Book of Abstracts

50th Meeting of the European Radiation Research Society

Oslo, Norway
6–9 September 2026

ERRS 2026
OSLO, NORWAY

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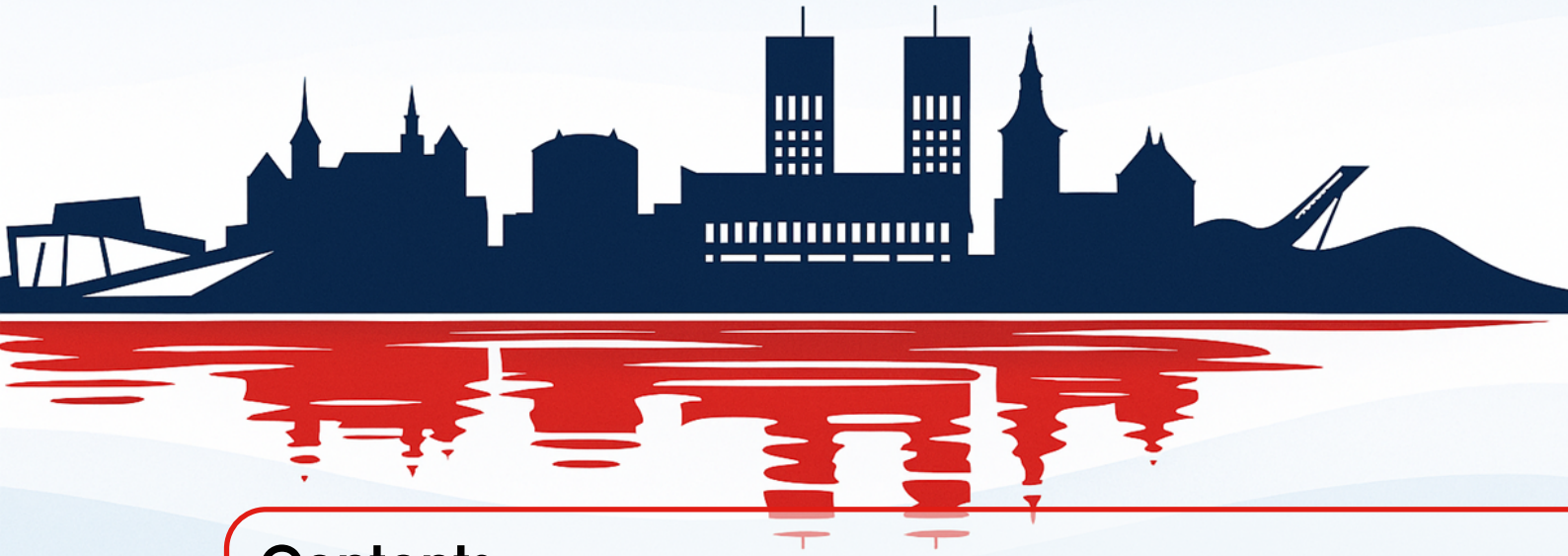

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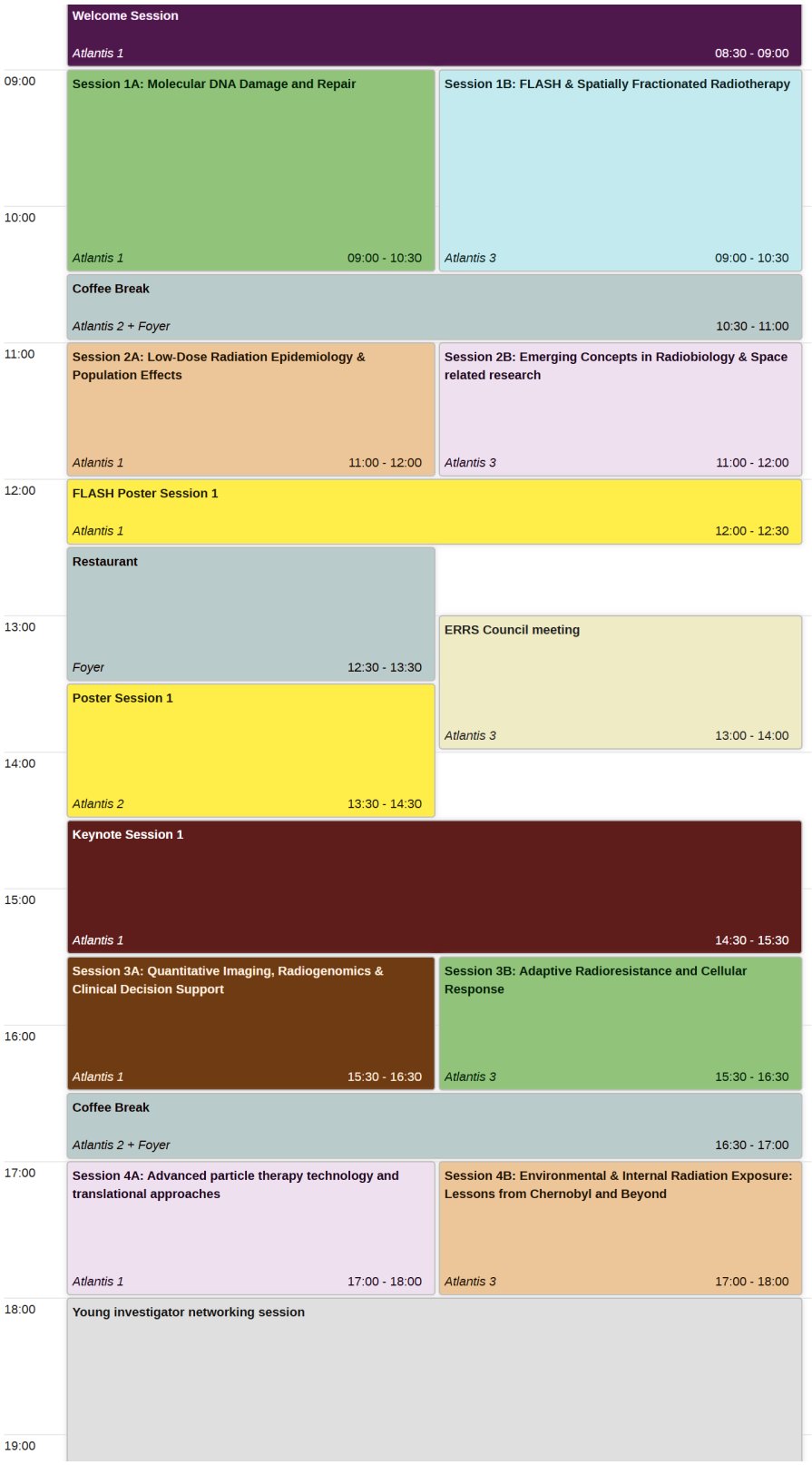
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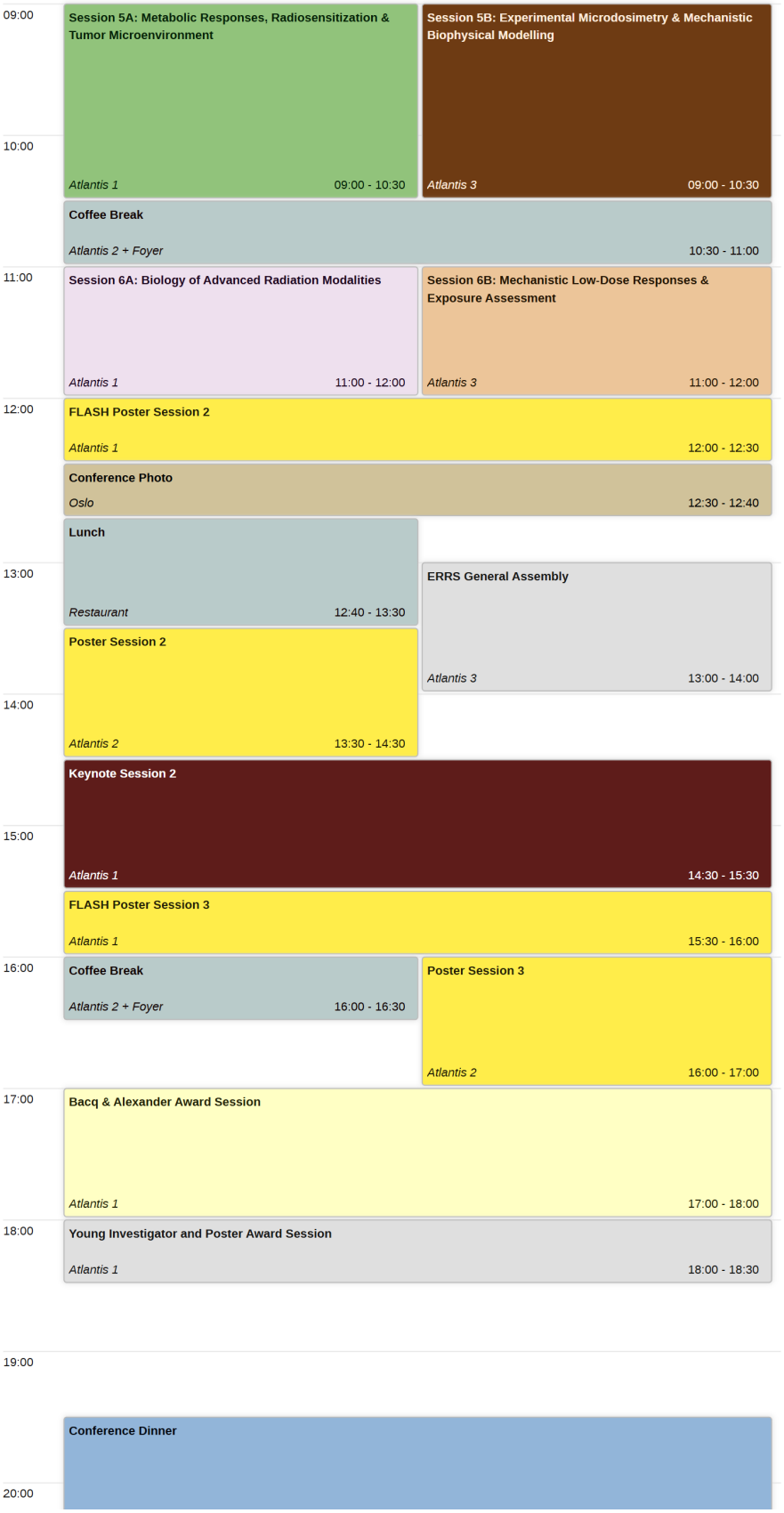
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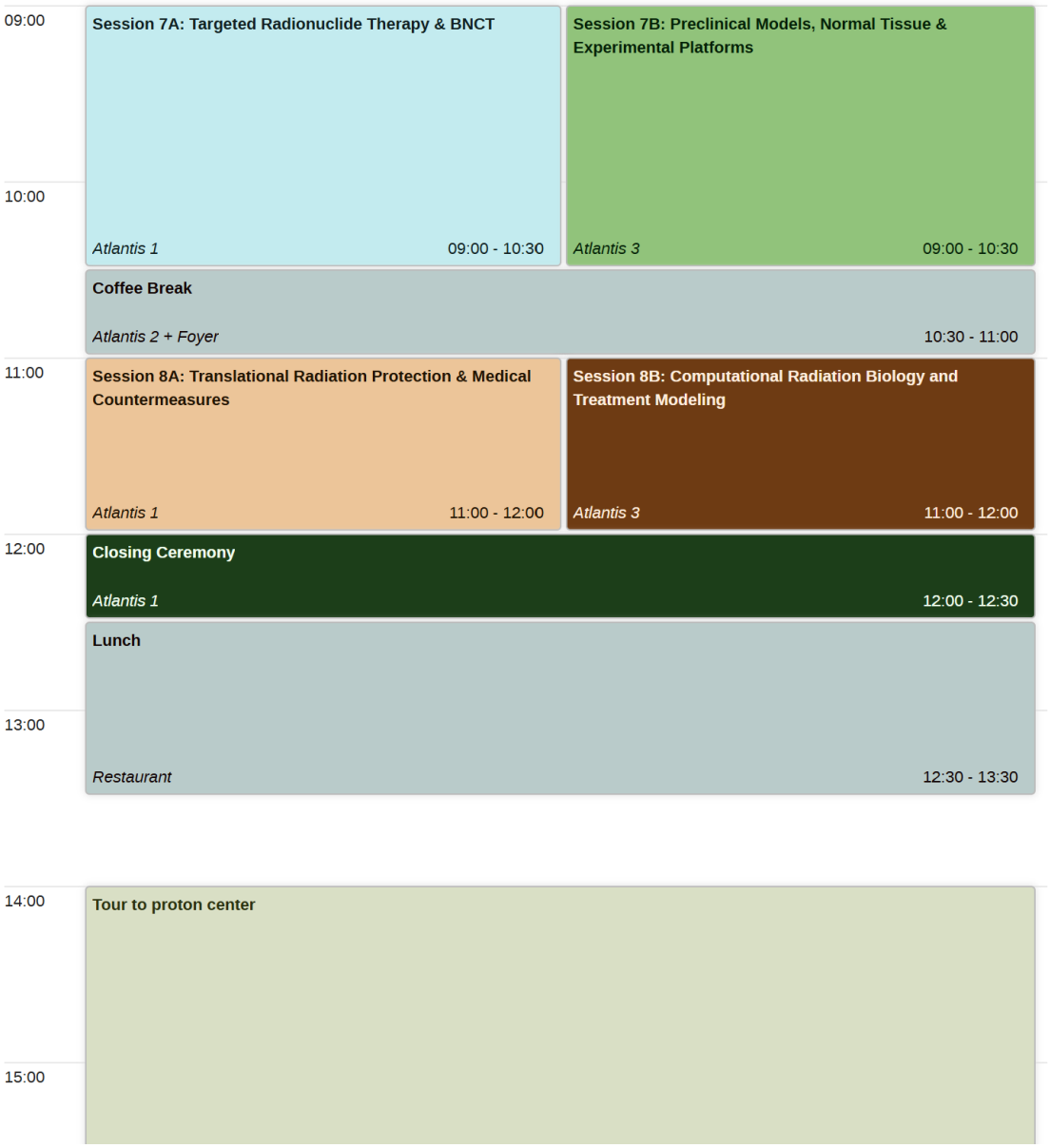
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Useful Information

FLASH presentations

Presenters should be ready at the stage **5 minutes before the session begins**.

Welcome Reception

Sunday, 6 September, 19:00
Hotel foyer
Sandwiches, wraps and cake will be served. Registered participants receive two complimentary drink vouchers.

Conference Run & Walk

Tuesday, 8 September, 07:00
Meet outside the hotel main entrance.
Run: 5.6 km, with three pace groups.
Walk: 2.4 km along the coastline.
Drinks will be provided. Participants wishing to join should inform the organisers.

Award Ceremony

Tuesday, 8 September, 18:00
Presentation of the Young Investigator Awards and Poster Prizes.

Gala Dinner

Tuesday, 8 September, 19:30
Hotel restaurant
A three-course seated dinner will be served to registered participants.

Guided Tour

Wednesday, 9 September, 14:00
Departure by bus from the hotel.
The tour includes Holmenkollen, Vigeland Park and the Proton Therapy Centre and ends in Oslo city centre. Participants with flights on Wednesday should contact the organisers. Further details will be provided during the conference.

Public transport

Use the **Ruter app** to plan journeys and purchase public-transport tickets. **To Oslo city centre:** Bus 31 runs every 6–10 minutes until midnight. The journey takes approximately 20 minutes to Nationaltheatret and 25 minutes to Oslo Central Station.

From Oslo Airport

Take train R10, R11 or R12 towards Oslo to **Lysaker**. Change to bus 31 towards Snarøya and get off at **IT Fornebu**. You can also change to the bus 31 at the main station **Oslo S**. This journey is covered by a public-transport ticket for zones 1, 2Ø, 3Ø and 4N. Flytoget is another option, but requires a separate Flytoget ticket and a zone 1 public-transport ticket.

Bikes

Bikes are available to borrow from the hotel. There is a pleasant cycle path to the city centre, and the Fornebu peninsula is also worth exploring by bike.

Swimming

The nearest beaches are south of the hotel and can be reached by walking through the apartment blocks. You can also enter the water from the pier in front of the apartment blocks, where ladders are available.

Allergies and dietary requirements

The hotel has been informed about registered allergies and dietary requirements. Please identify yourself to the waiting staff so that they can provide the appropriate meal.

Weather

September in Oslo can be mild, cold or rainy. Please check the official weather forecast at yr.no and bring warm, waterproof clothing.

In case of fire

Leave the building according to the hotel's evacuation instructions. Everyone should go outside to the terrace and assemble **by the tower**.

Special Issue at International Journal of Radiation Biology

All participants contributing an oral presentation or a poster to ERRS 2026 qualify to send a submission to „International Journal of Radiation Biology“. The goal is a joined ERRS 2026 Special Issue ready for print before the next annual ERRS meeting or similar. Hence, a 4-months deadline (until early January 2026) is required for the first submission of manuscripts. Prof. Dr. MSc. Michael Abend from ERRS council will contact them and coordinate this.

More information

For the latest information and updates, please visit:

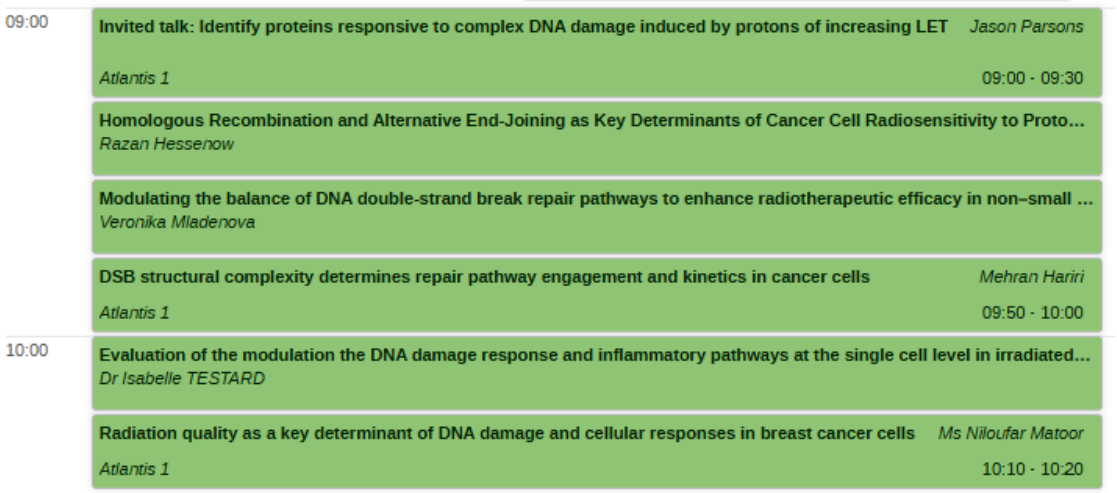
www.errs2026.com

Oral presentations

Monday, 07.09.2026

Session 1A: Molecular DNA Damage and Repair

Chairs: Randi Gussgard Syljuåsen and Samantha Cairncross
Room: Atlantis 1



Invited talk: Identify proteins responsive to complex DNA damage induced by protons of increasing LET

Author: Jason Parsons[1]

[1] *Department of Cancer and Genomic Sciences, University of Birmingham, UK; School of Physics and Astronomy, University of Birmingham, UK*

Radiotherapy using X-rays/photons is the cornerstone for treatment of 50% of all human cancers, where the therapeutic effect is driven through DNA damage, particularly DNA double strand breaks and complex DNA damage (CDD) where DNA lesions are induced in close proximity within the DNA. However, acute short and long-term adverse side effects in addition to tumour radioresistance remain common problems. The increased use of proton beam therapy enables more precise radiation delivery to the tumour via the Bragg peak, although the associated increased linear energy transfer (LET) driving increased CDD formation creates biological and clinical uncertainty. Despite this, we still have a lack of understanding of the precise biological effects of protons with increasing LET, and therefore how this can be optimised for effective use in the clinic.

