LUND UNIVERSITY

Swedish Radiobiological Society
Svensk förening för radiobiologi

European Radiation ERRS Research Society

Programme and abstract book

45th Annual Meeting of the European Radiation Research Society

September 13 - 17 2020
Lund, Sweden

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Welcome

We are delighted to welcome you to the 45th Annual Meeting of the European Radiation Research Society (ERRS2020)!

The ERRS meeting series belongs to a tradition of European conferences on radiation research initiated already in 1953. This year's meeting is jointly organised by the Swedish Radiobiological Society and Lund University.

With the pandemic situation in mind it was decided to reformat this year's meeting into a digital conference. As organizers, we are committed to bring you an enjoyable experience in an immersive and interactive meeting space. During the organization we have been excited to see the scientific programme grow into a list of contributions that we find truly state-of-the art, covering a wide range of aspects in the fields of radiobiology and radiation sciences, and with representation from about 30 different countries and five continents.

Many meetings were cancelled this year, and we are happy that it was possible to arrange the ERRS2020 event in an alternative manner. We would already now like to thank all participants for submitting their abstracts and adapting to this format. Please take this opportunity to interact with colleagues and friends around the globe, to engage and contribute to a pleasant and rewarding experience. We look forward to your contribution during live discussions, chats around posters and within the sponsor area. Enjoy!

Sincerely,
The Local Organizing Committee

Committees

The Local Organizing Committee (LOC)

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ERRS meetings

Tuesday 15, 11:00 – 12:00: ERRS Council meeting

Wednesday 15, 11:00 – 12:00: ERRS General Assembly

Awards

Bacq & Alexander Award

Gabriel Pantelias: Premature Chromosome Condensation as a powerful tool for dose estimation and individualised long-term risk assessment following exposure to different radiation qualities

Award lecture is held Wednesday 17, 15:30 – 16:10

Young investigator awards

Chiara Feoli
Annemarie Schröder
Verdiana Trappetti
Lisa Hintz
Biche Osong
Ségolène Ladaigue
Shari Wouters
Timo Smit
Aggeliki Nikolakopoulou
Martha Habibi
Alessio Parisi
Viacheslav Shcherbakov
Samia Chaouni
Sandra Bicher
Isabella Guardamagna
Anna Kirstein
Lorain Geenen
Magdalena Płódowska
Simon Sioen
Evi Duthoo
Valerio Ricciardi
Jade Monaghan
Milagrosa Lopez Riego
Delmon Arous
Gaia Pucci

Award ceremony is held Thursday 17, 15:30 – 16:10
Poster award ceremony is held Thursday 17, 16:50 – 17:10

Scientific Programme

Sunday 13: Conference opening and opening lecture

15:00 - 15:30	Conference Opening - Mats Helmfrid: Lund City Welcome Conference Opening - Fiona Lyng: ERRS President Address
15.30 - 16.10	Opening Lecture Pat Zanzonico: The validity and applicability of the Linear No-Threshold (LNT) model: Hypothesis for Use in Risk Assessment
16.10 - 16.30	How to access the ERRS2020 Digital Conference
16.30 - 16.50	Mingle in the Lounge
16.50 - 17.10	

Monday 14: Molecular and cellular effects

12.00 - 12.40	Keynote 1 Alexandros G. Georgakilas: Induction and repair of oxidative clustered DNA damage and its biological importance for humans	
12.40 - 13.00	Invited 1 Eva Forssell-Aronsson: Genome-wide multi-omics profiling in radionuclide therapy	
13.00 - 13.20	Invited 2 Kristian Unger: Integrative system biology	
13.20 - 13.40	Invited 3 Carmel Mothersill: Radiation-induced genomic instability as a driver for environmental evolution	
13.40 - 14.00	Coffee Break	
14.00 - 14.40	O1 - Lovisa Lundholm: Impact of ATM and DNA-PK inhibition on gene expression and individual response of human lymphocytes to mixed beams of alpha particles and X-rays	O5 - Raghda Ramadan: Blocking Connexin43 hemichannel alleviates radiation-induced endothelial cell damage
	O2 - Stephanie Vermeulen: RAD51 foci as biomarkers for HR efficiency and radiosensitivity in individuals with a BRCA1 or BRCA2 mutation	O6 - Jean-Pierre Pouget: Cell membrane and lipid raft are involved in targeted and non-targeted effects of Auger and alpha molecular radiotherapy
	O3 - Chiara Feoli (YIA): Chromosome aberration complexity revealed in proton-irradiated cells treated with boron carriers supports Proton-Boron Capture Therapy	O7 - Bjorn Baselet: Combination therapy: particle irradiation with the Hedgehog inhibitor GANT61 differently modulates the radiosensitivity and migration of cancer cells
	O4 - Annemarie Schröder (YIA): Endometrial stem cells isolated from menstrual blood - A better model for the radiobiology of mesenchymal stem cells?	O8 - Auchi Inalegwu: Identification of linear and circular RNA biomarkers of radiation resistance in MCF7 breast cancer cells
14.40 - 15.00	Panel Discussion	

Monday 14: Molecular and cellular effects, continued

15:00 - 15:30	Poster Viewing	
15.30 - 16.10	Keynote 2 Kevin Prise: Mechanistic modelling of non-target effects	
16.10 - 16.30	Invited 4 Roger Howell: Relative Biologial Effect (RBE) for alpha, beta and Auger emitters - implication for radionuclide therapy	
16.30 - 16.50	Invited 5 Olga Martin: Systemic effects of microbeam radiotherapy	
16.50 - 17.10	Invited 6 Valentin Djonov: Vascular effects of micro-beam radiotherapy	
17.10 - 17.30	Coffee Break	
17.30 - 18.10	O9 - Federica Ciamarone: Study of cytotoxic effects induced by carbon ions irradiation on U-251 Glioblastoma cell line after treatment with a new platinum(IV)-based prodrug	O13 - Yuting Jiang: Role of cellular senescence in radiation-induced cognitive dysfunction
	O10 - Nicole Matejka: Influence of Alpha-particle Radiation on Intercellular Communication Networks of Tunneling Nanotubes in U87 Glioblastoma Cells	O14 - Yi Wu: Role of microenvironment on the post-irradiation regenerative potential of salivary gland stem cells
	O11 - Paulo Godoy: Targeting NRF2, regulator of antioxidant system, to sensitize glioblastoma neurosphere cells to radiation-induced oxidative stress	O15 - Ingunn Hanson: Communication of radioprotective effects by TGF-β3
	O12 - Soile Tapio: CREB signalling in the irradiated hippocampus	O16 - Gabriel Adrian: FLASH-effect observed under normoxic conditions in vitro – mechanisms other than oxygen depletion?
18.10 - 18.30	Panel Discussion	

Tuesday 15: Translational and clinical research

12.00 - 12.40	Keynote 3 Ester Hammond: Tumour hypoxia and radiosensitivity	
12.40 - 13.00	Invited 7 Elisabeth Schültke: Spatio-temporal fractionation	
13.00 - 13.20	Invited 8 Bart Cornelissen: Imaging of tumour biology	
13.20 - 13.40	Invited 9 Karl Butterworth: Preclinical models of radiation response	
13.40 - 14.00	Coffee Break	
14.00 - 14.40	O17 - Justine Perrin: Reversing cold tumor microenvironment with targeted alpha-therapy	O21 - Elke Beyreuther: Normal tissue reaction following proton irradiation of the mouse brain
	O18 - Aisling Heeran: RIBE induction using human ex vivo explants causes alterations in mitochondrial metabolism in bystander cells	O22 - Daniëlle Voshart: Differential neurocognitive response after partial brain proton irradiation
	O19 - Verdiana Trappetti (YIA): First pre-clinical study for lung carcinoma employing Synchrotron Microbeam Radiotherapy at the Australian Synchrotron	O23 - Angelica Facoetti: The new experimental beam line and research facility at CNAO for radiobiological studies with charged particles
	O20 - Malin Larsson: Proteomic expression analysis of rat thyroid tissue 12 months after low-intermediate 131I exposure	O24 - Lisa Hintz (YIA): RBE-dependence on LET and fractionation in the rat cervical spinal cord after helium ion irradiation
14.40 - 15.00	Panel Discussion	

Tuesday 15: Translational and clinical research, continued

15:00 - 15:30	Poster Viewing	
15.30 - 16.10	Keynote 4 Marie-Catherine Vozenin: FLASH radiobiology	
16.10 - 16.30	Invited 10 Kristoffer Petersson: FLASH radiotherapy	
16.30 - 16.50	Invited 11 Elke Beyreuther: Proton FLASH	
16.50 - 17.10	Invited 12 Ana Carneiro: Combined radiotherapy and immunotherapy	
17.10 - 17.30	Coffee Break	
17.30 - 18.10	O25 - Sarah Rudigkeit: Proton-FLASH – Radiation effects of ultrahigh dose-rate irradiation	O29 - Aisling Heeran: Examining the effect of radiation on the secretome of normal and rectal cancer tissue and how this secretome interacts with the innate immune system
	O26 - Jia-Ling Ruan: Investigating FLASH irradiation on acute normal tissue toxicity in the murine gastrointestinal system	O30 - Alain Chapel: Clinical Trial Evaluating the Efficacy of Mesenchymal Stromal Cell Injections for the Treatment of Radiation Induced Chronic Pelvic Complications
	O27 - Elise Konradsson: First veterinary patient treated with electron FLASH radiotherapy at a clinical linear accelerator	O31 - Anna Sundlöv: Pituitary Function after High-Dose 177Lu-DOTATATE Therapy and Long-Term Follow-Up
	O28 - Julie Constanzo: Immunomodulatory effects of external and targeted radiotherapy depend on radiation type	O32 - Biche Osong (YIA): Decision tool for radiotherapy compliance in elderly cancer patients
18.10 - 18.30	Panel Discussion	

Wednesday 16: Health effects and radiation protection

12.00 - 12.40	Keynote 5 Udo Gaipl: Effects of low versus high doses of radiation on the immune system	
12.40 - 13.00	Invited 13 Charles Limoli: Neurocognitive effects of radiation	
13.00 - 13.20	Invited 14 Pawel Olko: Unwanted doses from stray radiation in proton therapy	
13.20 - 13.40	Invited 15 Lindsay Morton: Radiation carcinogenesis	
13.40 - 14.00	Coffee Break	
14.00 - 14.40	O33 - Ségolène Ladaigue (YIA): Characterization of monocytes-endothelium interactions after radiotherapy	O37 - Clément Brossard: Preclinical study of Chronic radiocystitis and cell therapy treatment
	O34 - Shari Wouters (YIA): Colonic and microbial alterations in a colorectal cancer mouse model provoked by pelvic fractionated X-ray treatment	O38 - Bjorn Baselet: Can rosiglitazone protect endothelial cells from irradiation-induced mitochondiral dysfunction?
	O35 - Roel Quintens: p53 drives premature neuronal differentiation in response to radiation-induced DNA damage during early neurogenesis	O39 - Ankit Chauhan: Evaluation of the radioprotective potential of a PTEN inhibitor bpV(HOpic)
	O36 - Lorena Giuranno: NOTCH inhibition promotes bronchial stem cell renewal and epithelial barrier integrity after irradiation	O40 - Timo Smit (YIA): Cardiomyocyte dysfunction upon radiation
14.40 - 15.00	Panel Discussion	

Wednesday 16: Health effects and radiation protection, continued

15:00 - 15:30	Poster Viewing	
15.30 - 16.10	Bacq & Alexander Award - Gabriel Pantelias: Premature Chromosome Condensation as a powerful tool for dose estimation and individualised long-term risk assessment following exposure to different radiation qualities	
16.10 - 16.30	Invited 16 Uta Eberlein: Biodosimetry and internal dosimetry in nuclear medicine	
16.30 - 16.50	Invited 17 Per Roos: Radionuclides in the environment and their radiobiological implications	
16.50 - 17.10	Invited 18 Deborah Oughton: Science, ethics and society lessons learnt from radioecology	
17.10 - 17.30	Coffee Break	
17.30 - 18.10	O41 - Mieke Verslegers: Early-life X-ray exposure accelerates brain aging in a 3xTg-AD mouse model	O45 - Kim Carola Roeder: Effects of α-particles and X-rays on human lung epithelium
	O42 - Christopher Rääf: The time-dependence of radiological benefit of decontamination of residential areas after a nuclear fallout for newborn and adults	O46 - Aggeliki Nikolakopoulou (YIA): G2/M checkpoint abrogation with selective inhibitors results in chromosome break repair defects in RPE and 82-6 hTERT cells
	O43 - Martin Andersson: Improved patient dosimetry at radioiodine therapy by combining the ICRP iodide compartment model and the EANM pre-therapeutic standard procedure	O47 - Martha Habibi (YIA): Detection of DNA damage and chromosomal aberrations after exposure to low ionizing radiation doses in interventional cardiology
	O44 - Charlotte Andersson: Coadministration of three antioxidants did not influence the tumour response to radiotherapy in GOT1 neuroendocrine tumour model	O48 - Sjors Stouten: Mathematical modelling of radiation-induced acute myeloid leukaemia incidence
18.10 - 18.30	Panel Discussion	

Thursday 17: Radiation physics and chemistry

12.00 - 12.40	Keynote 6 Mark Konijnenberg: Radionuclide therapy: biological, physical and medical aspects in preclinical and clinical settings	
12.40 - 13.00	Invited 19 Sauli Savolainen: Boron neutron capture therapy (BNCT) : Technological and physical prospects	
13.00 - 13.20	Invited 20 Stephen McMahon: Mechanistic Modelling of Intrinsic Radiation Sensitivity	
13.20 - 13.40	Invited 21 Olle Lundh: Progress towards using laser wakefield accelerators for radiotherapy	
13.40 - 14.00	Coffee Break	
14.00 - 14.40	O49 - Giulia Tamborino: Modeling early radiation damage occurring during [177Lu]Lu-DOTA-[Tyr3]octreotate radionuclide therapy with the Geant4-DNA toolkit	O53 - Viacheslav Shcherbakov (YIA): About the Absence of Reactive Oxygen Species Overproduction in the Presence of Gold Nanoparticles
	O50 - Mario P. Carante: RBE prediction by the BIANCA model for in vitro and in vivo irradiation by different hadron therapy ion-beams	O54 - Anouchka Gatin: Azide and hydroxyl radicals induce several di-tyrosine bridge isomers from the amino acid to the protein scale
	O51 - Alessio Parisi (YIA): Development of a new microdosimetric biological weighting function for the RBE assessment in case of the V79 cell line exposed to ions from 1H to 238U	O55 - Sergey Denisov: Presolvated electron attachement towards nucleotides in liquids: pulsed radiolysis studies
	O52 - Lovisa Waldner: EURADOS WG10 and RENEB WG2 exercise on retrospective dosimetry methods in a simulated small scale incident involving ionising radiation.	O56 - Sverker Werin: UrMAX - the light from Lund. Preservation of epoch-making scientific equipment illustrated by the evolution from UrMAX to MAX IV
14.40 - 15.00	Panel Discussion	

Thursday 17: Radiation physics and chemistry, continued

15:00 - 15:30	Poster Viewing
15.30 - 16.10	Young Investigator Awards
16.10 - 16.30	Invited 22 Marjolein Thunnisen: Life science at MAX IV
16.30 - 16.50	Invited 23 Sindra Petersson Årsköld: Life science at ESS
16.50 - 17.10	Poster Award and Closing Ceremony

Abstracts - Oral presentations

O1 - Impact of ATM and DNA-PK inhibition on gene expression and individual response of human lymphocytes to mixed beams of alpha particles and X-rays

Molecular and cellular effects

Lei Cheng[1]
Beata Brzozowska[2], Halina Lisowska[3], Andrzej Wojcik[1, 3], **Lovisa Lundholm**[1]
[1] Centre for Radiation Protection Research, Department of Molecular Biosciences - The Wenner-Gren Institute, Stockholm University, Sweden
[2] Biomedical Physics Division, Faculty of Physics, University of Warsaw, Warsaw, Poland
[3] Institute of Biology, Department of Radiobiology and Immunology, Jan Kochanowski University

Introduction: Accumulating evidence suggest a synergistic effect in cells exposed to different ionising radiation qualities with varying damage complexity and linear energy transfer (LET), originating from natural or medical exposure.
Methods: Here we aimed to analyse the effect of mixed beams on the expression of selected genes involved in DNA damage response in peripheral blood lymphocytes (PBL) isolated from 4 donors. Two donors were compared upon inhibition of ATM or DNA-PK and at different sampling times. qPCR was used to measure the relative expression levels of the genes FDXR, GADD45a, BBC3, MDM2, CDKN1A and XPC 24 hours following exposure to alpha particles, X-rays or mixed beams (1:1 dose of alpha particles and X-rays).
Results: Generally, alpha particles and mixed beams were stronger inducers of gene expression compared to X-rays and this difference was largest at low doses, displaying saturated versus linear dose response curves, respectively. Gene expression levels in three out of four donors showed a significant synergistic effect of mixed beams. Interestingly, when two of the donors were sampled again one year later, the former additive effect of mixed beams in one donor was now synergistic and the donors displayed no significant difference in intrinsic radiosensitivity as determined by gamma radiation-induced micronuclei. ATM, but not DNA-PK inhibition, reduced the radiation-induced gene expression, but differently for alpha radiation between the two donors.
Conclusion: In conclusion, synergy was present for all donors but the results suggest individual variability in the response to mixed beams, most likely due to life style changes.

O2 - RAD51 foci as biomarkers for HR efficiency and radiosensitivity in individuals with a BRCA1 or BRCA2 mutation

Molecular and cellular effects

Stephanie Vermeulen[1, 2]
Valerie De Mey[1], Evi Duthoo[1, 2], Anne Vral[1, 2], Ans Baeyens[1, 2]
[1] Ghent university, department of human structure and repair, radiobiology group, Ghent, Belgium
[2] Cancer research institute Ghent, Ghent, Belgium

Introduction: Breast cancer is the most common cancer in females. Known breast cancer predisposition genes are *BRCA1* and *BRCA2*. These genes are involved in the DNA damage response pathway, more specifically in homologous recombination (HR). HR is a DNA double strand break repair pathway active in S- and G2-phase of the cell cycle. Accumulation of RAD51 at the double strand break site is a hallmark of HR and could therefore be used to assess HR functionality and radiosensitivity in mutation carriers.
A recent study performed by our group showed that *in vitro* irradiation of MCF10A breast epithelial cells with reduced BRCA1 and BRCA2 protein levels resulted in a significant decrease in RAD51 foci.
Aim: Investigate if RAD51 foci can be used as biomarkers to assess HR functionality in peripheral blood mononuclear cells (PBMCs) of healthy and *BRCA1*/*BRCA2* mutation carriers.
Methods: PBMCs were isolated by density gradient centrifugation and cultured for 72h. The cells were irradiated with 5 Gy (220 kV X-rays). Identification of cells in S-phase at time of irradiation was achieved by EdU pulse-labelling. Thereafter RAD51 foci were detected by immunofluorescent staining and automatically scored by Metacyte software (Metafer 4, Metasystems).
Results: The functional RAD51 foci assay was optimized. Preliminary results comparing RAD51 foci between healthy individuals and mutation carriers will be presented.
Conclusion: As *BRCA1*/*BRCA2* mutation carriers might show increased risk for radiation-induced carcinogenesis, these results can ultimately contribute to personalized radiation regimens, both therapeutic as diagnostic.

O3 - Chromosome aberration complexity revealed in proton-irradiated cells treated with boron carriers supports Proton-Boron Capture Therapy

Molecular and cellular effects

Chiara Feoli[1]
Pavel Bláha[1], Roberto Catalano[2], Mario Ciocca[3], Angelica Facoetti[3], Giada Petringa[2], Valerio Ricciardi[1, 4], Enrico Rosa[5], Lorenzo Manti[1, 5]
[1] Istituto Nazionale di Fisica Nucleare (INFN), Sezione di Napoli, Naples, Italy
[2] Istituto Nazionale di Fisica Nucleare (INFN), Laboratori Nazionali del Sud (LNS), Catania, Italy
[3] Centro Nazionale di Adroterapia Oncologica (CNAO), Pavia, Italy
[4] Dipartimento di Matematica e Fisica, Università della Campania "L. Vanvitelli", Caserta, Italy.
[5] Dipartimento di Fisica "E. Pancini", Università di Napoli Federico II, Naples, Italy

Introduction: We investigated chromosome aberrations (CA) induced by the $p+^{11}B \rightarrow 3\alpha$ reaction (p-B). We already demonstrated enhancement of proton biological effectiveness by p-B [1]. The rationale underlying Proton-Boron Capture Therapy (PBCT) as a strategy to increase protontherapy tumour local control [2] hinges on the highly DNA-damaging high-LET α-particles from p-B, whose cross section peaks at 675 keV [3], i.e. for slowing down protons. To prove this, we analyzed the yield of complex CAs, a biomarker of high-LET exposure [4], in boron-treated cells along clinical proton Spread-Out Bragg Peaks (SOBPs).
Methods: MCF10A cells were irradiated at the SOBP entrance, mid and distal positions at two Italian protontherapy facilities, INFN-LNS and CNAO, with proton energies of 62 MeV and 131.5-164.8 MeV, respectively. In separate experiments, cells were treated with Sodium Boroncaptate BSH (80 ppm) or Boronophenylalanine BPA (120 ppm). After 36-48 h, chromosome spreads were obtained by calyculin A-induced Premature Chromosome Condensation [5]. Whole Chromosome Painting and mFISH karyotype reconstructions (Metafer, Metasystems, Germany) were performed. All aberration types were scored. Complex aberrations were classified as previously defined [6] and the degree of exchange complexity was evaluated by overall number of chromosome and breaks involved [7].
Results: CA frequency and degree of exchange complexity were consistently greater in boron-treated samples at all proton doses; more importantly, the proportion of complex exchanges increased moving from mid to the distal SOBP positions, with no effect observed at beam entrance.
Conclusion: Our findings confirm p-B-mediated enhancement of proton biological effectiveness and point to DNA damage complexity as the plausible explanation.
Selected references
[1] G. A. P. Cirrone, et al., *First experimental proof of Proton Boron Capture Therapy (PBCT) to enhance protontherapy effectiveness*. Sci. Rep., 8, 1141 (2018).
[2] Y. Do-Kun, et al., *Application of proton boron fusion reaction to radiation therapy: A Monte Carlo simulation study*. Appl. Phys. Lett. 105, 223507 (2014)
[3] M. H. Sikora, M. H. & H. R. Weller, *A new evaluation of the 11B(p, α)αα reaction rates*. J. Fusion. Energ. 35, 538–543 (2016).
[4] R. M. Anderson, et al. *Complex chromosome aberrations in peripheral blood lymphocytes as a potential biomarker of exposure to high-LET alpha particles*. Int. J. Radiat. Biol. 76, 31–42 (2000).

[5] L. Manti et al., *Chromosome aberrations in human lymphocytes from the plateau region of the Bragg curve for a carbon-ion beam*. Nucl. Instr. Meth B, 259, 884-888 (2007).
[6] M. N. Cornforth, Analyzing Radiation-Induced Complex Chromosome Rearrangements by Combinatorial Painting. Radiat. Res., 155, 643-659 (2001)

O4 - Endometrial stem cells isolated from menstrual blood - A better model for the radiobiology of mesenchymal stem cells?

Molecular and cellular effects

Annemarie Schröder[1]
Guido Hildebrandt[1], Katrin Manda[1]
[1] Department of Radiotherapy and Radiation Oncology, University Medical Centre Rostock, Suedring 75, 18059 Rostock, Germany.

Introduction: The endometrium is a highly remodeling tissue characterized by a large number of adult stem cells localized not only in the basalis but also in the functionalis. Due to its rejection during menstruation, it is possible to isolate mesenchymal stem cells (MSCs), here called Endometrial Regenerative Cells (ERCs) from the menstrual blood - a non-invasive, monthly repeatable, high-yield source of MSCs. These conditions are optimal and unique in stem cell research. Therefore, the aim of this study is to characterize ERCs for the first time in terms of their radiation response and to examine donor-specific influencing factors.
Methods: The successful isolation of ERCs was confirmed on the basis of their differentiation capacity and the panel of surface proteins. The donor-dependent proliferation rate, clonogenicity and differentiability were then examined. To assess radiation sensitivity, the clonogenic survival and the ability to repair DNA double-strand breaks were examined after irradiation.
Results: A total of 23 donors were registered. Donor-specific differences in growth kinetics and radiation sensitivity could not be attributed to the determined factors: body mass index, age, smoking behavior and number of pregnancies or miscarriages. Overall, the ERCs were characterized by a robust growth kinetic under norm- as well as hypoxic conditions with a population doubling time of 48 hours and a high repair capacity of DNA double-strand breaks leading to a moderate radiation sensitivity with an SF2 (survival fraction at 2Gy) of 34 %.
Conclusion: ERCs isolated from menstrual blood are very suitable as a model for the analysis of radiological examinations of MSCs.

O5 - Blocking Connexin43 hemichannel alleviates radiation-induced endothelial cell damage

Molecular and cellular effects

Raghda Ramadan[1, 2]
Els Vromans[3], Dornatien Chuo Anang[4], Ines Goetschalckx[5], Delphine Hoorelbeke[2], Elke Decrock[2], Sarah Baatout[1, 6], Luc Leybaert[2], An Aerts[1]
[1] Radiobiology Unit, Belgian Nuclear Research Centre (SCK CEN), Mol, Belgium;
[2] Department of Basic Medical Sciences, Physiology group, Ghent University, Ghent, Belgium;
[3] Centre for Environmental Health Sciences, Hasselt University, Hasselt, Belgium;
[4] Biomedical Research Institute, Hasselt university, Hasselt, Belgium;
[5] Protein Chemistry, Proteomics and Epigenetic Signaling Group, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Antwerp, Belgium.
[6] Department of Molecular Biotechnology, Ghent University, Ghent, Belgium

Introduction: Emerging evidence indicates an excess risk of late occurring cardiovascular diseases, especially atherosclerosis, after thoracic cancer radiotherapy. Ionizing radiation (IR) induces cellular effects which induces endothelial cell dysfunction, an early marker for atherosclerosis. In addition, intercellular communication through channels composed of transmembrane connexin proteins (Cxs), i.e. gap junctions (direct cell-cell coupling) and hemichannels (paracrine release/uptake pathway) can modulate radiation-induced responses and therefore the atherosclerotic process. However, the role of endothelial hemichannel in IR-induced atherosclerosis has never been described before.
Methods: Telomerase-immortalized human Coronary Artery/Microvascular Endothelial cells (TICAE/TIME) were exposed to X-rays (0.1 and 5 Gy). Production of reactive oxygen species (ROS), DNA damage, cell death, inflammatory responses, and senescence were assessed with or without applying a Cx43 hemichannel blocker (TAT-Gap19).
Results: We report here that IR induces an increase in oxidative stress, cell death, inflammatory responses (IL-8, IL-1β, VCAM-1, MCP-1, and Endothelin-1) and premature cellular senescence in TICAE and TIME cells. These effects are significantly reduced in the presence of the Cx43 hemichannel-targeting peptide TAT-Gap19.
Conclusion: Our findings suggest that endothelial Cx43 hemichannels contribute to various IR-induced processes, such as ROS, cell death, inflammation, and senescence, resulting in an increase in endothelial cell damage, which could be protected by blocking these hemichannels. Thus, targeting Cx43 hemichannels may potentially exert radioprotective effects.

Selected references

Ramadan, R., Vromans, E., Anang, D.C., Decrock, E., Mysara, M., Monsieurs, P., Baatout, S., Leybaert, L., and Aerts, A. (2019). Single and fractionated ionizing radiation induce alterations in endothelial connexin expression and channel function. *Scientific Reports* 9**,** 4643.

Decrock, E., Hoorelbeke, D., Ramadan, R., Delvaeye, T., De Bock, M., Wang, N., Krysko, D.V., Baatout, S., Bultynck, G., Aerts, A., Vinken, M., and Leybaert, L. (2017). Calcium, oxidative stress and connexin channels, a harmonious orchestra directing the response to radiotherapy treatment? *Biochim Biophys Acta* 1864**,** 1099-1120.

O6 - Cell membrane and lipid raft are involved in targeted and non-targeted effects of Auger and alpha molecular radiotherapy

Molecular and cellular effects

Jean-Pierre POUGET[1]
Riad Ladjohounlou[1], Alexandre Pichard[1], Julie Constanzo[1], Jihad Karam[1], Sara Marcatili[2], Manuel Bardiès[2], Frank Bruchertseifer[3], Alfred Morgenstern[3], Julien Torgue[4], Isabelle Teulon[1]
[1] IRCM, Institut de Recherche en Cancérologie de Montpellier, INSERM U1194, Université de Montpellier, Institut régional du Cancer de Montpellier, Montpellier, F-34298, France
[2] UMR 1037 INSERM/UPS, Centre de Recherche en Cancérologie de Toulouse, Toulouse F-31062, France
[3] Directorate for Nuclear Safety and Security, European Commission - Joint Research Centre, Karlsruhe, Germany
[4] AREVA Med, 4800 Hampden lane, Bethesda, MD 20814, USA

Introduction: With the development of new therapeutic radiopharmaceuticals in oncology, it is important to determine the contribution of targeted and non-targeted (bystander and systemic) effects of targeted radionuclide therapy.
Methods: Here, we investigated the contribution of non-targeted cytotoxic and genotoxic effects *in vitro* and *in* vivo (WT C57BL/6J and athymic nude mice) during alpha (^{212}Pb/^{212}Bi, ^{213}Bi) and Auger (^{125}I) radioimmunotherapy (RIT).
Results: *In vitro*, we showed that bystander effects contributed to 7-36% and 27-29% cell killing during alpha RIT and Auger RIT, respectively. We demonstrated that the bystander cell response was partly mediated by lipid raft-mediated activation of p38 kinase and c-JUN N-terminal kinases (JNK). We then showed that RIT efficacy was reduced *in vitro* and *in vivo* when RIT was combined with ASMase inhibitor (imipramine) or with drugs modifying cholesterol metabolism such as filipin, methyl-beta-cyclodextrin (or pravastatin). Reactive oxygen species also played a significant role in these bystander effects. Using autoradiography and voxel dosimetry, we confirmed the occurrence of bystander effects *in vivo* also, during Auger and alpha RIT. We isolated extracellular vesicles from the secretome of cells exposed to RIT and showed that they were responsible for clonogenic survival decrease *in vitro* and for tumor growth delay *in vivo* after intratumoral injection. We also showed that the latter therapeutic efficacy was enhanced in immune competent mice suggesting a role of immune cells.
Conclusion: We confirmed that non-targeted effects play a central role in Auger and alpha RIT and that drugs modifying cholesterol metabolism can modify RIT efficacy.
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O7 - Combination therapy: particle irradiation with the Hedgehog inhibitor GANT61 differently modulates the radiosensitivity and migration of cancer cells

Molecular and cellular effects

Bjorn Baselet[1]
Katrien Konings[1], Marjan Moreels[1], Sarah Baatout[1]
[1] Radiobiology unit, Interdisciplinary biosciences, Institute for environment, health and safety, SCK CEN, Mol, Belgium

Introduction: Due to the improved dose deposition and increased biological effectiveness advantage of charged particles, an increase is noted in the use of particle therapy in the clinic. Metastasis is an important cause of mortality in cancer patients and evidence has shown that conventional radiotherapy can increase the formation of metastasizing cells. An important pathway involved in the process of metastasis is the Hedgehog signaling pathway. Recent studies have demonstrated that activation of the Hedgehog pathway in response to X-rays, can lead to radioresistance and increased migratory and invasive capabilities of cancer cells.
Methods: The effect of X-rays, protons and carbon ions was investigated on cell survival, migration and Hedgehog pathway gene expression in prostate cancer (PC3) and medulloblastoma (DAOY) cell lines. In addition, the modulation of cell survival and migration by the Hedgehog pathway inhibitor GANT61 was investigated.
Results: We found that in both cell lines, carbon ions were more effective in decreasing cell survival and migration as well as inducing more significant alterations in the Hedgehog pathway genes compared to X-rays or protons. In addition, we show here for the first time that the Hedgehog inhibitor GANT61 is able to sensitize medulloblastoma cells to particle radiation (proton and carbon ion) but not to conventional X-rays.
Conclusion: This finding demonstrates that the results of combination treatment strategies with X-ray radiotherapy cannot be automatically extrapolated to particle therapy and should be investigated separately. In conclusion, combining GANT61 with particle radiation could offer a benefit for specific cancer types with regard to cancer cell survival.

O8 - Identification of linear and circular RNA biomarkers of radiation resistance in MCF7 breast cancer cells

Molecular and cellular effects

Auchi Inalegwu[1, 2]
Bart Cuypers[3], Jürgen Claesen[4], Sarah Baatout[1], Kris Laukens[3], Winnok H. De Vos[5], Roel Quintens[1]
[1] Radiobiology Unit, SCK•CEN, Belgian Nuclear Research Centre, 2400 Mol, Belgium.
[2] Department of Biomedical Sciences, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, 2610 Antwerp, Belgium.
[3] Adrem Data Lab, Department of Mathematics and Computer Science, University of Antwerp, 2020 Antwerp, Belgium.
[4] Microbiology Unit, SCK•CEN, Belgian Nuclear Research Centre, 2400 Mol, Belgium.
[5] Department of Veterinary Sciences, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, 2610 Antwerp, Belgium.

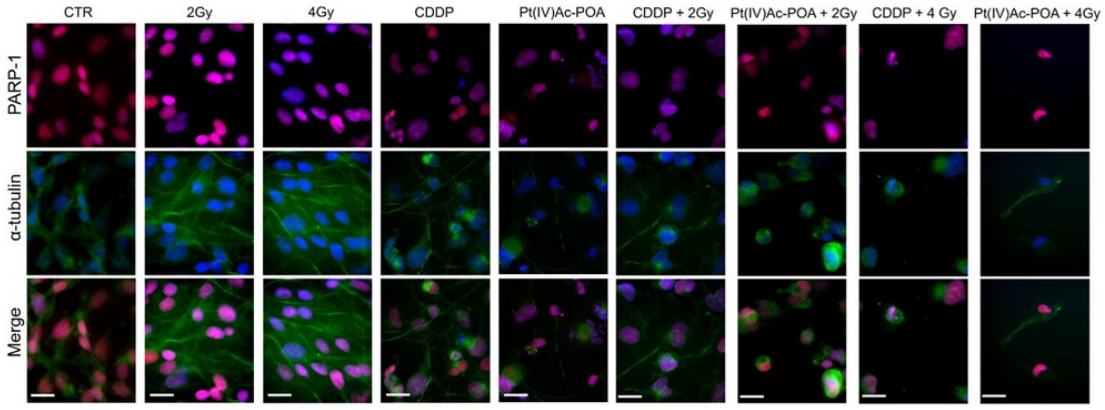
Introduction: Insights into the molecular mechanisms that determine radiation sensitivity of tumours can help optimize patient-specific treatment. The goal of the present study was to identify transcriptional biomarkers of radiation resistance in MCF7 breast cancer cells.
Methods: We generated a radiation resistant MCF7 breast cancer cell line (FIR20) through fractionated 2-Gy X-ray exposure of the parental (PAR) cell line. The radiation response of FIR20 cells was subsequently analysed with respect to a culture age-matched, sham-irradiated control (AMC) and PAR cells using the clonogenic survival assay, and live-cell fluorescence imaging. RNA-seq was also performed for linear and circular RNA (circRNA) detection.
Results: FIR20 cells showed increased clonogenic survival after irradiation as compared to PAR and AMC cells. Live-cell imaging revealed a reduction in proliferation and decreased susceptibility of FIR20 cells to radiation-induced apoptosis. RNA-seq analysis identified over 550 significantly differentially expressed genes (DEGs) in FIR20 cells, of which the up-regulated DEGs were mainly involved in inflammatory pathways, hypoxia, P53 signaling and epithelial-mesenchymal transition (EMT). In contrast, the down-regulated DEGs were mainly E2F and MYC targets and G2M checkpoint and mitotic spindle-associated genes indicating cell cycle deregulation; a commom hallmark of human malignancies. Additionally, using the CIRI2 and CircExplorer pipelines we detected ~40 differentially expressed circRNAs between FIR20 and the control cell lines.
Conclusion: The molecular mechanisms of radioresistance in the FIR20 cell line potentially attributable to the modulation of various immunoprotective pathways, altered proliferation and cell cycle deregulation may present an opportunity for therapeutic interventions.

