>gast</b>
<b>Profile:</b>	<b>www2uke</b>
<b>Start Time:</b>	<b>23-Aug-2017</b>
<b>End Time:</b>	<b>25-Sep-2017</b>

#### IMPORTANT!!!

If the LOG-IN window does not appear on your device, please type into your internet browser the following IP address: 1.1.1.1

Guests understand and acknowledge that we exercise no control over the nature, content and reability of the information and/or data passing through our network.

Als Nutzer des Wireless-LAN-Gastzugangs des Universitätsklinikum Essen, beachten Sie bitte dass wir weder Kontrolle noch Einfluss auf die Inhalte und Zuverlässigkeit von Informationen und Daten haben.