The University of Birmingham, as part of Cancer Research UK RadNet Birmingham, has unique resources in the UK/Europe for delivering protons and other high-LET radiation (including helium ions and boron neutron capture therapy) for research. We have recently characterised the MC-40 cyclotron using head and neck cancer (HNSCC) models to demonstrate that increased levels of CDD induced by protons at and around the Bragg peak drives HNSCC cell death. Through siRNA screening, we have also identified that poly(ADP-ribose) polymerase-1 (PARP-1), poly(ADP-ribose) glycohydrolase (PARG) and 8-oxoguanine DNA glycosylase (OGG1), and specific drugs against these proteins, enhance the radiosensitisation of HNSCC models to relatively high-LET protons through supressing CDD repair. Conversely, we have also demonstrated using inhibitors of ataxia telangiectasia mutated (ATM), DNA-dependent protein kinase (DNA-PK) and the cell cycle checkpoint proteins CHK1 and WEE1, that these can enhance HNSCC radiosensitivity to photons and protons irrespective of LET. Work is now underway to further explore the proteins, and inhibitors against these, in response to significantly higher LET helium ions and BNCT where the DNA damage is substantially more complex.

47. Homologous Recombination and Alternative End-Joining as Key Determinants of Cancer Cell Radiosensitivity to Proton Irradiation: Implications for Precision Proton Radiotherapy

Author: Razan Hessenow[1]
Co-authors: J. Matschke; E. Mladenov; X. Lin; F. Heinzelmann; J. Esser ; S. E. Afshar ; F. Li ; A. Soni ; Ch. Möllers ; M. Busch ; N. Dünker ; G. Pantelias ; Ch. Bäumer ; M. Stuschke ; G. Iliakis ; B. Timmermann ; V. Jendrossek
[1] *West German Proton Therapy Centre Essen (WPE), University Hospital Essen, Essen, Germany. Institute of Cell Biology (Cancer Research), University Hospital Essen, University of Duisburg-Essen, Essen, Germany.*

Background: Proton beam radiotherapy (PBT) enables superior dose conformity and sparing of surrounding normal tissues compared with photon radiotherapy. However, the molecular determinants of tumor response to proton irradiation remain insufficiently defined, limiting the development of biology-driven precision strategies. Building on our previous findings that proton irradiation differentially induces DNA double-strand breaks (DSBs) with distinct architectures, we investigated whether resection-dependent repair pathways, particularly homologous recombination (HR) and alternative end-joining (alt-EJ), represent key and targetable determinants of proton-specific radiosensitivity.
Methods: To dissect DSB repair pathway dependence after proton versus photon irradiation, we combined genetic and pharmacological approaches in A549 and HCT116 tumor models. CRISPR-Cas9-engineered ATM-, PARP1-, and BRCA2-deficient cell lines were studied alongside pharmacological inhibition of ATM and PARP using KU55933, AZD1390, and Olaparib. Repair pathway engagement was analyzed using U2OS DSB repair reporter assays, classical cytogenetics, and pulse-field gel electrophoresis (PFGE). Functional consequences were assessed by clonogenic survival and were further validated in the chorioallantoic membrane (CAM) assay.
Results: Proton induced a stronger shift toward resection-dependent DSB repair than photon irradiation, with enhanced activation of HR and alt-EJ. This distinct repair response was accompanied by increased structural chromosomal aberrations after proton exposure, consistent with greater engagement of error-prone alt-EJ. Notably, these aberrations were markedly reduced by PARP inhibition, but were largely unaffected by Polθ inhibition, identifying PARP1-dependent alt-EJ as a major repair route for proton-induced DSBs. Functionally, BRCA2-, ATM-, and PARP1-deficient tumor cells displayed significantly greater sensitivity to proton than to photon irradiation. Likewise, pharmacological inhibition of PARP or ATM selectively potentiated proton-induced tumor cell killing, including in repair-proficient models, whereas POLθ targeting failed to produce comparable radiosensitization. These findings were reproduced across multiple in vitro models with increasing complexity and supported by the CAM model.
Conclusions: Our study identifies HR and PARP1-dependent alt-EJ as key biological determinants of proton response and mechanistic features distinguishing proton from photon radiotherapy. The selective enhancement of proton radiosensitivity in genetically repair-deficient model systems and following PARP or ATM inhibition reveals actionable vulnerabilities with direct translational potential. These findings support further evaluation of combined treatment with PBT and clinically established PARP inhibitors such as Olaparib, and the significance of biomarker-guided patient stratification to advance precision PBT.
Acknowledgement: Some results presented here were published in: Hessenow, R. et al. International Journal of Radiation Oncology*Biology*Physics (2026) doi:10.1016/j.ijrobp.2026.02.234. This work was supported by grants from European Union's Framework under Marie Sklodowska-Curie actions (grant agreement no. 860245 [ITN THERADNET]), the German Research Association (DFG) grant no. GRK2762 and , the Wilhelm Sander Stiftung (grant no.2023.138-1).

109. Modulating the balance of DNA double-strand break repair pathways to enhance radiotherapeutic efficacy in non–small cell lung cancer

Author: Veronika Mladenova[1]
Co-authors: Emil Mladenov[1], Martin Stuschke, Georg Iliakis
[1] *UME Essen, Essen, Germany.*

Background: Lung cancer remains one of the leading causes of cancer-related deaths worldwide. Its prognosis is frequently unfavorable due to late-stage diagnoses and limited effectiveness of available treatments. Radiotherapy utilizing ionizing radiation (IR) is an essential modality in the treatment of non-small cell lung cancer (NSCLC), primarily exerting its cytotoxic effects through induction of DNA double-strand breaks (DSBs). Our study investigates the mechanistic basis of radiosensitivity variations in a panel of five NSCLC cell lines (H-1299, H-460, H-596, H-23 and H-820), with a focus on the functionality and balance between major DSB-repair pathways as a determinant of cellular radiosensitivity.

Materials and methods: To investigate the response of the selected NSCLC cell lines to ionizing radiation, we employ colony formation assay, multicolor fluorescence in situ hybridization (mFISH), Western blot analysis, immunofluorescence analysis of IR-induced foci (IRIF), quantitative image-based cytometry (QIBC).

Results: Our findings reveal marked cell type differences in radiosensitivity, due to varying engagement of individual DSB-repair mechanisms. Pharmacological inhibition of DNA-PKcs, a core component of canonical non-homologous end joining (c-NHEJ), almost uniformly sensitizes all tested cell lines to IR, underscoring the efficiency of c-NHEJ and its potential as a therapeutic target. Conversely, immunofluorescence analysis of IR-induced RAD51 foci combined with high-throughput image capturing via QIBC reveals homologous recombination (HR) supression in H-23 and H-820 cell lines. It is associated with impaired DNA end resection and increased incidence of structural chromosomal abnormalities (SCAs) — indicative of compensatory engagement of error-prone repair mechanisms. Notably, elevated PD-L1 expression in H-820 cell line was found to suppress HR, as PD-L1 downregulation partially restores RAD51 foci formation and confers increased radioresistance.

Conclusions: We hypothesize that the biological effects of IR could be effectively modulated by defining parameters that shift the balance between major DSB repair mechanisms. Our findings have translational potential in the clinic, which may be leveraged by combining IR with compounds that specifically target DDR/DSB repair factors.

Work supported by grant from BMFTR (02NUK082A)

112. DSB structural complexity determines repair pathway engagement and kinetics in cancer cells

Author: Mehran Hariri[1]
Co-authors: Bi Stenerlöw
[1] *Uppsala University, Uppsala, Sweden*

Background: DNA double-strand breaks (DSBs) are the most cytotoxic lesions induced by radiotherapy and many anticancer agents. However, DSBs differ widely in structural complexity, ranging from clean breaks to complex lesions associated with clustered damage. How DSB complexity influences repair pathway engagement, particularly non-homologous end joining (NHEJ), remains poorly understood.
Materials and Methods We investigated DSB repair responses in wild-type and NHEJ-deficient (XRCC4 and DNA-PKcs knockout) HCT116 cells following exposure to agents generating distinct DNA damage profiles: calicheamicin, X-rays, phleomycin, etoposide, and temozolomide. Clonogenic survival, prompt DSB induction, non-DSB clusters, and DSB repair kinetics were quantified using pulsed-field gel electrophoresis (PFGE).
Results DSB repair kinetics in wild-type cells displayed biphasic behavior with fast and slow components. In contrast, NHEJ-deficient cells exhibited only a rapid repair phase (within 30–60 min), followed by minimal repair over 1–24 hours. Repair capacity strongly depended on damage complexity: only ˜20% of DSBs induced by X-rays and calicheamicin were repaired in NHEJ-deficient cells, compared to ˜40–50% for phleomycin and ˜85% for etoposide. Temozolomide, which does not induce prompt DSBs, reduced cell survival independently of NHEJ status. Notably, non-DSB clustered lesions increased as the DSB:SSB ratio decreased and were rapidly resolved independent of NHEJ. Importantly, clonogenic survival did not directly correlate with DSB repair capacity, indicating differential contributions of NHEJ-independent pathways depending on lesion complexity.
Conclusions DSB structural complexity is a key determinant of repair pathway engagement and efficiency. NHEJ is essential for resolving highly complex DSBs, while alternative pathways can compensate for simpler lesions. These findings provide a framework linking DNA damage complexity to repair pathway choice and suggest new opportunities to exploit repair vulnerabilities for cancer therapy.

73. Evaluation of the modulation the DNA damage response and inflammatory pathways at the single cell level in irradiated human primary cells

Author: Testard Isabelle[1]
Co-authors: Alexandra Gattoni, Clément Rouichi, Elisabeth Charter-Garcia, Emmanuelle Soleilhac, Serge Candeias
[1] *UEA*

Background: The physical characteristics of ionizing radiation, such as dose, dose rate, and linear energy transfer, are critical determinants of cellular and tissue biological responses. Beyond the DNA damage response (DDR), which is primarily driven by ATM kinase activation (p53 pathway), radiation also triggers an inflammatory response (NF-κB pathway).
This study sought to compare how different radiation qualities affect key components of both pathways. Specifically, we investigated the dose dependence, kinetics, and cell-type specificity of radiation-induced responses in primary human cells following exposure to X-rays and high-linear energy transfer (LET) ions. **Methods:** We assessed the modulation of central effectors in the ATM-p53 and NF-κB pathways at both the protein (immunofluorescence) and mRNA (ViewRNA™ detection) levels, with single-cell resolution, for up to 24 hours after exposure to 0–10 Gy of X-rays or high-LET carbon ions. These *in situ* analyses were conducted on fixed cells, with imaging and quantification performed using an automated microscope equipped for high-content image analysis (CellInsight CX7). **Results:** Following X-ray exposure, our results align with previous findings based on qRT-PCR and cytokine secretion assays. Compared to X-rays, carbon ions elicit a similar inflammatory response but at elevated levels. DDR pathway modulation is comparable between the two radiation types, though it persists longer after high-LET irradiation. The use of pathway inhibitors revealed significant crosstalk between the DDR and inflammatory responses. **Conclusions:** This study confirms robust activation of both DDR and inflammatory pathways across different cell types and validates the in situ approach. The concurrent regulation of p53 and NF-κB in irradiated cells may be pivotal in determining cell fate by balancing survival and death signals.

Keywords: ionizing radiation, high-LET, inflammation, DDR, primary cells, *in situ*.

34. Radiation quality as a key determinant of DNA damage and cellular responses in breast cancer cells

Author: Niloufar Matoor[1], Alexandra Charalampopoulou[1]
Co-authors: Amelia Barcellini [2]; Baldassare ; Dall'Acqua ; Magro ; Mereghetti ; Pullia ; Facoetti
[1] IUSS & CNAO; [2] UNIPV & CNAO

Background: Radiotherapy represents a cornerstone of breast cancer management, contributing to local control and patient survival, particularly in breast-conserving and adjuvant settings. Although photon radiotherapy (XRT) remains the clinical standard, treatment efficacy may be influenced by inter-tumor heterogeneity and the therapy-resistant phenotypes. Acquired resistance to chemotherapy, constitutes a major clinical challenge and has been associated with reduced treatment outcomes. Particle therapy is increasingly being explored in breast cancer; however, radiation quality-dependent responses, in chemotherapy-sensitive and -resistant models, remain incompletely characterized. This study evaluated the effects of XRT, PRT, and CIRT on DNA damage, clonogenic survival, metabolic viability, migration, and invasion in Luminal A breast cancer T47D-S and T47D-R cells.

Materials and Methods: T47D-S and T47D-R Luminal A breast cancer sublines were exposed to 1–6 Gy of XRT, PRT, or CIRT, alongside controls. DNA damage was evaluated by γH2AX immunofluorescence at 30 min and 24 h post-irradiation. Metabolic-based viability was assessed using the MTT assay at 24 h and 6 days, while long-term reproductive capacity was determined by clonogenic assay. Cell motility was quantified using transwell migration and invasion assays. All experiments were conducted in triplicates and reproduced in three independent biological experiments.

Results: CIRT produced a marked reduction in metabolic viability in both sublines. In T47D-S cells, viability at 6 Gy (6 days) decreased by approximately 44% relative to XRT ($p < 0.01$). In T47D-R cells, CIRT reduced viability by ˜43% at 6 Gy compared with XRT ($p < 0.001$), with significant differences observed at 4 Gy ($p < 0.01$). Clonogenic survival analysis revealed a pronounced radiation quality effect. In T47D-S cells, CIRT reduced survival by ˜55% at 2 Gy and ˜94% at 6 Gy relative to XRT. T47D-R cells exhibited a similar pattern, with survival reductions of ˜46% at 2 Gy and ˜91% at 6 Gy. CIRT also significantly impaired cell motility. In T47D-S cells, migration decreased by ˜37% ($p < 0.05$) and invasion by ˜17% at 6 Gy. In T47D-R cells, migration was significantly reduced, with inhibition detectable at 4 Gy and a ˜42% decrease at 6 Gy ($p < 0.001$). Invasion analysis demonstrated significant effects of both dose and irradiation modality. Consistently, high-LET irradiation induced persistent DNA damage in both sublines, with >50% γH2AX signal observed at doses $\geq$4 Gy ($p < 0.001$).

Conclusions: Particle irradiation induced pronounced biological effects in Luminal A breast cancer cells, characterized by persistent DNA damage and reduced survival, viability, migration, and invasion. While PRT exhibited intermediate effect, CIRT reduced T47D-R cell survival, underscoring radiation quality as a critical determinant of therapeutic response and supporting further clinical evaluation of particle therapy in chemotherapy-resistant breast cancer.

Session 1B: FLASH Spatially Fractionated Radiotherapy

Chairs: Olga Martin and Cristian Fernandez Palomo
Room: Atlantis 3

09:00

Invited talk: Mechanistic Insights into FLASH Radiation for Clinical Translation *Kristoffer Petersson*
Atlantis 3 09:00 - 09:30

First irradiations of human cells and murine tissues with the CIX FLASH system adapted for Sub-Millimeter Spatially Fr...
Dr David Reparaz Pernaut

Establishing Submillimetre Spatially Fractionated Radiotherapy (μSFRT) as a Transformative Treatment for Hepatocellul...
Cheuk Ting Wu

FLASH proton irradiation of 2D and 3D cell models of triple-negative breast cancer cells *Carlota França*
Atlantis 3 09:50 - 10:00

10:00

Oxygen dynamics in hypoxic, normoxic, and hyperoxic murine skin: association with dose–response toxicity during FL...
Anna Holtz Hansen

A Combined Dose and Microdosimetric Framework Incorporating Volume Effects Correlates with Tissue Sparing in Mini...
Giulio Bordieri

Stochastic analysis of space-time proximity at ultra-high dose rate irradiation and interplay with radiation chemistry sc...
Lorenzo Castelli

Invited talk: Mechanistic Insights into FLASH Radiation for Clinical Translation

Author: Kristoffer Petersson[1]

[1] *Department of Oncology, University of Oxford, Oxford, United Kingdom*

FLASH radiotherapy has the potential to transform the way we treat cancer. It has been shown preclinically that FLASH significantly reduces treatment side-effects, the so-called FLASH effect, with a dose-modifying effect of 10-50% depending on the healthy tissue exposed. This could allow for treatment of tumours with higher radiation doses for better treatment response, more aggressive fractionation schemes or, alternatively, treatment with similar doses as today resulting in fewer and less severe side-effects. Our research aims to move us closer to a successful clinical translation of FLASH radiotherapy by addressing the remaining challenges on three fronts:

1. Through preclinical experiments we work to elucidate the biological mechanisms resulting in the FLASH effect, the magnitude of the effect and how it depends on physical beam characteristics and tissue environment. In particular, we determine the differential FLASH effect between different tumour and normal tissues, if the effect can be further expanded, through fractionation strategies and in combination with anti-cancer drugs to improve clinical efficacy, for a more effective clinical translation

2. We work towards solving part of the technical limitations by exploring the possibilities of using available linear accelerator components, namely electron gun, RF (radiofrequency) generation, and bremsstrahlung target & beam intensity modulation technologies, and optimising them for MV Photon FLASH delivery.

3. We carry out part of our research in a clinical setting, using a modified clinical linear accelerator for FLASH delivery, moving towards clinical levels of safety and dose delivery precision. Our goal is to validate our preclinical findings in a scenario that is closest to the clinical situation as possible, performing veterinary clinical studies on canines with spontaneous tumours.

16. First irradiations of human cells and murine tissues with the CIX FLASH system adapted for Sub-Millimeter Spatially Fractionated Radiotherapy

Author: David Reparaz Pernaut[1]
Co-authors: Verdiana Trappetti, Paolo Pellicioli, Mara Eugster, Francesca Andronico, Jakub Korzeniowski, Ting Cheuk Wu, Ismael Sanchez, Cristian Fernandez-Palomo, Valentin Djonov
[1] *University of Bern, Bern, Switzerland*

Aims:: Sub-millimeter Spatially Fractionated Radiotherapy (smSFRT) delivers highly heterogeneous radiation doses to targets and has demonstrated superior tumor control and survival in murine tumor models compared with conventional broad-beam (BB) radiotherapy (RT), with an homogeneous dose pattern. The production of optimal smSFRT beams requires ultra-high dose-rate (UHDR) delivery, a capability until now achievable only with synchrotron facilities. However, recent technological advances have enabled the development of compact UHDR X-ray sources capable of delivering smSFRT. Our group has acquired one of the first CIX FLASH prototypes from XStrahl, a system that has not yet been evaluated for smSFRT in biological models. We aim to demonstrate the feasibility and therapeutic effectiveness of smSFRT using this platform in human cancer and normal tissue cell lines and murine tumors and healthy tissues, and to establish robust, reproducible irradiation protocols applicable to multiple cancer models.
Methods: Hs294T/HTB-140 melanoma cells and HaCaT keratinocytes were seeded in two-well Permanox chamber slides to form semi-confluent monolayers. Cells were irradiated with either BB (2–8 Gy) or smSFRT (200 μm beam width, 800 μm spacing and 10 to 50Gy in the peak) using 20 × 20 mm² and 5 × 5 mm² fields for clonogenic and immunostaining assays, respectively. Clonogenic assays were initiated immediately after irradiation, while cells were fixed at 1, 2, 4, 6, and 24 h to evaluate smSFRT geometry by γH2AX staining. Murine tissues (skin, liber, melanoma and breast cancer) were irradiated under anesthesia with a peak dose of 50 Gy.
Results: A clear smSFRT pattern on the cell layer in vitro and tissues in vivo was confirmed by γH2AX staining. In 2D cell layers the pattern could be identified starting at 1h post-irradiation and up to 24h at the highest peak doses. In vivo smSFRT patterns were observed from the beam entry point through to deep regions of the organs. γH2AX positive areas appeared broader than the 200μm collimator aperture due to X-ray beam divergence. Irradiation with CIX FLASH shows a reduction of the surviving fraction of the cells as doses were escalated both in BB and smSFRT.
Conclusions: Irradiation of human cell lines in vitro and murine tissues in vivo using the CIX FLASH irradiation system was feasible and enabled clear visualization of the smSFRT pattern across both time and depth. Clonogenic assays revealed dose dependent effective killing of the tested cell lines.