O9 - Study of cytotoxic effects induced by carbon ions irradiation on U-251 Glioblastoma cell line after treatment with a new platinum(IV)-based prodrug

Molecular and cellular effects

Federica Ciamarone[1, 2]
Beatrice Ferrari[1], Mario Ciocca[2], Federica Carnevale[2], Chiara Rita Inguscio[1], Angelica Facoetti[2], Maria Grazia Bottone[1]
[1] Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia, Italy
[2] CNAO National Center for Oncological Hadrontherapy, Pavia, Italy

Introduction: The use of carbon ions for the treatment of high-grade cancer cells, has further advantages compared to protons, including a Bragg peak with a smaller width and better radiobiological effects. Cell apoptosis is one of the key mechanisms through which ionizing radiation kills tumour cells via the extrinsic or the intrinsic death pathway. The highly resistance and the unsuccessful treatment of Glioblastoma, remains a significant therapeutic challenge. The introduction of Pt(IV)-based compounds in combination with heavy charged particles, to overcome the gliomas intrinsic resistance, is still under investigation.
Methods: The U-251 glioblastoma cell line was treated with 48h-continuous treatment of cisplatin or with a new platinum(IV)-based prodrug, Pt(IV)Ac-POA, followed by 0, 2 or 4 Gy carbon ions irradiation. The cytotoxic effects induced by two different carbon ion doses and by the Pt(IV)Ac-POA, the morphological cell alterations and the activation of apoptotic mechanisms, trigger through caspase-3 and PARP1 activity, involved in several crucial cellular processes, were analysed by Western blotting and immunocytochemical techniques.
Results: The cytotoxic effect observed demostrated a caspase-dependent cell apoptosis in glioblastoma cell death involving the PARP1 signaling pathway not only at 48 h after carbon ions irradiation but even after 7 days, demonstrating a prolonged antitumor effect of the Pt(IV)Ac-POA. The efficacy was detected more intensely after combined treatment with carbon ion irradiation, showing a long-term cytotoxic effect.
Conclusion: The combined treatments with the Pt(IV)Ac-POA and carbon ions irradiation could be an important contribution to emerging therapeutic approaches in glioblastoma treatment and discusses the future challenges in improving antitumor directions.



O10 - Influence of Alpha-particle Radiation on Intercellular Communication Networks of Tunneling Nanotubes in U87 Glioblastoma Cells

Molecular and cellular effects

Nicole Matejka[1]
Judith Reindl[1]
[1] Institut für angewandte Physik und Messtechnik, Universität der Bundeswehr München, Werner-Heisenberg-Weg 39, 85577 Neubiberg, Germany

Introduction: Cellular communication plays a crucial role in the coordination and organization of cancer cells. Especially processes such as uncontrolled cell-growth, invasion and therapy resistance, which are features of malignant tumors as glioblastomas, are supported by an efficient cell-to-cell communication. One powerful way for cells to communicate are tunneling nanotubes (TNTs). These tiny cytoplasmic membrane bridges with a diameter from 50 to 1500 nm directly connect cells over long distances up to several cell diameters and serve as highways for information and material exchange between them. We study the response of TNT communication networks in glioblastoma cells on radiative stress induced by α-particle radiation. The aim was to figure out whether cell-to-cell connections via TNTs are influenced by radiation and if cellular communication was enhanced upon irradiation.
Methods: U87 glioblastoma cells were irradiated using high-LET α-particles to a dose of 1.2 Gy. After irradiation cells were labeled with CellMask™ Orange plasma membrane stain. The TNT network was examined using live-cell confocal microscopy up to 72 h after irradiation and compared to sham irradiated controls. We quantify the development of TNT networks and suggest an evaluation method to characterize these communication networks.
Results: Our results show that irradiated cells establish their network faster and have more cell-to-cell connections with a high TNT content than sham irradiated controls within the first 24 h.
Conclusion: These findings suggest that there is an additional trigger upon radiation damage which results in fast and intensive network formation by TNTs as a radiation damage response mechanism.

O11 - Targeting NRF2, regulator of antioxidant system, to sensitize glioblastoma neurosphere cells to radiation-induced oxidative stress

Molecular and cellular effects

Paulo Godoy[1, 2]
Ali Pour Khavari[1], Marzia Rizzo[1], Elza Sakamoto-Hojo[2, 3], Siamak Haghdoost[1, 4, 5]
[1] Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University
[2] Department of Biology, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo
[3] Department of Genetics, Faculty of Medicine of Ribeirão Preto
[4] University of Caen Normandy
[5] Advanced Resource Center for HADrontherapy in Europe (ARCHADE)

Introduction: The presence of glioma stem cells (GSCs), which are enriched in neurospheres, may be connected to the radioresistance of glioblastoma (GMB) due to their enhanced antioxidant defense and elevated DNA repair capacity. The role of ROS in GSCs still needs better characterization in response to different qualities of radiation .
Methods: U87MG cells were cultured in a 3D model and irradiated with low (24 mGy/h) and high (0.39 Gy/min) dose rates of low LET gamma and high LET carbon ions (1-2 Gy/min). Thereafter, expression of proteins related to oxidative stress response (NRF2, hMTH1, PRDX2, GSTO1, APE1, SOD1 and SOD2), stemness marker (MUSASHI-1), extracellular 8-oxo-dG, and neurospheres were determined. The NRF2 gene was knocked down by CRISPR/Cas9.
Results: LD50 for carbon ions was significantly lower compared to LD50 of high and low dose rate gamma radiation. A significantly higher level of 8-oxo-dG was detected in the media of cells exposed to a low dose rate as compared to a high dose rate of gamma or carbon ions. A downregulation of oxidative stress proteins was also observed (NRF2, hMTH1, and SOD1). The NRF2 gene was knocked down by CRISPR/Cas9 in neurosphere cells, resulting in less self-renewal, more differentiated cells, and less proliferation capacity after irradiation with low and high dose rate gamma rays.
Conclusion: NRF2 knockdown exerted a great impact in cellular responses to irradiation by decreasing the antioxidant properties of neurospheres leading to a lesser self-renewal capacity and increasing differentiation, thus indicating NRF2 as a molecular target for reducing GBM cell survival.

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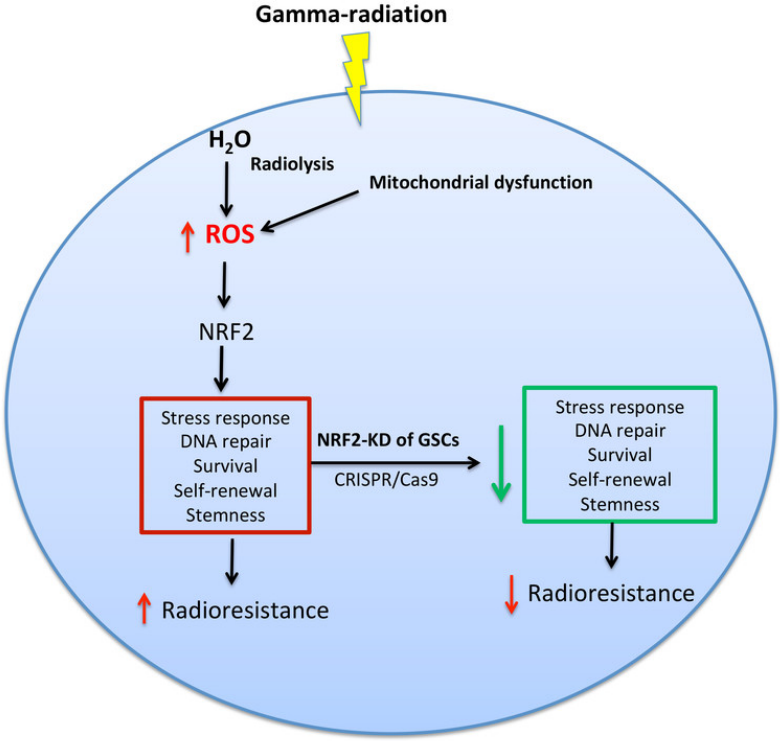
Gamma-radiation
H2O
Radiolysis
Mitochondrial dysfunction
ROS
NRF2
Stress response
DNA repair
Survival
Self-renewal
Stemness
NRF2-KD of GSCs
CRISPR/Cas9
Stress response
DNA repair
Survival
Self-renewal
Stemness
Radioresistance
Radioresistance

O12 - CREB signalling in the irradiated hippocampus

Molecular and cellular effects

Soile Tapio[1, 2]
Daniela Hladik[1, 2], Claudia Dalke[3], Christine von Törne[4], Omid Azimzadeh[1, 2]
[1] Institute of Radiation Biology, Helmholtz Zentrum München, German Research Center for Environmental Health GmbH (HMGU), 85764 Neuherberg, Germany
[2] Technical University Munich (TUM), 80333 Munich, Germany
[3] Institute of Developmental Genetics, HMGU
[4] Research Unit Protein Science, HMGU

Introduction: The impact of low-dose radiation on human brain has recently attracted attention due to its increasing medical use for diagnostic purposes. High doses of ionizing radiation are known to induce harmful effects in the central nervous system, whilst the effects of low doses are still controversial.
Methods: Female B6C3F1 mice were total body irradiated at the age of 10 weeks with doses of 0 (control), 0.063, 0.125 or 0.5 Gy (^{60}Co). Hippocampus was analysed 24 months post-IR by quantitative label-free proteomics. The results were validated by western blotting. The oxidative stress level was determined using carbonylation assay.
Results: The proteomics data showed that CREB signalling was affected at all doses. Notably, the lower doses of 0.063 Gy and 0.125 Gy seemed to induce the CREB pathway, whereas the exposure to 0.5 Gy deactivated CREB. Similarly, the lowest dose (0.063 Gy) had an anti-inflammatory effect reducing the number of activated microglia (IBA-1), whereas an induction of both activated microglia and reactive astroglia (GFAP) was found at the 0.5 Gy dose suggesting inflammation and astrogliosis, respectively. Apoptotic and oxidative stress markers were increased only at the highest dose (0.5 Gy).
Conclusion: The CREB pathway plays a central role in long-term memory formation. These data suggest neuroprotection at 0.063 Gy, but neurodegeneration at 0.5 Gy. These effects become significant first in old animals and support the hypothesis of radiation-induced accelerated aging in the brain.

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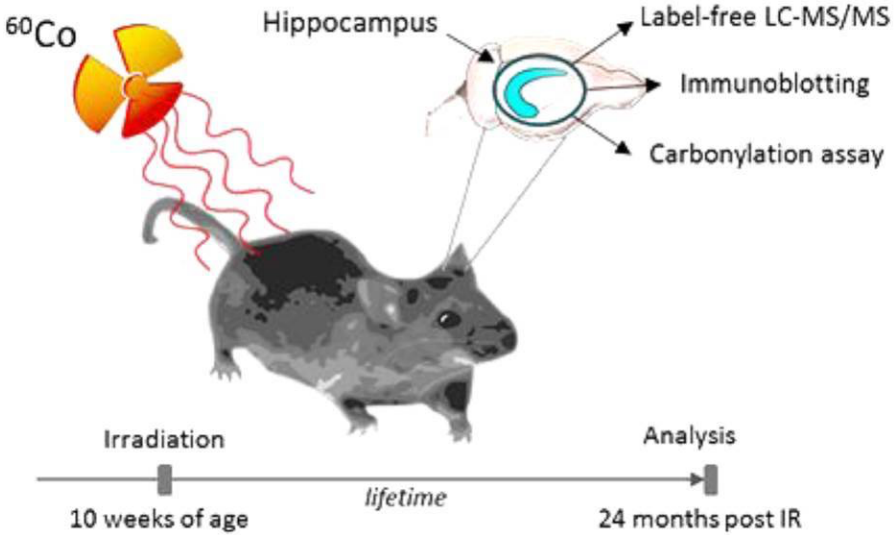


O13 - Role of cellular senescence in radiation-induced cognitive dysfunction

Molecular and cellular effects

Yuting Jiang[1, 2]
Daniëlle Voshart[1, 2], Fabian Hobbie[2], Maarten Hamberg[2], Fleur van Buuren-Broek[1, 2], Steven Bergink[2], Rob Coppes[1, 2], Lara Barazzuol[1, 2]
[1] Department of Radiation Oncology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.
[2] Department of Biomedical Sciences of Cells and Systems, Section of Molecular Cell Biology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.

Introduction: Radiotherapy causes cognitive dysfunction in 50-90% of brain tumour surviving patients. However, the underlying mechanisms are still unclear. Recently, cellular senescence has been reported to be involved in the pathogenesis of age-related neurodegenerative diseases by promoting protein aggregation. Thus, in this study we investigated the connection between cellular senescence and protein aggregation after irradiation.
Methods: Senescence and protein aggregation were measured both *in vivo* in 14 Gy irradiated rat brains and *in vitro* in mouse primary neurons/astrocytes and pluripotent stem cell-derived human cortical brain organoids after 5 or 10 Gy irradiation. The senolytic drug ABT-263 was used to selectively kill the senescent cells. β-Galactosidase staining and qPCR analysis of p16, p21 and senescence-associated secretory phenotype genes were used to evaluate cellular senescence. Accumulation of protein aggregates was assessed by immunofluorescence staining of markers like the autophagy receptor p62, the RNA/DNA-binding protein TDP43 and the aggresome dye Proteostat. Neuronal function was measured using live calcium imaging.
Results: Senescent cells and protein aggregates accumulated in the cortex of irradiated rat brains and in human brain organoids. Astrocytes accounted for about 60% of radiation-induced senescent cells. In a co-culture system of senescent astrocytes and non-irradiated neurons, astrocytes were shown to mediate neuronal protein aggregation. Additionally, treatment with ABT-263 could remove radiation-induced senescent cells and partially relieve the accumulation of protein aggregates improving calcium dynamics.
Conclusion: Our study suggests that cellular senescence plays a role in radiation-induced cognitive decline and senolytic drugs may be a promising therapeutic strategy.

O14 - Role of microenvironment on the post-irradiation regenerative potential of salivary gland stem cells

Molecular and cellular effects

yi wu[1, 2]
sarah pringle[3], uilke brouwer[1, 2], xiaohong peng[1, 2], arjan vissink[4], lara barazzuol[1, 2], rob coppes[1, 2]
[1] university medical center groningen, dept. of biomedical sciences of cells & systems, groningen, netherlands
[2] university medical center groningen, dept. of radiation oncology, groningen, netherlands
[3] university medical center groningen, dept. of rheumatology and clinical immunology, groningen, netherlands
[4] university medical center groningen, dept. of oral and maxillofacial surgery, groningen, netherlands

Introduction: Radiotherapy of head and neck cancer involves co-irradiation of salivary glands, often resulting in hyposalivation and consequential reduced quality of life. Radiation-induced microenvironmental changes may be unfavorable for regenerative potential of the tissue. The aim was to assess how these changes affects regenerative potential of salivary gland stem cells (SGSCs).
Methods: Mice were locally irradiated (IR) with 15 Gy on the salivary glands and salivary secretion was measured at distinct time points. Morphological changes and level of senescence were determined using IHC and SGSC organoid formation efficiency (OFE) was assessed up on several passages. Irradiated organoids derived conditioned medium was tested for naïve SGSC OFE.
Results: Hyposalivation, gland weight reduction, morphology decline and infiltration of inflammatory cells increased progressively from 30 days after IR onwards. The number of acini declined and an enhanced number of p21+ senescent cells were observed. The irradiated glands exhibited a lower number of CD24hi/CD29hi stem cells than the control. Interestingly, the OFE of SGSCs obtained up to 30-days post-irradiation was initially reduced but recovered at later passages. However, SGSCs obtained from 90 days irreversibly lost potential to form organoids, indicative of loss of regenerative potential. After incubation with the conditioned medium, OFE was significantly decreased, indicating the irradiated microenvironment compromised SGSC self-renewal potential.
Conclusion: The regenerative potential of surviving SGSCs early after irradiation seems to be comparable to unirradiated stem cells when taken out of a deleterious environment. However, at late phases, stem cells have lost regenerative potential indicating permanent environmental-related changes in stemness.

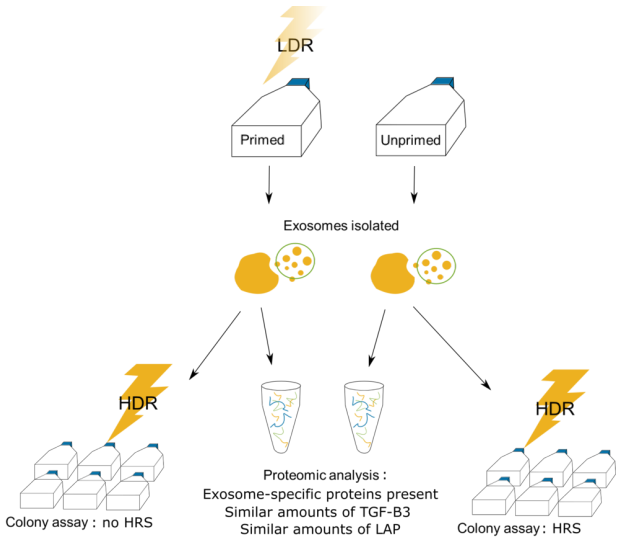
O15 - Communication of radioprotective effects by TGF-ß3

Molecular and cellular effects

Ingunn Hanson[1]
Ursula Altanerova[1], Bernd Thiede[2], Nina F. J. Edin[1]
[1] Department of Physics, University of Oslo
[2] Department of Biosciences, University of Oslo

Introduction: TGF-β3 is induced by low dose-rate priming irradiation (0.06-0.3 Gy/h) and removes low dose hyper-radiosensitivity (HRS) both in primed cells, and in un-primed reporter cells.
Methods: To examine whether TGF-β3 was transported between irradiated and reporter cells via exosome secretion, we isolated these from primed and wild-type T-47D cells. The exosomes were cocultured with reporter cells to determine their radioresponse, and MS was performed on the contents. To elucidate the mechanism with which TGF-β3 removed HRS from reporter cells, we added recombinant TGF-β3 together with inhibitors of various TGF-β receptors to reporter cell medium.
Results: Exosomes from primed, but not unprimed, cells removed HRS in reporter cells. Addition of TGF-β3 inhibitor to the medium restored HRS, indicating that active TGF-β3 is responsible for removal of HRS and is contained in exosomes from primed cells only. Exosome-specific proteins were confirmed present in the exosomes. Exosomes from both groups contained similar amounts of TGF-β3, along with inactivating latency-associated protein (LAP), indicating that TGF-β3 is secreted in inactive form in exosomes from primed and unprimed cells, and that the primed exosomes contains an un-identified activator of TGF-β3.

Inhibition of ALK5 did not affect removal of HRS in primed cells. Inhibition of ALK1 retained HRS in reporter cells, indicating that ALK1 alone mediates the removal of HRS by TGF-β3. Other results indicated a competition between ALK5 and ALK1 for TGF-β3 binding, where ALK5 has higher affinity, but ALK1 mediates removal of HRS.
Conclusion: TGF-β3 removes HRS in reporter cells by secretion in exosomes and binding to ALK1.



O16 - FLASH-effect observed under normoxic conditions in vitro – mechanisms other than oxygen depletion?

Molecular and cellular effects

Gabriel Adrian[1, 2]
Karl Butterworth[3], Elise Konradsson[4], Crister Ceberg[4], Mihaela Ghita[3], Stephen J. McMahon[3], Kristoffer Petersson[2, 5]
[1] Division of Oncology and Pathology, Clinical Sciences, Skåne University Hospital, Lund University, Lund, Sweden
[2] Department of Haematology, Oncology and Radiation Physics, Radiation Physics, Skåne University Hospital, Lund, Sweden
[3] Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland
[4] Department of Medical Radiation Physics, Clinical Sciences, Lund University, Lund, Sweden
[5] Department of Oncology, Oxford Institute for Radiation Oncology, University of Oxford, Oxford, United Kingdom; Department of Haematology, Oncology and Radiation Physics, Radiation Physics

Introduction: Radiotherapy at ultra-high dose rates (FLASH) have been shown in pre-clinical studies to spare normal tissues while maintaining efficient tumor control, compared with conventional radiotherapy (CONV). Oxygen depletion has been suggested as an underlying mechanism for the observed FLASH-sparing effect. Previously, we have shown that FLASH-sparing was apparent when cells were irradiated under hypoxic (1.6% oxygen) and physoxic (2.7, 4.4, and 8.3% oxygen) conditions, whereas we did not find a significant effect in normoxia (20% oxygen), comparing CONV to FLASH. In the current project, we aimed to expand our studies investigating any FLASH-sparing effect for several established cell lines, starting in normoxic conditions.
Methods: Breast cancer, glioblastoma, cervix cancer and fibroblasts cell lines were irradiated in normoxia with doses of 0-12Gy with an electron beam from a modified linear accelerator, providing dose rates of 14Gy/min (CONV) and 600Gy/s (FLASH). Survival was determined by colony formation assays.
Results: Surprisingly, we found a higher survival after FLASH compared with CONV irradiation in normoxia. For some cell lines, a low variability in the survival data allowed for this difference to be significant (Figure). The separation between FLASH and CONV survival curves was seen already seen at 3Gy and was significant at 6Gy and 9Gy, where oxygen depletion in normoxic cells is estimated to have no effect on cell survival (according to published models).
Conclusion: The FLASH-sparing effect occurs at moderate doses for cells in normoxic conditions. Consequently, oxygen depletion does not seem to be the sole underlying mechanism for the FLASH-sparing effect.

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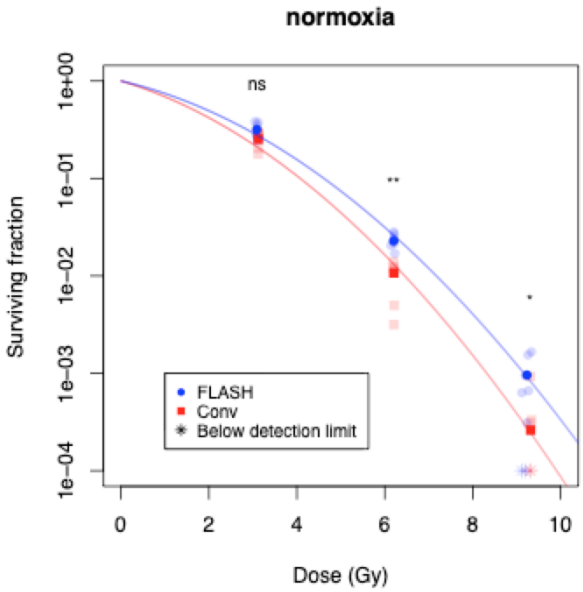

Figure

Cell survival after irradiation in normoxia with FLASH (blue line and circles) compared with conventional dose-rate (red line and squares). ns: not significant,** $p < 0.01$, * $p < 0.05$.

O17 - Reversing cold tumor microenvironment with targeted alpha-therapy

Translational and clinical research

Justine Perrin[1]
marisa capitao[1], sebastien gouard[1], catherine maurel[1], cedric louvet[2], mélanie lancien[2], franck bruchertseifer[3], alfred morgenstern[3], michel chérel[1, 4], joëlle gaschet[1], yannick guilloux[1]
[1] CRINA, INSERM, CNRS, Université d'Angers, Université de Nantes, Nantes, France
[2] Centre de Recherche en Transplantation et Immunologie, INSERM, Université de Nantes, Nantes, France
[3] European Commission, Joint Research Centre, Directorate for Nuclear Safety and Security, Karlsruhe, Germany
[4] Nuclear Medicine Unit, ICO Cancer Center Gauducheau, Saint Herblain, France

Introduction: Cancer therapies are facing challenges towards tumor cell destruction: tumor microenvironment (TME) involves immunoregulatory cells and cytokines, preventing anti-tumoral immune response. Therapeutic combination could turn these « cold » TME into « hot » ones. Therefore, this project focus on combining targeted alpha-therapy (TAT) and adoptive T-cells transfer (ACT).

This combination was conducted in a Multiple Myeloma murine model using a cell line expressing the CD138 antigen and $H_2K^b/OVA_{257-264}$ complexes grafted subcutaneously to mice. TAT was delivered through i.v. injection of a 213-bismuth radiolabelled anti-CD138 antibody. To reinforce its efficiency, TAT was combined with an ACT of tumor specific OT-1 T-cells. This combination resulted in a delayed tumor growth (1).
Methods: Based on these results, this project aims to understand the impact of TAT on the "cold" TME and on ACT efficacy. Tumor infiltrated cells were analysed by flow cytometry to identify *in situ* immune populations, and cytokines production were assessed by RT-qPCR on tumor fragment.
Results: Although OT-1 T cells infiltrated the tumor after ACT, only combination with TAT resulted in regulatory CD4 T cell drop and production of IL-2 and IFNg within the tumor. Futhermore, OT-1 T cells motility was increased on TAT treated tumor slices as observed by *ex vivo* time lapse.
Conclusion: Combining TAT and ACT appears to turn this "cold" tumor model into a "hot" one with regulatory T cells depleted, proportion and motility of tumor-specific CD8 T cells increased and IL-2 and IFNg increased production. Next, we will investigate impact of this combination on metabolism and hypoxia.

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O18 - RIBE induction using human ex vivo explants causes alterations in mitochondrial metabolism in bystander cells

Translational and clinical research

Aisling Heeran[1]
Helen Berrigan[1], Croí Buckley[1], Heleena Bottu[2], Amy Buckley[1], Niamh Clarke[1], Noel Donlon[1], Timothy Nugent[1], Michael Durand[3], Cara Dunne[3], John Larkin[3], Brian Mehigan[3], Paul McCormick[3], Lorraine Brennan[2], Niamh Lynam-Lennon[1], Jacintha O'Sullivan[1]
[1] Department of Surgery, Trinity Translational Medicine Institute, St. James's Hospital, Dublin 8, Ireland
[2] Institute of Food and Health and Conway Institute, UCD School of Agriculture and Food Science, UCD, Belfield, Dublin 4, Ireland
[3] GEMS, St. James's Hospital, Dublin 8, Ireland.

Introduction: Locally advanced rectal cancer is treated with neoadjuvant-chemoradiotherapy, however only 22% of patients achieve a complete response. Resistance mechanisms are poorly understood. Radiation-induced bystander effect (RIBE) describes the effect of radiation on neighbouring unirradiated cells. We investigated effects of *ex vivo* RIBE-induction from normal and rectal cancer tissue on bystander cell metabolism, mitochondrial function and metabolomic profiling. We correlated bystander events to patient clinical characteristics.
Methods: Human normal and rectal cancer tissue were cultured as *ex vivo* explants and either mock-irradiated or received 1.8Gy radiation. Following 24hours, the tissue conditioned media was harvested and the effect of RIBE on bystander rectal cancer cell metabolism was investigated using Seahorse. Metabolomic profiling was conducted using NMR. The effect of RIBE induction on reactive oxygen species and mitochondrial membrane potential was investigated using fluorescent probes.
Results: *Ex vivo* RIBE-induction caused metabolic alterations in bystander cells, specifically reductions in OXPHOS following RIBE-induction in normal (p=0.01) and cancer tissue (p=0.03) and reduced glycolysis in cancer (p=0.01). Visceral fat area correlated with glycolysis (p=0.02) and ATP production (p=0.03) following exposure of cells to TCM from irradiated cancer biopsies. Leucine levels were higher in the irradiated normal compared to the irradiated cancer secretome (p=0.04). ROS levels were higher in cells exposed to the cancer compared to the normal secretome (p=0.04).
Conclusion: RIBE-induction *ex vivo* causes alterations in the metabolome in normal and malignant rectal tissue along with alterations in bystander cellular metabolism. This may offer greater understanding of the effects of RIBE on metabolism, mitochondrial function and the secreted metabolome.

O19 - First pre-clinical study for lung carcinoma employing Synchrotron Microbeam Radiotherapy at the Australian Synchrotron

Translational and clinical research

Verdiana Trappetti[1]
Cristian Fernandez-Palomo[1], Lloyd Smyth[2], Mitzi Klein[3], Duncan Buttler[3], David Haberthür[1], Micah Barnes[3], Daniel Hausermann[3], Michael de Veer[4], Jean Laissue[1], Valentin Djonov[1]
[1] Institute of Anatomy, University of Bern, Switzerland
[2] Obstetrics And Gynaecology Royal Women'S Hospital/Mercy, Melbourne, Australia
[3] Australian Synchrotron ANSTO, Clayton, Australia
[4] Monash Biomedical Imaging Facility, Monash University, Clayton, Australia

Introduction: Synchrotron Microbeam Radiation Therapy (S-MRT) spatially fractionates Synchrotron X-rays into an array of micro-planar beamlets. This spatial fractionation together with a FLASH mode delivery of the radiation allows for minimal normal tissue toxicity while delaying tumour growth or even ablating malignancies. Here, we wanted to use S-MRT to treat lung carcinoma in mice for the first time.
Methods: Lewis lung carcinoma-bearing mice were irradiated with crossfired arrays of either S-MRT or Synchrotron Broad Beam (S-BB) 11 days after tumour cell injection in their right lung. The S-MRT field size was 7x7 mm (50 µm beam width spaced by 400 µm) with a peak-dose of 400 Gy delivered in 418 ms. While S-BB delivered a homogeneus dose of 5.16 Gy in 5.4 ms (does rate 957Gy/sec). Mice were sacrificed when human endpoints were reached.
Results: Both treatments significantly increased the survival of the animals relative to the control group, however there was no difference between S-BB and S-MRT. Pleural effusion was observed after S-MRT in tumor-bearing mice but not in sham-implanted mice. This suggests that the presence of a tumour changes the response of the lung to S-MRT.
Conclusion: We made a first step towards the use of S-MRT for lung cancer, targeting precisely a localized lung carcinoma. This study suggests that the S-MRT parameters (beam configuration, peak dose, and dose rate for a full FLASH effect) need to adapt in relation to the sensitivity of the organ bearing the malignancy, in order to reduce collateral effects and increase survival.

O20 - Proteomic expression analysis of rat thyroid tissue 12 months after low-intermediate ^{131}I exposure

Translational and clinical research

Malin Larsson[1]
Emman Shubbar[1], Johan Spetz[1], Britta Langen[1], Toshima Parris[2], Khalil Helou[2], Eva Forssell-Aronsson[1]
[1] Department of Radiation Physics, Institute of Clinical Sciences, Sahlgrenska Cancer Center, Sahlgrenska Academy at University of Gothenburg, SE-413 45 Gothenburg, Sweden
[2] Department of Oncology, Institute of Clinical Sciences, Sahlgrenska Cancer Center, Sahlgrenska Academy at University of Gothenburg, SE-413 45 Gothenburg, Sweden

Introduction: ^{131}I is released during nuclear accidents, with the thyroid being an organ at risk. Thyroid cancer incidence increased in children but not adults after the Chernobyl accident, possibly due to higher absorbed dose to the thyroid and higher radiosensitivity. The aim of this study was to identify potential age-dependent biomarkers for ^{131}I exposure, thyroid function and cancer induction by evaluating the long-term effects of ^{131}I irradiation in thyroid tissue in young and adult rats.
Methods: Male Sprague Dawley rats were divided into three groups (n=12/group): young (irradiated at 5 weeks), adult (irradiated at 17 weeks), and controls (mock treated). Six individuals from each age group (young and adult) were i.v. injected with 50 kBq or 500 kBq ^{131}I, with six controls per age group. The rats were killed twelve months after study start. LC-MS/MS analysis was performed on thyroid protein extracts. Statistical analysis and functional enrichment were performed using the Preseus software with a fold change cut-off set to ±1.
Results: In this study, age-related proteins that were identified only in young or adult rats, independent of absorbed dose were found. Dose-related proteins that were common for exposure to 50 or 500 kBq regardless of age were identified. Furthermore, unique proteins that were present only in one group were detected. These proteins were mainly related to RNA processing, protein cleavage and energy metabolism.
Conclusion: We have identified several age- and dose-related biomarker candidates. However, further validation is necessary.

O21 - Normal tissue reaction following proton irradiation of the mouse brain

Translational and clinical research

Elke Beyreuther[1, 2, 3]
T Suckert[1, 3], J Müller[1, 3, 4], B Azadegan[1], E Bodenstein[1], R Haase[5], M Schürer[1, 6], M Krause[1, 3, 4, 6, 7], A Lühr[1, 3, 8], C von Neubeck[1, 3, 9], A Dietrich[1, 3]
[1] 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
[2] Helmholtz-Zentrum Dresden – Rossendorf, Institute of Radiation Physics, Dresden, Germany
[3] German Cancer Consortium (DKTK), Partner Site Dresden, and German Cancer Research Center (DKFZ), Heidelberg, Germany
[4] Helmholtz-Zentrum Dresden - Rossendorf, Dresden, Institute of Radiooncology – OncoRay, Germany
[5] Myers Lab, Max Planck Institute CBG, Dresden, Germany
[6] National Center for Tumor Diseases (NCT), Partner Site Dresden, Germany: German Cancer Research Center (DKFZ), Heidelberg, Germany; Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, and; Helmholtz Association / Helmholtz-Zentrum Dresden - Rossendorf (HZDR), Dresden, Germany
[7] Department of Radiotherapy and Radiation Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany
[8] Medical Physics and Radiotherapy, Faculty of Physics, TU Dortmund University, Germany
[9] Department of Particle Therapy, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

Introduction: Due to the beneficial inverse physical depth-dose profile, proton radiotherapy (PT) offers the potential to reduce normal tissue toxicity by depositing the maximum dose within the tumor volume while sparing the surrounding tissue. However, range uncertainties and necessary clinical safety margins in combination with varying relative biological effectiveness (RBE) may result in a critical dose in the normal tissue. Dedicated preclinical studies are needed to assess and better understand potential adverse effects of PT and to develop potential biomarkers and countermeasures for backtranslation into clinics.
Methods: For this purpose, a high-precision image-guided proton irradiation setup for small animals was established at the University Proton Therapy Dresden that mimics the clinical workflow, including pre-treatment imaging, treatment planning and image-guided brain irradiation.
Results: The right hippocampus of C57BL/6 and C3H/HeN mice was irradiated to study the dose- and time-dependent radiation response of mouse brain tissue after short or long-term follow-up analyses. A Monte Carlo model of the proton irradiation field was designed in the simulation toolkit TOPAS to calculate the dose distributions *in vivo.* The observed radiation response was spatially correlated with the proton dose and linear energy transfer distributions.
Conclusion: The combination of geometric accuracy of proton irradiation, detailed dose simulations on mouse CT and cell-based assessment enable a biologically and spatially resolved analysis of short-term radiation response and RBE. In addition, the long-term

follow up over six months provides insights into the formation of normal tissue damage in mouse brain after PT.

O22 - Differential neurocognitive response after partial brain proton irradiation

Translational and clinical research

Daniëlle Voshart[1, 2]
Fleur van Buuren-Broek[1, 2], Peter van Luijk[1, 2], Rob Coppes[1, 2], Lara Barazzuol[1, 2]
[1] Department of Radiation Oncology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.
[2] Department of Biomedical Sciences of Cells and Systems, Section of Molecular Cell Biology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.