38. Establishing Submillimetre Spatially Fractionated Radiotherapy (μSFRT) as a Transformative Treatment for Hepatocellular Carcinoma: From Mechanistic Normal Tissue Healing Windows to Tumour Control

Author: Cheuk Ting Wu[1]
Co-authors: Cristian Fernandez-Palomo [2]; Deborah Stroka [3]; Jennifer Fazzari [4]; Nahoko Shintani [2]; Paolo Pellicioli [2]; Tural Yarahmadov [3]; Valentin Djonov [2]; Verdiana Trappetti [2]
[1] *Institute of Anatomy, University of Bern ;*[2] *Institute of Anatomy, Faculty of Medicine, University of Bern, Bern, Switzerland ;*[3] *Department for BioMedical Research (DBMR), Faculty of Medicine, University of Bern, Bern, Switzerland ;*[4] *Department of Radiation Oncology, Mayo Clinic – Rochester, Rochester, USA*

Aim: Conventional radiotherapy (CONV-RT) for hepatocellular carcinoma (HCC) is severely limited by the low tolerance of healthy parenchyma, often leading to radiation induced liver disease (RILD). Submillimetre spatially fractionated radiotherapy (μSFRT), including microbeam and minibeam approaches, has demonstrated a unique ability to spare healthy tissue1–3 while maintaining tumour control4–8 in various mouse models, but yet to be in any liver-related models. This study investigates the spatiotemporal mechanisms and alternative cell death modalities induced by μSFRT in the healthy liver and liver tumours.
Methods: Livers of C57BL/6 mice were irradiated using two distinct SFRT platforms:
1. Synchrotron based MRT (Healthy Liver): Performed at the European and Australian Synchrotrons (50μm beams, 400μm spacing, 400Gy peak) to map high resolution healthy tissue responses.
2. In house CIX MBRT (Healthy and HCC Tumour): Utilising our CIX FLASH system (200μm beams, 800μm spacing, 50Gy peak) compared against CONV RT (up to 50Gy) to evaluate therapeutic index and tumour control.
A high throughput methodology was employed, utilising Imaging Mass Cytometry (IMC) with a 30 plus liver specific marker panel complemented with multiplex immunofluorescence (mIF) for cell death markers. For longitudinal tumour growth, it was followed by Bioluminescence Imaging (BLI).
Results: Preliminary MRT data reveals that no fibrosis was detected 6-month post MRT. Our data indicated that there is a dynamic cell death and repair mechanism at peak region instead of valley within 6 days post-MRT. mIF profiling identified a specific positive and negative expression signature that defines a non-canonical programmed cell death pathway. IMC analysis defined a distinct Healing Window where a targeted recruitment of M1 and M2 macrophages at Day 3 is rapidly reduced by Day 6, accompanied by a regulatory T cell (Treg) influx. In synchronisation, hepatocyte proliferation only starts to increase on Day 6.***(See Attachment: Figure 1).*** Pilot BLI data suggest emerging trends toward improved tumour control with MBRT compared to CONV RT, with expanded longitudinal studies currently in progress.
Conclusion: Our findings suggest that μSFRT, particularly MRT, induces a non-canonical programmed cell death followed by an acute, resolving inflammatory phase that facilitates tissue repair without chronic inflammation or fibrosis. The temporal synchronisation of Treg infiltration and hepatocyte proliferation highlights a uniquely controlled regenerative response. By expanding this comparative analysis to MBRT and CONV RT, we aim to define the mechanistic foundation for a transformative clinical approach to HCC that enables aggressive dose escalation while preserving essential hepatic reserve.

113. FLASH proton irradiation of 2D and 3D cell models of triple-negative breast cancer cells

Author: Carlota França[1]

Co-authors: Ana Belchior[1]; Célia Tavares de Sousa[2]; Edgar Mendes[1]; Federico Picollo[3]; Maria Dolores Ynsa[2]; Paula Campello[1]; Pedro Assis[1]; Teresa Pinheiro[1]

[1] *Universidade de Lisboa,Portugal;*[2] *Universidad Autónoma de Madrid, Madrid, Spain;*[3] *University of Turin, Italy*

Background: Particle therapy has emerged as an innovative modality due to its favorable physical and radiobiological characteristics. Proton therapy has expanded worldwide, and ultra-high dose rate (FLASH) shows promise in reducing normal tissue toxicity, although radiobiological mechanisms, including radioresistance, DNA damage response, and increased linear energy transfer in hypoxic tumor regions remains unclear. This is highly relevant for triple-negative breast cancer (TNBC), marked by high intratumoral heterogeneity, hypoxia, early metastatic potential,and limited response to conventional therapies. The present study evaluates the radiobiological effects of proton FLASH irradiation using 2D adherent cells and 3D spheroids, and applies gold nanoparticle (AuNP)-based platforms as innovative therapeutic agents. **Materials and Methods**: The radiobiological effects of FLASH irradiation were evaluated in HCC1806 TNBC cells using 2D monolayers and 3D spheroids, each under two conditions: untreated cells and cells incubated with Gold-Coated Nanodiamonds (NDAus, 10 μg/mL)1. NDAu incubation was performed for 24 h in 2D cultures and 48h in 3D spheroids prior to irradiation. Irradiations were performed at the Bragg peak using a 10 MeV proton beam (1 nA) at CMAM under FLASH conditions at a dose rate of 40 Gy/s. For 2D, radiobiological endpoints included clonogenic survival, assessed at doses of 0.5, 1, 2, 4, 6 and 10 Gy, as well as DNA double-strand break (DSB) induction and reactive oxygen species (ROS) generation (DCFH-DA and DHE quantification), evaluated at 2, 6, and 10 Gy at 4 h and 24 h post-irradiation. For 3D, cells were irradiated with doses of 6, 10 and 20 Gy. Analyses included morphological evolution and growth, as well as cell viability, ROS production and DNA DSB, all evaluated at 24 h, 48 h, and 7 days post-irradiation. **Results**: Preliminary results indicated that FLASH proton irradiation induced a dose-dependent decrease in cell viability in both 2D HCC1806 monolayers and 3D spheroids, with 3D models showing measurable reductions in spheroid size and ATP levels. The presence of NDAu appears to enhance radiosensitivity in both models. **Conclusions**: A potential synergistic interaction between NDAu and FLASH proton irradiation is anticipated; however, further studies are required to clarify whether the observed increase in radiosensitivity translates into greater biological damage, particularly through disruption of DNA repair pathways. **Acknowledgements**: This work was supported by COMPETE2030-FEDER-00841000 https://doi.org/10.54499/2023.16391.ICDT, International Atomic Energy Agency (IAEA, CRP F11024), UIDP/04565/2025, LA/P/0140/2020 and UID/MULTI/04349/2020 projects. **References**:1 Mendes E, Aprà P, Belchior A, Picollo F, Alves MM, Dinica RM, Moura MJ, Sturari S, Pinheiro T, Campello MPC (2026) Green-synthesized gold-coated nanodiamonds as potential radiosensitizers for proton therapy. Nanoscale Horiz.2;11(3):883-898. doi: 10.1039/d5nh00424a

124. Oxygen dynamics in hypoxic, normoxic, and hyperoxic murine skin: association with dose–response toxicity during FLASH versus CONV irradiation

Author: Anna Holtz Hansen; Brita Sørensen[1]; Jacob Graversen[1]; Line Kristensen[1]; Morten Busk[1]; Per Poulsen[1]; Sara Poulsen[1]; Valentin Møller[1]

[1] *Aarhus University Hospital*

Background: FLASH irradiation, delivered at ultra-high dose rates (UHDR), has been shown to reduce normal tissue toxicity while main-taining tumor control. The radiolytic oxygen depletion (ROD) hypothesis was among the first proposed mechanisms un-derlying this effect, although its role remains debated. In this study, we investigate oxygen dynamics during irradiation in murine skin while systematically modulating baseline tissue oxygenation to better understand their contribution to the FLASH effect.

Materials and Methods: Oxygen dynamics in murine hind-limb skin were assessed using time-resolved phosphorescence-lifetime oximetry to measure tissue pO_2 during electron FLASH (231.4 Gy/s) and conventional (CONV) (0.26 Gy/s) irradiation under hypoxic, normoxic, and hyperoxic conditions. Hypoxia was induced by applying a thigh clamp to restrict blood flow prior to and during irradiation, while hyperoxia was achieved by carbogen breathing (95% O2+5%CO2). Real-time pO_2 was recorded throughout irradiation.

To evaluate the biological response, mice were irradiated with FLASH or CONV across a range of doses under each oxygen-ation condition. Acute skin reactions were scored from day 7–28 post-irradiation to generate dose–response curves. Real-time pO_2 measurements in normoxic and hyperoxic animals enabled correlation of irradiation depending oxygen dynam-ics with the resulting toxicity response at the individual level.

Results: Data demonstrated that FLASH reduces acute skin toxicity under normoxic conditions and increases toxicity under hypoxia compared with CONV irradiation. Although a sparing effect was observed under hypoxia for both FLASH and CONV, this effect was less pronounced for FLASH, leading to the FLASH sparing effect was abolished (1) . This was associated with cor-responding decreases and transient increases in tissue pO_2 during FLASH irradiation. These findings suggest that oxygen dynamics during irradiation, may influence radiosensitivity.

Hyperoxic conditions induced by carbogen breathing were introduced to enable direct comparison across oxygenation states. Real-time pO_2 measurements were obtained in individual animals during irradiation, allowing direct correlation of oxygen dynamics during irradiation with the resulting toxicity response at the individual level. Data acquisition for the hy-peroxic cohort is ongoing.

Conclusions: irradiation induced changes in tissue pO_2 are associated with acute normal tissue toxicity, with increases linked to higher toxicity and decreases to reduced toxicity under normoxic and hypoxic conditions. These findings highlight the importance of oxygen dynamics during irradiation, beyond baseline oxygenation alone, and suggest that real-time modulation of tis-sue oxygenation may contribute to the FLASH effect. Ongoing data collection from the same model and setup will reveal how the FLASH effect is influenced by increasing the oxygen level.

Reference:

1. Anna Holtz Hansen , P.R.P., Line Kristensen , and P.M.S. Valentin Vacari Møller, Jacob Graversen Johansen, Brita Singers Sørensen Impact of oxygen deprivation on the FLASH effect for skin toxicity in a murine model. Radiotherapy and Oncology, 2025. Volume 214111277January 2026

11. A Combined Dose and Microdosimetric Framework Incorporating Volume Effects Correlates with Tissue Sparing in Minibeam Radiotherapy: Integrated Modeling and Experimental Analysis

Author: Giulio Bordieri[1]
Co-authors: Anatoly Rozenfeld [2]; Emanuele Scifoni [3]; Enrico Verroi [3]; Francesco G. Cordoni [1]; Gianluca Lattanzi [1]; Giulio Magrin [4]; Linh Tran [2]; Marco Battestini; Marta Missiaggia [5]; Matthias Knopf [6]; Simon Waid [7]
[1] University of Trento [2] University of Wollongong [3] Trento Institute for Fundamental Physics and Applications (TIFPA) [4] EBG MedAustron GmbH [5] Louisiana State University [6] Technische Universität Wien [7] Marietta Blau Institute for Particle Physics

Background and Aims: Proton minibeam radiotherapy (pMBRT) delivers spatially alternating high-dose peaks and low-dose valleys to widen the therapeutic window. While experimental studies have shown promising normal-tissue sparing and improved tumor control, the biophysical mechanisms underlying pMBRT remain unclear, hindering clinical translation. This work develops a multiscale framework grounded in microdosimetry, combining Monte Carlo (MC) simulations, experimental measurements, and mathematical modeling to investigate the influence of beam-shaping parameters and tissue architecture on normal tissue complication probability (NTCP).
Materials and Methods: Microdosimetric spectra for 70 MeV protons were experimentally measured for four collimator configurations at the Centro di Protonterapia di Trento using a 10-µm-thick silicon-on-insulator (SOI) 3D mushroom microdosimeter with cross-sectional sensitive area of 1mm². Measurements were acquired for four minibeam collimator geometries characterized by different peak widths, center-to-center (ctc) spacings, and collimator thicknesses, respectively, to be (in mm): 1–2–5, 1–2–10, 1–2–50, and 1–4–10. Spectra were measured directly at the collimator exit, after 1cm WET, and after 3cm WET at the center of the central peak and of the adjacent valley. All measurements were benchmarked against MC simulations performed using Geant4.
A general NTCP model was then developed by coupling the critical-volume model with the Generalized Stochastic Microdosimetric Model (GSM²), explicitly accounting for non-uniform irradiation patterns characteristic of minibeam therapy. Tissues were represented as assemblies of functional subunits, and NTCP was evaluated across seriality parameters, from 0 (fully parallel) to 1 (fully serial).
Results: Both experimental and MC microdosimetric analysis showed marked differences between peaks and valleys at the entrance, with a progressive quality homogenization at 3cm depth, close to the Bragg peak. Microdosimetric spectra from the valleys exhibited a shift towards higher lineal energy values when compared to the spectra from the peaks at the same depth. This effect is progressively lower with increasing depth and dependent on the collimator. The collimator with the highest ctc spacing still shows difference in the microdosimetric spectra from peaks and valleys at 3cm depth.
From the NTCP results, the integral normal-tissue dose corresponding to 50% complication probability (D50) was extracted. pMBRT and conventional homogeneous fields (HF) were compared by defining the Minibeam Dose Ratio at 50% (MDR50) as the ratio between D50 for HF and D50 for pMBRT. NTCP predictions indicated substantial normal-tissue sparing from pMBRT, particularly for configurations with higher peak-to-valley dose ratio, including relatively highly serial organs with seriality values around 0.7.
Conclusions: This integrated microdosimetric–biological framework, supported by MC simulations and experimental validation, elucidates how spatial fractionation, radiation quality, and organ architecture collectively shape tissue sparing in pMBRT.

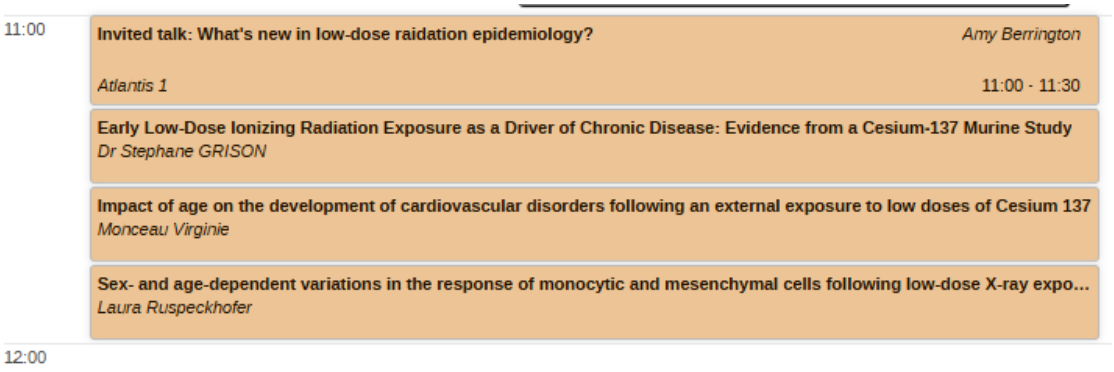
95. Stochastic analysis of space-time proximity at ultra-high dose rate irradiation and interplay with radiation chemistry scales.

Author: Lorenzo Castelli[1]
Co-authors: Emanuele Scifoni [2]; Valentina Tozzini [3]
[1] *Università di Trento;* [2] Trento Institute for Fundamental Physics and Applications (TIFPA)*;* [3] Istituto Nanoscienze CNR-Nano, lab NEST- Scuola Normale Superiore, Pisa, Italy

Among the leading hypotheses for the radiobiological origin of the FLASH effect, one key idea is that spatiotemporal clustering of particle tracks at ultra-high dose rates (UHDR) alters the local physicochemical conditions in the irradiated medium. These effects, controlled by the beam's temporal structure, involve not only direct interactions between chemical species from different tracks but also the cumulative buildup of chemically relevant conditions that can trigger specific biological responses. Here, we introduce an analytical framework based on a Poisson random measure to quantify track spatiotemporal proximity directly from the irradiation structure, thereby identifying the characteristic time and length scales relevant for modeling and characterizing FLASH-relevant irradiation conditions. Track arrivals are modeled as a spatiotemporal Poisson random measure, assuming statistical independence in time and spatial uniformity over the irradiated field, to account for stochastic fluctuations. The time-dependent intensity is constructed directly from irradiation parameters (dose, dose rate, linear energy transfer (LET), target density, and size) and extended to explicitly include pulsed delivery through periodic macro- and micro-pulse structures. Closed-form expressions are derived for the expected number of tracks within a prescribed spacetime proximity window. The proposed framework enables direct evaluation of track spatiotemporal proximity for arbitrary irradiation conditions, allowing systematic comparison of proximity regimes for electrons, protons, and heavy ions under relevant experimental parameters. It shows how beam structure, LET, dose rate, and pulse structure govern the likelihood of track co-occurrence. The analysis identifies the temporal and spatial scales at which clustering may emerge for different irradiation setups, indicating that collective effects mainly arise beyond early heterogeneous chemistry, likely in later, denser biochemical stages. As an initial case study, analytical predictions were combined with multi-track TRAX-CHEM simulations to examine the heterogeneous chemistry stage. Intertrack interactions are generally negligible for clinically relevant UHDR regimes, becoming significant only in limiting cases with low-LET radiation and extremely high instantaneous dose rates. This framework offers a general stochastic basis for deriving space and time scales relevant to FLASH irradiation directly from beam delivery conditions. It constrains the contribution of heterogeneous intertrack chemistry for protons and ions under FLASH-relevant regimes, while suggesting that later, densely populated biochemical stages are more important for collective effects. In this context, the local track arrival rate provides a meaningful metric to compare with the system's intrinsic chemical timescales, i.e., its capacity to evolve and readjust between successive track arrivals.

Session 2A: Low-Dose Radiation Epidemiology Population Effects

Chairs: Deborah Oughton and Katalin Lumniczky
Room: Atlantis 1



Invited talk: What's new in low-dose raidation epidemiology?

Author: Amy Berringtom[1]
[1] *Institute of Cancer Research, London, United Kingdom*

46. Early Low-Dose Ionizing Radiation Exposure as a Driver of Chronic Disease: Evidence from a Cesium-137 Murine Study

Author: Stephane Grinson[1]
Co-authors: Amandine Sache [1]; Chloé Brizais [1]; Christelle Elie [1]; Céline Gloaguen [1]; Dmitry Klokov [1]; Florence Bachelot [1]; Honoré Rakotoarivelonarivo [2]; Maâmar Souidi [1]; Meissa Chekri [1]; Raphaël Bo [2]
[1] Autorité de sûreté nucléaire et de radioprotection (ASNR) [2] Autorité de Sûreté Nucléaire et de Radioprotection (ASNR)

Background: Chronic metabolic diseases arise from interactions between genetic predispositions and environmental stressors. In the Developmental Origins of Health and Disease framework, prenatal exposure to low dose ionizing radiation acts as a subtle developmental modifier with the capacity to influence long term metabolic outcomes. Clarifying this contribution is important for radiation protection, especially when early exposure interacts with later life challenges that enhance metabolic vulnerability.
Materials and Methods: In this study, pregnant C57BL/6j mice ingested cesium-137 throughout gestation, resulting in an approximate maternal dose of 100 mGy. F1 offspring were subsequently fed either a standard diet or a lipid enriched diet to reveal susceptibility to chronic inflammatory stress. Biological endpoints were evaluated at 10 weeks and 15 months, with specific attention to liver injury assessed through clinical follow up, systemic lipid homeostasis, and metabolomic profiling at 10 weeks to identify pathway level disturbances.
Results: At 15 months, male offspring exposed prenatally to low dose ionizing radiation and challenged with the lipid enriched diet exhibited markedly increased liver toxicity compared with non-exposed controls and exposed animals maintained on a standard diet. This response included elevated transaminase levels, disrupted lipid homeostasis, and pronounced sex specific differences. Male offspring also showed increased expression of the proto-oncogene Myc and a higher incidence of steatosis liver tumours. At 10 weeks, before overt hepatic injury, metabolomic profiles already revealed persistent alterations in pathways associated with energy metabolism and inflammatory processes.
Conclusions: these findings demonstrate that early low dose ionizing radiation can contribute to adult metabolic disease through its interaction with environmental stressors. These results support a transition in radiation protection toward an integrative contribution-based framework that incorporates life stage, sex specific susceptibility, and combined environmental exposure contexts, to more accurately estimate the fraction of chronic disease risk attributable to low dose radiation.

56. Impact of age on the development of cardiovascular disorders following an external exposure to low doses of Cesium 137

Author: Thin Hinan Nabet[1]; Solyne Allirol[1]; Florence Bachelot[1]; Nathalie Mougenot[2]; Sophie Nadaud[2]; Monceau Virginie[1]

[1] PSE-SANTE / SESANE / LRTOX, Autorité de Sûreté Nucléaire et de Radioprotection -ASNR, France [2] UMR 1166, unité de recherche sur les maladies cardiovasculaires et métaboliques, INSERM, Paris, France.