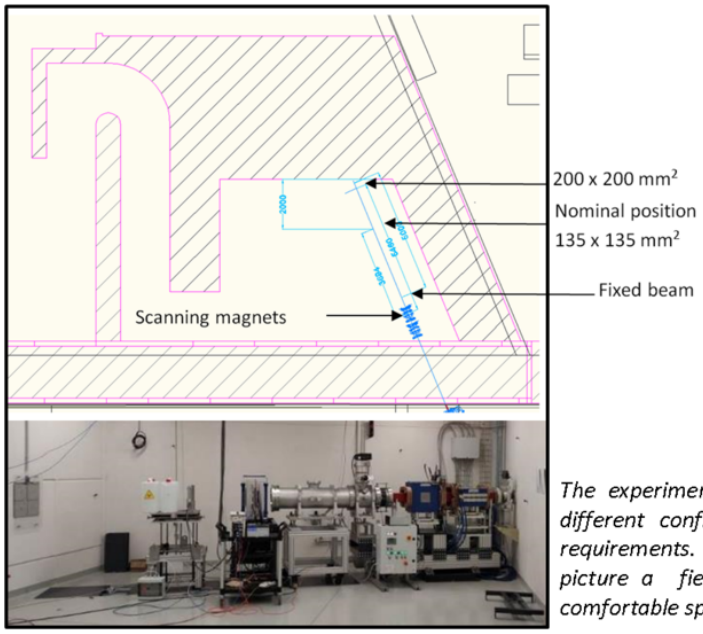
Introduction: Modern radiotherapy technologies, such as proton therapy, enable the potential sparing of brain regions that contribute most to radiotherapy-associated neurocognitive decline. However, current knowledge is largely limited to the role of the hippocampus. This study aims to identify regional contribution to the development of radiotherapy-induced neurocognitive decline.
Methods: High-precision brain irradiation with 14 Gy protons was delivered to the 100%, the 50% anterior and the 50% posterior sub-volumes of the rat brain. Cognitive function was measured at different time points using several behavioral tests, including the Novel Object Recognition test, the Barnes maze test and the Rotarod test.
Results: Preliminary results indicate that irradiation of the 50% anterior brain sub-volume leads to a greater loss in memory function and learning than the 50% posterior brain sub-volume, as measured by the Novel Object Recognition and Barnes Maze tests. Although this difference was evident at 12 weeks post irradiation, it largely resolved at 48 weeks post irradiation. Rotarod performance was similarly impaired in all treatment groups at 12 weeks post irradiation. However, at 48 weeks post irradiation, 50% anterior irradiated animals showed a significant improvement.
Conclusion: Our data indicate that irradiation of the 50% anterior brain sub-volume leads to a greater decline in memory and spatial learning. In contrast, the 50% posterior brain sub-volume seems to be more important for locomotor function and skill learning. This suggests a differential contribution of the anterior and posterior part of the brain to the development of neurocognitive dysfunction after radiotherapy.

O23 - The new experimental beam line and research facility at CNAO for radiobiological studies with charged particles

Translational and clinical research

Angelica Facoetti[1]
Marco Donetti[1], Marco Pullia[1], Simone Savazzi[1], Sandro Rossi[1]
[1] CNAO National Center for Oncological Hadrontherapy, Pavia, Italy

Introduction: CNAO is one of the four centers in Europe, and six worldwide, offering treatment of tumours with both protons and carbon ions. Although the Center is mainly dedicated to clinical irradiation, it also provides great opportunities to perform research related to radiobiology, biophysics, space, dosimetry, radiation detections. Besides three treatment rooms, a fourth room dedicated to experimental activities is now available.
Methods: The maximum energies available are up to 400 MeV/u (corresponding to a Bragg peak depth of up to 27 cm in water) for carbon ions and up to 230 MeV for protons (Bragg peak depth of up to 32 cm in water), minimum extraction energies are 60 MeV and 120 MeV/u for protons and carbon ions respectively. All the intermediate energies are possible and are distributed in steps of 1 mm range rather than in fixed energy steps.
Results: The experimental beamline can be arranged in different configurations according to the needs in term of space downstream the target or in terms of field size dimensions (figure). Within 2023, a third source will be installed and additional ions will be made available in the experimental room. For external researchers, the access to the cell laboratory is available.
Conclusion: In the next 2 years the research area will be expanded and the radiobiology laboratories foresee new premises for a total area of about 250 square meters. Thanks to a strong collaboration with the University of Pavia, it is possible to carry out *in vivo* irradiations, after approval by the local ethical committee.



The experimental room at CNAO can be arranged in different configurations according to the experiment requirements. In the nominal configuration shown in this picture a field of 135 x 135 mm² is available with comfortable space around the irradiation position.

O24 - RBE-dependence on LET and fractionation in the rat cervical spinal cord after helium ion irradiation

Translational and clinical research

Lisa Hintz[1, 2, 3]
Christin Glowa[1, 3, 4], Maria Saager[1, 3], Rosemarie Euler-Lange[5], Peter Peschke[1, 3], Stephan Brons[3, 6], Jürgen Debus[1, 3, 4], Christian Karger[1, 3]
[1] Department of Medical Physics in Radiation Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[2] Faculty of Biosciences, Heidelberg University, Heidelberg, Germany
[3] National Center for Radiation Research in Oncology (NCRO), Heidelberg Institute for Radiation Oncology (HIRO), Heidelberg, Germany
[4] Department of Radiation Oncology and Radiotherapy, University Hospital Heidelberg, Germany
[5] Division of Radiooncology/Radiobiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[6] Heidelberg Ion Beam Therapy Center (HIT), Heidelberg, Germany

Introduction: Helium (^{4}He) ions show less lateral scattering compared to protons and a lower variablility of the relative biologoical effectiveness (RBE) than carbon ion and therefore pose a promising alternative in ion beam radiotherapy. For patient treatments, the relative biological effectiveness (RBE) needs to be predicted with biophysical models and uncertainties in these predictions may lead to under- or over-dosages. Therefore, models have to be validated by experimental data. This study uses the rat cervical spinal cord (CSC) to investigate the RBE of late effects *in vivo* in dependence of the linear energy transfer (LET) and the fractionation.
Methods: The CSC of female Sprague Dawley (SD) rats was irradiated with increasing doses of ^{4}He-ions in 1 or 2 fractions (fx) at 4 different positions within a 6 cm Spread-out Bragg-peak (SOBP). Dose-response curves were measured for the endpoint paresis grade II (palsy of the forelimbs) within 300 days after irradiation. RBEs were calculated based on the TD_{50}-values (dose at 50% complication probability) and using previously measured values for photons [1].
Results: With increasing LET, the RBE increased from 1.1 to 1.5. No significant difference was observed between 1 and 2 fx.
Conclusion: We found a clear LET-dependence of the RBE for ^{4}He-ions, which is larger than for protons [2]. Similar to protons, no fractionation effect was observed for the applied high doses, however, the RBE might increase at higher fraction numbers. This study established the LET- and dose-dependence of the RBE in late-responding tissue after for ^{4}He-irradiation, which can be used to benchmark RBE-models.
Selected references
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O25 - Proton-FLASH – Radiation effects of ultrahigh dose-rate irradiation

Translational and clinical research

Sarah Rudigkeit[1]
Nicole Matejka[1], Ce-Belle Chen[2], Parisa Hosseinpour[1], Annique Hunger[3], Matthias Sammer[1], Thomas Schmid[3, 4], Günther Dollinger[1], Judith Reindl[1]
[1] Universität der Bundeswehr München, Institut für Angewandte Physik und Messtechnik, Neubiberg, Germany
[2] Centre for Ion Beam Applications, Department of Physics, National University of Singapore, 2 Science Drive 3, Singapore 117542, Singapore
[3] Helmholtz Zentrum München, Institute of innovative Radiotherapy, Neuherberg, Germany
[4] Klinikum rechts der Isar, Technische Universität München, Radiooncology, München, Germany

Introduction: Tissue sparing by irradiation with ultra-high dose-rates – the so-called FLASH-effect - is investigated since several years using electrons or x-rays. Since protons already show advantageous effects compared to conventional therapy, we designed a study to test the FLASH-effect with protons in-vivo and in-vitro.
Methods: We performed irradiation with 20 MeV protons at the ion microprobe SNAKE at the 14 MV tandem accelerator in Garching near Munich using three different dose-rates (2 Gy/min, 10 Gy/s and 1000 Gy/s). In the in-vitro experiments we compared genetic damage measured by micronuclei induction to cell survival using colony forming assay and cell death using a caspase 3/7-sytox assay on a flowcytometer. For the in-vivo study we irradiated the right ears of 63 Balb/c mice and measured the ear thickness, desquamation and erythema over 180 days.
Results: No difference in cell survival was visible. Whereas, early apoptotic and late apoptotic cells were reduced after irradiation with 1000 Gy/s to base level of sham irradiated controls. In the in-vivo study we obtained a 16 % reduction of the earthickness after 32 Gy irradiation with 1000 Gy/s and a 22 % reduction for 10 Gy/s compared to the conventional dose-rate of 2 Gy/min. Desquamation and erythema was reduced by half for both higher dose-rates.
Conclusion: By using FLASH dose-rates for low-LET proton irradiation a tissue sparing effect can be achieved. But especially the in-vitro experiments showed more diverse results than expected. Therefore, further investigations are necessary to understand the underlying mechanisms and interactions in the tissue after FLASH-irradiation.

O26 - Investigating FLASH irradiation on acute normal tissue toxicity in the murine gastrointestinal system

Translational and clinical research

Jia-Ling Ruan[1]
Iain DC Tullis[1], Sean C Smart[1], James Thompson[1], Mark A Hill[1], Anderson J Ryan[1], Ruth J Muschel[1], Borivoj Vojnovic[1], Anne E Kiltie[1], Kristoffer Petersson[1]
[1] Department of Oncology, CRUK/MRC Oxford Institute for Radiation Oncology, University of Oxford, Oxford, United Kingdom

Introduction: Gastrointestinal damage caused by ionising radiation of abdominal tissues is a dose-limiting factor in radiotherapy treatment. Recently, preclinical and clinical studies using ultra-high dose rate (FLASH) irradiation have shown reduced normal tissue toxicity in multiple organs compared to conventional dose rate radiotherapy.
Methods: In this study, we investigated acute normal tissue effects in C3H mice where the whole abdomen was irradiated with either single pulse 6 MeV electron FLASH irradiation (dose rate = 2-6*10^6 Gy/s) or conventional dose rate irradiation (15 Gy/min), delivered by a modified experimental linear electron accelerator. Mice were irradiated using either irradiation technique at various radiation doses and culled after 3.75 days. The small intestines were made into "Swiss rolls" for histological assessment. Normal tissue damage was quantified using a modified crypt assay.
Results: We found statistically significant differences in crypt survival between mice irradiated with doses between 7.5 and 12.5 Gy. Nonlinear regression analysis of the dose-response curves for both irradiation techniques showed a dose modifying factor of ≈ 1.1, i.e. a 10% higher dose was needed for FLASH compared conventional dose rate irradiation to achieve the same level of toxicity. Mice irradiated with FLASH also showed reduced weight loss compared to those groups that received conventional irradiation, but the effect was not statistically significant.
Conclusion: This study demonstrates that FLASH irradiation is a promising radiotherapy technique, capable of sparing gastrointestinal normal tissue. Further research will focus on identifying the optimal pulse structure for maximising the FLASH sparing effect.

O27 - First veterinary patient treated with electron FLASH radiotherapy at a clinical linear accelerator

Translational and clinical research

Elise Konradsson[1]
Maja L. Arendt[2], Kristine Bastholm Jensen[3], Betina Børresen[2], Crister Ceberg[1], Anders E. Hansen[4], Annemarie T. Kristensen[2], Per Munck af Rosenschöld[5], Kristoffer Petersson[5, 6]
[1] Medical Radiation Physics, Department of Clinical Sciences, Lund University, Lund, Sweden
[2] Department of Veterinary Clinical Sciences, University of Copenhagen, Frederiksberg, Denmark
[3] Veterinärhuset Öresund, Limhamn, Sweden
[4] Department of Biotherapeutic Engineering and Drug Targeting, DTU Health Tech, Kgs, Technical University of Denmark, Lyngby, Denmark
[5] Radiation Physics, Department of Hematology, Oncology and Radiation Physics, Skåne University Hospital, Lund, Sweden
[6] Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, Oxford, United Kingdom

Introduction: There is a growing interest in advancing ultra-high dose rate radiotherapy (FLASH-RT) towards clinical studies. However, the availability of accelerators capable of delivering ultra-high dose rates in a clinical setting is still limited. We have initiated a veterinary clinical study of FLASH-RT for clinical canine cancer patients with superficial tumors using the electron beam of our modified clinical linear accelerator. Here we present the treatment of the first patient.
Methods: A clinical canine cancer patient diagnosed with a grade 1 soft tissue sarcoma at the right forelimb, with incomplete excision after surgery, was treated with 15 Gy FLASH-RT using a field size of 8x4 cm^2 (Figure 1). The irradiation was delivered with a source-to-surface distance of 70 cm. Dosimetric equipment consisted of radiochromic film, an ionization chamber (for relative measurements) and phantom material mimicking the experimental setup for irradiation. *In vivo* dose measurements were performed with film to verify the delivered dose.
Results: For the canine patient, the prescribed dose was accurately delivered (14.8 ± 0.5 Gy) using 7 pulses in 0.03 s, i.e. with an average dose rate of 500 Gy/s. Only grade 1 cutaneous side effects were observed at 7 and 30 days post treatment.
Conclusion: We present irradiation parameters and toxicity data for the first clinical veterinary patient receiving electron FLASH-RT using a clinical linear accelerator. The treatment was feasible, safe, delivered with good dosimetric accuracy and successful in terms of the observed treatment toxicity.

Figure 1: Treatment setup for the first patient receiving FLASH-RT at our clinical linear accelerator.

O28 - Immunomodulatory effects of external and targeted radiotherapy depend on radiation type

Translational and clinical research

Julie Constanzo[1]
Jihad Karam[1], Julien Faget[1], Frank Bruchertseifer[2], Alfred Morgenstern[2], Isabelle Vila[3], Nadine Laguette[3], Nathalie Bonnefoy[1], Jean-Pierre Pouget[1]
[1] IRCM, Institut de Recherche en Cancérologie de Montpellier, INSERM U1194, Université de Montpellier, Institut régional du Cancer de Montpellier, Montpellier, F-34298, France
[2] Directorate for Nuclear Safety and Security, European Commission - Joint Research Centre, Karlsruhe, Germany
[3] IGH, Institut de Génétique Humaine, CNRS, Université de Montpellier, Molecular Basis of Inflammation Laboratory, Montpellier, France

Introduction: Beside conventional radiotherapy, TRT consists in the administration of radiopharmaceuticals made of monoclonal antibodies or peptides coupled to a radionuclide-emitting alpha, beta and Auger particles, specifically irradiating disseminated tumor cells. Here, we investigate the role of X-rays, alpha and Auger in triggering systemic effects through the cGAS-STING pathway.
Methods: B16F10 melanoma cells were subcutaneously injected in C57BL/6J and Athymic mice. Mice received intraperitoneal injections of anti-TA99 mAb targeting TYRP-1/gp75 tumor antigen radiolabeled either with ^{225}Ac (1× 9.25 kBq; 74MBq/mg, alpha-TRT) or ^{125}I (2× 27 MBq; 37MBq/mg, Auger-TRT), or with extracellular vesicles (EVs) purified from cells exposed either to 0.5 Gy X-rays or to 4 MBq/mL ^{125}I-anti-TA99.
Results: *In vivo,* T-cells contribute to both alpha- and Auger-TRT efficacy. Despite its short penetration range (≈ 1µm), Auger-TRT demonstrated significant tumor growth delay and survival (controls: 15 days versus ^{125}I-anti-TA99 : 29 days, $^{**}p = 0.0035$) in immunocompetent mice, while no difference was observed in Athymic mice. *In vitro,* B16F10 cells exposed to ^{125}I-anti-TA99, demonstrated a weak accumulation of cytosolic dsDNA compared to X-rays (Fig.1). However, an early and persistent activation of the cGAS-STING pathway (up-regulation of cGAS, p-STING, p-IRF3 and p-TBK1 expression) was observed, from 1h-48h following the beginning of Auger-TRT incubation. Finally, we showed that EVs purified from Auger TRT do not contribute to pro-immunogenic response *in vivo*, which was associated with absence of dsDNA in their content, while EVs purified from X-rays do carry dsDNA and induce tumor growth delay in immunocompetent mice.
Conclusion: Cytosolic dsDNA and dsDNA-containing EVs mediate radiation-induced systemic response *in vivo*.

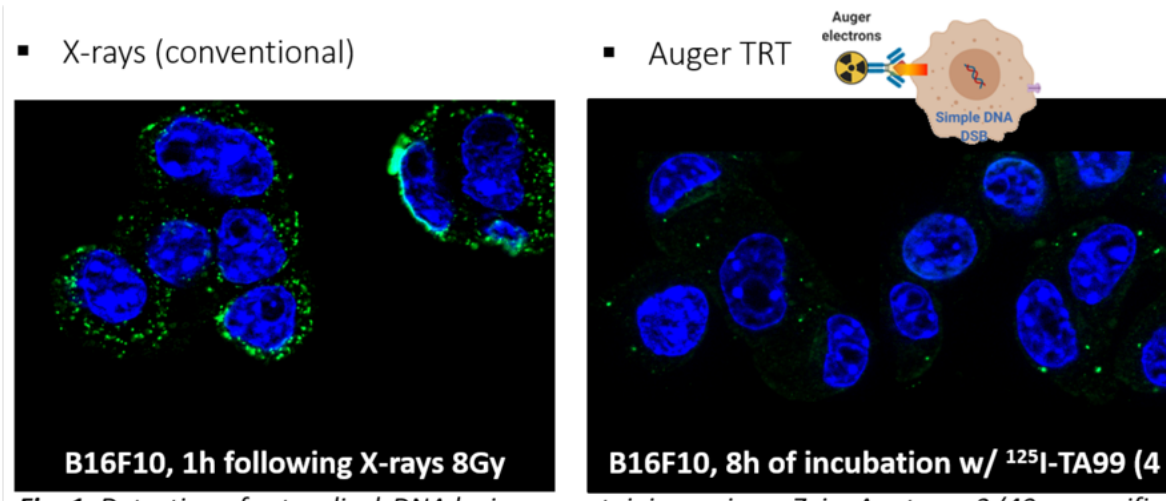

Fig. 1. *Detection of cytosolic dsDNA by immunostaining, using a Zeiss Apotome.2 (40x magnifica*

O29 - Examining the effect of radiation on the secretome of normal and rectal cancer tissue and how this secretome interacts with the innate immune system

Translational and clinical research

Aisling Heeran[1]
Margaret Dunne[1], Maria Morrissey[1], Croí Buckley[1], Niamh Clarke[1], Aoife Cannon[1], Noel Donlon[1], Timothy Nugent[1], Michael Durand[2], Cara Dunne[2], John Larkin[2], Brian Mehigan[2], Paul McCormick[2], Niamh Lynam-Lennon[1], Jacintha O'Sullivan[1]
[1] 1.Department of Surgery, Trinity Translational Medicine Institute, St. James's Hospital, Dublin 8, Ireland
[2] 2.GEMS, St. James's Hospital, Dublin 8, Ireland

Introduction: Locally advanced rectal cancer is treated with neoadjuvant-chemoradiotherapy, however only 22% of patients achieve a complete response. Resistance mechanisms are poorly understood. We profiled the inflammatory secretome of normal rectal and rectal cancer tissue pre- and post-radiation and investigated the effect of this secretome on immune cell function. We correlated findings with patient clinical characteristics.
Methods: Human normal and rectal cancer tissue were cultured as *ex vivo* explants and either mock-irradiated or received 1.8Gy radiation. Following 24hours, the tissue conditioned media was harvested and the *ex vivo* secretomes were profiled. The effect of these secretomes on innate immune cell function, specifically dendritic cell (DC) maturation was assessed by flow cytometry measuring CD86, CD80, CD83, PD-L1 and CD11c.
Results: Radiation increases the secretion of MDC, GM-CSF, IL-15 and IL-17A ($p<0.05$) in normal rectal tissue and IL-15 and TNF-β ($p=0.05$) in rectal cancer tissue. The secretome from the irradiated *ex vivo* rectal cancer tissue significantly enhanced DC maturation markers, specifically CD86, PD-L1 and CD11c compared to the secretome of irradiated normal tissue. Secreted levels of MIP3α, IL-7 and IL1RA following radiation correlated with patient's visceral fat area while secreted levels MIP3α, VEGF and IL1RA correlated with intermuscular fat.
Conclusion: Radiation causes significant alterations in the *ex vivo* inflammatory secretome of normal and rectal cancer tissue. The e*x vivo* secretome of rectal cancer tissue enhances DC maturation. This may offer greater understanding of the effects of radiation on inflammation and immunity and the connection with treatment response in rectal cancer patients.

O30 - Clinical Trial Evaluating the Efficacy of Mesenchymal Stromal Cell Injections for the Treatment of Radiation Induced Chronic Pelvic Complications

Translational and clinical research

Alain Chapel[1]
Alexandra Sémont[1], Christine Linard[1], Noëlle Mathieu[1], Christelle Demarquay[1], Claire Squiban[1], Fabien Milliat[1], Marc Benderitter[1], Hélène Rouard[2], Christophe Martinaud[3], Norbert-Claude Gorin[4], Mohamad Mohamad Mohty[4], Jean-Marc Simmon[5]
[1] Service de recherche en radiobiologie et en médecine régénérative (SERAMED), Laboratoire de Radiobiologie des expositions médicales (LRMED), Institut de Radioprotection et de Sûreté Nucléaire (IRSN), Fontenay, FRANCE
[2] Unité Bioingénierie Cellulaire, Tissulaire et Sanguine à Usage Thérapeutique (BCTSVT) - EA 3952, Hôpital Henry Mondor, Etablissement Français du Sang (EFS), Créteil, FRANCE
[3] Département des activités cliniques, Hôpital militaire de Percy, Centre de transfusion sanguine des armées (CTSA), Clamart, FRANCE
[4] Service : Hématologie clinique et thérapie cellulaire, Sorbonne Université, Assistance Publique-Hôpitaux de Paris, Hôpital Saint Antoine, FRANCE
[5] Service d'Oncologie Radiothérapie, Sorbonne Université, Assistance Publique-Hôpitaux de Paris, Groupe Hospitalier Pitié-Salpêtrière-Charles Foix, Paris, FRANCE

Introduction: Radiation cystitis or proctitis are pathologies resulting from pelvic radiotherapy that may be refractory to standard therapy. Our group has demonstrated that cell therapy can provide therapeutic benefit when other treatments have failed.
Methods: A phase 2 clinical trial is being enrolled in patients with post-radiotherapy abdominal and pelvic complications who did not improve their symptoms after conventional treatments (NCT02814864, Trial evaluating the efficacy of systemic mesenchymal stromal cells (MSC) injections for the treatment of radiation-induced abdominal and pelvic complications that are severe and chronic and refractory to standard therapy (PRISME). It involves the participation of 6 radiotherapy departments for the recruitment of 12 patients. They will all be treated and followed up in the haematology department of Saint Anthony's Hospital. The cells will be prepared in two centres (EFS Mondor and CTSA Clamart) belonging to the EcellFrance national network of regenerative medicine and MSC-based cell therapy. Treatment is a suspension of allogeneic stromal mesenchymal stromal stem cells from an intra-familial donor.
Results: Eligible patients must have a grade higher than 2 for rectorrhagia or hematuria at the inclusion. Each patient will receive 3 MSC injections, 7 days apart. Patients will be followed up over a period of 12 months. The main objective is decrease of one grade on the SOMA LENT scale for rectorrhagia or hematuria. The secondary goal is to reduce the frequency of diarrhea, analgesic consumption, pain and improve quality of life.
Conclusion: This clinical trial open new treatment for sequelae from radiotherapy for breast, prostate, bladder and uterus cancers.

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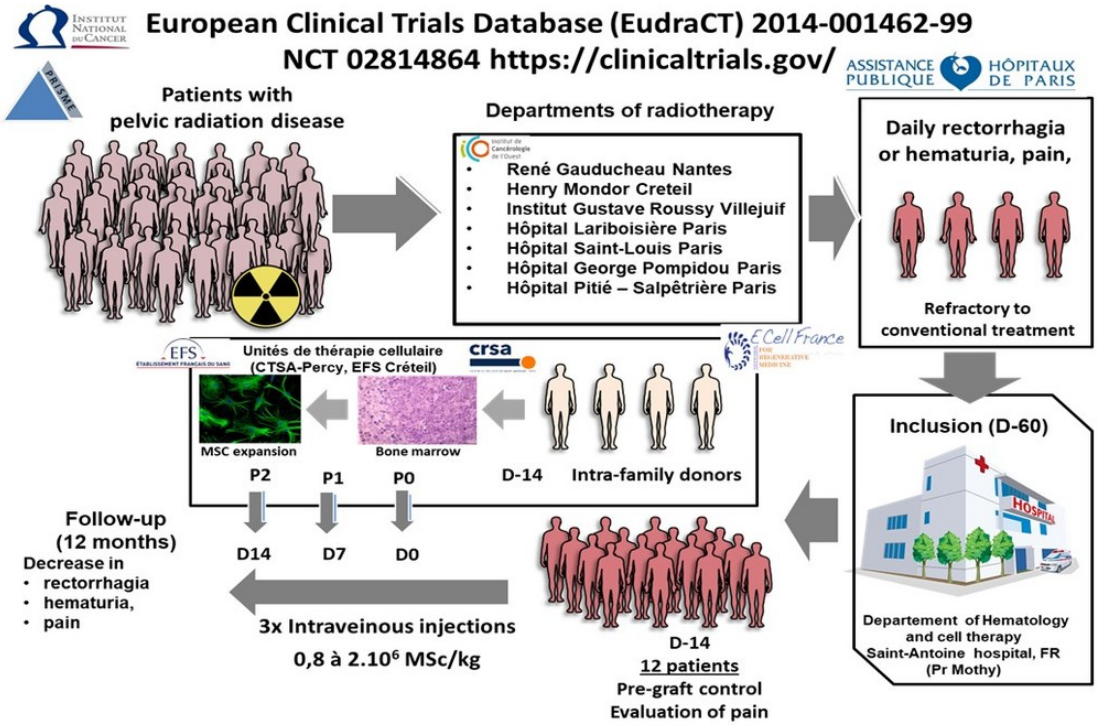
European Clinical Trials Database (EudraCT) 2014-001462-99
NCT 02814864 https://clinicaltrials.gov/
Patients with pelvic radiation disease
Departments of radiotherapy
René Gauducheau Nantes
Henry Mondor Creteil
Institut Gustave Roussy Villejuif
Hôpital Lariboisière Paris
Hôpital Saint-Louis Paris
Hôpital George Pompidou Paris
Hôpital Pitié – Salpêtrière Paris
Daily rectorrhagia or hematuria, pain,
Refractory to conventional treatment
Inclusion (D-60)
Departement of Hematology and cell therapy Saint-Antoine hospital, FR (Pr Mothy)
Unités de thérapie cellulaire (CTSA-Percy, EFS Créteil)
MSC expansion
Bone marrow
P2
P1
P0
D-14
Intra-family donors
D14
D7
D0
Follow-up (12 months)
Decrease in
rectorrhagia
hematuria,
pain
3x Intraveinous injections
0,8 à 2.10⁶ MSc/kg
D-14
12 patients
Pre-graft control
Evaluation of pain

O31 - Pituitary Function after High-Dose 177Lu-DOTATATE Therapy and Long-Term Follow-Up

Translational and clinical research

Anna Sundlöv[1]
Katarina Sjögreen Gleisner[2], Jan Tennvall[1], Ludvig Dahl[3], Johanna Svensson[4], Anna Åkesson[5], Peter Bernhardt[6], Ola Lindgren[1, 7]
[1] Department of Clinical Sciences, Oncology, and Pathology, Skåne University Hospital, Lund University, Lund, Sweden
[2] Medical Radiation Physics, Department of Clinical Sciences Lund, Lund University, Lund, Sweden
[3] Department of Hematology, Oncology, and Radiation Physics, Skåne University Hospital, Lund, Sweden
[4] Department of Oncology, Sahlgrenska University Hospital, Gothenburg, Sweden
[5] Clinical Studies Sweden, Forum South, Skåne University Hospital, Lund, Sweden
[6] Department of Radiation Physics, University of Gothenburg, Gothenburg, Sweden
[7] Department of Endocrinology, Skåne University Hospital, Lund University, Lund, Sweden

Introduction: The pituitary gland has a high expression of somatostatin receptors and is therefore a potential organ at risk for radiation-induced toxicity after 177Lu-DOTATATE treatment. Objective: To study changes in pituitary function in patients with neuroendocrine tumors (NETs) treated with dosimetry-based 177Lu-DOTATATE to detect possible late toxicity.
Methods: 68 patients from a phase II clinical trial of dosimetry-based, individualized 177Lu-DOTATATE therapy were included in this analysis. Patients had received a median of 5 (range 3–9) treatment cycles of 7.4 GBq/cycle. Median follow-up was 30 months (range 11–89). The GH/IGF-1 axis, gonadotropins, and adrenal and thyroid axes were analyzed at baseline and on a yearly basis thereafter. Percent changes in hormonal levels over time were analyzed statistically using a linear mixed model and described graphically using box plots. The absorbed radiation dose to the pituitary was estimated based on post-therapeutic imaging, and the results analyzed versus percent change in IGF-1 levels over time.
Results: A statistically significant decrease in IGF-1 levels was found ($p < 0.005$), which correlated with number of treatment cycles ($p = 0.008$) and absorbed radiation dose ($p = 0.03$). A similar decrease, although non-significant, was seen in gonadotropins in postmenopausal women, while in men there was an increase during the first years after therapy, after which the levels returned to baseline. No change was observed in the adrenal or thyroid axes.
Conclusion: No signs of severe endocrine disorders were detected, although a significant decrease in the GH/IGF-1 axis was found, where dosimetric analyses indicated radiation-induced damage to the pituitary gland as a probable cause.

O32 - Decision tool for radiotherapy compliance in elderly cancer patients

Translational and clinical research

Biche Osong[1]
Inigo Bermejo[1], Andre Dekker[1], Seok Ho Lee[2], Johan van Soest[1]
[1] Department of Radiation Oncology (Maastro), GROW School for Oncology, Maastricht University Medical Centre+, Maastricht, The Netherlands
[2] Department of Radiation Oncology, Gachon University, College of Medicine, Gil Medical Center, Incheon, Korea

Introduction: Radiotherapy is one of the most common treatment modality for cancer. However, treatment is often affected by unwanted interruptions (non-compliance), which ultimately affects the local control and overall survival of the patient.
Methods: Among the patients who received radiation therapy between 2005 to 2017 at our institution, 789 patients over 75 years of age were retrospectively analyzed. Radiotherapy compliance was determined by whether the scheduled radiotherapy plan was completed. Chi-square tests and 5-fold cross-validation repeated ten times was used to establish the decision tree model after a 70 – 30 split percentage of the original data to train and test the model, respectively. The discriminative performance of the developed tree distinguishing compliant and non-compliant patients was assessed measuring the area under the receiver operating characteristic curve (AUROC).
Results: The decision tree uses four predictors (Eastern Cooperative Oncology Group Performance Status Scale (ECOG PS), treatment aim, age, and gender) to make a decision, as shown in Figure 1. The mean AUROCs of the trees discriminating ability on the training, and test datasets are 0.71 (0.65 - 0.77), and 0.65 (0.55 - 0.76) respectively
Conclusion: We developed and internally validated a novel decision tree that could discriminate between patients who will or will not comply with radiotherapy treatment. This model could be used to guide caregivers and physicians to opt for alternative therapies, such as short-course radiotherapy, for patients who are predicted to be non-compliant. Alternatively, it could help target these patients with strategies to help them comply with radiotherapy treatment.

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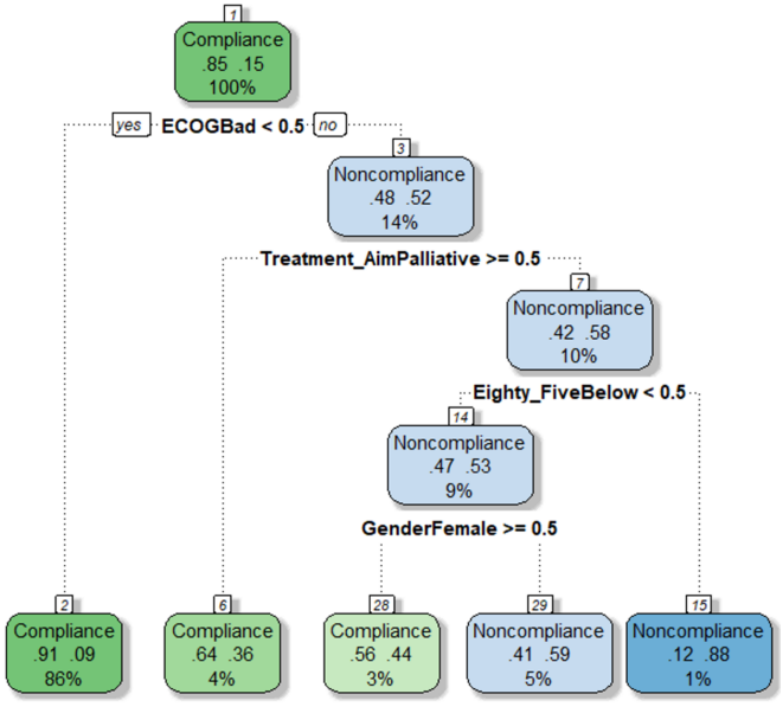


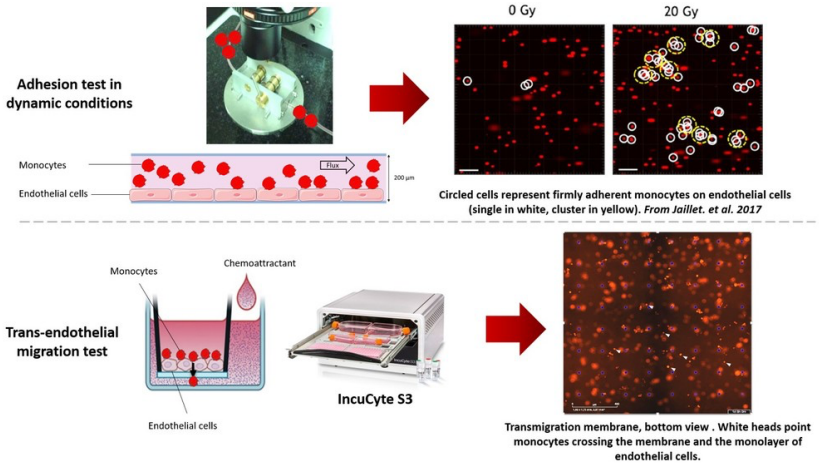
Figure 1: *Decision tree for predicting radiotherapy compliance in elderly cancer patients.*

O33 - Characterization of monocytes-endothelium interactions after radiotherapy

Health effects and radiation protection

Ségolène Ladaigue[1]
Anne-Charlotte Lefranc[1], Khadidiatou Baldé[1], Monica Quitoco[1], Valérie Buard[1], Fabien Milliat[1], Olivier Guipaud[1]
[1] Radiobiology of medical exposure laboratory, LRMED. French institute for radiological protection and nuclear safety, IRSN. Fontenay-aux-roses, France.

Introduction: Normal tissue damages associated with chronic innate immune cell infiltration is thought to drive late normal tissue injury induced by radiotherapy (RT). While macrophages may play a major role in these adverse effects, little is known about how their precursors, the monocytes (Mo), are recruited in irradiated tissues through the endothelium. This study aims to clarify the role of the different endothelial cell (EC)'s actors : high-mannose N-glycosylations (HMNG), CCR2/CCL2 and CX3CR1/CX3CL1 in Mo recruitment after irradiation.
Methods: *In vitro* interactions of Mo with irradiated ECs, i.e. firm arrests on ECs under flow and trans-endothelial migration, were evaluated by real-time imaging techniques (video-microscopy and IncuCyte live cell analysis system) using a human Mo cell line (THP-1) and human primary ECs (HUVECs). The roles of endothelial HMNG, MAN1C1, involved in the trimming of HMNG, CCR2/CCL2 and CX3CR1/CX3CL1 were evaluated using siRNA, lentiviral particles and molecular competitors.
Results: siRNA against *man1c1* in ECs increased Mo adhesion and the level of HMNG on ECs, suggesting a role of MAN1C1 in the recruitment of Mo. In addition, we developed a reliable transmigration test allowing us to show that irradiation of ECs stimulates Mo transmigration in a dose-dependent manner. We also showed that Mo transmigration and adhesion are regulated by CX3CL1 signaling in ECs using competitors and siRNA.
Conclusion: Our results suggest that CX3CR1 and MAN1C1 may regulate Mo recruitment at different steps following irradiation. Mo adhesion after endothelial MAN1C1 overexpression, as well as *in vivo* studies of leukocytes-endothelium interactions in specific murine models are now under investigation.