Background/Objectives: Populations living near areas contaminated following nuclear accidents, as well as nuclear workers, are chronically exposed to low doses (LD) of ionizing radiation (IR). However, the cardiovascular consequences remain poorly understood. Epidemiological data suggest a link between age and the onset of cardiovascular disease (CVD), with higher rates of cardiovascular morbidity and mortality among older adults than among younger individuals within exposed populations. Our objective is to examine the effect of low-to-moderate doses of gamma radiation on the development of late-onset CVD, particularly vascular remodeling, while highlighting age-related health effects.

Methods and Materials: Male C57Bl/6J mice aged 15 months were exposed via whole-body irradiation to gamma-ray doses ranging from 0.1 to 0.5 Gy to simulate cumulative doses of chronic radiation exposure over several years. Analyses were performed 24 hours, 15 days, and 3 months after IR. The effects of radiation on the vascular system were assessed 1) by Doppler ultrasound and Millar probe for functional parameters and 2) by immunolabeling and histological staining for morphology.

Results: 15 days post-exposure, a significant alteration of cardiac function was observed with an accelerated heart rate (472 BPM in non-irradiated mice (NIR) vs 517 BPM, p=0,04) and a decrease in left ventricular ejection fraction (69% vs 79% for NIR, p=0,026) at 100mGy. A significant enlargement of aortic diameter was also observed at 250mGy. Pulmonary artery systolic pressure increased significantly at 100 and 250mGy (24mmHg and 23mmHg respectively vs 17mmHg for NIR, P=0,02 and P=0,006), and this result was confirmed by analyzing pulmonary vascular remodeling. Immunolabelling of lung sections showed a significant increase in pulmonary arteries musculature at 100 and 250mGy (43% and 39% respectively vs 26% for NIR, p=0,004 and p=0,04).

Conclusion: One of our previous studies conducted on young irradiated mice revealed cardiac alterations that differed from those observed in older mice. Significant effects were detected at 100mGy in the older group and at an earlier stage, suggesting potential age-related susceptibility. These results indicate that in the elderly population, LD of IR (<500mGy) can cause early structural and functional changes, contributing to the development of cardiovascular pathologies in these populations. This study could also be used as a prospective human follow-up study for the development of long-term cardiovascular bioindicators in exposed cohorts.

75. Sex- and age-dependent variations in the response of monocytic and mesenchymal cells following low-dose X-ray exposure

Author: Laura Ruspeckhofer[1]
Co-authors: Lena Winterling [1]; Tom Unterleiter [1]; Michael Rückert [1]; Thomas Weissmann [2]; Rainer Fietkau [2]; Stefanie Corradini [2]; Udo Gaipl [1]; Lisa Weissmann [1]
[1] *Translationale Strahlenbiologie, Strahlenklinik, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg ;[2] Strahlenklinik, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg* Demographic differences in radiation-exposed individuals necessitate the individualization of radiation protection measures to minimize health risk. Although sex- and age-dependent differences in cellular (immune) responses are known, they remain largely neglected in radiation protection. Given that ionizing radiation influences various cellular properties such as collagen synthesis and fibrosis of fibroblasts (FBs), bone metabolism of osteoclasts (OC) or the immunological activation status of macrophages (MPH), they represent a critical target for research of (immune) responses following X-ray exposure. Consequently, sex and age-specific differences of these monocytic and mesenchymal cells after low to high doses of X-ray in vitro were investigated.

Monocytes from wild-type C57BL/6 mice of different sexes and ages were differentiated into the MPH subtypes M0, M1, and M2 or OCs by the addition of cytokines. FBs were isolated from lung, skin and ear. OCs and FBs were kept either at healthy or inflamed-like conditions (+/- TNF-α). Irradiation was performed using X-rays with single (all) or fractionated doses (OCs) ranging from 0 to 2 Gy. For OCs the maturation rate was determined via TRAP staining. The phenotype of FBs and MPHs was examined by analyzing surface markers. The levels of reactive oxygen species (ROS; OC and MPH) and the secretion and expression of pro-, anti-inflammatory and fibrotic proteins and genes (all) was measured.

Sex- and age-dependent differences are evident across all investigated cell types and in various processes, such as surface marker modulation, protein secretion, gene expression and OC differentiation. For instance, in MPHs a significant reduction in pro-inflammatory proteins of iNOS, IL-1β, IL-23, and IL-15 following irradiation with 0.5 Gy is exclusively detected in female M1-MPH, with effects diminishing with increasing age. Additionally, 0.5 Gy reduced pro- and anti-inflammatory cytokine transcription in M1 MPHs from 6-week-old female mice. Further, OC showed a significant increase in TRAP secretion following RT with 2 Gy in 6-week-old female mice and from 0.3 to 2 Gy in 14-week-old female mice while remaining mostly stable in males. Moreover, in FBs FAP, CD87, and CD49b displayed radiation-induced alterations, particularly in female mice and preliminary data suggest a potential phenotype switch with age towards a fibrotic, senescent and matrix producing phenotype.

These findings demonstrate that radiation responses do not uniformly alter (immune) modulatory effects but are shaped by both, age and sex within the examined monocytic and mesenchymal cell types. Female-derived cells, particularly at a younger age, show stronger radiation-induced, rather anti-inflammatory effects, while FB results also indicate a phenotype switch with age. Consequently, radiation protection and therapeutic strategies should account for these biological differences.

Session 2B: Emerging Concepts in Radiobiology Space related research

Chairs: Fieke Dekkers and Ans Baeyens
Room: Atlantis 3

11:00

Invited talk: Synthetic Hibernation: From Deep Space Exploration to the Future of Cancer Therapy *Walter Tinganelli*
Atlantis 3 11:00 - 11:30

Mechanistic insight into Radiobiological Response of Lung Cells to Thorium: Cross-talk between DDR, Senescence and...
Mr Sourav Kumar Das

Beyond Dose: Ion Beam Research as a Key to Mechanistic Radiobiology *Judith Reindl*
Atlantis 3 11:40 - 11:50

New models for the track-structure simulation of carbon and lithium ions in Geant4-DNA *Gabriele Parisi*
Atlantis 3 11:50 - 12:00

12:00

Invited talk: Synthetic Hibernation: From Deep Space Exploration to the Future of Cancer Therapy

Author: E. Piscitiello[1]
Co-authors: R. Chowdhury[2] M. Durante[3] M. Cerri[4] Walter Tinganelli[2]
[1] Biophysics Department- GSI Helmholtzzentrum für Schwerionenforschung GmbH, Darmstadt, Germany and Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy ;[2] Biophysics Department- GSI Helmholtzzentrum für Schwerionenforschung GmbH, Darmstadt, Germany; [3] Biophysics Department- GSI Helmholtzzentrum für Schwerionenforschung GmbH, Darmstadt, Germany; Technische Universität Darmstadt, Darmstadt, Germany;[4] Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy Hibernation is a natural physiological state adopted by several animal species in response to extreme environmental conditions, such as low temperatures, food scarcity, or prolonged drought (a condition known as aestivation). During hibernation, profound physiological adaptations occur. Despite prolonged immobility, hibernating animals do not experience significant bone demineralization or loss of muscle strength. Moreover, and perhaps most intriguingly, they exhibit increased resistance to ionizing radiation. The possibility of reproducing these physiological adaptations in humans could have important implications, particularly for space exploration. Inducing a hibernation-like state in astronauts could mitigate the detrimental effects of microgravity and space radiation, including exposure to galactic cosmic rays and solar particle events. In addition, it could alleviate psychological stress associated with long-duration missions, reduce energy consumption by lowering spacecraft cabin temperature, and decrease the requirements for food, water, and other life-support resources. Since every kilogram launched into space carries substantial costs, such reductions could significantly improve mission feasibility. In collaboration with the University of Bologna, we have developed a model of artificial hibernation in nonhibernating species, a condition we refer to as synthetic hibernation. Our studies demonstrate that synthetic hibernation is also capable of inducing radioresistance. More recently, we have begun exploring the potential medical applications of synthetic hibernation, particularly in the field of oncology. Our preliminary findings suggest that synthetic hibernation may represent a valuable adjunct to particle therapy, with the potential to improve treatment outcomes and reduce radiation-induced side effects. These early results open new perspectives for the translation of hibernation-inspired strategies from space research to clinical medicine

35. Mechanistic insight into Radiobiological Response of Lung Cells to Thorium: Cross-talk between DDR, Senescence and Inflammation

Author: Sourav Kumar Das[1]
Co-authors: Manjoor Ali [2]; Neena G. Shetake [2]; Badri N. Pandey [1]; Amit Kumar [1]
[1] *1. Radiation Biology & Health Sciences Division, Bhabha Atomic Research Centre, Mumbai-85, India. 2. Homi Bhabha National Institute, Anushaktinagar, Mumbai-94, India;* [2] *Radiation Biology & Health Sciences Division, Bhabha Atomic Research Centre, Mumbai-85, India*

Background: Thorium-232 (Th), the most abundant naturally-occurring nuclear fuel, is increasingly recognized as a sustainable energy source. However, large-scale utilization raises concerns regarding occupational and environmental exposure, particularly human evidence linking Th exposure to pneumoconiosis and lung carcinogenicity.
Objective: The present study investigated the mechanism underlying Th-induced inflammation, DNA damage response and senescence signaling in human lung cells and mice lung tissue following Th exposure.
Methods: AEPT cells were exposed to ThO_2 (1-25 μg/mL, 24-48h). Expression/secretion of inflammatory cytokines were analysed, and activation of NF-κB, JAK/STAT and DDR signaling pathways was examined. In vivo validation was performed using mouse nose- only exposure system.
Results: Depending on the concentration and exposure time, Th was found to increase the expression/secretion of IL-6, IL1β, IFNγ and TGFβ and, decrease the expression of anti-inflammatory cytokines (IL-4/10). Th exposure significantly altered the level of p-p65 and p-JAK1/p-STAT3, suggesting the role of NF-kB signalling as well as JAK-STAT pathway. Th-induced DNA damage response was assessed in-terms of γH2Ax and level of NHEJ (DNA-PK, Ku70/80) and HR (ATM, Rad51 and BRCA1) repair proteins. Results showed that Th exposure (1-10 μg/ml) significantly increased the level of DNA-PK foci in A549 and WI26 cells, suggesting the predominant role of NHEJ-DSB-repair pathway. However, Th decreased Rad51 foci, indicating HR impairment. Further, ATM inhibitor (KU55933) was found to significantly prevent Th-induced up-regulation of p-p65 (NF-kB) and p-STAT3, suggesting primary role of DDR in the activation of these inflammatory pathways. These results were corroborated in mice lung following exposure with Th-aerosols, which showed an irregular network of alveoli, decreased air sacs and infiltration of immune cells (macrophages), suggesting Th-induced inflammatory damage. Our data suggested the elevation of molecular markers of senescence (e.g. lamin B1) and fibrosis (TGFβ) in the lung tissues exposed to Th.
Conclusion: These results have delineated the mechanism of inflammation and its intracellular cross-talk with DDR and senescence. Present study has implications for identification of biomarkers of inflammation associated with Th exposure in lung as well as the therapeutic targets for management of respiratory symptoms.

125. Beyond Dose: Ion Beam Research as a Key to Mechanistic Radiobiology

Author: Judith Reindl[1,2]
Co-authors: Nina Edin [1];
[1] *Univeristy of Oslo, Oslo, Norway;* [2] *University of the Bundeswehr Munich, Munich, Germany*

Background: Understanding the biological effects of ionizing radiation remains a central challenge in radiation research. While classical radiobiology has largely been shaped by photon-based exposures and dose–response relationships, emerging applications in particle therapy, space exploration, and low-dose risk assessment increasingly involve complex radiation qualities that are insufficiently captured by these paradigms.
Methods: In particular, ion beams provide unique opportunities to systematically vary radiation quality, linear energy transfer (LET), spatial dose distributions, and dose rates. However, disentangling the underlying biological mechanisms—ranging from DNA damage complexity to immune modulation—requires experimental conditions that are both highly controlled and reproducible. Such conditions are typically only accessible at large-scale research facilities.
Results: Here, we argue that ion beam research at accelerator-based infrastructures is not merely complementary but essential for advancing mechanistic radiobiology. For example, recent studies using spatially fractionated proton irradiation have demonstrated differential immune activation between high-dose peak regions and low-dose valleys, highlighting how spatial dose heterogeneity can modulate systemic biological responses in ways not observable under conventional irradiation conditions.
Conclusion: We propose that integrating ion beam research with molecular, cellular, and computational approaches will be key to moving beyond phenomenological descriptions towards predictive models of radiation response. This shift is critical for improving radiotherapy, refining radiation protection, and addressing the challenges of human spaceflight.

77. New models for the track-structure simulation of carbon and lithium ions in Geant4-DNA

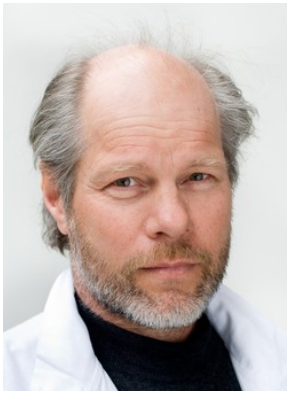
Author: Gabriele Parisi [1]
Co-authors: Naoki Dominguez Kondo [2]; Yann Perrot [1]; Sébastien Incerti [3]; Hoang Tran Ngoc [3]; Jose Ramos Mendez [2]; Alessandro Somenzi [4]; Nicoletta Protti [4]; Carmen Villagrasa [5]
[1] *ASNR - Autorité de sûreté nucléaire et de radioprotection;* [2] *University of California San Francisco;* [3] *IN2P3;* [4] *University of Pavia;* [5] ASNR **Background and aims** Radiation transport Monte Carlo (MC) codes are the gold standard for dose calculations in fields such as radiation therapy, radioprotection and space applications. MC codes can explore scenarios that are difficult to create experimentally and allow for estimating quantities that cannot be measured directly. In particular, track structure MC (TSMC) codes, simulating each particle interaction individually, allow for accurate simulations at nanometric scales and for the gain of a mechanistic understanding of the phenomena studied. The Geant4-DNA extension of Geant4 stands out among TSMC codes thanks to its open-source availability and its unique integration of physical interactions, radiolysis, DNA-scale geometries, and biological repair models. This enables the simulation of physical quantities, direct and indirect DNA damage, cell survival, and other radiobiological endpoints. In Geant4-DNA, however, only a generalised and simplified model exists for ions heavier than helium. This work aims to develop specific physics models for lithium and carbon ions in Geant4-DNA with direct impact to boron neutron capture (BNCT) and carbon ion therapy research. **Materials & Methods** The new models developed for ions heavier than helium include all the relevant interaction processes at low energy: ionisation, excitation, charge transfer and elastic scattering. The ionisation and excitation cross-sections (XS) were obtained using a novel scaling approach that improves the Born "Z^2" scaling, otherwise failing at high Z/v, and takes into account the different particles' charge states. Charge transfer and elastic scattering XS were calculated, respectively, using the Classical Trajectory Monte Carlo approach and by numerically solving classical mechanics equations.
Results The newly developed models are fully compatible with the latest Geant4 version (11.4.0). Compared to reference data from ICRU, they show lower stopping power (SP) values, in accordance with other recent works in the literature. This is particularly evident in the energy region where the ion is dressed and ionisation prevails. The limited availability of experimental cross sections, stopping power and range data in liquid water complicates the direct assessment of their accuracy. The impact observed on micro- and nano-dosimetric quantities, radiochemical yields, and DNA damage yields when using the new models is presented.
Conclusions The new models improve Geant4-DNA and extend its applicability to BNCT, carbon and multi-ion therapies, radioprotection studies involving heavier ions and neutron's secondaries, and cosmic rays studies for space. The software infrastructure and the flexibility of the approach used will facilitate their further extension to other ion species such as boron, oxygen and neon. Benchmark against micro- and nano-dosimetry experiments is underway to assess the models' accuracy for dosimetry applications.

Keynotesession 1

Chair: Julie Nonnekens
Room:Room: Atlantis 1

The speakers

Øyvind Bruland



Øyvind Bruland is Professor of Clinical Oncology at the Faculty of Medicine, University of Oslo, and a leading expert in the management of bone and soft-tissue sarcomas. His clinical and research career has focused on the development of improved diagnostic and therapeutic strategies for rare malignancies, with particular contributions in osteosarcoma, Ewing sarcoma, and bone metastases. Prof. Bruland has been instrumental in translating radiopharmaceutical research into clinical practice, including work that contributed to the development of targeted alpha-particle therapy using radium-223.

Asta Juzeniene

Asta Juzeniene is a senior researcher at the Department of Radiation Biology, Oslo University Hospital, and an adjunct Associate Professor at the University of Oslo. Her work focuses on targeted radiopharmaceutical therapy, with a particular emphasis on alpha-emitting isotopes and their application in preclinical cancer models. Dr. Juzeniene's research integrates radiochemistry, radiobiology, dosimetry, and translational studies to advance novel radionuclide-based treatments. She collaborates broadly with academic and industry partners and contributes to national and international efforts aimed at improving the precision, efficacy, and clinical readiness of next-generation radiopharmaceutical therapies.

From radium-223 to next-generation alpha therapies: bridging preclinical discovery and clinical translation

Author: Øyvind Bruland[1]; Asta Juzeniene[2]

[1] *Oslo University Hospital and University of Oslo;* [2] *Department of Radiation Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway.* The clinical success of the bone-seeking radionuclide radium-223 in metastatic castration-resistant prostate cancer with skeletal metastases demonstrated that alpha-emitting radionuclides can provide clinically meaningful therapeutic benefit while maintaining an acceptable safety profile. Developed in Norway, radium-223 (AlpharadinR/XofigoR) became the first, and remains the only globally approved alpha-emitting radiopharmaceutical for cancer therapy. This milestone established the clinical foundation for the rapidly expanding field of targeted alpha therapy and stimulated the development of next-generation alpha-emitting radiopharmaceuticals. Currently, this includes intravenously administered targeted ligands and antibodies, as well as locally delivered alpha therapies such as intraperitoneal microparticles and wire-based intratumoural approaches.

In this keynote lecture, we will discuss aspects of the translational journey from radium-223 to modern alpha therapies, focusing on how radiobiology, dosimetry, radionuclide chemistry and tumour biology collectively drive clinical implementation. The lecture will provide a broad overview of the field, spanning bone-seeking radionuclides, PSMA-targeting radioligands, antibody-based constructs and local alpha therapies developed to combat metastatic cancer.

We will further address major translational challenges facing the field. No currently available alpha-emitting radionuclide possesses ideal physical, chemical and biological characteristics. Important considerations include radionuclide half-life, production and availability, chelation stability, radiolabelling chemistry, daughter radionuclide redistribution, recoil effects, and compatibility between radionuclide half-life and tumour targeting kinetics. In addition, the short range and stochastic energy deposition of alpha particles create substantial challenges for dosimetry, especially at cellular and subcellular scales where heterogeneous radionuclide distribution, non-uniform target expression and varying biological assumptions may strongly influence absorbed dose estimates and therapeutic benefit.

Finally, we will discuss current methodological limitations, including the lack of standardised dosimetry approaches, uncertainty in activity quantification, limited quantitative imaging capabilities for alpha emitters with low gamma yields, and the need for improved preclinical models, instrumentation and microdosimetric tools. Addressing these challenges will be essential for robust clinical translation, biologically informed treatment planning and patient selection, and for advancing next generation of alpha-emitting radiopharmaceuticals in oncology.

Session 3A: Quantitative Imaging, Radiogenomics Clinical Decision Support

Chairs: Aidan Meade and Jessica Neubauer
Room: Atlantis 1

Invited talk: AI for radiomics and radiogenomics for outcome modeling in radiotherapy — *Issam El Naqa*
Atlantis 1 15:30 - 16:00

16:00

Radiogenomic Predictors of Toxicity in Head and Neck Cancer Patients — *Samantha Inga Cairncross*
Atlantis 1 16:00 - 16:10

Multimodal modeling of histopathology and transcriptomics reveals hypoxia-tolerant tumor programs in prostate cancer
Vilde Eide Skingen

MR imaging of radiation resistance during treatment in anal cancer patients — *Ms Tiril Hillestad*
Atlantis 1 16:20 - 16:30

Invited talk: AI for radiomics and radiogenomics for outcome modeling in radiotherapy

Author: Issam El Naqa [1]

[1] *Chair of Machine Learning Department, Senior member of Radiology, Radiation Oncology & GI Departments, Moffitt Distinguished Scholar, Moffitt Cancer Center, Tampa, FL, USA* Artificial Intelligence (AI) and Machine Learning (ML) are transformative technologies that have sparked both excitement and concern. In radiation oncology, AI has been successfully applied to automate tasks such as auto-contouring, geometric adaptation, radiation dose calculation, and quality assurance. However, its effectiveness in personalizing patient care using imaging (radiomics) and genetics (radiogenomics) for optimizing clinical decision-making remains limited. This is due to a range of technical, practical, ethical, and legal challenges associated with deploying AI/ML-driven technologies in clinical settings. Concerns include skepticism about commercialization hype, under-representation in data, inherent implementation biases, lack of robustness, and a lack of transparency in predictions. In this presentation, we delve into these challenges, outline the key requirements for the successful clinical implementation of AI/ML, and explore strategies that integrate physics, biology, and engineering to address these obstacles. We also demonstrate the application of these strategies in radiation oncology with examples from our work, discussing their broader implications for ensuring the safe and effective use of AI/ML in outcome prediction.