***In vitro* methods to assess monocyte-endothelium interactions after irradiation : adhesion under flow and trans-endothelial migration tests**

O34 - Colonic and microbial alterations in a colorectal cancer mouse model provoked by pelvic fractionated X-ray treatment

Health effects and radiation protection

Shari Wouters[1, 2]
Mieke Neefs[1], Sarah Baatout[1], Natalie Leys[1], Surbhi Malhotra-Kumar[2], Samir Kumar-Singh[2], Mieke Verslegers[1], Mohamed Mysara[1]
[1] Belgian Nuclear Research Centre, SCK•CEN, Interdisciplinary Biosciences group, Mol, Belgium
[2] University of Antwerp, Faculty of Medicine and Health Sciences, Antwerp, Belgium

Introduction: Radiotherapy plays an adjunctive role in colorectal cancer (CRC) treatment. However, (hypo)fractionated pelvic x-ray irradiation involves exposure to high cumulated doses (25-55 Gy), which leads to collateral damage of the surrounding intestinal tissue and drives substantial changes in the gut microbiome, termed dysbiosis. Consecutively, dysbiosis leads to epithelial destruction and mucosal ulceration which might require premature treatment interruption or additional symptomatic treatment. Hence, to reduce radiation-induced side effects, maintaining a healthy gut microbiome could be key.
Methods: Therefore, a reproducible CRC mouse model (AOM/DSS) receiving farctionated pelvic radiation (6x3 Gy) was established to represent clinical radiation-induced pathology. Upon dissection, tumour development was assessed and tissues were harvested to assess temporal radiation-induced damage and bacterial translocation. Fresh faeces were collected for microbial analysis.
Results: Mice showed consistent weight loss (<10%) following each session of radiation but quickly recovered three days post final irradiation. As anticipated, a significant decrease (19-33%) in number of macroscopically identifiable tumours was observed in irradiated animals as compared to sham-irradiated controls. Additionally, a significant overall decrease in spleen size was observed following irradiation, which might be indicative of radiation-induced atrophy. Finally, observed temporal changes in lymph node bacterial count are believed to be associated with increasing bacterial translocation and colon permeability post irradiation.
Conclusion: Altogether, our results support the successful development of a reproducible CRC mouse model receiving fractionated pelvic radiation. The analysis of the histological, inflammatory and dysbiotic status is ongoing to further validate the developed model. In future, our model can be employed to test potential prophylactic therapies.

O35 - p53 drives premature neuronal differentiation in response to radiation-induced DNA damage during early neurogenesis

Health effects and radiation protection

Roel Quintens[1]
André-Claude Mbouombouo Mfossa[1, 2], Mieke Verslegers[1], Tine Verreet[1, 3], Haris Bin Fida[1], Mohamed Mysara[1], Wilfred FJ Van Ijcken[4, 5], Winnok H De Vos[6], Lieve Moons[3], Sarah Baatout[1], Mohammed A Benotmane[1], Danny Huylebroeck[4, 5]
[1] Radiobiology Unit, Institute of Environment, Health and Safety, Belgian Nuclear Resreach Centre, SCK CEN, Mol, Belgium
[2] Laboratory of Molecular Biology (Celgen), Department Development and Regeneration, KU Leuven, Leuven, Belgium
[3] Laboratory of Neural Circuit Development and Regeneration, Department of Biology, Faculty of Science, KU Leuven, Leuven, Belgium
[4] Center for Biomics, Erasmus University Medical Center, Rotterdam, The Netherlands
[5] Department of Cell Biology, Erasmus University Medical Center, Rotterdam, The Netherlands
[6] Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp, Antwerp, Belgium

Introduction: p53 regulates the cellular DNA damage response (DDR). Hyperactivation of p53 during embryonic development, however, can lead to a range of developmental defects including microcephaly.
Methods: Here, we induce microcephaly by acute irradiation (1 Gy of X-rays) of mouse fetuses at the onset of neurogenesis (embryonic day 11). We used fluorescence microscopy and RNA sequencing to investigate radiation effects mostly at early time points after irradiation. Dorsal forebrain-specific *Trp53* knock-out (cKO) mice were generated by crossing *Trp53*fl/fl mice to *Emx1*-Cre mice.
Results: Besides a classical DDR culminating in massive apoptosis, we observe ectopic neurons in the subventricular zone in the brains of irradiated mice, indicative of premature neuronal differentiation. A transcriptomic study indicates that p53 activates both DDR genes and differentiation-associated genes. In line with this, *Trp53* cKO mice do not show this ectopic phenotype and partially restore brain size after irradiation. Irradiation furthermore induces an epithelial-to-mesenchymal transition-like process resembling the radiation-induced proneural-mesenchymal transition in glioma and glioma stem-like cells.
Conclusion: Our results demonstrate a critical role for p53 beyond the DDR as a regulator of neural progenitor cell fate in response to DNA damage.

O36 - NOTCH inhibition promotes bronchial stem cell renewal and epithelial barrier integrity after irradiation

Health effects and radiation protection

Lorena Giuranno[1]
Eloy Moreno Roig[1], Carolien Wansleeben[1], Annette Van Den Berg[1], Arjan Groot[1], Ludwig Dubois[1], Marc Vooijs[1]
[1] Maastricht University

Introduction: Tumor control by radiotherapy is limited by dose-limiting adverse effects, which negatively affect patients quality of life. NOTCH signaling pathway plays a key role in the regeneration of the airway epithelium. Deregulated NOTCH activity is associated with tumor growth and radiotherapy resistance and therefore is a potential therapeutic target. However, the mechanism through which NOTCH inhibition integrates with airway repair and treatment response is unknown. We therefore aimed to characterize the effect of inhibiting NOTCH signaling in the normal lung to investigate whether extra normal tissue toxicity derives from the use of NOTCH inhibitors.
Methods: We used an air-liquid interface pseudo-stratified culture derived from primary human bronchial epithelial cells (PBECs), and we blocked the NOTCH signaling pathway using the pan-NOTCH γ-secretase inhibitor DBZ alone and in combination with irradiation (2, 4 Gy).
Results: We found that stem cells (TP63+) proliferation decreases overtime but inhibiting NOTCH alone and in combination with radiotherapy increases TP63+cells proliferation and stemness capability. In irradiated cultures, we observed reduced 53BP1 foci 24 hours and 3days post-irradiation when NOTCH signaling was inhibited. The increased stem cells proliferation together with reduced damage contributed to a more intact epithelium, as shown by the upregulation of ZO1, AFADIN, CD2AP when NOTCH was inhibited and combined with irradiation. Comparable results were obtained after *in vivo* irradiation, where the combination of NOTCH inhibition and irradiation increased stem cell proliferation.
Conclusion: These data support the use of normal patient tissue for predictive toxicity screening of combination treatments and disclose novel interactions between NOTCH inhibition and radiotherapy.

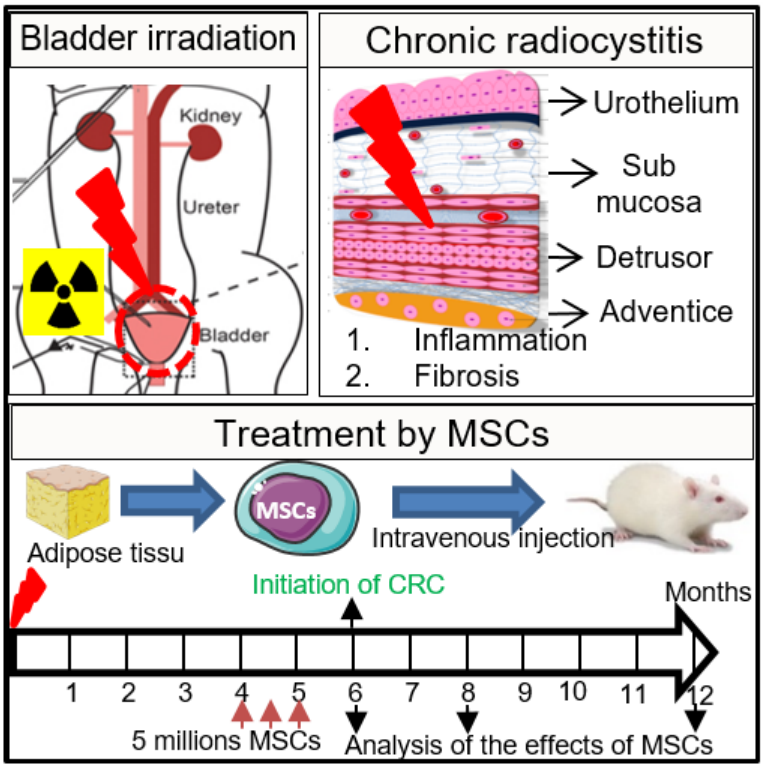
O37 - Preclinical study of Chronic radiocystitis and cell therapy treatment

Health effects and radiation protection

Clément BROSSARD[1, 2, 3]
Anne-Charlotte Lefranc[1, 2, 3], Morgane Dos Santos[1, 3, 4], Mohamedamine Benadjaoud[1, 3], Christelle Demarquay[1, 2, 3], Valérie Buard[1, 2, 3], Georges Tarlet[1, 2, 3], Claire Squiban[1, 2, 3], Christine Linard[1, 2, 3], Noëlle Mathieu[1, 2, 3], Romain Granger[3, 5], Amandine Sache[3, 5], Delphine Denais Lalieve[3, 5], Jean-Marc Simon[6], Marc Benderitter[1, 3], Fabien Milliat[1, 2, 3], Alain Chapel[1, 2, 3]
[1] Service de recherche en radiobiologie et en médecine régénérative (SERAMED)
[2] Laboratoire de Radiobiologie des expositions médicales (LRMED)
[3] Institut de Radioprotection et de Sûreté Nucléaire, Fontenay-aux-roses
[4] Laboratoire de radiobiologie des expositions accidentelles (LRAcc)
[5] Service de recherche sur les effets biologiques et sanitaires des rayonnements ionisants (SESANE), Groupe de support à la recherche et à l'éthique animale (GSEA)
[6] Département de radio-oncologie, Hôpital universitaire de la Pitié-Salpêtrière, Paris, France

Introduction: Chronic radiocystitis (CRC) is a pathology resulting from irradiation of pelvic area without effective treatment. CRC is characterized by chronic inflammation leads to fibrosis. We investigated whether Mesenchymal Stromal Cells (MSC) treatment could reverse CRC damages.
Methods: Our study is divided into two parts, the modelling of CRC and the effect of treatment with MSCs. Preclinical CRC modelling in rats was performed by CT-guided localized irradiation of the bladder from 20 up to 80 Gy with a follow-up of 3 to 15 months after irradiation. Concerning MSCs treatment, bladder was irradiated at 40 Gy. Four month later, three intravenous injections, every two weeks, of 5.10^6 of MSCs was performed. A physiological, histological and molecular follow-up was done until 12 months post- irradiation.
Results: The transient haematuria increasing with time and irradiation dose. Transcriptomic analysis indicates an acute inflammation at 3 months (CCL2, CCL5, TNFα and IL1β upregulation). Results are in favour of tissue and vascular regeneration (EGF and VEGF upregulation) coupled with Extra Cellular Matrix (ECM) remodelling with overexpression of metalloprotease MMP2 and inhibitors, TIMP1/2/4, collagens Col1α2, Col3α1 and proteoglycan Cspg4.

At 6 months, a second wave of inflammation (CCL5, IL1β, IL6 and HIF1α upregulation) is observed, correlated with urothelium disorganization but without tissue and vascular regeneration or remodelling of the ECM.
Conclusion: Results have shown an initiation of CRC at 6 months, with chronic inflammation, hematuria, disorganization of the urothelium and fibrosis. Study is in progress to evaluate the efficacy of MSC treatment.


Bladder irradiation
Kidney
Ureter
Bladder
Chronic radiocystitis
Urothelium
Sub mucosa
Detrusor
Adventice
1. Inflammation
2. Fibrosis
Treatment by MSCs
Adipose tissu
MSCs
Intravenous injection
Initiation of CRC
Months
1 2 3 4 5 6 7 8 9 10 11 12
5 millions MSCs
Analysis of the effects of MSCs

O38 - Can rosiglitazone protect endothelial cells from irradiation-induced mitochondiral dysfunction?

Health effects and radiation protection

Bjorn Baselet[1]
An Aerts[1], Sarah Baatout[1]
[1] Radiobiology unit, Interdisciplinary biosciences, Institute for environment, health and safety, SCK CEN, Mol, Belgium

Introduction: Up to 50–60% of all cancer patients receive radiotherapy as part of their treatment strategy. However, the mechanisms accounting for increased vascular risks after irradiation are not completely understood. Mitochondrial dysfunction has been identified as a potential cause of radiation-induced atherosclerosis.
Methods: Assays for apoptosis, cellular metabolism, mitochondrial DNA content, functionality and morphology were used to compare the response of endothelial cells to a single 2 Gy dose of X-rays under basal conditions or after pharmacological treatments that either reduced (EtBr) or increased (rosiglitazone) mitochondrial content.
Results: Exposure to ionizing radiation caused a persistent reduction in mitochondrial content of endothelial cells. Pharmacological reduction of mitochondrial DNA content rendered endothelial cells more vulnerable to radiation-induced apoptosis, whereas rosiglitazone treatment increased
Conclusion: Pre-existing mitochondrial damage sensitizes endothelial cells to ionizing radiation-induced mitochondrial dysfunction. Rosiglitazone protects endothelial cells from the detrimental effects of radiation exposure on mitochondrial metabolism and oxidative stress. Thus, our findings indicate that rosiglitazone may have potential value as prophylactic for radiation-induced atherosclerosis.

O39 - Evaluation of the radioprotective potential of a PTEN inhibitor bpV(HOpic)

Health effects and radiation protection

Ankit Chauhan[1]
Dhananjay Kumar Sah[1], Neeraj Kumari[1], Yogesh Rai[1], Namita Kalra[1], Ravi Soni[1], Anant Narayan Bhatt[1]
[1] Institute of Nuclear Medicine and Allied Sciences, Delhi, India.

Introduction: Ionizing radiation (IR) exposures during the events of medical or nuclear accidents pose a critical threat to human health. Hence, there is an urgent need for the development of potent and safe radioprotective agents for the management of radio-nuclear emergencies. This study was undertaken to investigate the radioprotective potential of a Phosphatase and tensin homolog (PTEN) inhibitor, bpV(HOpic).
Methods: The cell cytotoxicity, proliferation index, and clonogenic survival assays were performed for assessing the safe dose and radioprotective potential of bpV(HOpic). The IR-induced apoptosis, the kinetics of DNA repair, cytogenetic damage, and IR-induced oxidative stress were studied as the indices of modification of radiation response. Furthermore, the in-vitro observation was verified in-vivo.
Results: A safe dose of bpV(HOpic) was shown to be radioprotective in the cells of three radiosensitive tissue origin. Further, bpV(HOpic) significantly reduced the IR-induced apoptosis and associated pro-death signaling. A faster and better DNA repair kinetics and reduced cytogenetic damage was also observed in bpV(HOpic) pretreated cells exposed to IR. Additionally, bpV(HOpic) decreased the IR-induced oxidative stress and significantly enhanced the anti-oxidant defense mechanisms in cells. The radioprotective effect of bpV(HOpic) was found to be AKT dependant and primarily regulated by the enhanced glycolysis and associated signaling. Intraperitoneal administration of bpV(HOpic) in C57BL/6 mice resulted in AKT activation and conferred survival advantage against IR-induced mortality.
Conclusion: These results imply that bpV(HOpic) diminishes IR-induced oxidative stress and cell death by inducing AKT signaling mediated anti-oxidant defense system and DNA repair pathways, thus strengthening its potential to be used as a radiation countermeasure.

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O40 - Cardiomyocyte dysfunction upon radiation

Health effects and radiation protection

Timo Smit[1]
E. Schickel[1], S. Ritter[1], M. Durante[1], I. S. Schroeder[1]
[1] GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany

Introduction: The risk of developing cardiovascular disease (CVD) increases with the received radiation dose, even at doses as low as 0.5 Gy. Therefore, radiotherapy patients and astronauts on space missions are considered groups with increased CVD risk [1]. However, the underlying mechanisms are poorly understood.
Methods: We used cardiomyocytes (CM) generated from human embryonic stem cells as a 3D model, which mirrors key features of the myocardium such as contraction automaticity and a functional syncytium. Physiology was investigated by patch clamp technique and cardiac pharmaceutical response. Matured clusters (100 days after differentiation initiation) were irradiated with 0.5 or 2 Gy X-rays. Functional parameters such as beat rate and rhythmicity were assessed up to one week after irradiation using a video-based analysis developed at GSI [2].
Results: At the time of exposure, CM showed highly organized sarcomeric structures and responded physiologically to pharmaceuticals. The video analysis of irradiated clusters with 0.5 Gy X-rays (n=77) did not result in strong effects on the function compared to the controls (n=70). In contrast, clusters irradiated with 2 Gy X-rays (n=48) revealed an increase in the beat rate compared to the controls (n=49). Additionally, cardiac abnormalities such as arrhythmias were observed. To expand the model to space-relevant radiation quality an experiment with ^{56}Fe radiation (1 GeV/n) has been recently performed.
Conclusion: In summary, the effects/changes observed after X-ray irradiation reflect a broad range of cardiac dysfunctions. Thereby mechanisms of the CVD development and the role of cardiomyocytes upon radiation can be studied in detail.
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O41 - Early-life X-ray exposure accelerates brain aging in a 3xTg-AD mouse model

Health effects and radiation protection

Emma Coninx[1]
Amelie Coolkens[1], Sarah Baatout[1], Winnok De Vos[2], Lieve Moons[3], Roel Quintens[1], **Mieke Verslegers**[1]
[1] Radiobiology Unit, Institute for Environment, Health and Safety, SCK CEN, Mol, Belgium
[2] Laboratory of Cell Biology and Histology, University of Antwerp, Belgium
[3] Neural Circuit Development and Regeneration Research Group, University of Leuven, Belgium

Introduction: Cranial radiotherapy is inevitable to treat pediatric brain tumors, the second most common childhood cancer. Due to therapeutic advances, survival rates of pediatric brain cancer patients have increased, leading to a higher risk for developing long-term cognitive defects. As irradiation is speculated to accelerate cellular and tissue aging and given that the developing brain is particularly radiosensitive, we hypothesize that pediatric cranial radiotherapy patients are at risk for accelerated brain aging. Further, we want to elucidate whether this predisposes the irradiated brain to develop Alzheimer's disease (AD).
Methods: Ten-day-old female C57BL/6J and triple transgenic (3xTg) AD mice were exposed to 1.8 Gy X-rays. Acute as well as persistent radiation-induced defects that could potentially induce brain aging were investigated in the hippocampus and correlated to cognitive outcome. Additionally, a potential exacerbation of AD pathology was examined using 3xTg-AD mice.
Results: An acute radiation-induced increase in DNA damage and oxidative stress confirmed the radiosensitivity of the developing brain. Furthermore, a persistent reduction in the number of neuronal progenitors in the dentate gyrus and an impaired hippocampal long-term potentiation was found in irradiated 3xTg-AD mice, accompanied by changes in fear response and sociability. Yet, with a newly developed brain epigenetic clock, no change in hippocampal aging rate was observed following irradiation. Finally, tissue-clearing experiments revealed radiation-induced changes in AD pathology.
Conclusion: Our study showed that early-life radiation exposure directly damages the hippocampus, resembling impairments in neurogenesis and plasticity in the aging brain. This increased aging is potentially contributing to AD pathology.

O42 - The time-dependence of radiological benefit of decontamination of residential areas after a nuclear fallout for newborn and adults

Health effects and radiation protection

Christopher Rääf[1]
Johan Martinsson[1], Yvonne Hinrichsen[1], Robert Finck[1], Mats Isaksson[2]
[1] Medical Radiation Physics, ITM, Lund University
[2] Department of Radiation physics, Institute of Clinical Sciences, Sahlgrenska Academy. University of Gothenburg

Introduction: Experience from Japan after the Fukushima fallout shows that decontamination measures of residential areas may take up to several years to achieve. The averted cumulative lifetime attributable risk (CUMLAR) as a function of implementation time of decontaminating residential areas has been theoretically evaluated for a number of scenarios involving unfiltered releases of fission products (such as ^{137}Cs) by applying an existing exposure model designed to compute age and gender dependent time integrated cancer risk.
Methods: The scenarios are partly based on data from the Chernobyl and Fukushima releases and from theoretical source terms from Swedish Nuclear Power Plants.
Results: Our models predict that compliance with existing reference level of 20 mSv y^{-1} can be possible for ^{137}Cs deposition levels up to ca 3 MBq m^{-2}, resulting in averted CUMLAR of up to 20% for the most sensitive population group (newborn girls). It is, however, found that the averted CUMLAR per unit fallout of ^{137}Cs decreases rapidly with a half-time of 2-3 y, depending on initial ^{134}Cs to ^{137}Cs activity ratio and the ecological half-time of the external dose rate (<5 y). If the decontamination time is 5 y before evacuate residents can return, the averted CUMLAR for newborn girls will be about 10% of that obtained by evacuation alone during the same time.
Conclusion: We conclude that although decontamination may have societal benefits in terms of restored trust of the public, decision makers need to consider the short time-window of the radiological benefits of this protective action compared with just evacuation before return.

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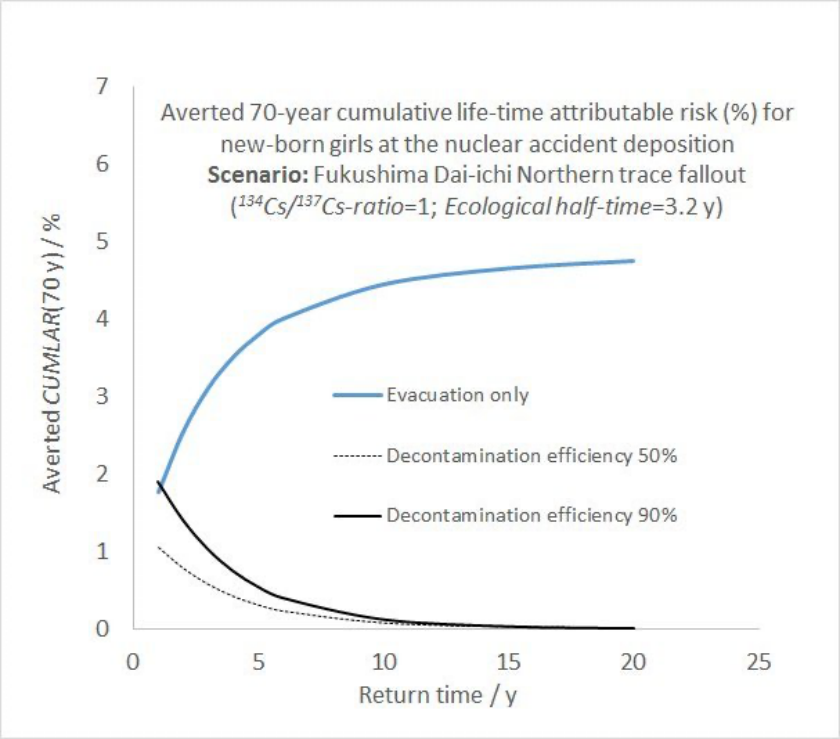
Averted 70-year cumulative life-time attributable risk (%) for new-born girls at the nuclear accident deposition
Scenario: Fukushima Dai-ichi Northern trace fallout
(134Cs/137Cs-ratio=1; Ecological half-time=3.2 y)
Averted CUMLAR(70 y) / %
Return time / y
Evacuation only
Decontamination efficiency 50%
Decontamination efficiency 90%
0
1
2
3
4
5
6
7
0
5
10
15
20
25

O43 - Improved patient dosimetry at radioiodine therapy by combining the ICRP iodide compartment model and the EANM pre-therapeutic standard procedure

Health effects and radiation protection

Martin Andersson[1]
Sören Mattsson[1]
[1] Medical Radiation Physics Malmö, Department of Translational Medicine, Lund University

Introduction: Radioactive iodine is commonly used for the treatment of different thyroid conditions since the 1940s. The EANM has developed a standard pre-therapeutic procedure to estimate patient specific thyroid uptake. The procedure which models the time dependent fractional thyroid uptake is a two compartment fitting system, one representing the thyroid and the other the blood. The absorbed dose is however only estimated for the thyroid and not for any other organ in the body. A more detailed biokinetic model for iodine is given by the ICRP and includes a systemic iodide transport in the whole body. The ICRP model has however fixed transfer coefficients and is only presented with three different thyroid uptake values (low, normal and high).
Methods: Combining the EANM method and the ICRP model gives both patient specific uptake estimation and include also most organs in the body. The ICRP model has 30 different compartments and 48 transfer coefficients to model the biokinetics of iodide and to model different transfer for inorganic iodide and organic iodine. However, as the ICRP model is a recirculation iodine model, the optimization is therefore performed on the whole model and not exclusively on the thyroid as in the EANM procedure.
Results: Fitted to data for a specific patient, the transfer coefficient from blood to thyroid was 16/day insted of the 7/day giving a 2.5 times higher thyroid uptake than with the ICRP normal uptake model.
Conclusion: Combining the ICRP and EANM methodologies gives a improve thyroid dosimetry and information on absorbed dose to radiosensitive organs.
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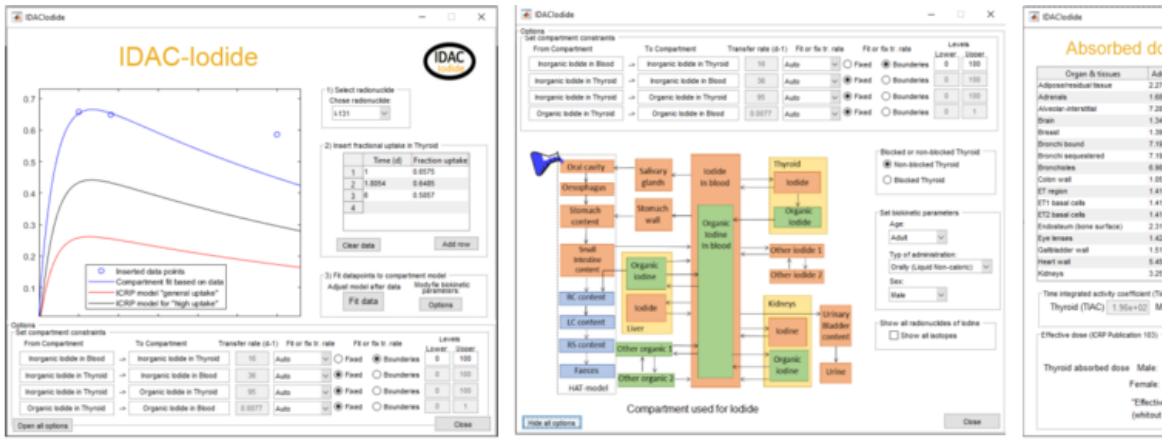

Figure: The iodine software. To the left image shows the patient specific actvivity measurements (circles), and the r
(red) and high (black) thyroid uptake. The blue line is the software generated comparmental fit according to the wh
the middle image for oral administration. The table in right image shows sex specific absorbed doses for both the t

O44 - Coadministration of three antioxidants did not influence the tumour response to radiotherapy in GOT1 neuroendocrine tumour model

Health effects and radiation protection

Charlotte Andersson[1]
Emman Shubbar[1], Lukas Lundholm[1], Bo Åkerström[2], Magnus Gram[3], Khalil Helou[4], Eva Forssell-Aronsson[1]
[1] Dept. of Radiation Physics, Inst. of Clinical Sciences, Sahlgrenska Cancer Center, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden
[2] Lund University, Dept. of Clinical Sciences, Infection Medicine, Lund, Sweden
[3] Lund University, Dept. of Clinical Sciences, Pediatrics, Lund, Sweden
[4] Dept. of Oncology, Inst. of Clinical Sciences, Sahlgrenska Cancer Center, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

Introduction: It is a common view that antioxidants can be used to protect against cancer, although there're studies that contradict this view and even show that antioxidants can increase cancer progression. These conflicting results should be considered when exploring combination treatment with radiotherapy together with an antioxidant that could be used to improve the cancer treatment. We previously demonstrated that the antioxidant rA1M, a potential kidney protector during treatment with ^{177}Lu-octreotate, did not negatively affect the therapeutic response of ^{177}Lu-octreotate in neuroendocrine tumour (NET) mouse model.

In this study we further investigated if rA1M interferes with the radiation induced response in NET. We also studied effects of two other antioxidants that previously showed to increase tumour burden and promote metastastatic growth in other tumour types.
Methods: Female BALB/c nude mice with GOT1 NET tumours were divided into 4 groups and treated with external beam radiotherapy (EBRT), 6 Gy to the tumour. Three of the groups also received antioxidant supplements: N-acetylcysteine, rA1M or vitamin E. The tumour response was monitored over time by measuring the tumour volume.
Results: During the first days after EBRT the mean tumour volume decreased to less than 10 % of the initial volume, and about a week later the mean tumour volume increased again. Statistical analysis of the area under the curves showed no difference between the EBRT only group and the antioxidant groups.
Conclusion: In conclusion, supplements of the antioxidants rA1M, N-acetylcysteine or vitamin E did not negatively affect the tumour response of EBRT in GOT1 NET mouse model.

O45 - Effects of α-particles and X-rays on human lung epithelium

Health effects and radiation protection

Kim Carola Roeder[1]
David Eilenstein[1], Michael Scholz[1], Marco Durante[1, 2], Sylvia Ritter[1]
[1] GSI Helmholtzzentrum für Schwerionenforschung, Darmstadt, Germany
[2] Technische Universität Darmstadt, Germany

Introduction: Radon is the second most common cause of lung cancer. To gain a deeper insight into the effects of α-particles and X-rays on lung epithelium, human bronchial epithelium cells (NHBE) were used. NHBE function as stem cells, ensure tissue homeostasis and repair. In vitro they form a functional epithelium consisting of basal, goblet, club and ciliated cells.
Methods: NHBE were irradiated with α-particles (0.25–1.5 Gy) from ^{241}Am or X-rays (0.5-5 Gy). Cell survival and stem cell identity of surviving cells (RT-qPCR analysis) were examined. Differentiation capacity of irradiated NHBE was analyzed via epithelium formation. Gene expression of cell type specific markers was quantified by RT-qPCR over 5 weeks. Additionally, for functional analysis (i.e. mucociliary clearance, MCC), micro-beads were placed onto the cells. Efficiency of MCC was assessed by an in-house designed video-based software.
Results: For NHBE α-particles were more effective in cell inactivation than X-rays. Both radiation types did not affect the expression of selected markers in surviving cells. Their capability to generate a functional epithelium was impaired. Most selected markers were dysregulated. First video-based analyses showed a disturbed ciliary beat (CB) pattern after X-ray irradiation; α-particle experiments are currently being carried out.
Conclusion: Per unit dose α-particles are more effective in killing NHBE than X-rays. Surviving progeny are able to form an epithelium. Yet, RT-qPCR and CB analyses suggest that the functionality of the epithelium is compromised. In the case of inhaled radon, MCC impairment can exacerbate the radiation damage, as the transport of α-decay products out of the lung is impeded.

O46 - G2/M checkpoint abrogation with selective inhibitors results in chromosome break repair defects in RPE and 82-6 hTERT cells

Health effects and radiation protection

Aggeliki Nikolakopoulou
Aashish Soni[1], Gabriel Pantelias[2], Martha Habibi[2], Pantelis Karaiskos[3], Georgia Terzoudi[2], George Iliakis[1]
[1] Institute of Medical Radiation Biology, University of Duisburg-Essen, Germany
[2] Laboratory of Health Physics, Radiobiology & Cytogenetics, Institute of Nuclear & Radiological Sciences & Technology, Energy & Safety (INRASTES), National Centre for Scientific Research ‘‘Demokritos’’
[3] Medical Physics Laboratory, Medical School, National & Kapodistrian University of Athens

Introduction: While technological advances in radiation oncology have led to a more precise delivery of radiation dose, there is a significant need to decrease risk of side effects and to overcome tumor resistance in radiation. Hence, there is a strong interest in combining radiotherapy treatment with new agents such as G2-M checkpoint inhibitors, which differentiate tumor’s radiosensitivity. It is necessary, therefore, to investigate the role of ATM, ATR and chk1 inhibitors in G2 regulation and to evaluate their radiosensitizing effect.
Methods: The contribution of ATR, ATM and chk1 inhibition in human cell lines by using G2-M checkpoint potent inhibitors was examined. RPE and 82-6 hTERT human cell lines were irradiated during G2- to M-phase transition. G2-checkpoint was inactivated by means of caffeine, VE-821 and UCN-1, the inhibitors were added 1h before irradiation, and the number of chromatid breaks was obtained using a modified G2-chromosomal radiosensitivity assay.
Results: The results obtained showed a substantial increase in the number of chromatid breaks after treatment with all three inhibitors, however, the ATRi inhibitor VE-821 showed the maximum effect with an increase of 60%.
Conclusion: The treatment of 82-6 hTERT and RPE cells with all three inhibitors led to abrogated G2 checkpoint after irradiation and the ATR appears potentially to play a bigger role in G2 regulation compared to ATM. The results are promising, since a defective regulation of the G2 checkpoint may contribute to the phenotype of radiosensitivity.

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O47 - Detection of DNA damage and chromosomal aberrations after exposure to low ionizing radiation doses in interventional cardiology

Health effects and radiation protection

Martha Habibi[1]
Panagiotis Karyofyllis[2], Aggeliki Nikolakopoulou[1], Panagiotis Papagiannis[3], Pantelis Karaiskos[3], Nikolaos Kollaros[4], Irene Mastorakou[4], Vasilis Voudris[2], Georgia Terzoudi[1]
[1] Laboratory of Health Physics, Radiobiology & Cytogenetics, INRASTES, National Centre for Scientific Research "Demokritos", Athens, Greece
[2] Department of Interventional Cardiology, Onassis Cardiac Surgery Center, Athens, Greece
[3] Medical Physics Laboratory, Medical School, National & Kapodistrian University of Athens
[4] Imaging Department, Onassis Cardiac Surgery Center, Athens, Greece

Introduction: Little information is available about the effect of reportedly low ionizing radiation doses, such as those very often delivered to patients in interventional cardiology. As interventional cardiac procedures are a major contribution to total collective effective dose, there is a growing concern about the safety of physicians and patients regarding radiation protection issues and the late health effects. The purpose of this study was to investigate the use of potential biomarkers for low ionizing radiation dose from exposure during interventional cardiac procedures, based on different molecular and cytogenetic endpoints.
Methods: Lymphocytes from whole blood samples were collected from 25 patients before and after interventional cardiac examination. Biomarkers based on DNA and cytogenetic damage and repair such as γH2AX foci, dicentric chromosomes and micronuclei were studied.
Results: The results obtained indicated that all three endpoints studied showed increased yields relative to the baseline ($p<0.001$) for all patients after their medical exposure. Furthermore, 24 hours after exposure, residual γH2AX foci were still detectable in irradiated lymphocytes with their decline found to vary significantly among the different individuals and their repair found to range from 20% to 97.2% of the initial γH2AX foci.
Conclusion: The three different endpoints examined can function as biomarkers of exposure after interventional cardiac procedures. However, the results illustrate a clear advantage of the use of γH2AX foci over the conventional dicentric and micronuclei assays after low dose exposure, as well as variability in the kinetics of the γH2AX foci among the different individuals.

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O48 - Mathematical modelling of radiation-induced acute myeloid leukaemia incidence

Health effects and radiation protection

Sjors Stouten[1, 2]
Sjoerd Verduyn Lunel[2], Rosemary Finnon[3], Christophe Badie[3], Fieke Dekkers[1, 2]
[1] The Dutch National Institute for Public Health and the Environment
[2] Utrecht University
[3] Public Health England

Introduction: Atomic bomb survivor studies have shown that the low-dose acute myeloid leukaemia (AML) response curve is probably nonlinear. Although different nonlinear curves can be used to adequately describe high-dose risk, they provide distinct low-dose risk estimates after extrapolation. Animal models are widely used to elucidate various pathways of leukaemogenesis. Murine radiation-induced AML can largely be explained by two mutations: an exposure-related deletion with Sfpi1/PU1 loss; and the occurrence of a specific point mutation in the remaining allele. This major pathway of leukaemogenesis is translated into a mathematical model in order to study the possible form of the low-dose response curve in CBA/H mice.
Methods: We have developed a stochastic model in which each simulation corresponds to a photon-irradiated *in silico* male CBA/H mouse capable of developing AML in a time-dependent manner. Besides quantifying AML incidence, the model also describes dose-dependent cell death, formation of Sfpi1 deletions and animal death due to non-AML causes.
Results: Model predictions are in accordance with experimental data on cell/animal survival, time of AML onset and high-dose AML incidence. Low-dose AML incidence was found to be proportional to the number of cells with Sfpi1 deletions, which is approximately linear-quadratic. A linear-quadratic function could be used to accurately predict modelled low-dose incidence when only high-dose model estimates were made available in a fitting procedure.
Conclusion: By translating the main pathway of leukaemogenesis into a mathematical model we have found a linear-quadratic low-dose response curve for AML incidence in photon-irradiated male CBA/H mice.

O49 - Modeling early radiation damage occurring during [177Lu]Lu-DOTA-[Tyr3]octreotate radionuclide therapy with the Geant4-DNA toolkit

Radiation physics and chemistry

Giulia Tamborino[1, 2]
Marijke De Saint-Hubert[1], Lara Struelens[1], Julie Nonnekens[2, 3], Mark W. Konijnenberg[2], Marion De Jong[2], Yann Perrot[4], Carmen Villagrasa[4]
[1] Research in Dosimetric Applications, Belgian Nuclear Research Centre (SCK•CEN), Mol, Belgium
[2] Department of Radiology & Nuclear Medicine, Erasmus MC, University Medical Center Rotterdam, The Netherlands
[3] Department of Molecular Genetics, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands
[4] IRSN, Institut de Radioprotection et de Sûreté Nucléaire, Fontenay aux Roses, France.

Introduction: The aim is to build a simulation framework for the number of DNA double strand breaks (DSBs) induced during *in vitro* targeted radionuclide therapy (TRNT).
Methods: A multiscale approach is implemented to simulate the number of DSBs produced by the cumulated decays of ^{177}Lu-DOTATATE, preliminary assumed as purely beta emitter, without including any repair. The approach involves 2 sequential simulations performed with Geant4/Geant4-DNA and accounts for a realistic geometry of the cell population and detailed sampling of the activity distribution within it.
To reproduce the exposure conditions of cells incubated 4h with ^{177}Lu-DOTATATE (2.5MBq/ml), a phase space is scored by recording particles that enter the nucleus of a cell belonging to a population modelled by polygonal mesh structures. The radioactive source is sampled according to dedicated uptake experiments evaluating the distribution of activities within medium and cells, thereby assuming instant and permanent internalization. The particles recorded in the phase space file are released within an ellipsoidal nucleus geometry, including a multi-scale description of the DNA. DnaFabric software is used to model the entire human genome with a continuous chromatin fiber per chromosome. This geometry is used to simulate the physical, physicochemical and chemical stages in Geant4-DNA and score the number of DSB/decay.
Results: Our results reveal induction of 6-10 DSBs/cell, depending on the cell confluence, compared to 11±2 DSBs/cell experimentally measured. The impact of physical/chemical parameters is currently under investigation.
Conclusion: This work represents the first step towards modeling DSBs during TRNT allowing a better understanding of underlying mechanisms for improved response prediction.

O50 - RBE prediction by the BIANCA model for in vitro and in vivo irradiation by different hadron therapy ion-beams

Radiation physics and chemistry

Mario P. Carante[1]
Giulia Aricò[2], Alessia Embriaco[1], Alfredo Ferrari[2], Christian P. Karger[3], Wioletta Kozlowska[2, 4], Andrea Mairani[5], Paola Sala[6], Francesca Ballarini[1, 7]
[1] INFN (Italian National Institute for Nuclear Physics), Sezione di Pavia, via Bassi 6, I-27100 Pavia, Italy
[2] CERN—European Organization for Nuclear Research, 1211 Geneva, Switzerland
[3] Department of Medical Physics in Radiation Oncology, German Cancer Research (DKFZ), 69120 Heidelberg, Germany
[4] Medical University of Vienna, 1090 Vienna, Austria
[5] HIT (Heidelberg Ion-Beam Therapy Center), 69120 Heidelberg, Germany
[6] INFN (Italian National Institute for Nuclear Physics), Sezione di Milano, via Celoria 16, I-20133 Milano, Italy
[7] Physics Department, University of Pavia, via Bassi 6, I-27100 Pavia, Italy

Introduction: BIANCA (*BIophysical ANalysis of Cell death and chromosome Aberrations*) is a two-parameter biophysical model assuming that radiation induces DNA "critical lesions" that produce chromosome aberrations, some of which lead to cell death.
Methods: In view of RBE prediction for hadron therapy, first we tuned the model parameters to produce a radiobiological database (cell survival alpha and beta coefficients as a function of ion type and energy) for V79 cells, chosen as a reference. Afterwards, we developed an approach to produce analogous databases for other cell lines, for which the photon response is known. This approach does not require any further parameter adjustment, thus providing full predictions for, in principle, any cell line of interest; these databases can be read by a radiation transport code and/or a treatment planning system (TPS).
Results: In this work BIANCA was interfaced to the FLUKA transport code, and was applied to predict cell survival and RBE in typical hadron therapy scenarios. More specifically, very good agreement was found with experimental data on the survival of CHO cells exposed *in vitro* at different positions along Spread Out Bragg Peaks of protons, C-ions (Figure) and He-ions [1]. Furthermore, good agreement was obtained with proton and carbon RBE data on late effects in the rat spinal cord, which represent a model for CNS effects in head-and-neck tumor treatment [2].
Conclusion: This work suggests that BIANCA, interfaced to a transport code and/or TPS, can be used to predict RBE for hadron therapy treatments, and poses the bases for applications to patient cases.
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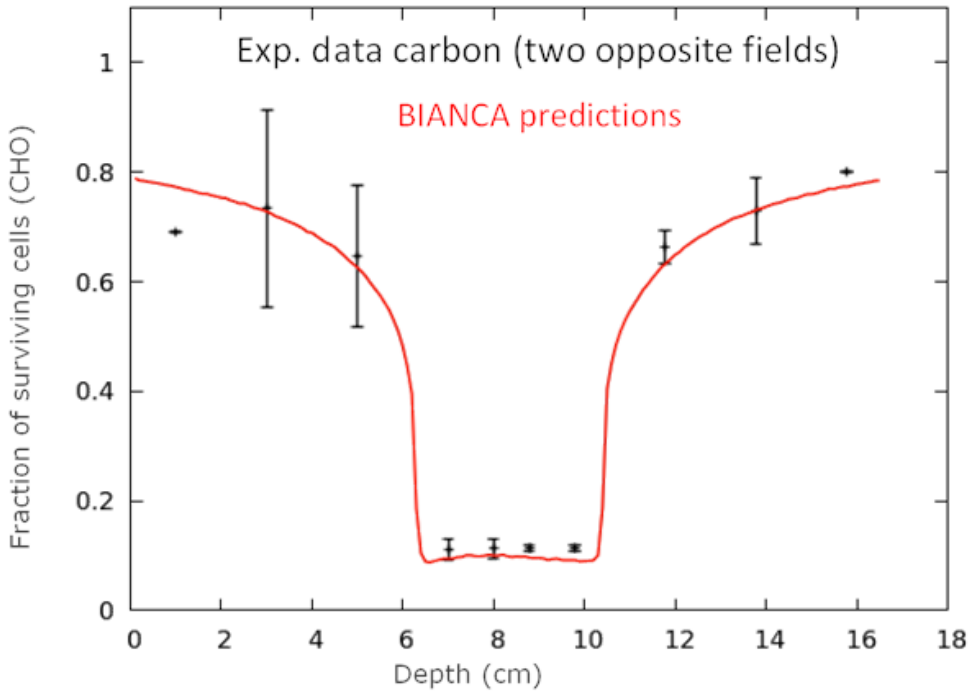
Exp. data carbon (two opposite fields)
BIANCA predictions
Fraction of surviving cells (CHO)
Depth (cm)
0
0.2
0.4
0.6
0.8
1
0
2
4
6
8
10
12
14
16
18

O51 - Development of a new microdosimetric biological weighting function for the RBE assessment in case of the V79 cell line exposed to ions from 1H to 238U

Radiation physics and chemistry

Alessio Parisi[1]
[1] Belgian Nuclear Research Centre (SCK CEN)

Introduction: With the increasing use of ions for cancer-radiotherapy it is of primary importance to model radiation-induced effects for treatment planning, quality control and research purposes.
In this work, a new biological weighting function (IBWF) is proposed to correlate microdosimetric[1] spectra with the RBE_{10} for the cell survival of the V79 cell line.
Methods: The IBWF was determined through an iterative deconvolution process between 592 PHITS-simulated[2] microdosimetric spectra and 267 *in vitro* survival-curves for V79 cells exposed to ions from ^{1}H to ^{238}U[3].
The IBWF results were compared with corresponding calculations performed using the modified MKM[4]. Furthermore, RBE values computed with the reference biological weighting function (BWF)[5] for the *in vivo* early intestine tolerance in mice were also included to investigate potential correlations between the two biological endpoints.
Finally, the IBWF was unchangingly applied to microdosimetric spectra experimentally measured with 8 different microdosimeters in 17 different ^{1}H and ^{12}C beams at 8 clinical facilities.
Results: The average deviation between IBWF-derived RBE values and the *in vitro* data was ~14%. Using the MKM, it was not possible to successfully reproduce the RBE_{10} data for ions heavier than ^{20}Ne.
The RBE values assessed by the IBWF using as input the measured microdosimetric spectra were found to be in agreement with the ones calculated in combination with computer-simulated spectra, with an average relative deviation of 0.8% and 5.7% for ^{1}H and ^{12}C ions respectively.
Conclusion:
The IBWF can be used as a fast and easy tool for intercomparing clinical beams or the results acquired with different microdosimeters.
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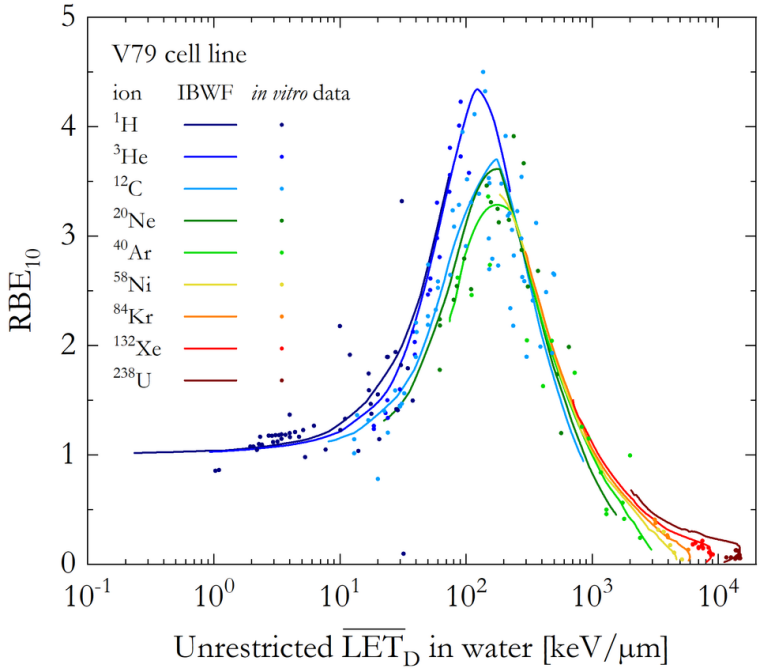

Overview of the RBE_{10} calculated by the IBWF (lines) for selected ions in comparison to *in vitro* data (dots) for the V79 cell line.

O52 - EURADOS WG10 and RENEB WG2 exercise on retrospective dosimetry methods in a simulated small scale incident involving ionising radiation.

Radiation physics and chemistry

Lovisa Waldner[1]
Christian Bernhardsson[1], Céline Bassinet[2], François Trompier[2], Eric Gregoire[2], Olivier van Hoey[3], Clemens Woda[4], Ursula Oestreicher[5], Ulrike Kulka[5], David Endesfelder[5], Andrzej Wojcik[6], Jonathan Ekins[7], Elizabeth Ainsbury[7]
[1] Lund University, Department of Translational Medicine, Medical Radiation Physics, Sweden
[2] Institut de Radioprotection et de Sûreté Nucléaire, Fontenay-aux-Roses, France
[3] Environment, Health and Safety Institute, Belgian Nuclear Research Center (SCK•CEN), Belgium
[4] Helmholtz Zentrum München, Institute of Radiation Medicine, Neuherberg, Germany
[5] Bundesamt für Strahlenschutz, BfS, Department of Radiation Protection and Health, Oberschleissheim, Germany
[6] Stockholm University, Department of Molecular Biosciences, The Wenner-Gren Institute, Sweden
[7] Public Health England, CRCE, Chilton, Didcot, Oxon, UK

Introduction: With the risk of accidents or malevolent use of ionising radiation, a number of methods have been developed to retrospectively reconstruct involuntary individual radiation exposures. Physical retrospective dosimetry is mainly done by EPR or luminescence (TL/OSL) methods and biological dosimetry methods include analysis of aberrations and damage of chromosomes and DNA. To ensure the quality and dependability of these methods and to study their compatibility, inter-laboratory comparisons (ILCs) are an important tool. The aim of this ILC was to estimate whole body, partial body, and organ doses to exposed anthropomorphic phantoms using the blood samples and physical materials placed on the phantoms.
Methods: Within the EURADOS network, Working Group 10 and RENEB Working Group 2 organised an ILC with the intention to simulate a small-scale incident involving ionising radiation. A 1.3 TBq ^{192}Ir source was used in an outdoor open-air geometry, to expose 4 phantoms in different geometrical configurations to absorbed doses up to several gray. Positioned on the phantoms were materials intended for accident dosimetry (e.g mobile phones, blood), and for reference dosimetry (LiF, NaCl, Glass rods).
Results: Absorbed doses from different materials were estimated at participating laboratories and will be put together for further analysis. The results were compared to Monte Carlo simulations and reference dosimeters.
Conclusion: Because of their value, many more ILCs will follow and the recent exercise provided useful experience in terms of planning and execution of future ILCs, with respect to radiation protection, time management, and reference dosimetry to be considered to obtain relevant data for analysis.

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O53 - About the Absence of Reactive Oxygen Species Overproduction in the Presence of Gold Nanoparticles

Radiation physics and chemistry

Viacheslav Shcherbakov[1]
[1] Institute de Chimie Physique (ICP), CNRS/Université Paris-Saclay, 91405 Orsay, France

Introduction: Gold nanoparticles (AuNPs) attract attention as a promising radiosensitizing agent for cancer treatment. Many *in vivo* and *in vitro* studies show that AuNPs increase the damage caused by ionizing radiation. However, the mechanism remains unknown. Now it is clear, that initially proposed a concept of high-Z materials, consisting of higher absorbed dose due to their larger cross-sections to ionizing radiation, cannot explain observed biological responses. Therefore, the last two decades many different biological and chemical explanations were proposed.[1]
Reactive oxygen species (ROS) overproduction in the presence of AuNPs were reported in many studies of both cell and molecular systems.[2] Usually, ROS are measured indirectly by fluorescent dyes.[3] The change in luminescence properties is assumed to be proportional to the concentration of $^{\bullet}OH$ radical or $O_2^{\bullet-}$. However, AuNPs catalytic effect on chemical reactions occurring in the transformation of initial dye molecule to final product with another fluorescent property is never considered.
Methods: Pulse radiolysis with time-resolved spectroscopy, Gamma radiolysis, HPLC, UV-vis spectroscopy. AuNPs were synthesized by reduction with $NaBH_4$ and Turkevich method.
Results: AuNPs do not cause primary radical overproduction at the concentration used *in vivo* or *in vitro* studies. Instead, drastic change in radical chemistry is observed by affecting the ratio and yields of the products. In addition, even without radiation in the presence of oxygen, the AuNPs can stimulate the degradation of stable molecules such as Vitamin C.
Conclusion: The catalytic activity of AuNPs must be taken into account in both ROS detection and explanation of radiosensitization.
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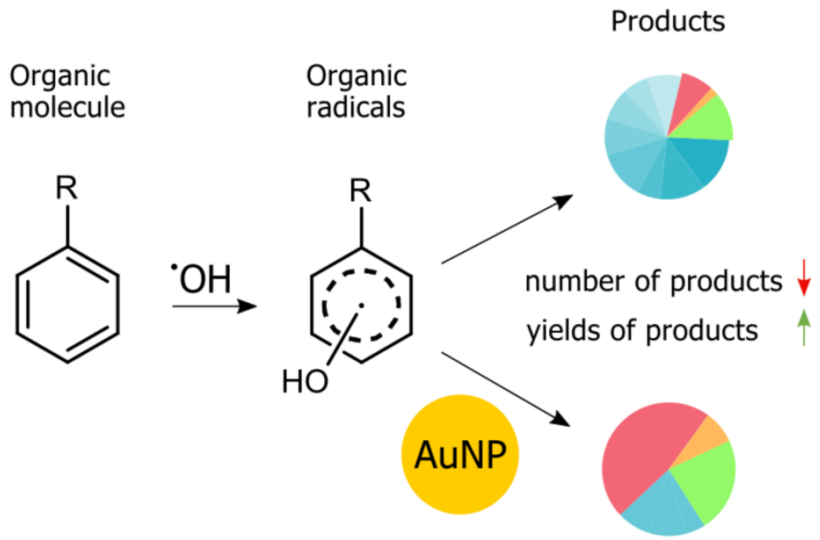
Products
Organic molecule
Organic radicals
R
R
•OH
HO
number of products
yields of products
AuNP

O54 - Azide and hydroxyl radicals induce several di-tyrosine bridge isomers from the amino acid to the protein scale

Radiation physics and chemistry

Anouchka GATIN[1]
Isabelle BILLAULT[1], Patricia DUCHAMBON[2], Guillaume VAN DER REST[1], Cécile SICARD-ROSELLI[1]
[1] Université Paris-Saclay, CNRS, Institut de Chimie Physique UMR 8000, 91405 Orsay Cedex, France
[2] CNRS UMR9187, INSERM U1196, Institut Curie, Université Paris Saclay, 91405 Orsay Cedex, France

Introduction: In neurodegenerative diseases, di-tyrosine bridge formation has been evidenced and is used as a biomarker[1,2] of oxidative pathologies. Herein, we bring into light new observations about the dimerization process.
Methods: Hydroxyl and azide radicals were produced by gamma radiolysis, then the induced biological oxidative damages were analysed by a specifically optimized chromatographic separation coupled to mass spectrometry, electrophoresis gels and fluorometry.
Results: We chose to focus our study on human centrin 2 which was shown to dimerize *via* its unique tyrosyl residue. With hydroxyl radicals, centrin dimerization appeared highly significant among other oxidative damages. Surprisingly, we highlighted that for human centrin 2 and a five amino acid peptide, up to five different dimers were highlighted. Though for free tyrosine, oxidation only leads to three different dimers. New dimers' structures were characterized coupling complementary approaches: isotopic labelling, mass spectrometry fragmentation and ionic mobility spectrometry.
Conclusion: Their evidence raises some questions: what is their respective role *in vivo* and hence their relative toxicity? Why do more complex and so more sterically hindered systems generate a higher number of di-tyrosine bridge isomers?

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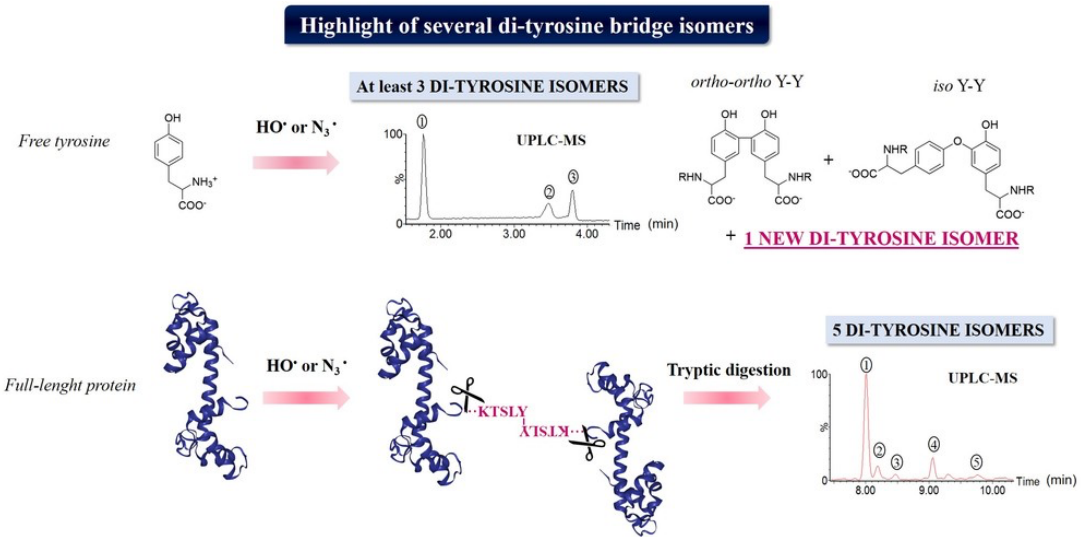

O55 - Presolvated electron attachement towards nucleotides in liquids: pulsed radiolysis studies

Radiation physics and chemistry

Sergey DENISOV[1]
Jun MA[2], Amitava ADHIKARY[3], Mehran MOSTAFAVI[1]
[1] Institute de Chimie Physique, UMR8000 CNRS/Université Paris-Saclay, Bâtiment 349, 91405 Orsay, France
[2] Department of Nuclear Science and Engineering, Nanjing University of Aeronautics and Astronautics, Nanjing 211106, China
[3] Department of Chemistry, Oakland University, 146 Library Drive, Rochester, MI, 48309, USA

Introduction: In the works of Leon Sanche it was shown that DNA radicals anions formed on electron attachment with certain kinetic energies in the gas phase lead to the formation of resonant DNA structures and rupture of the helix. Thus, such dissociative electron attachment (DEA) could be an unaccounted mechanism of DNA damage under ionizing radiation in cells. The DEA studies were limited to the gas and solid-state phases. After 10 years it is still under debate if it occurs in the liquid phase.
Methods: Herein we investigated non-equilibrium e_{pre}^- scavenging by nucleobases/sides/tides in water and such viscous solvent as diethylene glycol, allowing to slow down electron solvation using 5 ps electron (7 MeV) pulse radiolysis coupled with broadband transient absorption spectroscopy.
Results: The e_{pre}^- is more reactive with pyrimidine than purine bases/nucleotides with a reactivity order T > C > A > G. The signal due to the formation of the resulting anion radical directly correlates with the loss of the initial e_{hyd}^- signal.[2] In the case of uridine monophosphate (UMP), the hole formed by either direct-ionization or via reaction of UMP with the radiation-mediated water cation radical ($H_2O^{\bullet+}$) facilely localize on the ribose site, despite the fact that a part of them was initially created either on the phosphate or uracil.[3]
Conclusion: Our results indicate that DEA for nucleotides in water is not active (within 5% experimental error), while in more viscous solvent DEG case pieces of evidence are present for C-N bond cleavage between nucleobase and sugar moities.[4]
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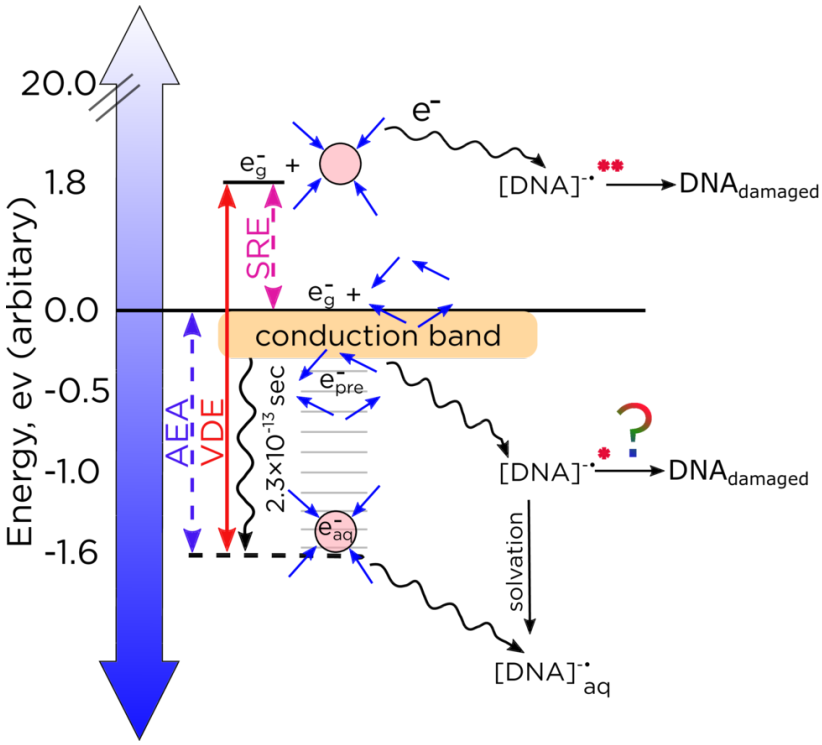
20.0
1.8
0.0
-0.5
-1.0
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Energy, ev (arbitary)
e-
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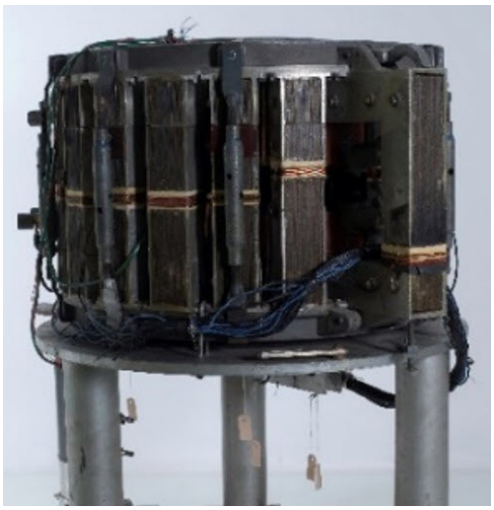
conduction band
AEA
VDE
2.3x10-13 sec
epre-
eaq-
solvation
[DNA]-•aq

O56 - UrMAX - the light from Lund. Preservation of epoch-making scientific equipment illustrated by the evolution from UrMAX to MAX IV.

Radiation physics and chemistry

Bengt Forkman[1, 2]
Bertil R Persson[1, 3], Lotta Elmros[4], **Sverker Werin**[1, 5]
[1] Lund University, Lund Sweden
[2] Nuclear Physics
[3] Medical Radiation Physica
[4] Exhibition producer, Sweden
[5] MAX IV, Lund

Introduction: This digital exhibition is a collaboration between Malmö Museum, the Conservation Group at Lund University*), and the Lund University of Technology, with funding from the Thora Olsson Foundation. The exhibition depicts the development of UrMAX, a small but 1.3-ton heavy electron accelerator that has become a historic goldmine, as well as the development that followed.
Methods: With UrMAX as a basis, researchers have paved the way for MAX IV, Sweden's largest research infrastructure, and the world's brightest synchrotron system, through groundbreaking experiments and theories. The exhibition also illuminates the UrMAX accelerator as an example of the value of preserving experimental equipment.
Results: There is a great interest in educationally described experiments and their importance for modern theories and modern technology. Luckily, the first electron accelerator in Lund was donated to Malmö Museum, when it was discarded in the 1960s, instead of ending up as rubbish. One could not then believe that it was the seed of a series of accelerators that led to the explosive development of the synchrotron light research in Lund and the MAX IV plant at Brunnshög. With this example, the exhibition also shows the importance of preserving objects to increase the possibility of future research and education to have a broader context and a historical scientific connection.
Conclusion: Through this virtual exhibition, you are able to see UrMAX, texts, and sample images. Click on each image and text to read more about the development of UrMAX from 1960 until today with MAX IV, as a chronological story.



Abstracts - Poster presentations

P1 - Development of a low-energy proton beamline for studies on the biological effectiveness of Proton-Boron Capture Therapy

Radiation physics and chemistry

Valerio Ricciardi[1, 2]
Giuseppe Porzio[1, 2], Raffaele Buompane[1, 2], Lucio Gialanella[1, 2], Lorenzo Manti[2, 3]
[1] Dipartimento di Matematica e Fisica, Università degli Studi della Campania Luigi Vanvitelli, 81100, Caserta (CE), Italy
[2] Istituto Nazionale di Fisica Nucleare - Sezione di Napoli, 80126, Naples (NA), Italy
[3] Dipartimento di Fisica E. Pancini, Università degli Studi di Napoli Federico II, 80126, Naples (NA), Italy

Introduction: Charged particle inverted dose-depth profile represents the physical pillar of protontherapy [1]. In the NEPTUNE project [2], new approaches to improve protontherapy effectiveness using nuclear reactions able to generate short-range high-LET alpha particles, are investigated. Significant enhancement of proton biological effectiveness has been demonstrated by exploiting the $p+^{11}B \rightarrow 3\alpha$ (pB) reaction [3], an approach termed Proton-Boron Capture Therapy (PBCT). The maximum of the cross section for the pB reaction occurs for proton energy of about 700 keV. The present work describes the implementation of an irradiation facility at the 3MV tandem accelerator of the CIRCE laboratory (Univ. Vanvitelli, Caserta), for the study of the pB radiobiological effects near cross section maximum.
Methods: The system mainly consists of a scattering chamber in whose centre it is possible to mount a target-holder, provided with beam collimators and a beam scatterer Au foil; proton irradiation occurs through Rutherford scattering. Live beam dosimetry is performed measuring protons energy by Silicon Surface-Barrier detectors placed at different angles [4]. Particle fluence and beam uniformity are measured and monitored by means of CR-39 detectors.
Results: The tests of the facility performance are discussed. With the scattering system, proton count rates corresponding to possible dose rates selectable in a range from 0.5-2 Gy/min were consistently obtained. Uniformity in energy and fluence was achieved with uncertainties of 2 and 5%, respectively.
Conclusion: The scattering chamber assembly and tests were completed. Proton beams with characteristics suitable for cellular irradiation at energies required by PBCT studies were obtained: first radiobiological experiments are in progress.
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P2 - Establishing a method for commissioning and validation of the micro-RayStation beam model of a 220 kV XenX small animal irradiator

Radiation physics and chemistry

Elise Konradsson[1]
Kristoffer Petersson[2, 3], Crister Ceberg[1]
[1] Medical Radiation Physics, Department of Clinical Sciences, Lund University, Lund, Sweden
[2] Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, Oxford, UK
[3] Radiation Physics, Department of Hematology, Oncology and Radiation Physics, Skåne University Hospital, Lund, Sweden

Introduction: To ensure high quality in the translation from pre-clinical radiotherapy research into clinical radiotherapy, a treatment planning system adapted for pre-clinical studies is of great value. The aim of this work was to establish a method for commissioning and validation of a beam model of the small animal irradiator XenX created in micro-RayStation, a new pre-clinical treatment planning system.
Methods: A method for commissioning and validation of the micro-RayStation beam model of a XenX 220 kV irradiator was established. The validation method is based on dose measurements in a 3D printed mouse phantom.
Results: Commissioning of the beam model involves import of profiles and depth dose curves for six collimator sizes (3x3 mm^2, 5x5 mm^2, 10x10 mm^2, 9x3 mm^2, Ø=5 mm^2 and Ø=10 mm^2) and a variable collimator, measured with EBT3 film at a source-to-surface distance of 33 cm in a solid water phantom. The beam model is manually tuned to achieve good agreement with the measurements. Reference dosimetry is performed using an ionization chamber at 2 cm depth in solid water. Validation of the beam model is done by comparing measured and calculated beam profiles and depth dose curves, as well as with different treatment plans delivered to a 3D printed mouse phantom based on a segmented CT image of a mouse.
Conclusion: We have established a method for commissioning and validating a new treatment planning system for pre-clinical radiotherapy using measurements in a solid water phantom as well as in a 3D-printed mouse phantom created using a CT image.

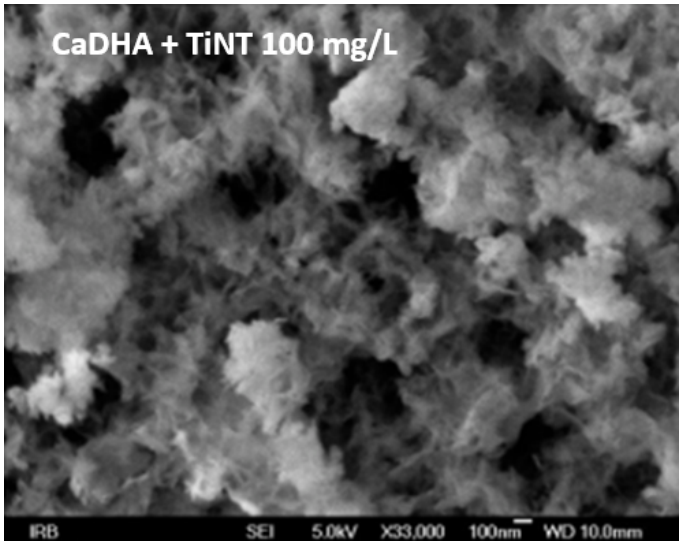
P3 - Evaluating the impact of gamma sterilization on calcium phosphates composites with different TiO2 nanomaterials

Radiation physics and chemistry

Ina Erceg[1]
Suzana Šegota[1], **Nadica Maltar-Strmečki**[1], Maja Dutour Sikirić[1]
[1] Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia

Introduction: Calcium phosphates (CaPs) composites with different TiO_2 nanomaterials (TiNMs) have recently attracted attention as promising implant materials for hard tissue regeneration. In this study, influence of TiO_2 nanoparticles (TiNPs) and titanate nanotubes (TiNTs) on CaPs spontaneous precipitation was investigated by AFM, SEM, FTIR, XRD and EPR.
Methods: EPR analyses were performed to monitor the possible difference in local structure after the formation of CaP in the presence of TiNPs and TiNTs. All results were compared with the control system. Before irradiation, no EPR signal was detected for any samples supporting the quality of synthesis. To monitor changes in the microenvironment, the radiation-induced centers were used. The samples were irradiated at 25 kGy, in order to simultaneously validate the suitability of the gold standard for gamma sterilization.
Results: In the presence of both TiNMs, at different concentrations, after one hour of reaction time CaDHA was the only formed crystalline phase. Experimental spectra of CaP formed in the presence of TiNPs and TiNTs and theoretical results show the same features and simulation parameters as control, indicating contributions of same type of paramagnetic center.
Conclusion: Therefore, it can be can concluded that the addition of the TiNPs and TiNTs does not induce changes in local structure of CaDHA. These findings are of importance for biomimetic preparation of CaP/TiNMs composites since they indicate that CaDHA composites with desired properties, could be prepared on different TiNMs without the need for a change of the experimental conditions.