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53. Radiogenomic Predictors of Toxicity in Head and Neck Cancer Patients

Author: Samantha Inga Cairncross [1]
Co-authors: Alistair Hunter [1]; Charlot Vandevoorde [2]; Julie Wetter [1]; Mongi Benjeddou [3]; Sameera Dalvie [1]
[1] *UCT ;*[2] *GSI Helmholtzzentrum für Schwerionenforschung;* [3] *UWC* **Background:** Radiotherapy is central to the management of head- and neck cancers, yet interindividual variability in treatment response often results in severe early and late toxicities. Genetic factors account for a substantial proportion of this variability. This study investigated genetic associations with radiotherapy-induced toxicities in a South African cohort to identify markers that may guide personalized treatment strategies. Furthermore, African populations—who harbour the greatest genetic diversity globally—remain critically underrepresented in radiogenomic studies and this work represents the first radiogenomic study that includes populations from sub-Saharan Africa.
Methods: Acute toxicities (mucositis, xerostomia, dysphagia) were assessed in 143 participants, and late skin fibrosis in 125 participants. Candidate SNPs in molecular and cellular radiation pathways were selected through a systematic review and database analysis. Genotyping was performed using MassARRAY, and associations were analysed using allelic, genotypic, additive, dominant, recessive and over-dominant models. Multivariate analyses were adjusted for clinical and lifestyle confounders.
Results: Multiple SNPs demonstrated significant toxicity associations across endpoints. ERCC5 rs17655 and CHEK1 rs11220184 increased mucositis risk, while XRCC1 rs3547 showed protective effects. Xerostomia was influenced by ERCC1 rs11615 and XRCC1 rs1799782 (protective), and BRCA2 rs1801406 (increased risk). Dysphagia risk was strongly associated with TANC1 rs6432512, rs7582141 and rs264588, while ERCC1 rs11615 and TGFβ1 rs2279744 were protective. Late fibrosis was associated with TP53 rs1042522, TGFβ1 rs1800469 and rs2241714, and XRCC1 rs3547, while CCND1 rs9344 and ERCC1 rs735482 showed protective effects.
Conclusion: This study identified several genetic variants associated with early and late radiotherapy-induced toxicities in a South African head- and neck cancer cohort. Furthermore, the findings highlight the value of including African populations in radiogenomic research and support the use of genomic information to better understand individual treatment responses and guide more personalised radiotherapy approaches.

114. Multimodal modeling of histopathology and transcriptomics reveals hypoxia-tolerant tumor programs in prostate cancer

Author: Vilde Eide Skingen [1]
Co-authors: SH Duran [1]; UB Salberg [2]; CS Fjeldbo [1]; H Helgeland [1]; T Hompland [1]; KF Valen [1]; HB Ragnum [3]; KH Hole [4]; T Seierstad [4]; Lj Vlatkovic [5]; Heidi Lyng
[1] Department of Radiation Biology, Oslo University Hospital ;[2] Department of Radiation Biology, Oslo University Hospital, ;[3] Department of Haematology and Oncology, Telemark Hospital Trust ;[4] Department of Radiology and Nuclear Medicine, Oslo University Hospital ;[5] Department of Pathology, Oslo University Hospital

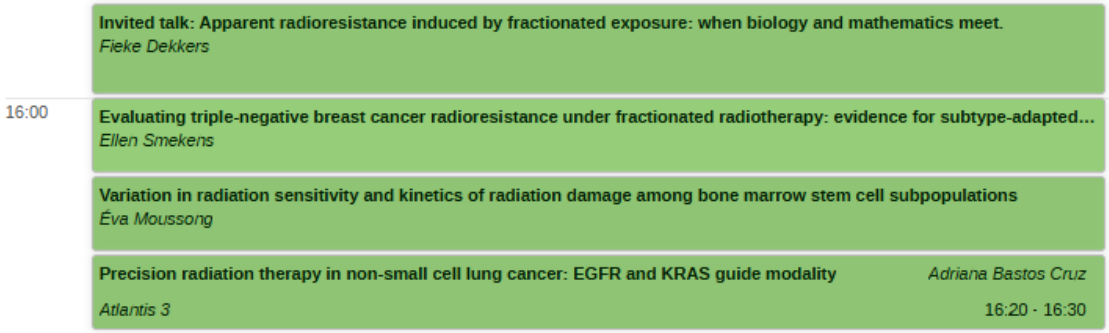
Background: Tumor hypoxia drives radioresistance in prostate cancer. Hypoxia-tolerant tumor cells adapt to oxygen deprivation through transcriptional reprogramming that promotes cell survival. Better understanding of this phenotype in patient tumors may reveal new therapeutic opportunities. We developed a framework to identify hypoxia tolerance in prostate cancer biopsies from histopathological features and applied integrative modeling of multimodal data to characterize transcriptional programs of these tumors.
Methods: For 119 biopsies from 76 patients, hypoxia-tolerant tumors were identified using image analysis of histological sections stained for pimonidazole (hypoxia) and CD31 (vasculature). The pipeline included co-registration of consecutive sections, computation of cell–vessel distance (CVD), and quantification of microregional features reflecting CVD and hypoxia. Spatial relationships between these features were assessed using microregional spatial correlation (MSC). Tumors were classified as hypoxia-tolerant when MSC showed a significant co-localization between CVD and hypoxia. The underlying transcriptomic program was revealed by identifying differentially expressed genes (DEGs) by Illumina bead arrays and by hallmark enrichment analysis (GSEA). To investigate spatial features, 12 additional biopsies from the cohort were analyzed using co-registered histopathology and spatial transcriptomic profiling (Visium, 10x Genomics). Findings were validated by histology (Ki67, PTEN, tryptase) and by RT-qPCR (MYC, PTEN).
Results: Thirty-four hypoxia-tolerant tumors were identified, with 850 up- and 692 downregulated DEGs. GSEA showed activation of 13 and suppression of 12 hallmarks, consistent with a highly proliferative program of these tumors marked by MYC overexpression, PTEN loss, and immune suppression. MSC revealed co-localization of proliferation (Ki67), PTEN depletion, and reduced mast cell activation (tryptase) within hypoxic regions. RT-qPCR confirmed MYC overexpression and PTEN suppression. Spatial transcriptomic analysis of hallmark scores revealed coordinated clustering of the hypoxia-tolerant program within hypoxic regions of solid tumor growth patterns across histopathological subtypes (cribriform, IDC-P). Comparison of a primary sample and a matched positive lymph node detected the same hypoxia-tolerant program in the two samples, in line with a hypothesis that hypoxia-tolerant cells drive tumor spread. Consistently, histology- and gene score–based classification of hypoxia tolerance predicted biochemical recurrence in our cohort and the TCGA cohort.
Conclusion: Hypoxia-tolerant tumor regions exhibit a proliferative gene expression program adapted to hypoxia and characterized by MYC activation, PTEN depletion, and solid growth patterns. This phenotype was associated with metastatic spread and biochemical recurrence. Targeting MYC-driven proliferation pathways in combination with radiotherapy may therefore improve outcomes in patients with prostate cancer.

119. MR imaging of radiation resistance during treatment in anal cancer patients

Author: Tiril Hillestad [1]
Co-authors: Eirik Malinen [2]; Marianne Guren [1]; Tord Hompland [1]
[1] *Oslo University Hospital* [2] *Department of Radiation Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway and Department of Physics, University of Oslo, Oslo, Norway* **Introduction:** Anal cancer is a rare malignancy with increasing incidence. Standard treatment is fractionated chemoradiotherapy, achieving a 3 year progression free survival of ˜70%. Tumour radioresistance contributes to treatment failure, highlighting the need for biomarkers to enable personalised treatment intensification. Tumour hypoxia is a known cause of radioresistance, but its prevalence and significance in anal cancer remain poorly characterised. Functional MRI, already used clinically, can probe tumour microenvironmental features related to radioresistance. This study investigated whether diffusion weighted (DW) MRI parameters can identify radioresistant anal tumours and explored the role of hypoxia.
Methods: Forty eight patients with T2–4 anal cancer enrolled in the ANCARAD study (NCT01937780) were included. All received 54–58 Gy in 2 Gy fractions. MRI was performed pre treatment (n=48) and after 20 Gy (n=27) using a 3.0 T scanner with multi b value DW MRI. Apparent diffusion coefficient (ADC; cell density) and fractional blood volume (fBV; perfusion) were derived using a simplified IVIM model. Median ADC, fBV, tumour volume, and MRI derived hypoxia levels were calculated for each tumour using a consumption and supply based hypoxia (CSH) model. Imaging and clinical parameters were compared between patients with and without relapse after four years.
Results: Eight patients experienced relapse. Most tumours were HPV positive (39/48). Clinical parameters, including T stage, nodal status, and HPV status, were not directly associated with outcome. Higher baseline tumour volume and hypoxia correlated with higher T stage and nodal involvement ($p<0.05$), but baseline imaging parameters did not predict outcome.
After 20 Gy, tumour volume decreased by a median of 54% ($p<0.001$), with increased ADC ($p<0.001$) and fBV ($p<0.01$), indicating reduced cell density and reperfusion. Consequently, tumour hypoxia decreased in nearly all cases ($p<0.001$). Greater increases in ADC and larger volume reductions were associated with improved outcome ($p<0.05$). Reduction in hypoxia was the strongest prognostic factor ($p<0.01$). HPV negative tumours showed limited volume and hypoxia reduction, indicating increased radioresistance.
Conclusion: DW MRI during radiotherapy provides valuable insight into radiation response in anal cancer. Early reductions in tumour hypoxia are strongly associated with favourable outcome and may serve as an early biomarker of radioresistance. Monitoring hypoxia during treatment offers a potential window for treatment intensification to improve cure rates.

Session 3B: Adaptive Radioresistance and Cellular Response

Chairs: Georgia Terzoudi and Sissel Hauge
Room: Atlantis 3



Invited talk: Apparent radioresistance induced by fractionated exposure: when biology and mathematics meet.

Author: Fieke Dekkers [1]
[1] *Utrecht University, Utrecht, The Netherlands*

18. Evaluating triple-negative breast cancer radioresistance under fractionated radiotherapy: evidence for subtype-adapted radiotherapy strategies

Author: Anne Vral[1]; Ans Baeyens[1]; Ellen Smekens[1]; Lama Ramadan[1]; Liv Veldeman[2,3]
[1] Radiobiology Lab, Department of Human Structure and Repair, Ghent University, Ghent, Belgium. ; [2] Radiation Oncology, Department of Human Structure and Repair, Ghent University, Ghent Belgium.; [3]Department of Radiation Oncology, Ghent University Hospital, Ghent, Belgium.

Triple-negative breast cancer (TNBC) is a clinically aggressive subtype of breast cancer characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression. Due to its distinct molecular profile and lack of receptor-targeted treatment options, radiotherapy (RT) remains a key component of TNBC management. However, emerging evidence suggests that TNBC tumors may be less responsive to radiation than other breast cancer subtypes. Despite this, current RT protocols remain uniform across all types of breast cancer, with no adjustments made for TNBC's potential radioresistance.

Previous in vitro studies exploring TNBC radioresistance have predominantly used single-dose irradiation, which do not accurately reflect clinical practice, where fractionated dosing is standard. To better approximate these clinical conditions, this study aimed to compare the radiobiological response of TNBC (MDA MB 231) and luminal A (MCF 7) cells under two clinically relevant fractionation schemes. Using γH2AX foci formation to track DNA double strand breaks and the cytokinesis-block micronucleus assay to measure chromosomal damage, we assessed both immediate and persistent radiation-induced effects.

Our preliminary data indicate that MDA MB 231 cells exposed to a fractionated irradiation protocol show higher γH2AX foci levels and slower DNA repair kinetics compared with MCF 7 cells. As expected, increasing the dose per fraction leads to more DNA damage in both cell lines. However, the increase in initial foci levels in MCF 7 cells is roughly twice as large when increasing the dose per fraction, while MDA MB 231 cells display a less pronounced increase in γH2AX foci. Examination of persistent damage reveals that low dose fractionation allows almost complete resolution of foci in both lines, whereas high dose fractionation leads to an accumulation of unrepaired DNA damage, with TNBC cells retaining substantially greater residual damage. Interestingly, radiation-induced chromosomal aberrations were comparable between the two cell types across both fractionation schemes, as reflected by similar frequencies of micronucleus formation.

These preliminary findings suggest that TNBC radioresistance persists under fractionated RT and is not limited to single dose exposure. They also imply that TNBC cells may better tolerate higher radiation doses per fraction. Ongoing analyses of cell death and clonogenic survival aim to provide a more comprehensive understanding of TNBC radiation response after fractionated RT. Overall, these insights support the need for subtype adapted RT strategies in breast cancer treatment.

36. Variation in radiation sensitivity and kinetics of radiation damage among bone marrow stem cell subpopulations

Author: Éva Moussong[1];
Co-authors: Ilona Csordás [1]; Katalin Balázs [1]; Katalin Lumniczky ; Martina Forgács [1]; Soma Hartai [1]
[1] *National Centre for Public Health and Pharmacy*

The diverse group of cells in the bone marrow (BM) consists of numerous subpopulations, including hematopoietic stem and progenitor cells (HSPCs) essential in hematopoiesis and blood cell homeostasis, and mesenchymal stem cells (MSCs) that can differentiate into a variety of cell types. Ionizing radiation (IR) can have significant damaging effects on tissues and organs, with the BM being one of the most sensitive areas. Persistent radiation-induced DNA damage in the BM may facilitate the development of senescent and pre-leukemic cellular states and increase the likelihood of leukemias. Although such processes have been observed at the BM level, there is limited research identifying the vulnerability of specific cell subpopulations. In this study, we investigated the time kinetics of cellular damage in BM stem and progenitor cells in mice, after low and high dose irradiation or extracellular vesicle (EV) injection.

Young CBA mice were total-body irradiated with 0.1Gy or 3Gy, or injected with BM-derived EVs from 0.1Gy- or 3Gy-irradiated animals. BM cells were isolated, and by applying flow cytometry, persistence of DNA double-strand breaks (γH2AX) and proliferation potential (Ki-67) were quantified in a large panel of BM stem and progenitor cells mainly within the myeloid lineage. Following treatment, measurements were performed at multiple time points between 4 hours and 6 months.

Our results suggest that BM cell subpopulations are affected by both low and high irradiation doses and EV treatment, however, their sensitivity and response kinetics vary. In general, within stem cell populations (HSCs and MSCs), the proportion of γH2AX+ cells after irradiation is highest at the earliest time point investigated (4 hours), is still elevated at 3 months, and falls back to baseline only at 6 months. However, IR response follows a different pattern in some of the progenitors where DNA damage level peaks later, at 24-48 hours, and drops back to control level at 3 months. EV treatment also induces DNA damage in these populations, leading to elevated γH2AX signal. In stem cells and early progenitors, Ki-67 levels increase after both low and high dose, with a peak either at 4 hours or at 24-72 hours. Some of the late progenitors show a temporary decrease in the proportion of Ki-67+ cells upon IR. As progenitors are cycling more actively, we assume that many committed progenitors undergo apoptosis or senescence and surviving ones are held in G1 arrest, which causes reduced Ki-67 levels.

In conclusion, we examined specific cell subpopulations distinctively within the BM niche, at several time points over a 6-month period. Our studies help in a better understanding of the kinetics and extent of DNA damage in BM stem and progenitor cells upon IR and EV treatment. Our findings contribute to the identification of the cell groups most sensitive to radiation damage within the BM. Our ongoing measurements aim to determine to what extent IR-induced persistent DNA damage is associated with cell cycle arrest, and to assess the mechanism how EVs transmit DNA damaging effects.

108. Precision radiation therapy in non-small cell lung cancer: EGFR and KRAS guide modality

Author: Adriana Bastos Cruz[1]; Mario Salmerón Merino[2]; Pablo de la Fuente[2]; Clara Garcia[2]; Belén Cortés-Llanos[2]; Gastón Garcia Lopez[2]; Maria Dolores Ynsa[3]; Célia T. Sousa[3]

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Background. Lung cancer remains the leading cause of cancer-related mortality worldwide 1, largely due to its pronounced molecular heterogeneity, which significantly influences prognosis and therapeutic response. Clinical screening routinely identifies common driver mutations [2,3], particularly in EGFR and KRAS, to guide targeted therapies. However, the mutational profile is rarely considered when defining radiation therapy strategies, potentially due to the perception that such interventions are primarily palliative in advanced-stage patients.

Methods. In this study, we present a systematic evaluation of the impact of EGFR and KRAS mutations on radiation response, with a particular focus on proton therapy modalities. Four non-small cell lung cancer (NSCLC) cell lines harboring these mutations were analyzed (NCI-358KRASG12C, PC-9EGFREx19Del), alongside a wild-type cell line (H292). Cells were exposed to both conventional and FLASH proton irradiation, and the subsequent effects were assessed with respect to metabolic activity, cell cycle progression, and clonogenic survival.

Results. Preliminary results indicate distinct responses between wild-type and mutant cell lines after 48h pos irradiation, as well as differential effects between conventional and FLASH proton therapy. Notably, EGFR- and KRAS-mutated cells exhibited enhanced G2/M cell cycle arrest following FLASH irradiation at equivalent doses, whereas the wild-type model showed increased arrest in the S phase. Furthermore, clonogenic assays revealed a greater sensitivity of mutant cell lines, with FLASH irradiation consistently exerting a stronger inhibitory effect on colony formation across all models. Regarding these results, at 48h post-irradiation, cellular viability assays revealed that all cell lines (wild-type and mutated) maintained good viability following conventional proton therapy.

Conclusions. These preliminary findings indicate a possible association between mutational status and response to advanced radiotherapy techniques, such as proton and FLASH therapy, suggesting potential relevance for personalized radiation treatment strategies.

Session 4A: Advanced particle therapy technology and translational approaches

Chairs: Pavel Blaha and Matilde Helgesen
Room: Atlantis 1

25. SHARP: A Compact Focusing System for Emerging Particle Therapies using an Active Plasma Lens

Author: Abélia Ellingsen[1]
Co-authors: Erik Adli [1]; Pierre Drobniak [1]; Carl Lindstrøm [1]; Elisabeth Rød-Lindberg [1]; Vilde Rieker [2]; Anthony Dyson [3]; Fardous Reaz [4]; Roberto Corsini [5]; Wilfrid Farabolini [5]; Antonio Gilardi [5]; Alexander Malyzhenkov [5]; Giacomo Tangari [6]; Kyrre Sjobak [1]
[1] *Department of Physics, University of Oslo ;*[2] *Center for Proton Therapy, PSI ;*[3] *University of Oxford ;*[4] *Department of Clinical Medicine, Aarhus University and Danish Centre for Particle Therapy, Aarhus University Hospital ;*[5] *CERN ;*[6] *CERN and Sapienza University of Rome*

Background: Improved control of the beam envelope in particle radiotherapy could enhance the precision of dose delivery. A novel approach is to incorporate an active plasma lens (APL) into the beamline. This compact device is used for strong and radially symmetric defocusing, giving rapid and controlled beam expansion. Such an expanded beam is of interest in itself, as it is generated by beam optics rather than through scattering, enabling large high intensity fields that may be relevant for FLASH applications. Beam expansion can also be followed by downstream refocusing to produce a sharp waist at the target. This may reduce entrance dose to healthy tissue while enhancing dose deposition at depth. Such beam geometries from a compact system could benefit precision conformal radiotherapy and spatial fractionation. The SHARP project is in the process of demonstrating these ideas experimentally with 200 MeV very high-energy electrons (VHEE), supported by simulations of beam optics and dose distributions.
Materials and Methods: Experiments are being conducted at the CLEAR facility at CERN. The system combines a defocusing APL with quadrupole magnets for final focusing onto the target. Beam enlargement, without focusing, is evaluated on a downstream profile monitoring screen. The refocused beam is subsequently characterized with screens downstream of the quadrupoles. Radiation transport simulations in Geant4 are used to model dose deposition at the target and outside the focal region. Simulations with ImpactX are used for designing beam optics and assessing the influence of e.g. energy spread.
Results: Beam enlargement from the APL is experimentally observed to be linear and symmetric in both transverse planes. Simulations show that quadrupoles can modify these beams into large, quasi-parallel fields for broad irradiation of higher intensity than passive scattering, which could be useful to investigate further for certain FLASH applications. For small deep targets, simulations show that increasing the APL current coupled with refocusing quadrupoles reduces focal beam size while increasing divergence, producing a sharp Bragg-like peak. Larger opening angles upstream and downstream of the target have also been confirmed experimentally. **Conclusions:** SHARP investigates a compact hybrid APL-quadrupole system to achieve precise focal spots and controlled dose distributions. The technique could serve as a versatile research platform for SFRT and FLASH applications. The project's first experimental results represent a promising step towards improved dose delivery by demonstrating both controlled beam enlargement and refocusing. Ongoing work to refine the concept includes simulations and experiments of scattering in water phantoms, nonlinear APL defocusing for generating enlarged flat fields, spot scanning, and extension of the concept to protons.

92. Construction of a proton minibeam beamline for preclinical experiments in small animals – Minibee Project

Author: Aikaterini Rousseti[1]
Co-authors: Jessica Neubauer[1] ; Georgios Kourkafas [2]; Minkyu Kang [2]; Alina Dittwald [2]; Jürgen Bundesmann [2]; Andrea Denker [2]; Günther Dollinger [1]; Judith Reindl[1,3]
[1] Universität der Bundeswehr München, Munich, Germany ;[2] Helmholtz-Zentrum Berlin, Berlin, Germany ;[3] University of Oslo, Oslo, Norway **Background:** Preclinical studies of proton minibeam radiotherapy (pMBT) have highlighted its potential to improve normal tissue sparing while maintaining, or even enhancing, tumor control. However, several key questions remain unresolved, including optimal strategies for minibeam generation and delivery, the underlying biological mechanisms, and the types of cancer most likely to benefit from this approach. Constructing a proton minibeam beamline for preclinical experiments (MINIBEE) at the Helmholtz Zentrum Berlin (HZB) will give the opportunity for in-depth and systematic research.
Materials and Methods: A proton beam of 68 MeV, suitable for small animal irradiation, can be extracted by the cyclotron. If required, the beam energy can be degraded downstream of the cyclotron, and a range shifter can further adjust it to generate a spread-out Bragg peak (SOBP). Minibeams will be generated via magnetic focusing using a quadrupole triplet. The beamline has a Small Animal Radiation Research Platform (SARRP), for animal imaging and positioning, and a biolab will be available on-site to support the studies. Additionally, FLASH irradiation can be performed at the beam entrance in single energy mode, with an average dose rate of kGy/s and local peak dose rates up to several MGy/s. The beamline construction is nearing completion.
Results In December 2025, the beam was injected into the beamline for the first time and transported from the cyclotron to the final diagnostics station, confirming safe and successful beam transport. The next phase will involve beam characterization measurements at the isocenter.
Conclusions A pMBT facility will give multiple users the opportunity to conduct experiments and intensify the research in the field while offering the opportunity to develop new technologies and better understand the underlying biological mechanisms.

76. Dose delivery in the presence of the magnetic field of an in-beam MRI scanner for MR-integrated proton therapy

Author: Jörg Pawelke[1]

Co-authors: Aswin Hoffmann [1]; Felix Horst [1]

[1] *Helmholtz-Zentrum Dresden - Rossendorf, Institute of Radiooncology - OncoRay*

Background: The planned clinical implementation of proton therapy combined with magnetic resonance imaging (MRiPT) is expected to increase the targeting accuracy. When treating patients inside an in-beam MRI scanner, the effects of the magnetic field (MF) on proton beam trajectories via the Lorentz force must be taken into account during treatment planning. In addition, MF-compatible devices for dosimetric verification and a daily workflow for machine- and patient specific- (PS) quality assurance (QA) are required. We report the results of experimental testing of the different components and workflow through an end-to-end (E2E) evaluation.

Materials and Methods: A mobile 0.32 T open in-beam MRI scanner is positioned in front of a horizontal pencil beam scanning (PBS) beamline, both systems have been commissioned 1. A research version of a commercial Monte Carlo-based proton treatment planning system (TPS) accounting for MF-induced beam deflection [2] and an energy-layer-wise deflection correction method [3] were developed and validated. For daily QA, first standard beam machine QA tests are performed without the MR scanner [4]. Subsequently, the scanner is positioned in front of the beamline and MRI QA tests are performed. Finally, an MRI-beam collinearity test is conducted using an in-house developed phantom before authorizing clinical use on the respective day.

For the E2E test, the whole workflow was executed with a dedicated CT- and MR-compatible E2E phantom (Fig. 1) containing inserts for dosimeters (radiochromic film, ionization chamber): CT and MR image acquisition for target volume delineation and treatment planning, dose calculation accounting for MF-induced beam deflection, MRI-guided phantom positioning at the beamline, and irradiation. Additionally, PSQA dose measurements inside the MF of the MRI scanner were performed.

Results: The daily QA tests, performed multiple times over nine months, consistently passed. The absolute dose output was stable within ±1% and the PBS characteristics were all within clinical tolerances. The MRI isocenter was stable within ±0.5 mm and the image distortion within a 20 cm diameter spherical volume around it was less than ±3 mm. The positioning accuracy and co-localization of MRI and beam coordinates was within ±1 mm. The overall setup uncertainty (2σ) was quantified as 6 mm.

In the E2E test, the measured dose inside the phantom was in excellent agreement with the TPS calculation (deviation <0.5%). Furthermore, a radiochromic film measurement showed good spatial agreement (deviation <2 mm). The PSQA tests passed with absolute dose deviations <0.5% and 100% gamma pass rates.

Conclusions: The developed components, daily workflow and QA program ensure the safety and accuracy of MRiPT treatment, confirmed by the satisfactory dosimetric results of the E2E test. In conclusion, the technical requirements for accurate dose delivery within the intended clinical application of MRiPT are now fulfilled.

References 1 F. Horst et al. (2025) Front. Oncol. 15:1594973; [2] F. Horst et al. (2025) Radiother. Oncol. 206(Suppl.1):S3590; [3] K. Godina Padre et al. (2025) Radiother. Oncol. 206(Suppl.1):S3478; [4] F. Horst et al., accepted abstract for ESTRO 2026, May 15th-19th

6. Towards a Widened Therapeutic Window for Aggressive Breast Cancer: A Comprehensive Radiobiological Characterization of Helium Ion Therapy

Author: Francesca Fede[1,5]

Co-authors: Giulia Acocella [2]; Alexandra Charalampopoulou [3,4]; Emilia Formicola [5,6]; Valerio Cosimo Elia [5]; Antonio Antoccia [2]; Francesco Berardinelli [2]; Valentina D'Alesio [7]; Saleh Eskandarian [8,9,10]; Angelica Facoetti [3]; Domenico Ivan Filosa [8,9,10,11]; Yasmin Hamad [9,10,11]; Nora Schuhmacher [9,10,11]; Marcello Serra [7]; Thomas Tessonnier [9,10,11]; Andrea Mairani [8,9,10,11]; Lorenzo MANTI [6]

[1] University of Naples Federico II,Italy; [2] Università Roma Tre, Roma, Italy; [3] CNAO National Center for Oncological Hadrontherapy, Pavia, Italy; [4] University School for Advanced Studies (IUSS), Pavia, Italy; [5] Istituto Nazionale di Fisica Nucleare (INFN), Naples, Italy; [6] University of Campania "Luigi Vanvitelli", Caserta, Italy; [7] Istituto Nazionale Tumori-IRCCS-Fondazione G. Pascale, Napoli, Italy; [8] Heidelberg University, Heidelberg, Germany; [9] German Cancer Research Center (DKFZ), Heidelberg, Germany; [10] Heidelberg University Hospital, Germany; [11] National Center for Research in Radiation Oncology (NCRO), Heidelberg, Germany

Background: While survival rates for Breast Cancer (BC) have significantly improved, managing aggressive subtypes and ensuring long-term patients' welfare remain critical issues. ^{12}C ion-therapy promises to increase tumor control and normal tissue sparing. However, lighter ions such as ^{4}He may also be suitable: they maintain proton ballistic precision with improved dose conformation and enhanced biological effectiveness, without the uncertancies of ^{12}C ions. In light of radiotherapy-induced cardiovascular complications, patients with left-sided BC, where sparing the heart and left anterior descending artery is mandatory, may thus benefit from these novel ions. Within the BIOphysical characterization of Helium and Oxygen ion beams for hadronTherapy (BIOHOT) collaboration, we investigated several pre-clinical radiobiological endpoints in BC and normal cells exposed to therapeutic ^{4}He beams to validate the rationale for introducing these ions as a hadrontherapy modality.

Methods: Irradiations were performed at the Heidelberg Ion-beam Therapy (HIT) Center along a ^{4}He ions SOBP (4cm, max energy 119.15MeV/u) to simulate different clinical scenarios, using 6-MV photons as a reference. Therapeutic efficacy was evaluated in MDA-MB-231 (Triple Negative BC, p53-mutated) and MCF7 (Estrogen Receptor +, wt-p53) cells via clonogenic survival, migration assay, and AnnexinV/PI staining for apoptosis detection. HMVEC (Human Microvascular Endothelial Cardiac) and MCF10A (breast epithelial) cells were used as a surrogate for normal-tissue response. Specifically, HMVEC were used to gain insights into in-vivo cardiovascular complications through measurement of radiation-induced premature senescence (histochemical β-galactosidase assay) and persistent oxidative stress. Possible bystander effects were also evaluated by medium-transfer experiments. Secondary cancer risk was assessed in MCF10A cells by mFISH labelling of structural chromosomal aberrations.

Results: ^{4}He ions demonstrated a significant therapeutic advantage, with RBE values ranging from 1.1 to 1.6. They decreased cell migration, outperforming photons in the inhibition of this behaviour, and increased apoptosis suggesting a p53 independent pathway in the aggressive MDA-MB-231 model. Crucially, in normal cells, ^{4}He ions did not exacerbate ROS and premature senescence, when applying clinically-scaled doses to cardiac cells, or chromosomal instability in breast epithelial cells, suggesting no added risk of late-term complications compared to conventional radiotherapy.

Conclusions: Our findings support the clinical transition to ^{4}He ion beams for aggressive or radioresistant BC particularly when p53-mediated resistance is a factor. By maintaining a safety profile for healthy tissues equivalent to that seen with photons regarding cardiovascular and secondary-cancer risks while increasing anticancer effectiveness, ^{4}He ion-therapy may provide widened therapeutic window for a disease that has a strong societal impact.

41. Hypofractionated proton therapy combined with dual LAG-3/PD-1 blockade induces durable tumor control and immune memory in a murine colorectal cancer model

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Co-authors: Carole MURA [1]; Céline MIRJOLET [2]; Georges NOEL [3]; Hélène BURCKEL [1]
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Background: Ionizing radiation (IR) modulates the tumor immune microenvironment, potentially synergizing the immune checkpoint inhibitors (ICI). Proton therapy (PT) offers distinct physical and biological advantages over photon radiotherapy, but preclinical PT+ICI data remain limited. In a CT26 colon carcinoma model, we previously observed that PT (3×8 Gy) combined with anti-PD-L1 increased complete responses (CR) without improving overall survival, while RNA-Seq showed PT-induced upregulation of PD-1 and LAG-3, identifying them as potential targets. Therefore, combining hypofractionated PT with dual LAG-3/PD-1 blockade could enhance local tumor control and long-term immune memory. Comparing concomitant versus sequential ICI schedules with tumor rechallenge was performed to identify the most effective regimen at inducing durable systemic antitumor immunity.
Materials and Methods: Female BALB/c mice bearing subcutaneous CT26 tumors were divided into five groups: control, PT (3×8 Gy), dual ICI (anti-PD-1, 10 mg/kg + anti-LAG-3, 1 m/kg) or concomitant PT+ICI and sequential PT+ICI. PT was delivered with a 25 MeV proton beam extracted from the CYRCé Cyclotron (Strasbourg, France). Antibodies were given intraperitoneally twice weekly for three weeks. Tumor growth was monitored up to day 100 and survival was analyzed by Kaplan–Meier and log-rank tests. Longitudinal blood counts were assessed to evaluate systemic hematologic toxicity. Mice in CR were rechallenged on the contralateral flank with CT26. Results: Dual ICI alone delayed CT26 tumor growth significantly compared to control. PT alone significantly improved survival versus dual ICI and yielded a 64% survival plateau at day 100 with 7/11 CRs. Combining dual ICI to PT produced almost equivalent survival curves for concomitant and sequential schedules, with plateaus of ˜90% at day 100. Although not statistically superior to PT alone, these combinations increased CR frequency and rescued a subset of tumors escaping PT. Blood counts showed no radiation-induced lymphopenia and preserved platelet counts. Upon rechallenge, 85–90% of cured mice rejected CT26 (PT 85.7%; sequential PT+ICI 88.9%; concomitant PT+ICI 100% at day 40), indicating robust and durable systemic immune memory.
Conclusions: Hypofractionated PT alone was effective in CT26 and its combination with dual LAG-3/PD-1 blockade further increased complete responses and controlled PT-resistant tumors without hematologic toxicity. Concomitant and sequential combinations of PT and dual ICI schedules achieved similar long-term efficacy, with overlapping survival and CR rates in this model. The high rechallenge rejection rate across combination groups demonstrated that these combinations elicit strong, long-lasting immunogenic memory. Ongoing multiplex cytokine analyses of plasma samples will further characterize treatment-induced systemic inflammation and immune activation and refine PT+ICI combination strategies for clinical translation.

90. Effects of metformin in cervical cancer: results of a phase II, chemoradiotherapy trial

Authors: Christina S Fjeldbo[None]; Tord Hompland[None]; Kjersti Skipar[None]; Kjersti Vassmo Lund[None]; Kari-Anne Frikstad Valen[None]; Kristina Lindemann[None]; Taran Hellebust[None]; Kjersti Bruheim[None]; Lyng Heidi[None]

Background: Metformin, a widely used diabetic drug, has gained attention for potential anticancer effects. Its antitumor activity is thought to arise from metabolic modulation, particularly inhibition of mitochondrial complex I in oxidative phosphorylation (OXPHOS). However, most mechanistic insights come from pre-clinical studies, and the molecular effects in patients are still not well defined. We investigated the effect of adding metformin to standard chemoradiotherapy in cervical cancer.

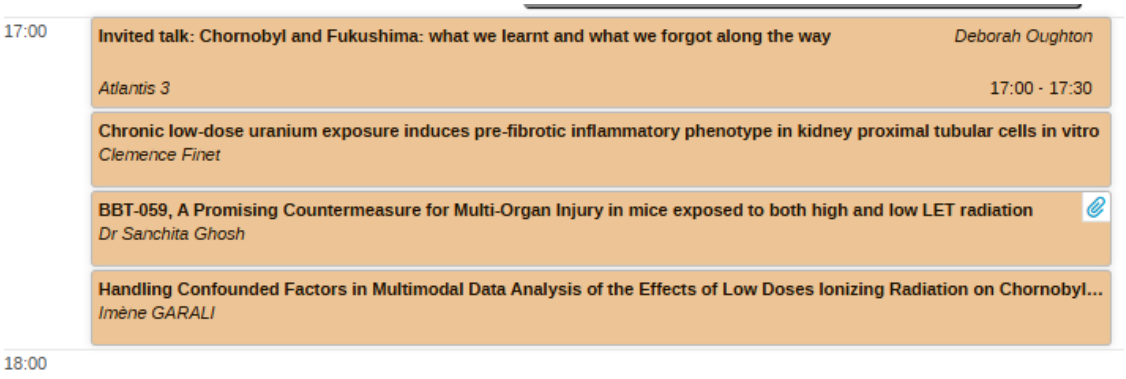
Materials and Methods: Forty-one non-diabetic patients with locally advanced cervical cancer were enrolled in a phase II randomized, open-label trial assigned 1:1 to standard chemoradiotherapy with or without metformin. Eighteen patients received metformin (850 mg twice daily) initiated one week before chemoradiotherapy and continued through treatment; 23 patients received standard therapy alone. Tumor biopsies and MRI were obtained around one week before start of metformin (baseline), after one week of metformin (oneweek), and at the first brachytherapy fraction. DCE-MRI was analyzed using the Tofts model, classifying voxels with Ve = 0 or > 10 as necrotic. Tumor volume was measured by MRI. Bulk RNA-seq (QuantSeq, Lexogen) was performed on all biopsies, and gene expression changes from baseline to oneweek were correlated with changes in necrotic fraction. Gene set enrichment analysis (GSEA) on genes ranked by these correlation coefficients identified pathways associated with necrotic expansion.

Results: A significant between-arm difference in tumor volume change was observed from baseline to oneweek (P =0.00072). The metformin arm showed a median 9.7% volume reduction (P=0.016), whereas the control arm showed a median 7.9% increase (P=0.023). This difference persisted at brachytherapy, where metformin-treated tumors exhibited greater regression (median -95.7% vs -86.5%, P=0.025). At oneweek, the necrotic fraction increased significantly in the metformin arm (median +2.3 percentage points, P=0.0079) but not in controls (P=0.62), indicating early metformin-induced cell death. In the metformin arm, increases in necrosis were associated with greater tumor shrinkage (rho=-0.52, P=0.035), and tumors with larger necrotic expansion showed reduced expression of metabolic pathways, including OXPHOS in GSEA. These associations were not seen in the control arm.

Conclusions: This study provides clinical evidence that metformin enhances tumor regression and induces early cell death in locally advanced cervical cancer at standard therapeutic doses. These findings support further evaluation of metformin in larger combination trials with radiation.

Session 4B: Environmental Internal Radiation Exposure: Lessons from Chernobyl and beyond

Chairs: Amy Berrington and Daniele Di Chio
Room: Atlantis 3



Invited talk: Chornobyl and Fukushima: what we learnt and what we forgot along the way

Author: Deborah Oughton [1]
[1] *NMBU, Oslo Norway*

24. Chronic low-dose uranium exposure induces pre-fibrotic inflammatory phenotype in kidney proximal tubular cells in vitro

Author: Clemence Finet[1]
Co-authors: Céline Bouvier-Capely [1]; Sabine Santucci [2]; Yann Guéguen [1]
[1] *Autorité de Sûreté Nucléaire et de Radioprotection (ASNR), Fontenay-aux-Roses, France* [2] *Université Côte d'Azur (CNRS), Nice*

Uranium is a radioactive heavy metal with known nephrotoxicity following acute exposure 1. As an α-emitting radionuclide and a heavy metal, it exhibits both radiological and chemical toxicities. Due to its widespread use in civilian and military activities, uranium represents a potential source of environmental and occupational exposure. However, the mechanisms underlying renal toxicity, particularly in the context of chronic or repeated low-dose exposure, remain incompletely understood [2]. Uranium preferentially accumulates in the kidneys, more specifically in proximal convoluted tubules. The effects of repeated low-dose uranium exposure on renal target cells were deciphered thanks to an experimental approach using renal proximal tubule epithelial cells (RPTECs).

hRPTEC-TERT1 cells are used as a reference model of proximal tubule epithelial cells [3]. A protocol was developed to enable the functional differentiation of these cells and their repeated exposure to low doses of uranium. The hRPTEC cells were exposed to uranium for 8 days, with medium changes every 2-3 days. Under these conditions and using uranium concentrations of either 10 µM, 100 µM or 200 µM, no necrotic cell death was detected, although a dose- and time-dependent increase in apoptosis was measured. Gene expression assays were used to study markers of mitochondrial integrity (MFN1/2, TFAM), oxidative stress (NOX4, NQO1, SOD2, CAT), and inflammation (TNF-α, IL-6, MCP-1, CXCL8) at different time points (days 2, 5 and 8) : a dose dependent progressive induction of oxidative stress and inflammation biomarkers was observed over time. In addition, protein array screening and western-blot analyses confirmed the induction of inflammation through the NFχB pathway, leading to the accumulation of specific biomarkers such as p62, CCL2, ICAM-1 or CXCL8. Finally, we were also able to show a functional alteration of proximal tubule cells by measuring γ-GT activity and its impact on epithelial phenotypic markers. In conclusion, repeated exposure of hRPTEC to low doses of uranium induces a kinetic response leading to an inflammatory, pre-fibrotic phenotype. Thus, the model sensitivity is greater after chronic exposure (100 µM) than after acute exposure (300-500 µM). Future analyses in more complex models (organoids and mice) will help us to gain a more comprehensive understanding of potential renal adverse effects. This work will contribute to a more accurate assessment of the risk of renal pathologies associated with chronic low-dose uranium exposure and to the identification of potential biomarkers that could be useful in developing prevention strategies and therapeutic approaches.