The study was supported by Croatian Science Foundation, Grant HRZZ- IP-2018-01-1493.



P4 - Machine learning for automated assessment of in vitro T-47D colonies using principal component based watershed segmentation

Radiation physics and chemistry

Delmon Arous[1]
Stefan Schrunner[1], Ingunn Hanson[2], Nina J. Edin[2], Eirik Malinen[2]
[1] Department of medical physics, Oslo University Hospital, Oslo, Norway
[2] Department of physics, University of Oslo, Oslo, Norway

Introduction: For in vitro cell culture assays, manual identification of viable colonies is time-consuming with potentially large inter-observer variations. However, by optical imaging, dishes with stained colonies can be assessed digitally. Image processing of such pictured/scanned assays depends upon several factors like background noise, clustering of cells/colonies, variable staining and cell line specific characteristics.
Methods: A robust machine learning procedure is presented that circumvents these issues by characterizing, extracting and segmenting inquired colonies through implementation of three pillar techniques: principal component analysis, k-means clustering and a modified watershed segmentation algorithm, respectively. For this purpose, an imageset consisting of 16 cell culture flasks used for clonogenic assay of the T-47D (breast) cancer cell line was deployed. To validate the segmentation quality, automated colony count (ACC) delivered by the algorithm was compared to manual colony count (MCC) facilitated by 3 independent human observers. Additionally, an extra independent observer established a ground truth - manual counting during a microscopic analysis of the culture dishes.
Results: There was a high correspondence between the automated and manual count as the ACC obtained slightly lower F1 scores relative to the MCC, but the absolute ranges for both procedures were on a very high level (F1 score > 0.9) which underlines the ability of the algorithm to align with manual observers. Also, the algorithm produced a low relative error (< 10%) with a small tendency to underestimate the ground truth.
Conclusion: By agreement with ground truth data, the proposed method accurately maps cell colonies and produces a precise quantitative estimate of number and localization.

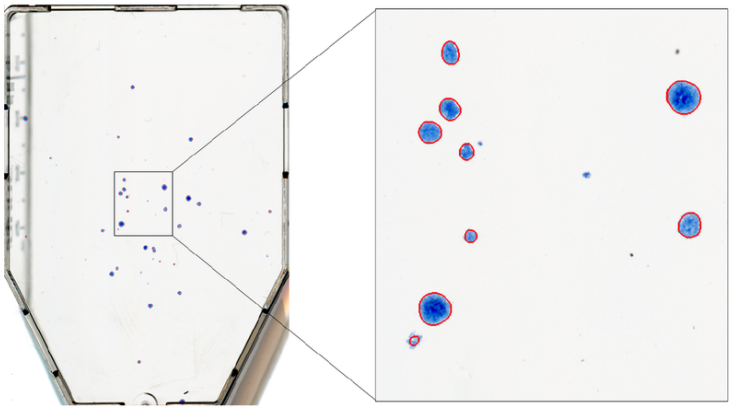


Fig. 1: Example image from the T-47D cell line. The segmentation suggested by our algorithm is outlined in red.

P5 - Modeling of the radiation situation in the rooms at BNCT research post using the MCNP code.

Radiation physics and chemistry

Martyna Araszkiewicz[1, 2]
Agnieszka Korgul[1], Kamila Maliszewska-Olejniczak[2], Katarzyna Tymińska[2], Grzegorz Wojtania[2]
[1] University of Warsaw, Faculty of Physics, Warsaw, Poland
[2] National Centre for Nuclear Research, Świerk, Poland

Introduction: Boron Neutron Capture Therapy (BNCT) consists of administering to the patient boron agents with a stable isotope ^{10}B that selectively accumulate in cancerous tissues. Then the tumor is irradiated with a beam of neutrons with epithermal or thermal energy. The capture of thermal neutrons by the ^{10}B nuclei results in the production of the high-energy α particle and ^{7}Li nucleus. The range of these particles is comparable with the size of a single cell. Thanks to this, only cancer cells are destroyed. In the National Centre for Nuclear Research in Świerk a research post for BNCT is created. The source of neutrons is the MARIA research reactor.
Methods: Before starting the research, it is necessary to assess the radiation situation in the rooms in the planned laboratory and to design radiation shielding to ensure safe working conditions. For this purpose, a three-dimensional model of rooms was created in the MCNP (Monte Carlo N-Particle Transport Code System) program which is used to analyze the transport of neutrons and photons.
Results: The calculations allows to determine the spatial distribution of neutron and photon flux in all research rooms. Additionally, thirteen cubic cells with an edge of 20 centimeters were defined in the most important spots in the laboratory. The cells played the role of ideal detectors in which the particle flux and the dose of ionizing radiation were calculated.
Conclusion: The calculated values will be compared with the results of planned measurements.

P6 - Up to speed – Real-time dosimetry for FLASH radiotherapy

Radiation physics and chemistry

Alessia Gasparini[1]
Piet Stevens[1], Giuseppe Felici[2], Emiliano D'Agostino[3], Dirk Verellen[1]
[1] GasthuisZusters Antwerpen/Iridium network, Antwerp, Belgium
[2] Sordina IORT Technologies, R&D Department, Aprilia, Italy
[3] DoseVue, Diepenbeek, Belgium

Introduction: FLASH Radiation Therapy (RT) is a recently introduced modality that could potentially modify radiation treatments. It uses ultra-high dose over very short time (above 40 Gy/s). Unexpectedly, FLASH RT increases the therapeutic window between tumour and healthy tissue, allowing the use of higher radiation doses to the tumour with the same normal tissue response.
Dosimetry for high dose-rates is not standard and highly challenging. Studies using an adapted LINAC for dose-rates up to 1050 Gy/s, compared alanine, Gafchromic films and thermoluminescent dosimeters[1]. The detection methods agreed within 3%, showing no dose-rate dependency. The most commonly used real-time detectors (ion chamber and semiconductors) fail to accurately measure dose at high dose-rates, due to saturation issues.
Methods: Scintillator detectors are possibly independent from saturation issues and have the potential to be used as real-time detectors for high dose-rate systems, calibrated against films or alanine. We tested the system DoseWire, a multi-channel scintillating optical fiber system from DoseVue. Current measurements have been performed on a LINAC dedicated for Intra-Operative electron RT (LIAC HWL, SIT), which employs high dose per pulse with low pulse frequency (PRF), corrisponding to dose-rates higher than 15 Gy/s at PRF of 400 Hz.
Results: DoseWire showed an agreement with the advanced Markus chamber up to 4.5 cGy/pulse at 12 MeV, with an accuracy of 5%.
Conclusion: Scintillation dosimeters, and specifically DoseWire, are promising candidates for real-time dosimetry with high dose per pulse. In the next step, the DoseWire system will be tested on the ELECTRONFLASH4000, the dedicated Flash electron unit SIT developed.
Selected references
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P7 - A lineage tracing tool to map the fate of hypoxic tumour cells

Molecular and cellular effects

Jonathan Ient[1]
Jenny Vermeer[2], Bostjan Markelc[2, 3], Jakob Kaeppler[2], Lydie Barbeau[1], Arjan Groot[1], Ruth Muschel[2], Marc Vooijs[1]
[1] Department of Department of Radiation Oncology (MAASTRO)/GROW - School for Oncology, Maastricht University Medical Center + , Maastricht, The Netherlands
[2] CRUK/MRC Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford
[3] Department of Experimental Oncology, Institute of Oncology Ljubljana, Slovenia

Introduction: Hypoxia is known to play a role in many types of cancer, and is linked to metastasis, genetic instability, resistance to therapy and poor prognosis. Hypoxic modification has been tested in clinical trials but results have been inconclusive. This is in part due to a knowledge gap into the characterization and behaviour of hypoxic tumour cells. In this study, hypoxic cells are lineage traced and they are analysed both *in vivo* by intravital imaging as well as by IHC to investigate their behaviour.
Methods: To explore this issue in a spatial and temporally-controlled manner we developed a genetically encoded sensor by fusing the O_2-labile Hypoxia-Inducible Factor 1α to eGFP and tamoxifen-regulated Cre recombinase. Under normoxic conditions HIF-1α is degraded but under hypoxia, the HIF-1α-GFP-Cre-ER^{T2} fusion protein is stabilised and in the presence of tamoxifen activates a tdTomato reporter gene that is constitutively expressed in hypoxic progeny.
Results: We visualise the random distribution of hypoxic tumour cells from hypoxic or necrotic regions and vascularised areas using immunofluorescence and intravital microscopy. Using this system, we could show that the post-hypoxic cells were more proliferative *in vivo* than non-labelled cells.
Conclusion: Our results demonstrate that single-cell lineage tracing of hypoxic tumour cells can allow visualisation of their behaviour in living tumours using intravital microscopy. Furthermore we are developing a model that allows selective ablation of labelled hypoxic cells; to investigate their role in treatment resistance further. These tools should prove valuable to study dissemination and treatment response of post-hypoxic tumour cells *in vivo* at single-cell resolution.

P8 - A novel EdU-based protocol for the investigation of cell cycle kinetics of irradiated human lymphocytes

Molecular and cellular effects

Evi Duthoo[1, 2]
Anne Vral[1, 2], Ans Baeyens[1, 2]
[1] Radiobiology group, Department of Human Structure and Repair, Ghent University, Ghent, Belgium
[2] Cancer Research Institute Ghent, Ghent, Belgium

Introduction: In radiation biology, gaining insights into the effects of irradiation on the proliferation and kinetics of cells is imperative. A powerful tool to accurately quantify cell cycle kinetics is the use of nucleoside analogues, such as 5-ethynyl-2'-deoxyuridine (EdU). EdU is a thymidine analogue that can be incorporated into DNA during replication. Via bivariate analysis of cellular DNA content and EdU incorporation, a distinction between cell cycle phases can be made. By performing a time-lapse analysis of EdU pulse-labeled cells, kinetics of the cell cycle can be investigated. Lymphocytes are widely used in radiation research. However, knowledge of the effects of radiation on their cell cycle kinetics is still limited. In this study, we optimized an EdU-labeling protocol on whole blood cultures to study the cell cycle progression of irradiated human lymphocytes.
Methods: Whole blood was cultured and cell division was stimulated by the addition of phytohaemagglutinin (PHA). After 4 days of culture, the cells were pulse-labeled with EdU and irradiated *in vitro* with 1, 2 and 4 Gy of 220 kV X-rays. Time-lapse analysis was performed from 0 up to 25 hours of incubation, with one-hour intervals. After counterstaining with DAPI to measure DNA content, the cells were analyzed by flow cytometry.
Results: G2-arrest after irradiation of the lymphocytes could be detected with our EdU-based cell cycle analysis protocol. The length of the G2 arrest depends on the irradiation dose.
Conclusion: We propose a novel protocol of EdU-based cell cycle analysis to determine the proliferation kinetics of human lymphocytes in whole blood cultures.

P9 - A Raman spectroscopy-based alternative approach for the analysis of X-ray irradiated SH-SY5Y human neuroblastoma cells

Molecular and cellular effects

Valerio Ricciardi[1, 2]
Ines Delfino[3], Lorenzo Manti[2, 4], Giuseppe Perna[5, 6], Maria Lasalvia[5, 6], Vito Capozzi[5, 6], Maria Lepore[7]
[1] Dipartimento di Matematica e Fisica, Università degli Studi della Campania Luigi Vanvitelli, 81100, Caserta (CE), Italy
[2] Istituto Nazionale di Fisica Nucleare - Sezione di Napoli, 80126, Naples (NA), Italy
[3] Dipartimento di Scienze Ecologiche e Biologiche, Università degli Studi della Tuscia, 01100, Viterbo (VT), Italy
[4] Dipartimento di Fisica E. Pancini, Università degli Studi di Napoli Federico II, 80126, Napoli (NA), Italy
[5] Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Foggia,71122, Foggia (FG), Italy
[6] Istituto Nazionale di Fisica Nucleare - Sezione di Bari, 70126 Bari (BA), Italy
[7] Dipartimento di Medicina Sperimentale, Università degli Studi della Campania Luigi Vanvitelli, 80138 Napoli (NA), Italy

Introduction: Raman spectra of cytoplasm and nucleus region of single cells indicate differential changes induced by X-rays as for DNA/RNA, lipid, and protein contributions [1,2]. The present work aims to introduce a new approach to deepen radiobiological investigation on single SH-SY5Y human neuroblastoma cells. To this end, the difference spectra obtained by subtracting each cytoplasm-related Raman spectrum from the corresponding one acquired for the nucleus were examined using multivariate analysis.
Methods: Raman micro-spectroscopy was performed on in-vitro single cells after irradiation by graded X-ray doses (2, 4, 6, 8 Gy). Spectra from nucleus and cytoplasm regions were acquired and the difference spectra were examined by interval Principal Component Analysis (i-PCA).
Results: The analysis by i-PCA of the nucleus-cytoplasm difference spectra allowed us to shed light on the modifications, due to X-ray irradiation, of Raman features due to components with different relative contents in the two regions or modes with a low intensity that are not appreciable in the simple spectra [3]. In particular, the effects on DNA/RNA backbone and single nucleobases and those occurring on lipids and proteins are discussed.
Conclusion: The proposed approach allowed us to highlight newly identified specific characteristics not previously reported**.**
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P10 - Bi-modal treatment using radiation therapy and drug delivery nano-systems for enhanced cytotoxicity in radio-resistant tumor models

Molecular and cellular effects

Roxana-Cristina Popescu[1, 2, 3]
Mihai Straticiuc[4], Cosmin Catalin Mustaciosu[1, 2], Roxana Trusca[2], Hiltraud Hosser[5], Carsten Herskind[3], Mihai Radu[1], Marlon R. Veldwijk[3], Diana Iulia Savu[1]
[1] "Horia Hulubei" National Institute for Research and Development in Physics and Nuclear Engineering, Department of Life and Environmental Physics
[2] University Politehnica of Bucharest, Department of Science and Engineering of Oxide Materials and Nanomaterials
[3] University of Heidelberg, Medical Faculty Mannheim, Universitätsmedizin Mannheim, Department of Radiation Oncology
[4] "Horia Hulubei" National Institute for Research and Development in Physics and Nuclear Engineering, Department of Applied Nuclear Physics
[5] University of Heidelberg, Medical Faculty Mannheim, Universitätsmedizin Mannheim, Center for Biomedicine and Medical Technology, Department of Anatomy and Developmental Biology

Introduction: This study aims to investigate whether ionizing radiation combined with doxorubicin-iron oxide nano-systems (DOX-NP) improves the cytotoxic effects of the nano-carrier-mediated drug delivery in radio-resistant tumor models (MG-63, human osteosarcoma and respectively HeLa, human cervical adenocarcinoma).
Methods: For this, two approaches have been investigated: the application of nanoparticles (NP) before irradiation and respectively after.
Results: The nanoparticle internalization was evaluated qualitatively through fluorescence and electron microscopy emphasizing efficient loading through macro-pinocytosis and localization in the peri-nuclear area. Quantitative measurements were performed using Particle Induced X-Ray Emission showing that previous exposure to ionizing radiation significantly improved the internalization of the nanoparticles. Cells that underwent irradiation and NP treatment proved a statistically significant reduction in their clonogenic survival. 100 μg/mL DOX- free NP enhanced the radio-sensitivity of 50 kV X-Rays with a $DMF_{SF0.1}$= 1.13±0.06, but no additional effect to 6 MV X-Rays, while DOX-NP resulted in a $DMF_{SF0.1}$=1.3±0.1 at 6 MV X-Ray. Genotoxicity evaluation showed that DNA breaks increased with NP concentration and irradiation at 48h in the case of radiation treatment followed by NP exposure. Initial NP incubation followed by radiation showed that the enhanced radio-modulatory effect of the NP was not linked to the induction of DNA double strand breaks in tumor cells.
Conclusion: These results conclude that DOX-NP are good candidates for the controlled delivery of DOX to enhance the cytotoxic effects of ionizing radiation.

P11 - Causes and Conseqences of telomere instability

Molecular and cellular effects

Cecile Herate[1]
Laure Sabatier[1]
[1] PROCyTox, French Alternative Energies and Atomic Energy Commission (CEA), Paris-Saclay University, Fontenay-aux-Roses, France.

Introduction: Telomeres control chromosomal integrity and stability by forming a protective structure that avoids the signaling of DNA damage. By shortening with each cell division, they act as a lifeguard, driving aged and damaged cells towards death. Telomeres are composed of DNA repeat sequences at the ends of chromosomes that recruit a multitude of proteins to form a complex loop structure at each extremity. The integrity of this structure is critical and correct conformation of the loop is essential for the protection of chromosome ends from DDR signaling. Many external factors, such as irradiation, cellular stress, trigger cell-cycle dysfunction and, in some cases, enable the survival of cells with threateningly short telomeres.
Methods: The genome instability generated by telomere dysfunction mostly promotes cell death. Destabilized loops at chromosome ends can then lead to dramatic consequences, by a butterfly effect such as multiple chromosomal fusions and rearrangements causing large chromosomal deletions, XXL-LOH, the expression of recessive mutations, and potential cell transformation.
Results: Carcinogenesis due to multiple Telo-LOH is still an exceptional event, with dramatic consequences although telomere insults are frequent. A recent study from our lab showed telomeres to be hypersensitive to DNA damage, which is over-signaled as DSBs by γ-H2AX foci, even after DNA repair [Ricoul et al, 2019]. Moreover, subtelomeric regions also show elevated sensitivity to DNA DSBs.
Conclusion: These observations support the hypothesis that IR causes irreversible damage and stress, specifically at the telomere regions, that can still be detected long after IR.
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P12 - Cell-cycle perturbation, atypical mitosis and micronuclei in Caco-2 cells as indicator of radiation-induced genomic instability

Molecular and cellular effects

Isabella Guardamagna[1]
Leonardo Lonati[1], Andrea Ottolenghi[1], Monica Savio[2], Lucia Anna Stivala[2], Giorgio Baiocco[1]
[1] Radiation Biophysics and Radiobiology Group (RadBioPhys), Physics Department, University of Pavia, Pavia, Italy
[2] Immunology and General Pathology Unit, Department of Molecular Medicine, University of Pavia, Pavia, Italy

Introduction: We have started an extensive experimental characterization of the response to X-rays of Caco-2: this cell line is derived from human colorectal adenocarcinoma, usually adopted as intestinal barrier model and recently characterized as radioresistant (1, 2). Colorectal cancer is the fourth most common cancer worldwide, commonly treated with radiotherapy and chemotherapy before surgery or as adjuvant therapy following surgery.
Methods: Studied endpoints include: cell survival, cell cycle distribution, necrosis and apoptosis, micronuclei and atypical mitosis. Combined techniques of flow-cytometry and immunofluorescence microscopy were used.
Results: The clonogenic assay confirmed a radioresistant behaviour of Caco-2 up to 5 Gy. Cells exposed to 10 Gy showed an initial ability to proliferate, creating small colonies that can be observed only for a period of about a week. Flow-cytometry analysis showed dose- and time-dependent modulation of cell-cycle distribution and activation of death mechanisms. Cells showed an increase in atypical mitosis (**Figure**) with increasing dose, at 48 hours after irradiation. Micronuclei formation was found to increase up to 5 Gy, while at 10 Gy we were not able to distinguish micronuclei from early necrosis events (coherently with results from the clonogenic assay and flow-cytometry measurements).
Conclusion: The integration of results from different endpoints allows to achieve a more detailed picture of radiation effects to Caco-2 cells, both in terms of survival and consequences leading to genomic instability as suggested by induction of atypical mitosis and micronuclei (3). Knowledge acquired in this experimental campaign can be exploited in perspective to address the effectiveness of different therapeutic strategies.
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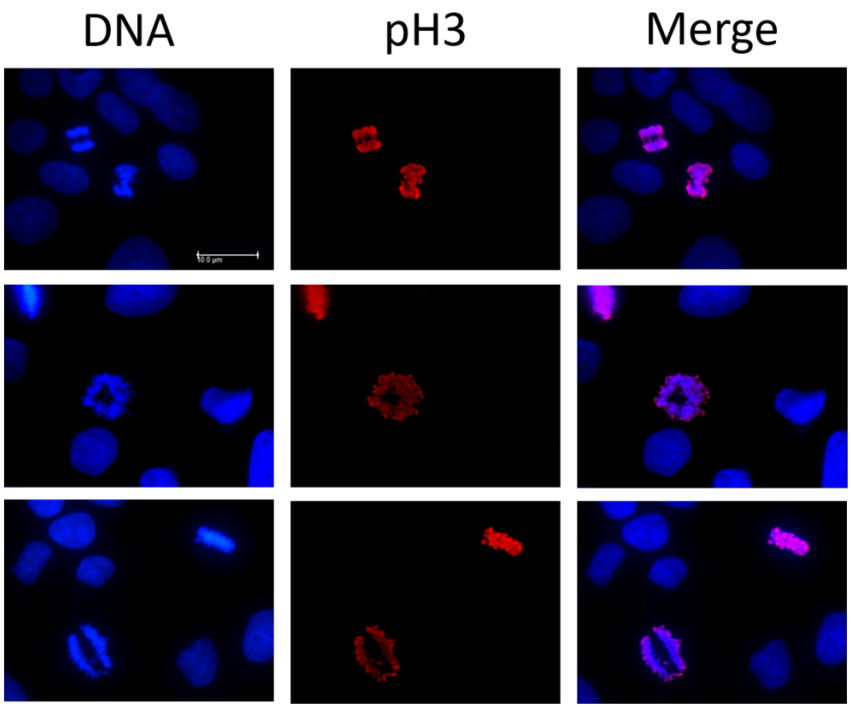

Representative images of atypical mitotic figures of Caco-2 cells. DNA is marked with Hoechst dye (blue fluorescence) and phospho-H3 (Ser 10) is a marker of mitosis (red fluorescence). Scale bar 10 µm.

P13 - Cytotoxicity study of peptide receptor radionuclide therapy using [177Lu]Lu-DOTA-TATE for the treatment of neuroendocrine tumours

Molecular and cellular effects

Lorain Geenen[1, 2]
Sarah Baatout[1, 3], Julie Nonnekens[4], Marion De Jong[2], An Aerts[1]
[1] Radiobiology Unit, Interdisciplinary Biosciences (BIO), Institute for Environment, Health and Safety, SCK CEN | Belgian Nuclear Research Centre, Mol, Belgium
[2] Department of Radiology and Nuclear Medicine, Erasmus MC, Rotterdam, The Netherlands
[3] Department of Molecular Biotechnology, Ghent University, Ghent, Belgium
[4] Department of Molecular Genetics, Department of Radiology and Nuclear Medicine, Oncode Institute, Erasmus MC, Rotterdam, The Netherlands

Introduction: Radiopharmaceuticals for peptide receptor radionuclide therapy (PRRT) consist of a cancer-seeking molecule labeled with a radionuclide to deliver therapeutic doses of ionizing radiation directly to the cancer sites, both the primary tumour and metastatic lesions. This cancer treatment modality holds promise to be more effective and to reduce the detrimental effects on the healthy tissues compared to external beam radiotherapy. However, much of the radiobiology is not fully investigated for radionuclide therapy and (long term) cytotoxic effects are yet not well understood. Therefore, in this project we aim to obtain a better understanding of the cellular and molecular mechanisms underlying the cytotoxic responses of PRRT. We will focus on the radionuclide [^{177}Lu]lutetium coupled to the somatostatin analogue DOTA-TATE ([^{177}Lu]Lu-DOTA-TATE) for the treatment of neuroendocrine tumours, which have overexpression of the somatostatin receptor.
Methods: Currently we are conducting in vitro studies in neuroendocrine tumour cell models (AR42J and CA20948) as well as normal human kidney (HK-2), liver (THLE-2) and microvascular endothelial (TIME) cells. The expression of somatostatin receptor type 2 (SSTR2) is being investigated by western blot analysis. Furthermore radiolabeling studies have been performed and the binding capacity of the radiopeptide will be evaluated for the different cell lines, followed by the comparison of the membrane-bound fractions and internalized fractions. In complement we are optimizing different models for the determination of cell viability, clonogenic survival and apoptosis after incubation with [^{177}Lu]Lu-DOTA-TATE.
Results:
Conclusion: This way we hope to contribute to the optimization of PRRT by further reducing side effects while maintaining efficient tumour targeting.
Selected references

P14 - Deep learning-based approach for the segmentation of human carcinoma cells

Molecular and cellular effects

Bahareh Omidali[1]
Alexey Sofiev[1], **Tuomas Kaseva**[2], Eero Hippeläinen[3], Vappu Reijonen[4], Ulla Wilppu[2], Marjo Yliperttula[5], Sauli Savolainen[1], Eero Salli[2]
[1] Department of Physics, University of Helsinki, POB 64, FI-00014 Helsinki, Finland.
[2] HUS Diagnostic center, Radiology, University of Helsinki and Helsinki University Hospital, POB 340, FI-00290 Helsinki, Finland.
[3] HUS Diagnostic center, Clinical Physiology and Nuclear Medicine, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[4] Comprehensive Cancer Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[5] Centre for Drug Research, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Finland.

Introduction: Assessment of absorbed radiation dose at cellular level is believed to be a crucial part of optimizing molecular radionuclide therapy. In that process three-dimensional cell segmentation is a predominant step. The segmentation can also be utilized in analyzing morphological features of cells and tissues efficiently. Although a variety of different algorithms exists for the automated segmentation of the cells, they are often specialized to certain cell types and may fail in conditions that are more adverse.
Methods: In this study, we propose a novel deep learning-based method for three-dimensional cell segmentation. The method is trained and evaluated with 12 human hepatocellular carcinoma HepG2 spheroids, which consist of densely distributed and arbitrary shaped and sized cells. Each spheroid has been manually segmented to obtain ground truth labels. In the method, the neural network is first applied for the segmentation of axial slices of the chosen spheroid, and then these slices are combined to form a final 3D-segmentation
Results: The method is validated using SEG-score and compared with multiple different well-established cell segmentation algorithms. In the experiments, we split our dataset to 11 training spheroids and to one evaluation spheroid. In this preliminary setting, our method outperforms the reference algorithms and reaches 0.55 SEG-score
Conclusion: We developed and validated a promising deep-learning based method for advanced 3D cell segmentation
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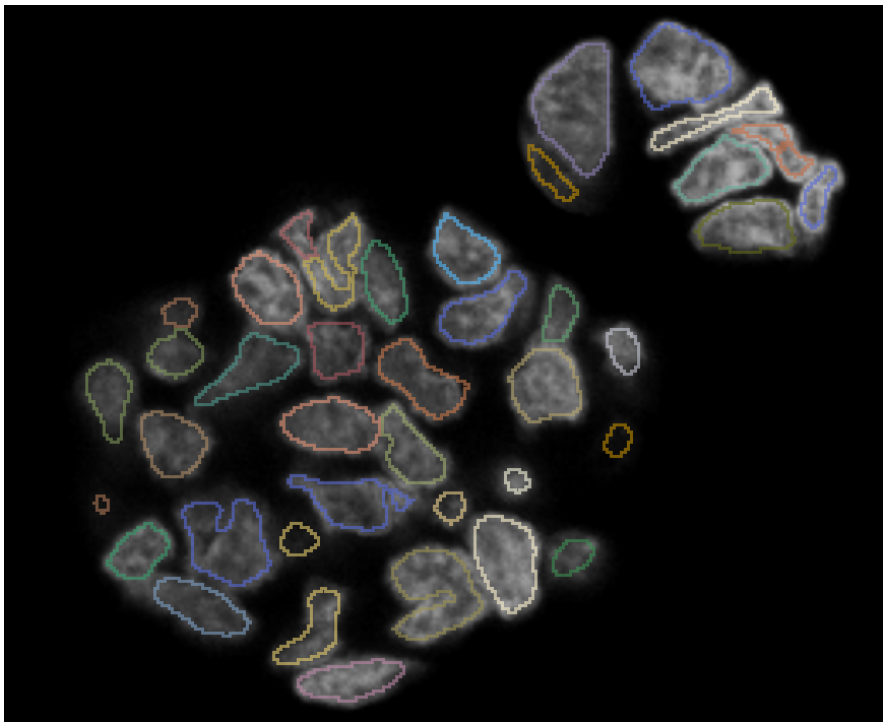

P15 - Ephrin receptors take part in cellular DNA damage response after ionizing radiation and regulate cell viability of non-small lung cancer cells.

Molecular and cellular effects

Vitaliy Kaminskyy[1]
Petra Hååg[1], Metka Novak[1], Vasiliki Arapi[1], Rolf Lewensohn[1, 2], Kristina Viktorsson[1]
[1] Karolinska Institutet, Department of Oncology/Pathology, SE-171 76, Stockholm, Sweden.
[2] Theme Cancer, Patient area head and neck, lung, and skin, Karolinska University Hospital, Stockholm, Sweden.

Introduction: Receptor tyrosine kinases (RTK) regulate various pro-survival pathways in tumor cells. Besides the conventional role of RTK in transduction of kinase signaling via ligand-receptor interactions, understanding of their other functions may help us to reveal novel targets to overcome therapy resistance. Thus, besides a well-known function of EGFR as a tyrosine kinase, this receptor has been reported to be engaged in DNA damage repair (DDR) upon cellular response to ionizing radiation (IR) via its interaction with DNA-PK[1]. Ephrin family receptors is the largest family of RTK that regulate cell proliferation, migration, invasion and angiogenesis in tumors. Recent reports have demonstrated the involvement of EphA5 receptor in DDR via its accumulation at DNA foci and interaction with ATM[2].
Methods: Immunoprecipitation, cell fractionation, mass spectrometry, gene silencing, immunoblotting
Results: We demonstrate that some Ephrin family members correlate to some extent with IR sensitivity of non-small cell lung cancer cells(NSCLC). By gene silencing we show that targeting the EphA2 receptor sensitizes NSCLC to IR and reduces clonogenic potential. Furthermore, we demonstrate that the EphA2 receptor is partially localized in nuclei of NSCLC cells either prior or post IR. Using immunoprecipitation of EphA2 from nuclear extracts and mass spectrometry analysis, we identified components of DDR signaling that suggest EphA2 to be linked to the cellular DDR response.
Conclusion: Taking into account our data with responsiveness to IR and previous reports showing upregulation of some Ephrins in various tumor malignancies, understanding their role in DDR can help us to explore significance of targeting these receptors in cancer therapy.
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P16 - Experimental database for research on low dose hyper-radiosensitivity and induced radioresistance

Molecular and cellular effects

Szabolcs Polgár[1]
Balázs Madas[1]
[1] Radiation Biophysics Group, Centre for Energy Research, Budapest, Hungary

Introduction: One of the most frequently applied models describing low-dose hyper radiosensitivity (HRS) and induced radioresistance (IRR) is the induced repair (IR) model. While the model usually fits graphically well to experimental data, its parameters as well as the numerical values of experimental data are not given in many publications. The aim of this study was to set up a database of experimental data and compare it with the IR model.
Methods: From 38 articles, we collected 90 data sets i) showing low dose HRS, ii) containing readable data points, and iii) providing error bars. The publication date varied between 1993 and 2018. Besides the data points with the error bars, radiation parameters, the cell type, and parameters of the fitted IR or LQ model (if given) were also recorded in the database.
Results: We found that the IR model parameters were given for 53 datasets from 90. Fitting again the IR model to the collected datasets and comparing the results with the parameters given in the papers, we found a similarly good fit in 39 cases. In 14 cases, however, we could not reproduce the same results.
Conclusion: In the future, the database can be used for additional tests of the IR model or other mathematical models aiming to describe low dose HRS.

P17 - Gene expression during radiation-induced differentiation of human fibroblasts in vitro

Molecular and cellular effects

Carsten Herskind[1]
Carsten Sticht[2], Ahmad Sami[1], Frank A. Giordano[1, 3], Frederik Wenz[1, 4]
[1] Dept. of Radiation Oncology, Universitaetsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Germany
[2] Centre for Medical Research, Universitaetsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Germany
[3] Dept. of Radiation Oncology, University Medical Centre Bonn, Faculty of Medicine, University of Bonn, Germany
[4] University Medical Centre, Freiburg, Germany

Introduction: Fibroblasts play a major role in radiation-induced fibrosis. However, most studies on radiation-induced gene expression in fibroblasts have focused on early time points (0-24h). The purpose was to study differential gene expression during radiation-induced fibroblast differentiation *in vitro*.
Methods: The fibroblast phenotype of three skin fibroblast strains (GS3-5) was determined in the colony formation assay, and the myofibroblast marker α-smooth muscle actin was detected by immunofluorescence microscopy. Differential gene expression was analysed on microarrays and validated by real-time qRT-PCR. Pathway analysis was performed using Reactome. 6 MV X-rays were used for irradiation.
Results: 163 genes were up- and 253 down-regulated by >4-fold in GS4 at least once on day 2, 3 or 5 after irradiation with 4 Gy. The upregulated extracellular matrix (ECM) gene, *COL11A1*, showed maximum expression on day 5. Several of the down-regulated genes were related to cell division and cell-cycle progression. 216/268 downregulated pathways were common to all three time points with more than a third relating to the cell cycle. 45/106 upregulated pathways were common to all time points with ~60% relating to ECM proteins or glycosaminoglycans. Independent microarray experiments for day 3, showed 243/319 down-regulated and 59/133 up-regulated pathways common to all three strains. Up-regulated pathways were dominated by ECM synthesis and processing, and inflammatory responses. Independent real-time PCR on day 1-6 confirmed upregulation of several collagen genes, *ACTA2*, and signalling genes, and downregulation of a matrix metalloproteinase, *MMP12*.
Conclusion: Differential gene expression reflected premature differentiation of fibroblasts and represented aspects of inflammation, wound healing and fibrogenesis.

P18 - Identifying the cellular response to complex DNA damage induced by high-LET protons

Molecular and cellular effects

Rachel Carter[1]
Catherine Nickson[1], Andrzej Kacperek[2], Mark Hill[3], **Jason Parsons**[1, 2]
[1] Cancer Research Centre, University of Liverpool, UK
[2] The National Eye Proton Therapy Centre, Clatterbridge Cancer Centre, Bebington, UK
[3] CRUK/MRC Oxford Institute for Radiation Oncology, University of Oxford, UK

Introduction: Proton beam therapy (PBT) is a precise and effective cancer treatment that is increasingly being used worldwide. However, the radiobiology of PBT is not entirely understood due to increases in linear energy transfer (LET) at and around the Bragg peak where the radiation dose is deposited [1]. Here, there are increases in formation of complex DNA damage (CDD), containing multiple DNA lesions within close proximity, that can significantly contribute to cell death. The cellular response to CDD, however, is not entirely understood.
Methods: Utilising the 60 MeV cyclotron at the Clatterbridge Cancer Centre, we have analysed clonogenic survival of HeLa and head and neck cancer cells following low-LET (1 keV/µm) protons at beam entrance, versus high-LET (12 keV/µm) protons at the Bragg peak distal end. Data has been correlated with high-LET α-particles and low-LET x-rays. CDD repair was monitored using an enzyme-modified neutral comet assay. siRNA screening has also been used to identify enzymes crucial for cell survival post-irradiation.
Results: We have demonstrated that high-LET protons generate increased amounts of CDD triggering a specific cellular DNA damage response [2]. siRNA screening has identified that USP6 is essential for maintaining cell survival and cell cycle progression following high-LET protons (and α-particles), mediated through stabilisation of PARP-1 required for efficient CDD repair [3]. Further preliminary screening experiments have also exposed other key DNA repair proteins and pathways important for cell survival following high-LET protons.
Conclusion: CDD induced by high-LET protons is repaired through a specific DNA damage response mechanism that promotes cell survival.
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P19 - Increased immunogenic signaling in terms of calreticulin expression after x-ray irradiation

Molecular and cellular effects

Anne Marit Rykkelid[1]
Martine Ruud[1], Adrian Eek Mariampillai[2], Randi Syljuåsen[2], Eirik Malinen[1], Nina Jeppesen Edin[1]
[1] University of Oslo
[2] Oslo University Hospital

Introduction: Immune checkpoint inhibitors have emerged as an efficient cancer treatment for some patients, but the effect appears to depend on a cancer specific immunogenic response. Such a response is associated with damage associated molecular patterns (DAMPs). One such DAMP is calreticulin, a protein that is translocated from the cytoplasm to the cell surface membrane as a response to cellular damage in a process called immunogenic cell death (ICD). Here, we investigate the effect of ICD induced by x-ray irradiation on calreticulin expression in lung cancer cells.
Methods: 220kV x-rays were used for irradiation of A549 lung cancer cells with different doses in one or two fractions. The cells were analyzed for calreticulin expression 48H post-irradiation using flow cytometry. Barcoded controls were added to irradiated samples. A fluorescence metric was calculated, taking unspecific binding of secondary antibody and background control levels into account.
Results: A significant increase in calreticulin expression was found for all doses tested (2-12 Gy). The increase seemed to stabilize at two levels with a single dose threshold between 4 and 6 Gy. For single doses of 4 Gy and lower a fold change of about 1.5 was found independent of number of fractions. For single doses above 4 Gy a fold change of about 2 was found, independent on number of fractions or further increase in dose.
Conclusion: This indicates that the eco-calreticulin may have a threshold dose for efficient activation, and that a further increase in dose does not influence the strength of the signal.