2. BBT-059, A Promising Countermeasure for Multi-Organ Injury in mice exposed to both high and low LET radiation

Author: Sanchita Ghosh[1]
[1] *Armed Forces Radiobiology Research Institute (AFRRI)*

The detonation of a nuclear weapon will result in the production of various qualities of radiation; initially producing neutrons with relatively high linear energy transfer (LET), which will result in the activation of environmental materials and the sustained presence of relatively low LET gamma irradiation in the wake of the nuclear fallout. Development of radiation countermeasures should be framed in the context of radiation of different qualities such that they are effective regardless of the LET associated with the exposure. Depending on the absorbed dose, the symptoms of Acute Radiation Syndrome (ARS) and long-term effects can appear within hours to months. Depending on the region of exposure, one or more organs may be affected. Damage to the gastrointestinal system (GI) should be evident within days and pulmonary damage takes months, which usually requires a relatively higher dose of radiation than that needed to affect the hematopoietic system. Currently, no prophylactic countermeasures are available for ARS. We present for the very first time BBT-059 as a new promising countermeasure for multiple organ injuries including GI.

BBT-059, developed by Bolder Biotechnology (BBT), is a long-acting PEGylated IL-11 analog with a site-specific PEGylation. Previously we have shown that a single dose of BBT-059, administered prior to total body irradiation accelerated recovery from radiation-induced peripheral blood cytopenia, restored sternal bone marrow, protected bone marrow progenitor cells, as well as attenuated protein biomarkers of hematopoietic acute radiation syndrome in mice exposed to total body low LET gamma radiation.

Here we demonstrate the efficacy of BBT-059 in mice exposed to high LET mixed-field neutron-gamma (65% to 35%) in AFRRI TRIGA facility. A single subcutaneous administration of BBT-059 prior to 14 Gy PBI significantly improved overall survival from GI-ARS in mice exposed to partial body (sparing one hind leg and tail) radiation using LINAC (low LET). BBT-059 treated animals showed histologically better structural integrity of the villi and crypts. Bacterial translocation to nearby organs upon damage to the intestines was considerably decreased by BBT-059. Metabolomic dysregulation was found because of radiation exposure peaks 3 days after exposure, with overall metabolomic profiles moving closer to baseline by Day 7. Treatment of the drug, BBT-059, does seem to somewhat reduce the intensity of dysregulation overall. In addition, BBT-059 modulated tissue as well as circulatory biomarkers from inflammation and endothelial dysfunction pathways which could be utilized as disease-specific biomarkers.

In conclusion, BBT-059 is a novel prophylactic and mitigating countermeasure of H-ARS and GI-ARS with significant survival benefit in both high and Low LET radiation.

The views expressed here are those of the authors and do not reflect the official policy of AFRRI, USUHS, DoD or the US Government. There are no conflicts of interest. (Supported by JPC6 and AFRRI Intramural Research). There are no conflicts of interest.

7. Handling Confounded Factors in Multimodal Data Analysis of the Effects of Low Doses Ionizing Radiation on Chornobyl tree frogs

Author: Elen GOUJON[1]
Co-authors: Olivier ARMANT [2]; Sandrine FRELON [2]; Arthur TENENHAUS [3]; Imène GARALI [1]

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Background: More than 30 years after the nuclear power plant accident in Chornobyl, wildlife in the Chornobyl Exclusion Zone (CEZ) is still chronically exposed to low doses of ionizing radiation. In 2018, populations of the Eastern tree frog Hyla orientalis were sampled inside the CEZ along a gradient of radiocontamination and in nearby control sites. We wish to better understand the health status of tree frogs from the Chornobyl region through the identification of exposure signatures at the level of the gene and protein expression. However, the geographical site of origin of the frogs is a confounding factor for the analysis, and its severe confoundedness with the dose rate makes its handling challenging. This multi-omics study therefore requires the selection of appropriate statistical tools.

Materials and Methods: Analyzing RNA-seq and proteomics data could reveal radiation-specific molecular signatures; however, this can only be accomplished if confounding factors are properly accounted for. Transcriptomic data were obtained from 87 subjects and proteomic data from 76 subjects, collected across eight independent sites. Firstly, we present our strategy to correct the confounding effect associated with the frogs' sampling site using the following batch effect-correction algorithms: ComBat-seq, linear residualization, and Surrogate Variable Analysis. We show that the severe confounding between the site and radiocontamination level makes the correction step challenging. Secondly, multi-modal data integration was chosen to leverage the relationship between omics layers and extract common information between modalities, while also indirectly handling the confounding factor. Combining multi-omics datasets into a holistic analysis can increase statistical power, hopefully counterbalance the influence of confounding variations, and provide more comprehensive and interpretable results. Here, we study two integration methods based on joint dimensionality reduction (R/SGCCA and MOFA). Finally, we investigate the site-to-site variability and successfully deconvolute the batch variable from the radiation level by adjusting for the population genetic structure.

Results and Conclusions: In the presence of a confounded batch effect, we were able to deconvolute the batch variable from the variable of interest through the integration of additional information. Our correction strategy based on genetic distance allowed us to correct the confounding effect of the frogs' collection site. Here, we hypothesize that removing population structure effects, which we associate with historical exposure, allowed us to better investigate the impact of current exposure to low-dose radiation. We were able to identify distinctive changes in gene and protein expression associated with chronic radiation exposure in Chornobyl tree frogs. Our current work includes the development of a new method for the incorporation of confounding factors into multimodal analyses.

Tuesday, 08.09.2026

Session 5A: Metabolic Responses, Radiosensitization Tumor Microenvironment

Chairs: Heidi Lyng and Inga Juvkam
Room: Atlantis 1

09:00

Invited talk: Exploiting Metabolic Vulnerabilities for Cancer Radiosensitization *Dr Johann Matschke*
Atlantis 1 09:00 - 09:30

JNJ-26366821 attenuates radiation-induced inflammatory cytokines and miRNAs and triggers TR/RXR signaling pathwa
Roopa Biswas

Radiation-induced antitumor immunity and type I interferon response in syngeneic cancer models *Carine Michiels*
Atlantis 1 09:40 - 09:50

Radiation treatment curtails pro-tumorigenic functions from cancer-associated fibroblasts in preclinical models
Inigo Martinez

10:00

Enhancing antitumor immunity by targeting cancer-associated fibroblasts with radiation and ATM inhibition *Sissel Hauge*
Atlantis 1 10:00 - 10:10

A 3D Co-Culture Model with Fibroblast-Restricted FAP Expression for Cancer Therapy Using [212Pb]Pb-FAPI-46
Sandra Kristiansen

Involvement of KANK4 in the pathophysiological reprogramming of dermal fibroblasts associated with sustained devel...
Juliette Montanari

Invited talk: Exploiting Metabolic Vulnerabilities for Cancer Radiosensitization

Author: Johann Matschke[1]

[1] *University clinics Essen, Essen, Germany*

Radiotherapy induces a wide range of adaptive responses in tumour cells that extend beyond DNA damage processing and include substantial metabolic reprogramming. These changes affect mitochondrial function, redox homeostasis, substrate utilisation, and cellular stress tolerance, thereby influencing how tumour cells respond to irradiation. Increasing evidence suggests that such metabolic adaptations are not merely consequences of treatment, but important components of radiation response biology that may shape survival under genotoxic stress and contribute to radioresistance. This presentation will focus on the biological basis of metabolic responses to radiotherapy and on the question of how irradiation reshapes tumour cell metabolism over time. Particular attention will be given to dynamic changes in mitochondrial activity, oxidative metabolism, redox balance, and metabolic plasticity as part of the cellular adaptation to radiation-induced stress. The aim is to highlight metabolism as a functional layer of radiation response that interacts with broader stress response programmes in tumour cells. The talk will further address how adaptive metabolic states may help explain heterogeneity in radiation response and treatment resistance across tumour models. By considering metabolism as a dynamic rather than static feature of tumour biology, the presentation will outline a mechanistic framework for understanding how irradiated tumour cells adapt to stress and how these adaptive processes may create distinct biological dependencies. Overall, the presentation will provide a preclinical and mechanistic perspective on the role of tumour metabolism in radiotherapy response. By integrating concepts of metabolic adaptation, stress tolerance, and targetable vulnerability, it aims to outline a framework for understanding how modulation of tumour metabolism may enhance radiosensitivity and support the development of more effective radiotherapeutic strategies.

1. JNJ-26366821 attenuates radiation-induced inflammatory cytokines and miRNAs and triggers TR/RXR signaling pathway

Author: Roopa Biswas[1]

Co-authors: Bernedette Hritzo [2]; Dharmendra Soni [1]; Greogory Holmes-Hampton [2]; Sanchita Ghosh [2]; Venkateshwara Dronamraju [2]; Vidya Kumar [2]

[1] *Uniformed Services University of the Health Sciences;* [2] *AFRRI, Uniformed Services University of teh Health Sciences*

JNJ-26366821, a novel thrombopoietin mimetic peptide (TPOm), has been shown to protect mice from radiation-induced neutropenia and thrombocytopenia when given 24 h prior to total-body irradiation. TPOm is shown to increase platelets (PLT) transiently in peripheral blood. We hypothesized that increases in PLT counts may involve stimulation of hematopoiesis via cytokines, growth factors and microRNAs. Hence, we measured various cytokines, chemokines and growth factors in serum. The current data shows a strong induction of various cytokines and chemokines. TPOm pre-treatment resulted in significant increases in G-CSF, IL-6, MIP-1α and MIP-1β levels within 12–72 h after administration in a dose dependent manner. Time-course analysis (days 1, 3, 7, 15, and 30 post-7 and 9.5 Gy exposure) of G-CSF, IL-5, IL-6, IL-9, IL-10, TNFα, IL-1α, and IL-1β expression was significantly altered in vehicle the control group at 9.5 Gy compared to lower sublethal dose of 7 Gy on days 7 to 15 post-exposure. TPOm pre-treatment significantly ameliorated the changes in expression of these inflammatory cytokines and growth factors. We also performed microRNA (miRNAs) profiling to further understand the mechanism of action of TPOm in rescuing mice from radiation-induced hematopoietic damage and lethality. We show that TPOm differentially modulates the miRNA expression profiles in the spleen of irradiated mice compared to controls at both early times (day 1 and 7), as well as later times (day 15 and 30) after total-body irradiation. These results suggest a possible role of TPOm in protecting animals from radiation-induced thrombocytopenia and lethality by attenuating radiation-induced inflammatory cytokines and miRNAs.

Conflict of interest: There are no competing financial interests in relation to the work described.

Disclaimer: The opinions and assertions expressed herein are those of the author(s) and do not reflect the official policy or position of the Uniformed Services University of the Health Sciences or the Department of War.

8. Radiation-induced antitumor immunity and type I interferon response in syngeneic cancer models

Author: Carine Michiels[1]

Co-authors: Christoph Schiflers [1]; Emma Lambert [1]; Manon Van Den Abbeel [1]

[1] *University of Namur*

Background: Triggering the immune system to eliminate cancer cells throughout the body is a highly promising avenue in modern cancer research. The induction of adaptive antitumor immunity is essential for specific and systemic eradication of cancer cells and for the formation of a long-term antitumor immune memory to prevent cancer relapse. A growing number of studies propose that specific parameters of radiotherapy such as the type of radiation (X-rays vs. charged particles), dose and fractionation may tune the immune responses. While irradiation with charged particles provides advantages over X-ray irradiation such as better ballistics and enhanced cancer cell death, it also induces different cellular responses which could ultimately lead to a stronger immunogenic response.

The aim of this project was therefore to study and compare the immunogenicity induced by X-ray and high LET proton irradiation at different doses in two syngeneic murine cancer models.

Materials and Methods: SSC-VII cells implanted in C3H mice and MTCQ1 cells injected in C57bl/6 mice after being irradiated with X-rays or equivalent doses (RBE-adjusted) of high-LET protons. After 14 days the tumor was resected and unirradiated cells were injected in the contralateral flank. The growth of the secondary tumor was then followed. In parallel, the effects of the irradiation were assessed by immunofluorescence labelling of cytosolic dsDNA, western blotting for phosphorylated TBK1 and RT-qPCR for IFN responsive genes and immunosuppressive genes.

Results: The accumulation of cytosolic dsDNA, the activation of the cGAS/STING pathway, the IFN type 1 response and the release of IFN-ß were analyzed in vitro. The results showed cGAS/STING pathway activation following X-ray and proton irradiations in both cell types. In parallel, the expression of immunosuppressive genes was also assessed. We evidenced that they were differentially regulated according to the gene of interest and according to the type of irradiation. For example, IDO1 was upregulated in both cell lines following X-ray irradiation, whereas IL-10 overexpression was detected only in MTCQ1 cells after proton irradiation.

Secondly, an in vivo vaccination assay by injecting X-ray or proton irradiated cells was performed to evaluate the induction of systemic anti-tumor immunity. The development rate of secondary tumors indicate that the effector immune response was similarly effective after X-ray or proton irradiation in C53bl/6 mice. However, irradiations did not show any benefit on vaccination rates in C3H mice.

Conclusion: These preliminary results suggest that X-ray and proton irradiation have a similar effect in our model. However, the response differs depending on the cell lines and the irradiation doses.

14. Radiation treatment curtails pro-tumorigenic functions from cancer-associated fibroblasts in preclinical models

Author: Inigo Martinez[1]

Co-authors: Indusmita Routray [1]; Kristin Lode [1]; Lorenzo Ragazzi [1]; Rodrigo Berzaghi [1]; Thomas Kilvaer [2]; Turid Hellevik [2]; Vera Maia [1]

[1] *UiT The Arctic University of Norway;* [2] *University Hospital of Northern Norway*

Background: Cancer-associated fibroblasts (CAFs) are key components of the tumor microenvironment and active drivers of tumor progression, metastasis, and resistance to therapy. However, the effects of radiotherapy (RT) on CAFs and the consequent impact on tumor cell behavior remain controversial.

Methods: Primary cultures of human CAFs isolated from non-small cell lung cancer (NSCLC) tumor samples were exposed to ionizing radiation by a single-high dose or a hypofractionated regimen. Different lung tumor cell lines were exposed to control or irradiated CAFs in direct co-cultures or to CAFs conditioned medium (CM), and changes in tumor cell proliferation, migration, epithelial-mesenchymal transition (EMT) and clonogenic survival were measured. The impact of RT on intratumoral CAF levels in different subcutaneous tumor models was measured by α-SMA expression in excised tumor specimens. In addition, pirfenidone, a CAF-reprogramming agent, was investigated for its capacity to influence tumor responses to radiation. Finally, correlations of CAF markers with treatment outcomes was studied in a cohort of RT-treated non-small cell lung cancer (NSCLC) patients.

Results: Ionizing radiation induced premature senescence in CAFs; however, the expression of established CAF activation markers remained unchanged. Exposure of lung tumor cell lines to CM from irradiated CAFs did not alter their proliferation, migratory capacity, or clonogenic survival. Conversely, CAF-driven epithelial–mesenchymal transition (EMT) was attenuated in all tumor cell lines after incubations with CM from irradiated CAFs.

Radiation delivered to subcutaneously transplanted tumors (1x12 Gy) did not change intra-tumoral CAF abundance in the LLC and CT26 murine models, whereas CAF numbers were significantly reduced in the stroma-rich 4662PDA pancreatic model. Pirfenidone-treated animals exhibited enhanced tumor radio-resistance, along with decreased collagen levels within tumors, and unchanged numbers of aSMA+CAFs.

In clinical specimens, baseline tissue expression levels of the CAF markers FAP and aSMA were not associated with disease-specific survival.

Conclusions: Our findings suggest that ionizing radiation may lessen certain pro-tumorigenic CAF functions—such as EMT induction—, whereas pharmacologic CAF reprogramming with pirfenidone paradoxically confers increased tumor radio-resistance, highlighting potential negative implications from CAF-targeted therapies.

116. Enhancing antitumor immunity by targeting cancer-associated fibroblasts with radiation and ATM inhibition

Author: Sissel Hauge[1]

Co-authors: Chloé Müller [1]; Emilie Mosaker [1]; Inigo Martinez-Zubiaurre [2]; Turid Hellevik [3]; Randi G. Syljuåsen [1]

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Radiotherapy is a cornerstone of treatment for non-small cell lung cancer (NSCLC), and its combination with immune checkpoint inhibitors is an emerging strategy to improve therapeutic efficacy. However, treatment response is often limited by the tumor microenvironment, including cancer-associated fibroblasts (CAFs), which promote tumor cell survival and immune suppression. Here, we investigate whether targeting the DNA damage response in CAFs can reprogram their response to ionizing radiation toward immunostimulatory signaling. Building on our previous findings that cell cycle checkpoint inhibition enhances radiation-induced type I interferon (IFN-I) signaling in cancer cells through cytosolic DNA sensing, we examined whether a similar approach could trigger an IFN-I response in CAFs and shift their phenotype toward supporting antitumor immunity.

Patient-derived CAFs from four NSCLC donors were exposed to X-rays (2, 6, or 18 Gy) in combination with inhibitors targeting ATR, ATM, CHK1, or WEE1. IFN-I responses measured 3-8 days post-treatment showed that combining radiation with the ATM inhibitor AZD1390 induced IFN-β release from CAFs, with response magnitudes varying in a donor-dependent manner. Co-culturing CAFs with NSCLC cell lines further amplified this response. Multiplex cytokine analysis revealed increased secretion of additional immune-stimulating factors, including GM-CSF and CXCL-10, in irradiated CAF-cancer cell co-cultures treated with ATM inhibition.

Mechanistically, cell cycle analyses showed that ATM inhibition abrogated the radiation-induced G1 checkpoint in CAFs. Experiments using CDKN1A knockdown to abrogate the G1 checkpoint, or, conversely, contact inhibition to prevent cell cycle entry, clearly linked the IFN-I response to aberrant cell cycle progression post-irradiation. Knockdown of the cytosolic DNA sensor cGAS and RNA sensor RIG-I indicated that IFN-I signaling was partially dependent on cytosolic nucleic acid sensing pathways. Functionally, conditioned media from irradiated CAF-cancer cell co-cultures treated with ATM inhibition enhanced dendritic cell activation, evidenced by CD80 and CD40 expression. In parallel, live cell flow cytometry analyses showed that the combination treatment increased MHC-I expression on tumor cells in co-culture, consistent with enhanced IFN-I signaling.

Beyond its well-established role in tumor cell radiosensitivity, this study identifies ATM as a key regulator of the radiation-induced type I interferon response in CAFs, linking DNA damage checkpoint control to innate immune signaling in the stromal compartment. Altogether, our findings suggest that targeting the DNA damage response in stromal cells may enhance the immunogenic effects of radiotherapy and provide a mechanistic basis for improving radiotherapy-immunotherapy combinations in NSCLC.

52. A 3D Co-Culture Model with Fibroblast-Restricted FAP Expression for Cancer Therapy Using (212Pb)Pb-FAPI-46

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Co-authors: Nurtene Dernjani [2]; Øyvind Bruland [1]; Nina Frederike Jeppesen Edin [3]; Asta Juzeniene [1]
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Introduction: The tumor stroma is increasingly recognized as a therapeutic target across cancer types. Fibroblast activation protein (FAP) is abundantly expressed by cancer-associated fibroblasts (CAFs) and forms the basis for FAP-directed radioligand therapies. However, several preclinical strategies rely on tumor cell models with artificial FAP overexpression, limiting clinical relevance. To better reflect physiological FAP distribution, we developed a 3D co-culture system with fibroblast-restricted FAP expression to assess the effects of the alpha-emitting radioligand [212Pb]Pb-FAPI-46.