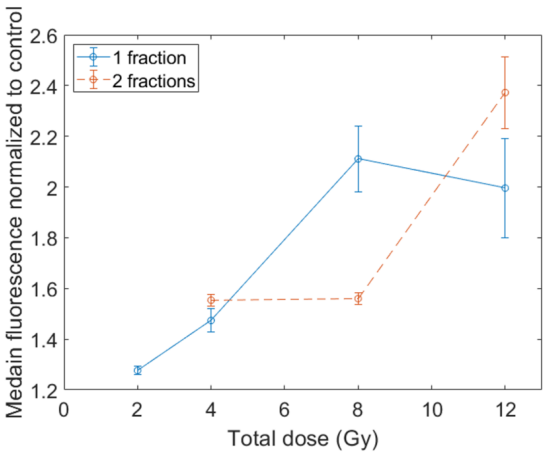


Fig. 1 Calreticulin expression in A549 cells 48H after x-ray irradiation. Signal was found as median fluorescence corrected for unspecific binding and normalized to control.

P20 - Ionizing radiation, psychological stress, and microgravity in space: hind limb unloading animal model in mice

Molecular and cellular effects

Bjorn Baselet[1]
Mieke Neefs[1], Sarah Baatout[1]
[1] Radiobiology unit, Interdisciplinary biosciences, Institute for environment, health and safety, SCK CEN, Mol, Belgium

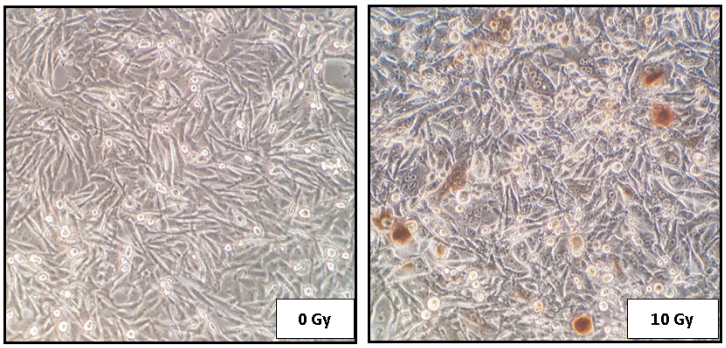
Introduction: Space travel comprises a unique and complex stress model composed of physical (i.e. radiation and microgravity) and psychological stressors. These extreme conditions can induce specific responses in the human body that will ultimately affect several organ systems. The precise nature of these health effects is not completely understood, and multiple underlying causes might be involved. In view of future interplanetary travel, studies onboard the International Space station (ISS) will help to answer many critical questions. However, due to financial and technical restrictions of these flight experiments, ground-based analogues are required for researchers to test theories without launching experiments into space.
Methods: The Radiobiology Unit of SCK•CEN has implemented multiple ground-based *in vitro* and *in vivo* experiments using space flight analogues, like the hind limb unloading (HLU) model in mice. This rodent ground-based analog model was developed to study mechanisms, responses, and treatments for the adverse consequences of microgravity conditions during spaceflight.
Results: Currently, we are looking into the combined effects of psychological stress, ionizing radiation and microgravity on a multitude of organ systems, such as the eyes, the bones, the muscles and the immune organs.
Conclusion: The HLU model in mice as a rodent ground-based analog model has been optimized. It will serve it's use to gain insights into the mechanisms, responses, and treatments for the adverse consequences of microgravity conditions in combination with ionizing radiation exposure and psychologucal stress during spaceflight.

P21 - Melanin production and radiobiological features of mucosal melanoma cells

Molecular and cellular effects

Angelica Facoetti[1]
Amelia Barcellini[1], Federica Carnevale[1], Alexandra Charalampopoulou[1], Mario Ciocca[1], Michele Ferrarini[1], Giovanni Battista Ivaldi[2], Marianna La Spada[1], Marco Liotta[2], Ester Orlandi[1], Marco Pullia[1], Paola Tabarelli de Fatis[2], Francesca Valvo[1]
[1] National Center for Oncological Hadrontherapy, Fondazione CNAO, Pavia, Italy
[2] I.C.S. Maugeri, Pavia, Italy

Introduction: Primary vaginal malignant mucosal melanoma is a rare and highly malignant disease intrinsically chemo- and radio-resistant.
Methods: In this study we evaluated morphology, melanin production and migration capacity of HMVII cells exposed to 6MV photons beams with doses between 0.5 and 10 Gy. Cells were observed under a phase contrast microscope and images captured at 2, 22 and 44 h.
Results: 22 hours after photon radiation the dendrite number and total dendrite length increased, particularly in samples irradiated with 8 or 10 Gy. Interestingly, we observed the appearance of brown cells from the second day after irradiation. The maximum number of brown cells was reached 12 days after 10 Gy-irradiation (Figure below). Subsequently the melanin enriched cells gradually decreased. These morphological observations were confirmed also by the pellets: higher doses corresponded to darker pellets. Then the influence of irradiation on the migration capability was evaluated by scratch assay: irradiation with either 2 or 4 Gy was able to increase the efficiency with which these cells restore the monolayer. This effect was dependent on the dose of radiation: the higher the dose, the greater the efficacy.
Conclusion: The formation of new dendrites together with the enhancement in melanin enriched cells are in agreement with the fact that melanocyte dendrites serve as the principal conduit for melanosome transfer. It is known that melanin protects normal melanocytes from ultraviolet radiation and oxidative stress, this is the first time that an induction of melanogenesis as protective mechanism after exposure to ionizing radiation was demonstrated in human cells.



Representative images of HMVII cells 12 days post exposure to 0 or 10 Gy of photons. Melanin enriched cells are clearly visible in irradiated samples (Magnification 200X).

P22 - Microbeam radiation therapy shows a sparing effect in normal tissue cells

Molecular and cellular effects

Sandra Bicher[1, 2]
Leo Schröpfer[3], Stephanie E. Combs[1, 2], Thomas E. Schmid[1, 2], Stefan H. Bartzsch[1, 2]
[1] Institute of Radiation Medicine, Helmholtz Zentrum München, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
[2] Department of Radiation Oncology, School of Medicine, Technical University Munich (TUM), Ismaninger Str. 22, 81675 Munich, Germany
[3] Department of Mechatronics and Medical Engineering, Ulm University of Applied Sciences, Albert-Einstein-Allee 55, 90091 Ulm, Germany

Introduction: Microbeam radiation therapy (MRT) is an innovative method in radiation oncology that delivers high doses in spatial fractions. This approach divides the radiation field into peak-regions of 25-50 µm width, receiving doses of up to several hundred Gy and valley-regions of 200-800 µm width, receiving doses below the tissue tolerance. Up to date, the biological mechanism behind the differential response to MRT in tumor and normal tissue cells is poorly understood. It is hypothesized that bystander signaling or a faster DNA damage repair plays a critical role.
Methods: Human tumor and normal tissue cell lines, A549 and MRC5 were irradiated using broad beam radiation (BB) or MRT. Clonogenic cell survival and DNA repair were analyzed after MRT and BB irradiation in both cell lines.
Results: There was no significant difference in the cell survival after MRT for the lung adenocarcinoma cells A549 compared to BB. However, the lung fibroblasts MRC5 showed an increased survival of 15.6% ± 0.9% at 4 Gy MRT compared to 2.2% ± 0.9% at 4 Gy BB ($p = 0.0137$). These data correlate well with DNA double strand breaks in the yH2AX assay. In MRC5 MRT produced 42% less residual DNA damage than BB irradiation.
Conclusion: Our results evidence a normal tissue sparing after MRT *in-vitro*. MRT caused less DNA double strand breaks and increased the survival of normal tissue cells compared to BB, whilst achieving equal tumor cell killing at equivalent dose levels. The wider therapeutic window makes MRT a promising novel radiotherapy approach.

P23 - Mitochondria nucleus communication is involved in DNA damage response following exposure to genotoxic stress

Molecular and cellular effects

Mihaela Temelie[1]
Rubab Talpur[2], Nicoleta Moisoi[2], Diana Savu[1]
[1] National Institute of Physics and Nuclear Engineering "Horia Hulubei", Magurele, Romania
[2] Leicester School of Pharmacy, Faculty of Health Sciences, De Montfort University, Leicester, United Kingdom

Introduction: Cellular ageing is known as a process associated with persistent DNA damage, besides well-known mitochondrial damage and accumulation of dysfunctional proteins. Few very recent studies are focused on connection of mitochondrial pathways with DNA damage response (DDR) in neurodegeneration. Radiation effects on CNS are mainly studied in the context of space radiobiology, where the main component of radiation is represented by charged particles – protons or heavy ions. Proton radiation was found as a candidate for rapid aging/neurodegeneration induction. HtrA2 is a mitochondrial serine-protease that induces expression of transcription factor CHOP, specifically in the brain, leading to upregulation of components of the integrated stress response.

Our aim was to evaluate how mitochondria-nucleus communication operates in mammalian cells in the context of mitochondrial / genotoxic stress.
Methods: We used Mouse Embrionary Fibroblasts (MEF) obtained from Wild-type (WT) mice and mitochondrial dysfunctional genetically modified mice – HtrA2 Knock Out (KO), CHOP KO, or double KO. Genotoxicity was induced by physical (X-rays, proton beam) and chemical factors (bleomycin - BLM).
Results: We proved exacerbated sensibility to all DNA-damaging factors in CHOP KO and HtrA2/CHOP KO cells. Protons exposure exhibited a slightly higher genotoxic effect in all cell lines. CHOP KO MEFs cells proved to be more sensitive to DNA damage, independently of HtrA2. Moreover, altered genotypes interfered with induction of molecular markers of compartmental stress responses.
Conclusion: Our study showed therefore that mitochondrial signaling pathways of HtrA2/CHOP are involved in DDR following exposure to physical and chemical genotoxic agents.

P24 - On to the molecular mechanisms of therapeutic and toxic responses of prostate cancer targeted radionuclide therapy

Molecular and cellular effects

Nathalie Heynickx[1, 2]
Sarah Baatout[1, 2], An Aerts[1]
[1] Radiobiology Unit; Interdisciplinary Biosciences; Institute for Environment, Health and Safety; Belgian Nuclear Research Centre (SCK•CEN), Mol, Belgium
[2] Department of Molecular Biotechnology; Ghent University, Ghent, Belgium

Introduction: Each year, worldwide, over 1 million men are diagnosed with prostate cancer, which is the second leading cause of cancer related death in men. Prostate specific membrane antigen (PSMA) is overexpressed in prostate cancer. Due to this overexpression, PSMA targeted radionuclide therapy is emerging as a treatment option for patients with advanced disease.
In PSMA targeted radionuclide therapy, a PSMA-targeting molecule is labeled with a radionuclide to specifically target prostate cancer cells. Clinical trials using the beta-emitter ^{177}Lutetium (^{177}Lu) showed promising results. However, approximately 30% of patients either showed no response or relapsed after treatment. Mechanisms underlying this radioresistance remain unclear and need to be investigated. Alpha-emitting particles are currently being investigated for targeted alpha therapy of prostate cancer. With their higher linear energy transfer, alpha particles are believed to induce more specific and efficient tumor cell killing, while sparing surrounding tissues. Clinical trials using ^{225}Actinium (^{225}Ac) therapy showed good patient response, but some patients experienced severe, irreversible xerostomia (i.e. dry mouth), which is the dose-limiting side-effect. Xerostomia was also reported to a less severe extent with radionuclide therapy using beta emitters. Besides being extremely uncomfortable, xerostomia can also be dangerous due to the absence of protective saliva, making patients more susceptible for bacterial infections. Therefore, there is a high need to investigate the mechanisms underlying this salivary gland toxicity (xerostomia), as a basis to develop possible countermeasures.
With this project, we aim to gain a better understanding of PSMA-targeted radionuclide therapy to increase safety and to improve clinical efficacy.
Methods:
Results:
Conclusion:
Selected references
Acknowledgements. *This project is funded by ANMI SA – Advanced Nuclear Medicine Ingredients. NH is a recipient of a SCK CEN/UGhent doctoral grant.*

P25 - Proteomic analysis of bystander effects in chondrosarcoma cells

Molecular and cellular effects

Gilbert Antoine[1, 2]
Tudor Mihaela[3, 4], Lepleux Charlotte[1, 2], Rofidal Valérie[5], Hem Sonia[5], Almunia Christine[6], Armengaud Jean[6], Brotin Emilie[7], Haghdoost Siamak[1, 2], Savu Diana[3], **Francois Chevalier**[1, 2]

[1] LARIA, CEA-DRF-iRCM, François JACOB Institute, Caen, France
[2] CIMAP UMR6252, Normandie Univ, ENSICAEN, UNICAEN, CEA, CNRS, Caen, France
[3] Department of Life and Environmental Physics, Horia Hulubei National Institute of Physics and Nuclear Engineering, Magurele , Romania
[4] University of Bucharest, Faculty of Biology, Splaiul Independentei 91-95, Bucharest R-050095, Romania
[5] BPMP, Mass Spectrometry Proteomics Platform, Montpellier, France
[6] Innovative technologies for Detection and Diagnostics, CEA-DRF-Li2D, Marcoule, France
[7] ImpedanCELL Platform, Federative Structure 4206 ICORE, Normandie Univ, UNICAEN, Inserm U1086 ANTICIPE, Biology and Innovative Therapeutics for Ovarian Cancers group (BioTICLA), Comprehensive Cancer Center F. Baclesse, 14000, Caen, France.

Introduction: Experimental evidences show that, in addition to direct and targeted effects of ionizing radiations, another effect is observed within the surrounding un-irradiated area; irradiated cells relay a stress signal in this close proximity, the bystander effect. Neighbouring un-irradiated cells react to this bystander signals with a specific response, characteristic of a biological context, but with a close relationship to the biological response typically associated with direct radiation exposure. Bystander responses and bystander factors secreted by irradiated cells were observed and studied in many physical and biological conditions, in vitro and in vivo [1].
Methods: In the present study, we investigated the capacity of chondrocytes in responding to bystander factors released by irradiated chondrosarcoma cells using a medium transfer protocol.
The cells were irradiated with low doses X-rays and the bystander supernatant was transferred on non-irradiated cells. Survival and proliferation assays were performed to study the effects of this treatment on the bystander cells [2]. In order to study the impact of these treatments on the cellular proteome, we carried out proteomic analysis starting from the cellular protein lysates and the conditioned medium.
Results: The proteomic analysis showed the effect of irradiation on the secretome of chondrosarcoma cells as well as the bystander effect on the proteome of bystander chondrocytes. We then selected variant spots that were analysed by mass spectrometry.
Conclusion: An in-depth analysis of the identified proteins provides a better understanding of the mechanisms involved in cell irradiation as well as the radio-induced Bystander effect.
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P26 - Proton boron capture therapy (PBCT) approach to enhance radiobiological effectiveness of proton beams on cell culture models

Molecular and cellular effects

Gaia Pucci[1]
Luigi Minafra[2, 3], Marco Calvaruso[2, 3], Fransesco P. Cammarata[2, 3], Gaetano Savoca[2, 3], Valentina Bravatà[2, 3], Guiseppe A.P. Cirrone[3], Giada Petringa[3], Vincenzo Cavalieri[1], Lorenzo Manti[4, 5], Giusi I. Forte[2, 3], Ciacomo Cuttone[3], Giorgio Russo[2, 3]
[1] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STeBiCeF), University of Palermo
[2] Istituto di Bioimmagini e Fisiologia Molecolare-Consiglio Nazionale delle Ricerche (IBFM-CNR), Cefalù
[3] Laboratori Nazionali del Sud (LNS), INFN, Catania
[4] Istituto Nazionale di Fisica Nucleare (INFN), Sezione di Napoli, Naples
[5] Radiation Biophysics Laboratory, Dipartimento di Fisica "E. Pancini", Università di Napoli Federico II, Naples

Introduction: Protontherapy (PT) represents an important radiation treatment modality used to treat cancer for several decades already, thanks to a better ballistic precision and a higher dose conformity than conventional radiotherapy (RT). A further improvement in the Relative Biological Effectiveness (RBE) of proton radiation, with a significant increase in tumor cell killing, may be obtained by exploiting the $p + {}^{11}B \rightarrow 3\alpha$ nuclear fusion reaction, to generate high-LET alpha particles through the use of the sodium borocaptate (NA2B12H11SH or "BSH"). This approach is known as the Proton Boron Capture Therapy (PBCT) and is expected to play a strategic role in medical applications, in particular to treat radioresistant tumors [Cirrone GAP et al, 2018].
Methods: To better investigate the radiobiological response at molecular level following PBCT in inducing a greater DNA Damage Response (DDR), we tested the enhancement effect of BSH in the human non-tumorigenic breast MCF10A cell line, commonly used as an healthy control epithelial cell line. After pretreatment with 80 ppm of BSH, cells were irradiated with 2 Gy dose at the middle position of the 62-MeV clinical Spread-Out Bragg Peak (SOBP) by using the proton beam at the superconducting cyclotron of the INFN-LNS facility (Catania, Italy). We evaluated the expression of the histone H2AX (γH2AX) as foci formation after irradiation, by immunofluorescence analysis, since it represents a well-known early marker of DNA break. We also studied by Western Blot analyses the expression of 5 proteins related to a DDR and involved in specific DNA repair pathways: the X-Ray Repair Cross Complementing 6 (XRCC6/KU70), the Xeroderma Pigmentosum Group A-Complementing Protein (XPA), the Polymerase Beta (POLB), the Ataxia Telangiectasia and Rad3-Related kinase (ATR) and, also, γH2AX to better quantify its expression levels.
Results: Overall, the results obtained revealed a synergic effect of BSH in inducing a higher DNA damage repair response. In addition, we evaluated whether the BSH is able to induce an enhancement of cancer cell killing on a radioresistant cell type, the human pancreatic carcinoma cell line Panc-1, after irradiation with increasing doses of proton beam at the mid-SOBP position in the same condition described above. We observed through dose response curves as the BSH pretreatment plays a radiosensitizing effect, reducing significantly cell survival compared to proton irradiation alone.

Conclusion: Our data confirm the key role of BSH in increasing the radiobiological effectiveness of PT and highlight some molecular mechanisms involved in the cell response to PBCT.

Selected references

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Nazionale 5 (CSN5) Call ‘NEPTUNE’.

P27 - Puzzling enhancement of proton-induced cellular damage by boron

Molecular and cellular effects

Kateřina Pachnerová Brabcová[1]
Anna Michaelidesová[1], Jana Vachelová[1], Oldřich Zahradníček[1], Irina Danilová[1], Vladimír Vondráček[2], **Marie Davídková**[1]
[1] Dept. of Radiation Dosimetry, Nuclear Physics Institute of the CAS, Prague, Czech Republic
[2] Proton Therapy Center Czech, Prague, Czech Republic

Introduction: For a long time, boron has been used as radiosensitizer in Boron Neutron Capture Therapy, producing high LET secondary particles by neutron nuclear reaction with ^{10}B isotope. Analogous idea has recently attracted attention in the proton therapy community, suggesting that the boron could amplify cell killing by producing low-energy alpha particles either via reaction with protons themselves or with capture of secondary thermal neutrons. Our goal is to elucidate the underlying mechanism of the observed enhanced biological effects of proton irradiation with the presence of boron.
Methods: Glioblastoma astrocytoma U87 MG and human prostate cancer cells DU145 were doped with 40 ppm of ^{11}B by cultivation with a delivery agent BSH (mercapto-undecahydro-dodecaborate, $Na_2B_{12}H_{11}SH$). Cell monolayers were irradiated with monoenergetic pencil scanned beam of 200 MeV in a plateau and in a Bragg peak position. The possible boron-radiosenzitizing effect was monitored by clonogenic assay.
Results: The results were expressed as dose modifying factors (DMF), the ratio of radiation doses with and without the boron agent causing the same effect, in this case 50%-survival doses. Enhancing effect was observed for U87 MG in the Bragg peak (DMF 1.47±0.2), while no increase was observed in the plateau (DMF 0.94±0.1). No DMF changes were observed for DU145 under the similar experimental conditions (1.10±0.1 plateau, 1.05±0.09 Bragg peak).
Conclusion: Different results for the studied cellular lines might suggest that the underlying mechanism of the enhanced efficiency is likely caused by a biochemical or biological process rather than by a local enhancement of absorbed dose.

P28 - Quantitative Modelling of Bystander Effects Within Spatially Fractionated Exposures

Molecular and cellular effects

Paul Cahoon[1]
Valentina Giacometti[1, 2], Conor McGarry[1, 2], Emily Russell[1], Kevin Prise[1], Stephen J. McMahon[1]
[1] Patrick G Johnson Centre for Cancer Research, Queen's University Belfast, Belfast, Northern Ireland, United Kingdom;
[2] Radiotherapy Physics, Northern Ireland Cancer Centre, Belfast Health and Social Care Trust, Northern Ireland, United Kingdom

Introduction: Radiation-induced bystander effects (RIBEs) damage cells located outside the directly irradiated field. This has led to increased interest in spatially fractionated radiotherapy, where through proper exploitation of RIBEs, non-uniform dose exposures could be used to enhance tumour control. However, this is challenging to optimise, as the mechanisms underlying RIBEs are not yet fully understood. We have created a mathematical model that captures the behaviour of RIBEs in *in vitro* experiments, simulating both direct damage as well as indirect damage mediated by signalling molecules. By supplementing experimental study with mathematical modelling techniques, it's possible to predict how different dose exposures influence cell survival, and identify optimal treatment approaches.
Methods: Using a clinical Linac, we delivered four plans to *in vitro* DU145 cells: a uniform plan and three heterogeneous exposures (Figure 1). In all plans the mean dose to the population was identical, with approximately 50% of the flask exposed in non-uniform plans.
Results: The clonogenic survival resulting from these exposures was well predicted by our mathematical model, and showed that all plans had similar effects at lower doses, while uniform exposure only proved most effective at higher doses.
Conclusion: This indicates that uniform exposures do not necessarily maximise cell kill at clinically relevant doses. With RIBEs considered it's possible that maximising the mean dose applied to the tumour would, in some cases, be a better use of resources than ensuring a fully uniform field. Future work will evaluate these effects in clinical treatment plans.

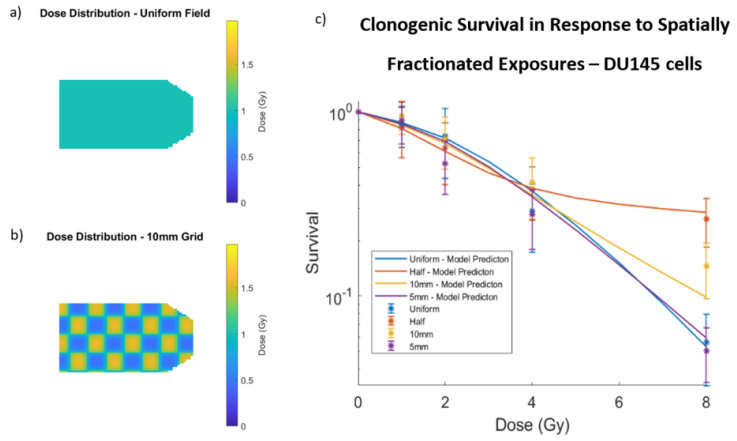

Figure 1: Example dose distributions for the (a) Uniform field and (b) 10 mm grid pattern exposures where the mean dose delivered to each T25 flask is 1 Gy. (c) The clonogenic survival resulting from each of the plans at the 1, 2, 4 and 8 Gy dose points is compared to the predictions made by the mathematical model.

P29 - Response of medulloblastoma cells to ionizing radiation

Molecular and cellular effects

Jana Klementová[1, 2]
Martina Zíková[2], Markéta Hurychová[1], Irina B. Danilová[1], Šárka Jarošová[2], Marie Davídková[1]
[1] Czech Academy of Sciences, Nuclear Physics Institute, Prague, Czech Republic
[2] Czech Academy of Sciences, Institute of Molecular Genetics, Prague, Czech Republic

Introduction: Brain and nervous system cancers in children represent the second most common neoplasia after leukemia. About 8-10 % of childhood brain tumors are medulloblastomas (MBs), embryonal tumors of the cerebellum (1). MB is not a single disease but is comprised of at least four subgroups, which are termed wingless (WNT), sonic hedgehog (SHH), group C, and group D (2). These subgroups differ in their molecular characteristics, clinical course, and are associated with different prognosis.
Methods: The established SHH MB cell lines DAOY (3) and ONS-76 (4) were cultured *in vitro* in standard adherent conditions or as neurospheres (medullospheres). The irradiation was performed by Co-60 gamma radiation and by X-ray beam, doses of 0 to 8 Gy. We determined tumorogenicity of cells by measuring their ability to proliferate, to form medullospheres, and to initiate colonies after irradiation.
Results: *TP53* gene mutations occur in a specific group of SHH MBs. We confirmed that DAOY cells express mutated *TP53* mRNA and ONS-76 cells express wild-type form of this gene. Both cell lines were able to grow as medullospheres in serum-free medium and moreover we verified the changes in the expression levels of stem cell markers in medullospheres compare to cells cultured as a monolayer. Additionally, we measured differences in colony formation after irradiation either in adherent or in attachment-independent conditions.
Conclusion: Because a significant part of cancer patients receive radiation as a critical part of the treatment regimen, the aim of our project is to characterize the functional impact of radiation on SHH MB cells.
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P30 - Side effects of scattered versus scanned proton beams on normal tissues in total body irradiated mice: preliminary results

Molecular and cellular effects

Samia Chaouni[1]
Ludovic De Marzi[2], Fréderic Pouzoulet[3], Jean-Louis Habrand[1, 4], François Sichel[1, 4], Dinu Stefan[1, 4], Delphine Lecomte[4], Carine Laurent[1, 5]
[1] ABTE(EA4651)-ToxEMAC, Caen University
[2] Curie Institute, Protontherapy center, Orsay,France
[3] Curie Institute, Translationnel Recherche Department, RadeXP plateform
[4] CLCC François Baclesse, Caen , France
[5] ARCHADE-SAPHYN, Caen , France

Introduction: Protons are now widely used instead of photons due to their ballistic properties which should allow to treat tumors localized near organs at risk (OAR) without leading to toxicities in these OAR. Nowadays, the state of knowledge is limited mainly to relative biological efficiency (RBE) on observables related to cell death. Moreover, difficulty of access to beam line facilities, initially dedicated to physics research, and their inadequacy with radiobiology experiments have limited the quantity and the quality of available and homogenous biological data. Therefore, only few studies have been performed on proton effects on normal tissues or cells in comparison with the large number of studies on conventional radiotherapy. In this way, there is a lack of data relative to biological effects of scattered versus scanned proton beams on normal tissues.
Methods: The present study aimed at evaluating the response of healthy tissues (skin, lung, heart and blood) after scattered or scanned proton beam irradiation. For this purpose, C57Bl6 mice were total body irradiated by DS (Double Scattering) or PBS (Pencil Beam Scanning) at different proton doses in the plateau phase of the Bragg peak. Blood and organs were collected 3 months after irradiation.
Results: First results showed differences between both types of proton delivery in terms of survival but also DNA damage, biomarkers of oxidative stress and inflammation
Conclusion: Experiments are in progress concerning other biomarkers of oxidative stress and inflammation on the collected blood and organs. Results will be then compared to normal tissues response after X-ray irradiation

P31 - Small is beautiful: low activity alpha and gamma sources for small-scale radiation protection research experiments

Molecular and cellular effects

Magdalena Plodowska[1]
Milagrosa Lopez-Riego[2], Pamela Akuwudike[2], Daniel Sobota[3], Janusz Braziewicz[3], Mateusz Filipek[4], Urszula Kazmierczak[5], Beata Brzozowska-Wardecka[4], Halina Lisowska[1], Lovisa Lundholm[2], **Andrzej Wojcik**[2]
[1] Institute of Biology, Jan Kochanowski University, Kielce, Poland
[2] Centre for Radiation Protection Research, MBW Department, Stockholm University, Sweden
[3] Institute of Physics, Jan Kochanowski University, Kielce, Poland
[4] Institute of Experimental Physics, Warszawa University, Warszawa, Poland
[5] Heavy Ion Laboratory, Warszawa University, Warszawa, Poland

Introduction: Uncertainties regarding the magnitude of health effects following exposure to low doses of ionising radiation remain a matter of concern both for professionals and for the public. There is consensus within the international radiation research community that more research is required on biological effects of radiation doses below 100 mGy applied at low dose rates. Moreover, there is a demand for increasing education and training of future radiation researchers and regulators. Research, education and training is primarily carried out at universities but university-based radiation research is often hampered by limited access to radiation sources. The aim of the present report is to describe small and cost effective low activity gamma and alpha sources that can easily be installed and used in university laboratories.
Methods: A gamma radiation source was made from an euxenite-(Y) rock $(Y,Ca,Ce,U,Th)(Nb,Ta,Ti)_2O_6$) that was found in an abandoned mine in Sweden. It allows exposing cells grown in culture dishes to radiation at a dose rate of 50 µGy/h and lower. Three alpha sources were custom-made and yield a dose rate of 1 mGy/h each.
Results: The construction and dosimetry of the sources is described.
Conclusion: We hope that the report will stimulate research and training activities in the low dose field by facilitating access to radiation sources.

P32 - The cytokinesis-block micronucleus assay on isolated fresh and frozen peripheral blood mononuclear cells.

Molecular and cellular effects

Simon Sioen[1]
Karlien Cloet[1], Anne Vral[1], Ans Baeyens[1]
[1] Radiobiology Research Group, Department of Human Structure and Repair, Ghent University

Introduction: The cytokinesis-block micronucleus (CBMN) assay is a standardized method used to evaluate genomic damage after exposure to various genotoxic agents such as ionizing radiation. Next to conventional whole blood cultures (WBC), also isolated peripheral blood mononuclear cells (PBMCs) cultures are used for the CBMN assay. However, there is no extensive investigation of a standardized protocol for the PBMCs CBMN assay. The aim of this study was to work out a reliable CBMN assay protocol for fresh and frozen isolated PBMC. Furthermore, we analyzed if PBMCs, isolated out of exposed whole blood, lead to representative MN data.
Methods: Blood samples of 10 donors were collected. Each donor's blood was used to set up a G_0 CBMN assay on whole blood, on isolated fresh and frozen PBMCs. In these assays isolation of PBMCs occurred before *in vitro* irradiation. Additionally, PBMCs were isolated from irradiated whole blood samples and MN were scored. Cells were exposed to *in vitro* doses ranging from 0,5 to 2 Gy of 220 kV X-rays.
Results: The CBMN assay on PBMCs, isolated both pre and post- irradiation, showed a high reproducibility, sensitivity and similarity with the conventional WBC CBMN assay. After freezing, cells showed no significant differences in MN counts until the time point of 2 weeks, where after significant elevated MN counts were assessed.
Conclusion: A reliable CBMN assay protocol for PBMCs, isolated both pre and post-irradiation, will be presented. Extra attention is needed when freezing isolated PBMCs for a longer period than 2 weeks.

P33 - The influence of MGMT expression on radiation responses in human glioblastoma multiforme cell lines

Molecular and cellular effects

Anna Kirstein[1, 2]
Daniela Schilling[1, 2], Stephanie E. Combs[1, 2, 3], Thomas E. Schmid[1, 2]
[1] Institute of Radiation Medicine (IRM), Helmholtz Zentrum München, Neuherberg, Germany
[2] Department of Radiation Oncology, School of Medicine, Klinikum rechts der Isar, Technical University of Munich (TUM), Munich, Germany
[3] Deutsches Konsortium für Translationale Krebsforschung (DKTK), Partner Site Munich, Germany

Introduction: GBM exhibit high resistance to radiochemotherapy. MGMT counteracts the therapeutic efficacy of temozolomide and is therefore a biomarker for chemotherapy outcome. As the influence of MGMT in radiotherapy is unknown the aim is to identify the correlation between MGMT and radiation response.
Methods: Radiosensitivities were determined by colony forming assay (CFA), MGMT promoter methylation was quantified by MethyQESD. Western Blots showed MGMT expression while DNA repair capacities were investigated by 53BP1 foci and correlated to cell cycle analyses.
Results: U251 are more radioresistant, than LN18 and LN229 cells. MethyQESD revealed unmethylated promoter regions in LN18 (0.3%), hemi-methylated in U251 (29.7%) and methylated in LN229 (165.2%). MGMT expression levels vary between cell lines, which is in accordance to MethyQESD. Western Blots showed a successful MGMT knockdown in LN18 with a significant reduction in MGMT. 24 hours after irradiation residual damages are increased (LN18: 1.7-fold±0.2; LN229: 2.7-fold±0.4; U251: 1.1-fold±0.2), which corresponds with similar doubling times for LN18 (21h±1.7) and LN229 (19h±1) but increased doubling time in U251 (27h±0.6). Cell cycle analysis revealed 29%±0.04 LN18, 30%±6.6 LN229 and 47%±0.02 U251 cells in G_2/M arrest 24 hours after irradiation.
Conclusion: Our results demonstrate increased repair capacity, slower growth and higher G_2/M arrests in the radioresistant cell line. No correlation between MGMT status and radiosensitivity was found in parental cells. Further in vitro experiments to characterize MGMT knockdown cells are ongoing and in vivo experiments are planned. First CFA results indicate a radiosensitizing effect of MGMT in knockdown cells.

P34 - The specific role of DNA-PKcs in DNA-DSBs repair induced by the neutron-mixed beam

Molecular and cellular effects

Kamila Maliszewska-Olejniczak[1]
Agnieszka Dróżdż[1], Martyna Waluś[1], Michał Dorosz[1], Maciej Wiliński[1], Szymon Domański[1], Martyna Araszkiewicz[1, 2], Katarzyna Tymińska[1], Agnieszka Korgul[2], Maciej Maciak[1], Michał A. Gryziński[1]
[1] National Centre for Nuclear Research, Nuclear Facilities Operations Department, Otwock, Poland
[2] University of Warsaw, Faculty of Physics, Warsaw, Poland

Introduction: The main goal of our research is to investigate the mechanisms of DNA-DSBs repair after exposure to the neutron-mixed beam used in boron neutron capture therapy (BNCT). The impact of a mixed beam on the activation of the DNA damage response is poorly understood. Our interest lies in neutron-gamma mixed beam which can induce another type of DNA damage, such as complex DNA damage, as indicated for high LET particles. The author assumes that after exposure to a neutron-mixed beam the reduced repair capacity will be observed, less efficient repair response will occur and may promote genome instability and cell death. Which repair pathway is involved and which proteins it is not clear in the case of the neutron-mixed beam.
Methods: We used *in vitro* model – colon cancer cell line and plan to introduce cell lines deficient in repair proteins and *C. elegans* which emerged recently as a suitable *in vivo* experimental model for studying the DNA damage response. Moreover, we have developed and introduced a reliable immunofluorescence staining protocol for the detection of radiation-induced DNA damage response with antibodies specific for repair factors from NHEJ and HRR pathways (Maliszewska-Olejniczak, *et al.*, 2020; *J. Vis. Exp.*).
Results: We observed the occurrence of the higher expression level of DNA-PKcs from NHEJ in the form of foci by immunofluorescence technique after neutron-mixed beam irradiation in comparison with other tested proteins from each repair pathway.
Conclusion: Our research will provide new knowledge about molecular mechanisms in the process of DNA damage formation and repair.

P35 - The temperature effect at the level of DNA damage foci and micronucleus frequency in U2OS-53BP1 and U2OS-NBS1 cells.

Molecular and cellular effects

Magdalena Płódowska[1]
Wiktoria Krakowiak[1], Aneta Węgierek-Ciuk[1], Andrzej Wójcik[1, 2], Anna Lankoff[1], Karol Szary[3], Krzysztof Lis[4], Halina Lisowska[1]
[1] Department of Medical Biology, Institute of Biology, Jan Kochanowski University, Kielce, Poland
[2] Centre for Radiation Protection Research, MBW Department, Stockholm University, Sweden
[3] Department of Atomic Physics and Nanophysics, Institute of Physics, Jan Kochanowski University, Kielce, Poland
[4] Holy Cross Cancer Center, Artwińskiego 15, 25-406 Kielce, Poland

Introduction: The radioprotective effect of hypothermia, termed the temperature effect (TE), was mainly observed in human peripheral blood lymphocytes (PBL) as reduced levels of chromosomal aberrations and micronuclei. TE was not visible at the level of gamma H2AX focus formation and decay. The mechanisms of TE are not known. The aim of the study was to analyze the effect of hypothermia at the level of formation and decay of 53BP1 and NBS1 foci and of micronuclei (MN) in cells other than PBL. 53BP1 and NBS1 foci were analysed in order to observe differences in early and late cellular responses. Another aspect was the observation whether cells left on ice after irradiation would at all repair DNA damage.
Methods: U2OS were exposed at 0.8°C and 37 °C to 2 Gy of gamma radiation. Kinetics of foci formation was analyzed afer 0, 5, 10 ,15, 30 and 60 minutes of repair time. Cells were also set up for MN, were harvested after three fixation time points: 20h, 26h and 32h of culture time. Three independent experiments were performed.
Results: The analysis of foci and the micronucleus scoring has not been completed at the time of abstract submission. Preliminary results show that the formation and decay of 53BP1 foci was delayed in cells exposed at 0.8°C as compared to 37°C. Cells left on ice formed 53BP1 foci.
Conclusion: Preliminary results demonstrate that, in contrast to human peripheral blood lymphocytes, TE is observed in U2OS cells. At present, we do not know the reason for the difference.
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P36 - Therapeutic potential of Hedgehog signaling pathway modulation for muscular repair after high local dose radiation exposure

Molecular and cellular effects

Nicolas Jullien[1]
Jérôme Pateux[1], Michel Drouet[1], Diane Riccobono[1]
[1] French Armed Forces Biomedical Research Institute (IRBA), Brétigny-sur-Orge, France

Introduction: Civilians but also the armed forces can be injured by high dose radiation exposure, in connection with terrorist acts or accidentally. In such a situation, one of the first physiological barriers is subcutaneous musculature. Consecutive lesions to this tissue can be severe, strongly inflammatory and degenerative (cutaneous radiation syndrome; CRS). However, no satisfactory pharmacological solution is available to treat victims. It is therefore necessary to develop new therapeutic strategies to improve post-irradiation muscle regeneration.
Methods: Here, the objective is to evaluate the benefit of a pharmacological protocol, based on the use of recombinant Sonic Hedgehog (Shh; agonist) or Cyclopamine (antagonist), to modulate the pro-myogenic Hedgehog (Hh) signaling pathway[1,2] in differentiating mouse myoblasts (C2C12 cells) exposed to radiation (X-rays; 5 Gy). Proliferation, metabolism and myogenesis genes/proteins expression have been evaluated.
Results: This study shows a significant negative impact of a high radiation dose in our *in vitro* model of mouse muscle progenitors differentiation at days 0 to 7 after irradiation. Interestingly, both the activation and the inhibition of Hh pathway appear to have a therapeutic potential in post-irradiation muscle regeneration: Shh promotes the proliferation of myoblasts and their survival while Cyclopamine significantly increases cell differentiation toward mature myotubes.
Conclusion: The best activation/blocking sequence of this metabolic pathway remains to be investigated to stimulate both progenitors preservation and new muscle fibers synthesis after irradiation.
A major scientific interest lies in such a project to improve the understanding of Hh involvement in muscular regeneration after CRS and to accumulate data for further *in vivo* studies.

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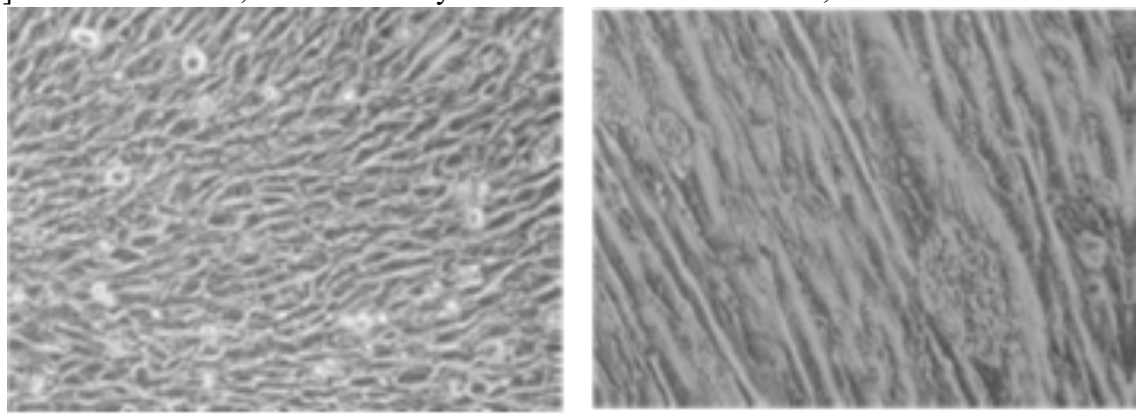

C2C12 cells in non differentiating (left)
and differentiating culture conditions (right) at day 7

P37 - Unravelling the potential interplay of simulated spaceflight conditions: how is the skin affected?

Molecular and cellular effects

Eline Radstake[1, 2]
Bjorn Baselet[1], Sarah Baatout[1, 2], Mieke Verslegers[1]
[1] Institute for Environment, Health and Safety, Radiobiology Unit, Belgian Nuclear Research Centre SCK CEN, Mol, Belgium
[2] Faculty of Bioscience Engineering, Department of Biotechnology, Ghent University, Belgium

Introduction: During space travel, astronauts are continuously exposed to microgravity, ionizing radiation, and increased psychological stress. One of the organs affected by this spaceflight environment is the skin, which is subject to rashes, itches, and delayed wound healing. Furthermore, alterations in extracellular matrix proteins, thinning of the epidermis, and loss of elasticity are found after spaceflight. Yet, there is still lack of understanding how the different spaceflight stressors interact to induce these defects.
Methods: We used cultured primary human dermal fibroblast to investigate the cellular effects after exposure to combined spaceflight stressors, where we first exposed fibroblasts to X-rays and hydrocortisone. Endpoints included wound healing capacity, expression of type I collagen and DNA damage repair.
Results: We found a decreased cell migration upon wound induction and lowered expression of type I (pro)collagens (indicative for skin aging) in response to hydrocortisone, but not following radiation with 0.1-2 Gy of X-rays. Furthermore, preliminary results show increased DNA damage after irradiation which was exacerbated in irradiated fibroblasts incubated with hydrocortisone, suggesting a synergistic effect of these stressors.
Conclusion: Besides hydrocortisone and ionizing radiation (low- vs. high- LET), fibroblasts will be exposed to microgravity to investigate the possible interactive effects on wound healing. Additional endpoints will include cell survival and an in-depth investigation of extracellular matrix proteins and cytoskeleton components. Altogether, the results of this PhD project will give more insights into the effects of combined spaceflight stressors on skin dermal cells, and as such might improve the risk assessment for human deep space exploration.

P38 - Comparison of the immuno-biological response in the tumor microenvironment after FLASH or conventional electron irradiation

Translational and clinical research

Anja Brus[1]
Sophie Eriksson[2], Elise Konradsson[2], Gabriel Adrian[3], Ana Carneiro[3], Kristoffer Petersson[4,5], Anders E. Hansen[1], Thomas L. Andresen[1], Crister Ceberg[2]
[1] Department of Health Technology, Biotherapeutic Engineering and Drug Targeting, Technical University of Denmark, Kgs. Lyngby, Denmark
[2] Medical Radiation Physics, Department of Clinical Sciences, Lund University, Lund, Sweden
[3] Department of Haematology, Oncology and Radiation Physics, Skåne University Hospital, Lund University, Lund, Sweden
[4] Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, Oxford, UK
[5] Radiation Physics, Department of Hematology, Oncology and Radiation Physics, Skåne University Hospital, Lund, Sweden

Introduction: Ultra-high dose rate radiotherapy (FLASH-RT) has been demonstrated to mediate a potent anti-cancer activity with reduced normal tissue toxicity in preclinical models. However, the biological effect on the immune composition in the tumor microenvironment (TME) has not been clarified. It has been demonstrated that conv-RT causes immunogenic cell death, releasing tumor antigens and increases the infiltration of cT cells, making radiation therapy a potent primer of anti-cancer immune response in combination with immunotherapy. Considering the observed tissue sparing properties of FLASH-RT we hypothesized that the effect on non-cancerous cells in the TME could be different compared to conv-RT.
Methods: The effects of FLASH-RT and conv-RT on the anti-cancer immune response were compared in a syngeneic murine cancer model, CT26. Tumors were irradiated with 8Gy of conv-RT or 8Gy FLASH-RT and the immune infiltration in the TME were evaluated 8 days after irradiation by flow cytometry.
Results: There was no significant difference in the viability of cancer cell between conv-RT and FLASH-RT. However, we found a significantly higher infiltration of cDC1 and cT cells in tumors treated with conv-RT compared to FLASH-RT. Interestingly, this was not associated with an increased activation based on CD86 and MHC-II expression and there was no difference in infiltration of MDSCs in the TME.
Conclusion: These preliminary results could indicate that FLASH-RT induces cell death by a level or mechanism that is less immunogenic. Follow-up studies to elucidate the underlying biological mechanisms and immune modulating properties of FLASH-RT at multiple time point and higher irradiation doses are therefore ongoing.

P39 - Identification of Raman spectral biomarkers of treatment response in high risk localised prostate cancer patients receiving SABR.

Translational and clinical research

Jade Monaghan[1, 2]
Daniel Cullen[1, 2], Dinesh Medipally[1, 2], Kelly Redmond[3], Ciaran Fairmichael[3, 4], Suneil Jain[3, 4], Aidan Meade[1, 2], Fiona Lyng[1, 2]
[1] Radiation and Environmental Science Centre, FOCAS Research Institute, Camden Row, Dublin 8, Ireland
[2] School of Physics, Technological University Dublin, Kevin Street, Dublin, Ireland
[3] Centre for Cancer Research and Cell Biology, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK
[4] Northern Ireland Cancer Centre, Belfast, 51 Lisburn Road, Belfast BT9 7AB, UK

Introduction: High risk localised prostate cancer (PCa) accounts for 15% of patients diagnosed with PCa. Stereotactic ablative body radiotherapy (SABR) offers an opportunity to increase the dose delivered to the prostate while sparing the surrounding normal tissues. After treatment, PCa patients can experience gastrointestinal and genitourinary toxicity and there is patient variability in response due to individual radiosensitivity. There is an unmet need for biomarkers to predict treatment response and potential toxicity from this treatment. Optical spectroscopic methods such as Raman spectroscopy can provide a unique biochemical fingerprint for molecules in biological samples. This study aims to identify Raman spectral biomarkers for monitoring treatment response and toxicity in SABR treated PCa patients.

Methods: PCa patients (n=30) were recruited as part of the SPORT High-Risk trial and blood samples were collected at baseline and at 8 time points up to 3 months post-SABR. At follow up, clinical details including prostate-specific antigen (PSA) and radiation toxicity were recorded. Raman spectra were recorded from lymphocytes and the data was analysed using MATLAB software.
Results: Through principal component analysis, differences in spectral features between samples before treatment and at subsequent time points were found in lipids and nucleic acids. Partial least squares-discriminant analysis provided sensitivity and specificity in the ranges of 88-100%.
Conclusion: Future work with these spectral signals involves modelling with treatment type and toxicity. Identification of biomarkers would allow stratification of high risk localised PCa patients according to risk of developing radiation toxicity and could provide individualised patient radiotherapy treatment.

P40 - Nomogram for predicting overall survival in patients diagnosed with spinal bone metastases

Translational and clinical research

Biche Osong[1]
Ilknur Sanli[1], Paul C. Willems[2], Leonard Wee[1], Andre Dekker[1], Seok Ho Lee[3], Johan van Soest[1]
[1] Department of Radiation Oncology (Maastro), GROW School for Oncology, Maastricht University Medical Centre+, Maastricht, The Netherlands
[2] Department of Orthopaedics and Research School Caphri, Maastricht University Medical Center, Maastricht, the Netherlands.
[3] Department of Radiation Oncology, Gachon University, College of Medicine, Gil Medical Center, Incheon, Korea

Introduction: The spine is the third most common site for cancer cells to metastasize, after lung and liver. About 30 - 70% of patients with a primary tumor have metastatic spinal disease at autopsy.
Methods: A total of 250 patients with spinal bone metastases admitted to our institution from January 2014 to April 2016 were reviewed for this study. The primary tumor was restricted to breast, prostate, colon, rectal, and lung. A 5-fold cross-validation Cox proportional hazard regression model with the lasso penalty was employed for the feature selection process before establishing the prognostic nomogram. The discrimination was measured by the concordance index (C-index). A bootstrap calibration plot was used to ascertain the model's accuracy.
Results: Six independent prognostic factors, including age, the presence of visceral metastasis, spinal cord compression, brain metastasis, WHO performance status, and primary tumor were identified during the feature selection process for building the nomogram with the manual addition of gender. The median follow-up time for this study was 46.8 months with a 1, 3, and 6-months overall survival probability of 88%, 67%, and 53%, respectively. The C-index of the nomogram was 0.720, with a standard error of 0.02.
Conclusion: We established a novel nomogram that could be used to predict the survival probability of patients with spinal metastasis. We provided a digital version for flexible and easy usage (https://bich.shinyapps.io/SpinalMets/), thus helping physicians with their (shared) decision-making process and the individualized care planning of such patients.
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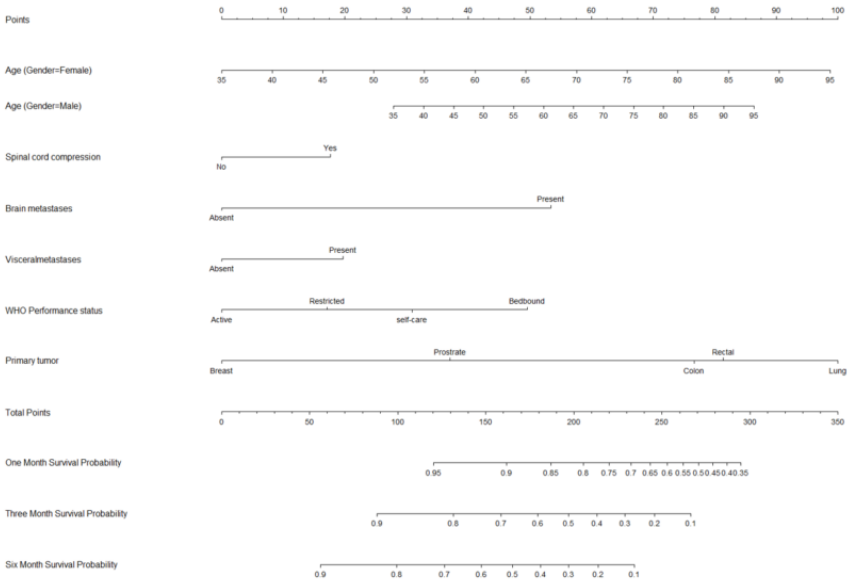

Figure 1. *Developed nomogram to predict 1, 3, and 6-months overall survival for metastatic spinal bone patients using six clinical characteristics. To use the nomogram, locate the patient's variable on the corresponding axis and draw a vertical line to the points axis, then sum the points, and bring a vertical line from the total points axis to the 1, 3, or 6 - months overall survival probability axis.*

P41 - Search for Biomarkers of Radiation-Induced Cardiovascular Disease and Pediatric Tumors

Translational and clinical research

Magy Sallam[1, 2]
An Aerts[2], Mohammed Abderrafi Benotmane[2], Sarah Baatout[2, 3], Pieter-Jan Guns[1]
[1] University of Antwerp
[2] Belgian Nuclear Research Centre (SCK•CEN)
[3] Ghent University

Introduction: Radiation therapy (RT) in breast cancer has significantly improved patient treatment outcomes contributing to a 5-year survival of around 90% in many countries. However, breast RT can cover part of the heart and major blood vessels which has been correlated with an increased cardiovascular morbidity and mortality as eventually leading to radiation-induced cardiovascular disease (CVD). Interestingly, DNA methylation alteration have been linked to both radiation and cardiovascular disease seperately. However, no previous research has focused DNA methylation alterations associated with radiation-induced CVD.

On the other hand, diagnostic computed tomography (CT) imaging ,while highly valuable, delivers significant radiation doses. Children are particularly at risk as they are more sensitive to radiation-induced cancer compared with adults and have a longer lifespan to express the delayed harmful effects. In fact, an increased risk of glioblastoma (GBM) was found in children after radiation exposure from CT scans. Hence, biomarkers for early detection of GBM are of crucial importance. Non-coding RNA have been shown to be differentially expressed in a variety of cancers including GBM. Consequently, a combined GBM biomarker panel of noncoding RNA could significantly alter disease diagnosis and monitoring.
Methods: NA
Results: NA
Conclusion: Consequently, our research aims to discover DNA methylation biomarkers for a more accurate risk estimation of early radiation-induced CVD as well as to identify and measure microRNA/ long non coding RNA (lncRNA) markers of GBM. This research is part of MEDIRAD project which has received funding from the Euratom research and training programme 2014-2018 under grant agreement No 755523.

P42 - Gene expression based signatures to predict the acute radiation syndrome after ionizing radiation - developments and challenges

Health effects and radiation protection

Matthias Port[1]
Patrick Ostheim[1], Matthäus Majewski[2], Michael Abend[1]
[1] Bundeswehr Institute of Radiobiology affiliated to the University of Ulm, Munich, Germany
[2] Department of Urology, Bundeswehr Hospital Ulm, Ulm, Germany

Introduction: Reliable diagnosis and triage of radiation injury patients is the key for determining appropriate guidance and therapeutic interventions after ionizing radiation accidents or attacks. Increasing threats due to proliferation of nuclear weapons, and even the possibility of improvised nuclear devices operated by terrorists challenged the conventional CBRN protection standard set within the last decades. Significant medical advances like usability of growth factors on the other hand underline the need for very early diagnosis.
Methods: Here we present our recent research strategy ranging from theoretical considerations on effect prediction to advanced gene signatures to predict the acute radiation syndrome after high dose ionizing radiation. Third generation gene expression signatures based on non-human-primate experiences were validated in human models.
Results: A small set of genes enabled us to set up a high-throughput diagnostic pathway which might be used even in large scale scenarios.
Conclusion:
First results in the development of point of care devices will be presented. Strategies to overcome challenges like cross species differences or exon specific markers will be discussed.

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P43 - Health effects of cardiac fluoroscopy and modern radiotherapy in paediatrics; Harmonic

Health effects and radiation protection

Siamak Haghdoost[1, 2, 3]
Brice Freneau[4, 5], Nadia Haddy[4], Maria Grazia Andeasi[6], Francois Chevalier[2], Mats Harms-Ringdahl[3], Isabelle Thierry-Chef[7, 8, 9]
[1] 1 Université de Caen Normandie (UNICAEN)/Archade, Caen, France
[2] 2 Cimap/Laria, Ganil facility Caen, France 3
[3] 3 Centre for Radiation Protection Research Stockholm University (SU), Stockholm, Sweden
[4] 4 Gustave Roussy (GR), Université Paris-Saclay, Villejuif, France
[5] 5Paris-Saclay University, Paris-Sud University, CESP, INSERM, Villejuif, France
[6] 6Genetics Research Unit, CNR Institute of Clinical Physiology, Pisa- Italy
[7] 7Institute for Global Health of Barcelona (ISGlobal), Barcelona, Spain
[8] 8Ciber Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
[9] 9Universitat Pompeu Fabra (UPF), Barcelona, Spain

Introduction: The use of radiation for medical diagnosis and treatment procedures has had a major impact on the survival of paediatric patients. However, it comes at the expense of exposure of healthy tissues to low and moderate doses of ionising radiation, which long term effects remain to be investigated in the context of rapid technology improvements. HARMONIC is a European project that started in June 2019 and aim at investigating the long term health effects of ionizing radiation exposure in children and young adults. The project encompasses six work packages (WP) including dosimetry, biology, radiotherapy and cardiac catheterization.
Methods: HARMONIC will set up the first European cohort with biosamples from children and young adults treated with modern radiotherapy techniques and cardiac fluoroscopy. In this presentation, the planned activities in the biology work package will be discussed. Blood and saliva samples will be collected prospectively. Plasma and saliva protein profiles will be established for each patient before, after 3 months and finally 1 year after the last exposure.
Results: Biomarkers of immune response, oxidative stress, ageing and activities of some DNA repair/damage signalling and oxidative stress response/immune response pathways will be investigated. These results will be related to development of adverse health effects e.g. second primary cancers, as well as cardiovascular and neurovascular disorders with the aim to provide a better understanding of the mechanisms behind the adverse response as well as to identify biomarkers that can be used to optimize the clinical outcome.
Conclusion: No conclussion yet.

P44 - Inter-comparison of OSL response of irradiated salted crackers between Croatian and Italian laboratories for retrospective dosimetry purposes

Health effects and radiation protection

Nadica Maltar-Strmečki[1]
Maja Vojnić Kortmiš[1], Ina Erceg[1], Sara Della Monaca[2], Paola Fattibene[2], Maria Cristina Quattrini[2], Emanuela Bortolin[2]
[1] Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia
[2] Istituto Superiore di Sanità, Viale Regina Elena 299, I-00161 Rome, Italy

Introduction: In retrospective dosimetry, for the radiation and nuclear (R/N) emergency if uncontrolled radiation affected many people or the environment, the information about radiation doses allows for the prediction of the biomedical consequences. Therefore, when professional dosimeters are not available, objects of everyday use can be applied as dosimeters. In such cases, the method of optically stimulated luminescence (OSL) has proved very promising.
Methods: The salt, following the exposure to ionizing radiation, exhibits a particularly high OSL response compared with many other materials. In this study, radiation sensitivity of salty crackers available on the market was monitored with two PSL systems in two physical dosimetry laboratories (ISS, Italy and RBI, Croatia) using the validated PSL methods. The response at two stimulation 890 nm and 470 nm have been compared.
Results: The results indicate that salty snacks can be used in accident dosimetry. The stimulation by 470 nm has better response, but for both stimulations detect absorbed doses are below 100 mGy.
Conclusion: The obtained data, showed good agreement between both laboratory OSL readout suggesting additional benefit to the use of salty crackers in the retrospective dosimetry.
The study was supported by NATO Science for Peace and Security Programme, grant No. G5684.

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P45 - Investigation of the Self-cleaning Processes in Lake Drūkšiai from Anthropogenic Origin 14C in the Cooling Pond of the Ignalina Nuclear Power Plant

Health effects and radiation protection

Algirdas Pabedinskas[1]
Evaldas Maceika[1], Rūta Barisevičiūtė[1], Žilvinas Ežerinskis[1], Justina Šapolaitė[1], Laurynas Butkus[1], Laurynas Juodis[1], Rūta Druteikienė[1], Vidmantas Remeikis[1]
[1] Nuclear research department, Center for physical science and technology, Lithuania

Introduction: Considerable amounts of ^{14}C in the nuclear reactor is generated by neutrons. It accumulates in reactor components, coolant and cleaning systems, and partly is released into environment as gaseous releases and as liquid effluents (IAEA 2004). RBMK-1500 type reactors were exploited at Ignalina NPP (Lithuania): Unit 1 - 1983-2004; Unit 2 - 1987-2009. Releases from NPP accumulate in local biosphere by photosynthesis including aquatic media, as INPP used Lake Drūkšiai as a cooling pond.
Methods: Temporal variation of ^{14}C in lake ecosystem was examined by analysing measured radiocarbon concentration of the organic compounds (Alkali soluble-AS and alkali insoluble-AIS) derived from the layers of the lake bottom sediments (Brock et al. 2010). A sediment core was sampled in 2019 at the deepest depression of Lake Drūkšiai by using a Kajak gravity corer, sliced to 1 cm layers and ^{14}C concentration was measured of every layer. AS and AIS organic fractions of sediment samples were extracted by using acid-base-acid method, were graphitized and measured by SSAMS at Vilnius Radiocarbon facility.
Results: Increase of ^{14}C concentration by 60 pMC in the AS fraction and only by 5 pMC in AIS fraction was observed corresponding to the period about year 2000, followed by gradual decrease. Estimated effective half-life of the self-cleaning is 8 years.
Conclusion: ^{14}C concentration profile analysis of the lake bottom sediments core revealed significant impact of the Ignalina NPP on the Drūkšiai Lake ecosystem. Critical period was in 2000s, when maintenance works of the reactors were performed, followed by gradual lake recovery.

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P46 - Long-Term 14C Activity Measurements in Tree Rings Near Ignalina Nuclear Power Plant: How it Helps to Monitor Safety of Our Environment

Health effects and radiation protection

Evaldas Maceika[1]
Algirdas Pabedinskas[1], Žilvinas Ežerinskis[1], Justina Šapolaitė[1], Dominykas Žiemys[1], Laurynas Butkus[1], Laurynas Bučinskas[1], Vidmantas Remeikis[1]
[1] Nuclear research department, Center for physical science and technology, Lithuania

Introduction: Atmospheric ^{14}C is produced by natural process of cosmic radiation interaction with atmosphere as well as by anthropogenic human activities.
Methods: 9 pine tree cores around the INPP and 3 tree cores in background area were extracted in order to examine the annual variation and dilution peculiarities of the released radiocarbon gaseous effluents from Ignalina NPP with RBMK-1500 reactors . ^{14}C concentration in tree rings were measured covering time span of 1980-2017.
Samples were physically and chemically (BABAB) prepared, graphitized with AGE-3 coupled with elemental analyzer and measured at Vilnius Radiocarbon SSAMS facility. Paired tree core samples, taken at the unidirectional sampling sites (located to the South- 1.8 and 5.1 km; West- 2.6 and 4 km; North-East- 1.9 and 6.6 km), were examined in details by considering meteorological data records from the Ignalina NPP local meteorological station (2004-2015) in order to trace atmospheric dilution effectiveness of ^{14}C released from the 150 m height INPP ventilation stacks.
Results: The results showed pronounced increase of ^{14}C up to 17.8 pMC in the tree rings during INPP exploitation as well during decommission periods and allowed to trace history of elevated release events.
Conclusion: The constructed Gaussian atmospheric model and analysis of unidirectional samples revealed relatively high year-by-year variation of released amounts and the atmospheric dilution conditions (in average about 130%), which were caused by different frequency of atmospheric stability classes occurrence (annually averaged dilution as typical for the C and D classes).

This research was funded by a grant (No. S-MIP-19-16) from the Research Council of Lithuania.

P47 - Low dose alpha, gamma and mixed beam radiation gene expression effects at low and high dose rates in human VH10 fibroblasts and AHH1 lymphoblasts

Health effects and radiation protection

Milagrosa Lopez Riego[1]
Pamela Akuwudike[1], Lovisa Lundholm[1], Andrzej Wojcik[1]
[1] Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University

Introduction: Low and high linear energy transfer ionising radiations (IR) from natural background, occupational or medical exposures occur at low doses (LD, <100 mGy) and dose rates (DR, <6 mGy/h). Such exposures have been associated with an increased incidence of solid tumours and leukaemia, but the shape of the dose response curve is not known. Using unique radiation sources at our facility, we aim at better understanding the effects of chronic exposure to different radiation qualities at low doses and different dose rates.
Methods: VH10 and AHH1 cells were chronically exposed to final doses of 50-200 mGy at 1 mGy/h ^{241}Am alpha, 1.6 mGy/h ^{137}Cs gamma or a 1:1 mixed exposure and analysed just after irradiation. These were compared to the same doses after acute 13.4 Gy/h alpha and 4.1 Gy/h X-rays, analysed 24 h post-IR. Six radiation responsive genes, i.e. *BBC3*, *CDKN1A*, *FDXR*, *GADD45A*, *MDM2*, and *XPC* were analysed by qRT-PCR.
Results: Gene expression analyses revealed a dose dependent upregulation pattern after chronic alpha exposure in VH10, not observed after mixed beam or gamma. Interestingly, there was a trend towards upregulation of some genes in acutely alpha-irradiated VH10, yet with a lower fold change than chronic exposure. In AHH1, we observed the opposite effects, here X-rays triggered a weak dose dependent upregulation after acute but not chronic exposure.
Conclusion: Further experiments are needed, but we believe that in vitro experiments with low activity sources can contribute to radiation protection and knowledge of potential health effects of different radiation qualities at LD and LDR.

P48 - Macrophage subpopulations in stereotactic radiation-induced lung injury in mice.

Health effects and radiation protection

Sarah Braga-Cohen[1]
Jan Baijer[2], Morgane Dos Santos[3], Valérie Buard[1], Christelle Demarquay[1], Fabien Milliat[1], Agnès Francois[1]
[1] Laboratoire de Radiobiologie des Expositions Médicales, IRSN
[2] UMRE008 Stabilité Génétique Cellules Souches et Radiations, CEA
[3] Laboratoire de Radiobiologie des Exposition Accidentelles, IRSN

Introduction: Stereotactic radiotherapy is a therapeutic alternative for 20-30% of patients with localised primary bronchial cancer and considered at high surgical risk. It is a technique of high ballistic precision, using converging small beams irradiating very small volumes. This technique allows the use of ablative doses per fraction, from 6 to 20 Gy. Despite accurate targeting, some patients develop inflammatory or fibrotic pneumopathies. The laboratory has developed a model of stereotactic pulmonary irradiation in mice allowing us to acquire anatomopathological features and to decipher mechanisms's involved. We observed an important macrophage infiltrate at the site of the focal lesion. Macrophages are immune cells which can evolve into several functional phenotypes, and are known to be involved in radiation-induced fibrotic processes. Therefore, we are interested in subpopulations of macrophages in the development of radiation-induced lung lesions under stereotactic conditions in mice.
Methods: To this end, lung lesions in wild type mice and CCR2 deficient mice, in which macrophage recruitment is compromised, will be compared following two radiation doses : 60 Gy and 80 Gy (3x3mm²) ; and at different times : 1 month and 3 months. On one hand, immunohistochemistry will determine the spatial location of macrophage sub-populations and on the other hand, by flow cytometry, we will quantify these populations.
Results: The flow cytometry panel has already been improved to fully identify subpopulations of macrophages in the lungs.
Conclusion: Moreover, we will used single cell RNAseq (10X Genomics methodology) to decipher in depth the phenotypic diversity and heterogeneity of macrophages after lung stereotactic irradiation.

P49 - The competitive relationship between cell killing and induction of carcinogenic mutations in normal cells exposed to fractionated radiation

Health effects and radiation protection

Pamela Akuwudike[1]
Milagrosa López Riego[1], Lovisa Lundholm[1], Andrzej Wojcik[1]
[1] Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University

Introduction: Predicting the risk of radiation-induced carcinogenesis at very high doses as encountered during radiotherapy is complicated by a lack of understanding of the competitive relationship between cell killing and the induction of carcinogenic mutations. It has been suggested that solving this dilemma requires consideration of both dose fractionation and the heterogeneous dose distribution across normal tissues exposed during radiotherapy. Here, we investigated this dual effect on the competition between cell death and the induction of stable carcinogenic mutations.
Methods: An *in vitro* experiment involving a fractionation scheme similar to clinical radiation exposure during conventional radiotherapy was designed. Two normal human cell lines, fibroblasts (VH10) and lymphoblasts (AHH-1) were irradiated at four different dose gradients (mimicking the heterogeneous dose distribution across normal tissue for each fraction), 0.25, 0.5, 1.0 and 2.0 Gy per fraction. Post fractionated radiation exposure, the effects on cell growth and cell survival, DNA damage repair kinetics via gamma H2AX assay as well as accumulation of stable chromosomal rearrangements using 3-color FISH were determined.
Results: Cell growth was inhibited with increasing dose, yet cells recovered completely after fractionated exposure ended, also at the highest dose level. A dose-dependent increase in markers of genomic instability which are indicative of the initiation of carcinogenic events was observed, such as accumulation of unrepaired DNA double-strand breaks and accumulation of micronuclei and nuclear buds at high doses.
Conclusion: In conclusion, the results so far indicate that events preceding cell death and induction of carcinogenic mutations steadily increase with increasing dose without a plateau.

P50 - The "BioPhyMeTRE" project: novel biological and physical methods for triage in radiological and nuclear (R/N) emergencies

Health effects and radiation protection

Clarice Patrono[1]
Dina Biyakhmetova[2], Emanuela Bortolin[3], Sara Della Monaca[3], Ina Erceg[4], Paola Fattibene[3], Laura Kenzhina[2], Nadica Maltar-Strmecki[3], Aigul Mamyrbaeva[2], Valentina Palma[1], Maria Cristina Quattrini[3], Maja Vojnič-Kortmiš[4], Fail Zhamaldinov[2], Antonella Testa[1]
[1] Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Via Anguillarese 301, 00123 Rome, Italy
[2] Institute of Radiation Safety and Ecology (IRSE) of National Nuclear centre of Kazakhstan, Krasnoarmeyskaya st.2, 071100 Kurchatov city, Kazakhstan
[3] Istituto Superiore di Sanità (ISS), Viale Regina Elena 299, 00161 Rome, Italy
[4] Ruder Boskovic Institute (RBI), Bijenička c. 54, 10000 Zagreb, Croatia

Introduction: The project entitled "Novel biological and physical methods for triage in radiological and nuclear (R/N) emergencies" (BioPhyMeTRE) has been recently approved within the NATO Science for Peace and Security Programme. The project focuses on innovative biological and physical dosimetry methods allowing a rapid screening/triage of potential victims by using inexpensive and user-friendly analytical procedures and devices.
Methods: In the "BioPhyMeTRE" project, a multi-parametric approach, by both biological and physical dosimetry methods, is used.

The biological method combines the two most standardised biodosimetry methods into a more exhaustive "two-in-one" assay.

The physical method focuses on the use of a low cost, portable mini photo-luminescence reader for the individual dose assessment by using personal objects.
Results: The novel biological and physical methods have been developed and partially tested by the laboratories participating in the project.

The biological combined protocol offers the advantage of simultaneous scoring of chromosome aberrations and micronuclei on the same slide, it is therefore time-saving and inexpensive.

The physical method system, designed and commercialized for irradiated food analysis, allows rapid measurements, is transportable and usable in site, even by not skilled operators.

Both methods will be validated through the set-up of calibration curves and inter-laboratory comparisons to verify their reliability for triage in R/N emergencies. Moreover, automation systems for the novel biological protocol will be evaluated.
Conclusion: The biological and physical dosimetry methods proposed in the "BioPhyMeTRE" project, once they have been fully developed and validated, could represent useful tools for the categorization of subjects overexposed to ionising radiation in R/N emergencies.

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