Materials and Methods: Spheroids consisting of FAP-negative prostate cancer cells co-cultured with either immortalized CAFs or immortalized normal fibroblasts (NFs) were generated using the liquid overlay technique and characterized by brightfield and fluorescence microscopy. Radiolabeling efficiency and stability were evaluated in vitro. FAP expression and radioligand uptake were assessed in 2D monocultures using flow cytometry and binding assays, while spatial localization of cell types within spheroids was confirmed using lipophilic tracers. Treatment response was evaluated by monitoring spheroid growth at varying fibroblast compositions.

Results: [212Pb]Pb-FAPI-46 was radiolabeled with >97% radiochemical purity and remained stable for up to 48 h in PBS, serum, and medium. CAFs and NFs exhibited high levels of FAP expression along with specific radioligand binding, while prostate cancer cells demonstrated negligible FAP expression and binding. Co-culture spheroids formed compact 3D structures, with fibroblasts localized in the spheroid core. Exposure to [212Pb]Pb-FAPI-46 reduced spheroid growth relative to untreated controls in a fibroblast-dependent manner. Growth inhibition was more pronounced in NF-containing spheroids than in CAF-containing spheroids. No treatment effect was observed in tumour cell monoculture spheroids.

Conclusion: This study introduces a 3D co-culture spheroid model that more accurately mimics stromal-restricted FAP expression and enables evaluation of FAP-targeted radioligand therapy in vitro. Despite limited proliferative capacity of fibroblasts in 3D monoculture, their presence in co-culture supported sustained spheroid growth and mediated sensitivity to targeted irradiation. The absence of effects in FAP-negative tumor monocultures suggests that therapeutic efficacy arises through indirect mechanisms linked to stromal targeting rather than direct tumor cell irradiation. Differences in treatment response between the two fibroblast co-culture systems further indicate that fibroblast lineage and 3D microenvironmental interactions influence therapeutic outcome. Collectively, this model provides an in vitro platform for evaluating FAP targeted therapies under stromal conditions not captured by tumour monoculture systems, with potential for further optimization and application across additional cancer types.

59. Involvement of KANK4 in the pathophysiological reprogramming of dermal fibroblasts associated with sustained development of cutaneous fibrosis

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Background: Skin fibrosis is a common late complication of radiotherapy, characterized by the progressive replacement of a functional parenchymal tissue with a non-functional connective tissue. A central cellular event involved in this process is the uncontrolled and persistent activation of fibroblasts into fibrotic myofibroblasts, leading to excessive extracellular matrix (ECM) deposition, thus altering tissue architecture and function. Transforming growth factor-β1 (TGFB1) is a driver of this pathophysiological process, promoting the transition from healthy fibroblasts towards hyper-contractile, ECM-producing myofibroblasts. Transcriptional analyzes having identified the KN Motif and Ankyrin Repeat Domains 4 (KANK4) gene as a candidate effector, our objective is thus to investigate its role in the fibroblast-to-myofibroblast switch, and evaluate its relevance as a target for the mitigation and correction of fibrosis traits.
Materials and methods: Cellular models in 2D culture and 3D dermal organoids were developed using primary human skin fibroblasts or TGFB1-induced myofibroblasts, to mimic fibrogenic and healthy contexts. A functional genomics approach centered on KANK4 has been developed, including bulk and single-cell transcriptomic profiling, and the implementation of RNA interference-mediated (siRNA) knockdown.
Results: TGFB1-induced myofibroblasts exhibited functional and molecular hallmarks of the transition from a healthy to a fibrogenic state, including increased contractility, modified secretion of ECM components, and informative molecular signatures on the phenomenon of fibrogenic reprogramming. Of note, a significant upregulation of KANK4 expression correlated with the TGFB1-induced fibrogenic switch of dermal fibroblasts, which could be consistent with a role as a fibrosis-promoting effector. Data supporting this hypothesis were obtained by repressing KANK4 using siRNAs. In particular, targeted repression of KANK4 appeared effective in attenuating hypercontractility and increased expression of ECM protein fibronectin 1 (FN1), two characteristics of the fibrosis pathological context.
Conclusion: The results obtained document KANK4 as a regulatory element of the fibroblasts-to-myofibroblast transition, which is associated with the establishment of cutaneous fibrosis, and position this molecule as a relevant target for the design of preventive or curative approaches against fibrotic traits. A multi-OMICs approach integrating genomic and transcriptome levels (ATAC-seq and RNA-seq), and proteome parameters, is being undertaken to gain an understanding of the mechanisms of the fibroblast reprogramming phenomenon leading to the fibrogenic state.

Session 5B: Experimental Microdosimetry Mechanistic Biophysical Modelling

Chairs: Giuseppe Schettino and Aikaterini Rousseti
Room: Atlantis 3

09:00

Invited talk: Advanced Dosimetry and Microdosimetry: Silicon Carbide as an Enabling Technology for Next-Generation Radiation Detection
Giada Petringa

Assessment of clinically relevant endpoints in particle therapy accounting for tissue heterogeneities and stochastic eff...
Marco Battestini

SMART-DNA: Simulation and Machine-learning Assisted Rapid predicTion of DNA damage across H to Fe ions from 1 to...
Chia-Wei Huang

Time-Resolved Dose-Rate Modeling with Geant4-DNA *Nicolas Sterckx*
Atlantis 3 09:50 - 10:00

10:00

Quantitative assessment of enhanced biological effectiveness of ion radiation using Deep Learning
Laura Quintero Velasquez

Modelling Radium 223 radiobiological response as a function of PC3 cell morphology *Joana Antunes*
Atlantis 3 10:10 - 10:20

FORRCE project: Development of a Neural Network-Driven computational framework for the simulation of the biological...
Omar Rodrigo Garcia Garcia

Invited talk: Advanced Dosimetry and Microdosimetry: Silicon Carbide as an Enabling Technology for Next-Generation Radiation Detection

Author: Giada, Petringa[1]

[1] *INFN, Italy* The rapid development of advanced radiotherapy and radiation-based technologies is creating increasingly demanding requirements for radiation detectors. Conventional dosimetry is progressively being challenged by small fields, steep dose gradients, high dose rates, ultra-high dose-rate (UHDR) irradiation, and the complex radiation quality of particle therapy. At the same time, a growing interest in understanding the microscopic patterns of energy deposition calls for detector technologies capable of bridging the gap between macroscopic absorbed dose and the stochastic nature of radiation interactions at cellular and sub-cellular scales.

Silicon carbide (SiC) has emerged as a particularly attractive material for next-generation radiation detectors. As a wide-bandgap semiconductor, SiC combines high radiation hardness, low leakage current, fast charge collection, high thermal stability and the possibility of fabricating very small sensitive volumes. Its physical properties also make it attractive for radiation environments where conventional semiconductor detectors may suffer from increased leakage, radiation damage or response limitations. These characteristics have stimulated the development of SiC-based detectors for a broad range of applications, from conventional radiotherapy dosimetry to particle therapy and FLASH irradiation. This talk will provide an overview of the evolution of SiC from a robust radiation sensor towards a versatile platform for advanced dosimetry and microdosimetry. The versatility of SiC detectors will be illustrated through their application to different radiation fields, including photon and electron beams, proton therapy, helium and carbon ion beams, and laser-driven particle sources. In conventional radiotherapy, SiC detectors have demonstrated promising characteristics for dose measurements, beam characterization and real-time monitoring. In particle therapy, the high spatial resolution and radiation hardness of SiC become particularly relevant for the characterization of steep dose gradients and changing radiation quality along therapeutic beams. SiC detectors have been investigated in proton and carbon ion fields for relative dosimetry, depth-dose measurements and LET-related studies, while their application to helium beams further extends the perspective towards emerging ion-therapy modalities.

A further step is represented by the development of SiC-based microdosimeters. By reducing the sensitive volume to micrometre dimensions, the detector response can be related to the stochastic energy deposited by individual radiation tracks, providing access to quantities such as lineal-energy distributions and dose-mean lineal energy. Recent experiments with a 10 μm-thick SiC p–n junction detector in proton beams at 30, 70 and 150 MeV demonstrated depth-dependent microdosimetric spectra consistent with the expected evolution of LET and in agreement with Monte Carlo simulations and independent microdosimetric detectors. The combination of macroscopic dose measurement, LET characterization and microdosimetry therefore represents a particularly promising direction for SiC technology. Rather than providing only a measurement of how much energy is deposited, advanced SiC detectors could provide information on how that energy is deposited and how radiation quality changes within a treatment field. This perspective is especially relevant for high-LET radiation, biologically optimized particle therapy and emerging treatment modalities in which conventional dose alone may not fully describe the biological impact of the radiation field.

17. Assessment of clinically relevant endpoints in particle therapy accounting for tissue heterogeneities and stochastic effects through a mechanistic radiation biophysical model

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Co-authors: Giulio Bordieri [2]; Marta Missiaggia [3]; Emanuele Scifoni [4]; Francesco G. Cordoni [2]
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Background and Aims: The biological effect of treatments in particle therapy (PT) is assessed using Tumor Control Probability (TCP) and Normal Tissue Complication Probability (NTCP) models. However, most of them have been developed specifically for photon radiotherapy and lack a robust radiation biophysical basis, neglecting tissue heterogeneities and key radiation-induced stochasticities, affecting energy deposition, damage formation, and their temporal evolution. The main goal of this work is to investigate the effect of PT, from single-cell responses to whole organ endpoints, under various tissue and irradiation conditions, considering heterogeneities in dose, oxygenation, and radiosensitivity.

Materials and Methods: Starting from the Generalized Stochastic Microdosimetric Model (GSM2) [Cordoni et al., Phys.Rev.E(2021), Rad.Res.(2022), Int.J.Radiat.Biol.(2023), Battestini et al., Front.Phys.(2023), Radiother.Oncol.(2025)], a probabilistic radiation-biophysical model that describes the induction, interaction, and evolution of DNA damage at the single-cell level, we extended its mechanistic formulation to whole-organ endpoints [Battestini et al., Comput.Biol.Med.(sub.)]. Specifically, we embedded GSM2 within a tissue-level TCP/NTCP framework by incorporating the organ's characteristic volume effect and representing the tissue as an assembly of functional subunits [Kallman, Int.J.Radiat.Biol.(1992)]. This approach enables GSM2's cell-by-cell predictions to propagate to clinical-scale biological outcomes.

Results: Using the GSM2-driven TCP/NTCP model, we study the induction of side effects in PT across diverse particles, radiation qualities, fractionations, and partial irradiated volumes, considering various oxygenations, cell lines, and tissue architectures (Fig.1), while accounting for the tissue's characteristic volume effect. Starting from single-cell resolution, we investigate the macroscopic biological impact of physical and biochemical heterogeneities in healthy and cancer tissues, such as distributions of energy, dose, oxygen concentration, and cell line, i.e., co-cultures that mimic the tumor microenvironment (Fig.2), across different radiation qualities and fractionations. We highlight the impact of considering oxygenation gradient in spheroids compared to uniform oxygenation, from single-cell responses to macroscopic effects (Fig.3). We show that both the oxygen gradient and tissue heterogeneity affect the TD50 and the slope of the TCP curves.

Conclusions: The GSM2-driven TCP/NTCP model can describe different tissue environments and irradiation modalities, with a single-cell resolution, reproducing the typical experimental trend in terms of oxygenation, cell type, and volume effect. This model will enable a deeper level of radiobiological assessments in treatment plan evaluation for PT.

19. SMART-DNA: Simulation and Machine-learning Assisted Rapid predicTion of DNA damage across H to Fe ions from 1 to 1000 MeV/u

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Background: Accurate prediction of DNA damage induced by light and heavy ions is essential for space radiation risk assessment and particle therapy. However, systematic datasets remain limited, particularly for ions heavier than Ne. Monte Carlo track structure simulations usefully complement experimental data but are computationally intensive. Coupling simulations with machine learning offers a pathway to rapid, physics-grounded damage prediction without requiring additional Monte Carlo calculations.

Materials and Methods: DNA damage induction was simulated using the Monte Carlo track structure code PARTRAC for Mg, Si, Ca, Ti, and Fe ions from 1 to 1000 MeV/u. These data were combined with previous simulations for H to Ne. Electronic stopping powers were benchmarked against ICRU and SRIM reference data. Double-strand break (DSB) yields were compared with pulsed-field gel electrophoresis experiments, and the formation of radiation-induced foci was simulated. The damage yields were described using an analytical formula. A Gaussian Process Regression (GPR) model was trained using ion species together with either energy, linear energy transfer (LET), or $(Z_{\mathrm{eff}}/\beta)^2$ as input parameters, capturing the nonlinear dependence of DNA damage on radiation quality across the full ion range.

Results: Simulated stopping powers closely matched reference data, confirming the physical reliability of the calculations. Simulated DSB yields were consistent with experimental data after accounting for assay detection limits. DNA damage yields showed clear and structured trends with radiation quality across all ions and energies, with increasing linear energy transfer leading to enhanced damage clustering and the formation of short DNA fragments and multi-DSB foci along ion tracks.

The Gaussian Process Regression model outperformed the analytical formula and reproduced these trends with high fidelity. Leave-one-out cross-validation demonstrated strong predictive accuracy and robust generalization across ions and energies. A web-based application, SMART-DNA, has been developed to make the results accessible to the community.

Conclusions: This work provides a systematic track-structure-based dataset of DNA damage from H to Fe across 1 to 1000 MeV/u, combined with a validated and publicly available machine learning model that enables rapid predictions. The model achieves high accuracy across energies and ion species from H to Fe. The database and SMART-DNA web application provide useful tools that complement experimental data, with potential applications in light and heavy ion radiobiology, radiotherapy, and space radiation risk modeling

62. Time-Resolved Dose-Rate Modeling with Geant4-DNA

Author: Nicolas Sterckx[1]
Co-authors: Anh Le Tuan [2]; Carmen Villagrasa [1]; Christelle Huet [1]; Hoang Tran Ngoc [3]; Sébastien Incerti [3]; Yann Perrot [1]
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Background: Emerging modalities in radiation therapy, such as hypofractionation and ultra-high dose rates (UHDR) delivery, challenge classical assumptions of dose-rate impact suggesting that the timing of energy deposition may influence biological outcomes. Track structure Monte Carlo codes must therefore be capable of modelling the dose rate, beyond the usual assumption of independent radiation tracks. The goal of this work is to contribute to the further development of Geant4-DNA(1) in order to overcome this challenge and enable the temporal structure of the beam to be taken into account when studying the impact of irradiation, in terms of chemical yields and biological damage.
Materials and Methods: A modified version of the "molecularDNA"(2) example of Geant4 DNA was used. This version was adapted to support DNA geometries in the DNAFabric format, which had previously been handled only by the "dsbandrepair"(3) Geant4-DNA example. The example can also handle ultra-high dose rate (UHDR) irradiation by adopting the approach available in the Geant4-DNA "UHDR" example. This adaptation enables an explicit and flexible multiscale representation of DNA organization while integrating dose rate modeling. Simulations were performed for the irradiation of HUVEC cells, including their complete genome, using electrons and protons. By varying beam parameters such as duration, interpulse interval, and frequency, this study investigates how temporal irradiation structure affects radiochemical yields and the induction of early DNA damage.
Results: Early DNA damage was compared at constant absorbed dose across different dose rates by varying pulse structure parameters. The analysis highlights the role of dose-rate–dependent radical dynamics, particularly their influence on indirect DNA damage pathways.
Conclusions: This work establishes for the first time a framework for exploring dose-rate–dependent mechanisms in complex DNA cellular geometries and for evaluating the impact of pulse structure on radiobiological outcomes. By combining full-scale modeling of human DNA geometry with different beam parameters, including UHDR and CONV, the study sheds light into the role of temporal beam structure in shaping radiochemical processes and DNA damage.
Keywords: UHDR, dose rate, DNA damage, inter-track chemistry, reactive oxygen species, DNA geometry, DNAFabric, molecularDNA, Monte Carlo simulation, Geant4-DNA, time-resolved modeling (1) 10.1002/mp.17256; 10.1002/mp.13048; 10.1016/j.ejmp.2015.10.087;10.1118/1.3476457; 10.1142/S1793962310000122; (2) 10.1002/pro6.1186;
(3) 10.1016/j.cpc.2016.02.019

66. Quantitative assessment of enhanced biological effectiveness of ion radiation using Deep Learning

Authors: Laura Quintero Velasquez[1];

Co-authors: Nikolaos Mallousis[1];Jessica Neubaue[1]; Selina Onoh [2]; Judith Reindl[1,3]

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Background: Fundamental radiobiological research aims to understand the behaviour of cells or biological tissues after irradiation with different radiation qualities and doses. It provides a better understanding of healthy tissue response and late effects in radiotherapy and helps improve radiation protection strategies. Ionizing radiation induces different types of DNA damage, which depend on the dose and radiation quality measured by Linear Energy Transfer (LET). The effectiveness of radiation is commonly assessed using the survival fraction of cells measured by the colony forming assay (CFA). High-LET radiation shows enhanced biological effectiveness. The CFA is used on populations of cells and does not provide insight into single-cell behaviour or the spatial distribution of ion hits within the cell. BioMICRO is a project that integrates ion microbeam technology for fundamental radiobiological studies, to investigate single-cell response to radiation. In this phase, the aim is to combine microscopy-based single-cell analysis in live cell videos with deep learning tools to quantitatively investigate radiation-induced biological responses at the single-cell level across multiple generations.

Materials and Methods: U2OS MDC1-GFP cell were used, where cell cultures at different densities were irradiated with different doses and radiation types (X-rays, 10 MeV p, 16 MeV He and 42 MeV C). Post-irradiation, cells were monitored using phase-contrast microscopy to visualize cell-cycle progression for several days. In the first day after irradiation ionizing radiation induced foci are monitored together with the phase-contrast imaging, to see damage location and repair. The phase-contrast data are used to train and improve the Cell Classification and In-Vitro Lifecycle Evaluation (CeCILE)1 model, originally developed for CHO and HeLa cell lines.

Results: CeCILE shows good performance on CHO and HeLa cell lines. However, its application to the U2OS MDC1-GFP cell line reveals reduced performance, including lower detection rates and increased misclassification. Initial X-ray irradiation experiments generated a dataset specific to U2OS MDC1-GFP, highlighting differences in cell morphology and image quality that limit model generalisation. Improved prediction accuracy was observed under low-density conditions, indicating that cell overlap, cell density, morphological variability, and image quality significantly affect model performance.

Conclusions: These results demonstrate the limited transferability of pre-trained deep learning models to new cell lines. The study emphasises the need for cell line specific training datasets to achieve reliable singlecell analysis. The ongoing development of a dedicated ground truth dataset represents a key step toward adapting CeCILE for U2OS MDC1-GFP cells and enabling robust single-cell analysis of radiation-induced responses across generations.

1. Rudigkeit, S. et al. CeCILE - An Artificial Intelligence Based Cell-Detection for the Evaluation of Radiation Effects in Eucaryotic Cells. Front. Oncol. 11, (2021).

85. Modelling Radium 223 radiobiological response as a function of PC3 cell morphology

Author: Joana Antunes[1]
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Background: Targeted radionuclide therapy relies on the interplay between radionuclide distribution, cellular morphology, and the resulting patterns of energy deposition. In this work, we investigate how PC3 prostate cancer cell models respond to the alpha emitter radium 223, and whether the radiobiological response of one culture type (suspension or adherent) can be predicted from the other.

Materials and Methods: Monte Carlo (MC) simulations were performed using realistic 3D cell geometries of the PC3 cell line in suspension and adherent culture. The geometries were reconstructed from confocal microscopy images[1]. The full Ra-223 decay chain was simulated, with source distributions defined according to experimentally measured internalization fractions[2]. From the simulations, the deposited dose in the nucleus was determined and the Survival Fraction (SF) was estimated using the Linear Quadratic (LQ) model. Alternatively, SF was estimated by applying the DBSCAN algorithm[3] to the MC ionization events, where clusters of dense energy deposition were classified as lethal lesions and their frequency converted into a predicted probability of cell death.

Results: The simulations reproduced experimental SF trends through the LQ model. However, they could not predict SF across culture types without experimentally determined α parameters, highlighting the limitations of dose based approaches. In contrast, the DBSCAN derived lethal cluster model enabled accurate cross prediction between suspension and adherent cultures. By fitting the lethal cluster model to suspension cell data and transferring the resulting parameters to adherent cell simulations, we achieved predictive agreement with the SF estimated using the LQ model without requiring new calibration. This suggests that mechanistic, track structure based descriptors of DNA damage can bridge morphological differences between cell models.

Conclusion: These findings highlight the importance of incorporating track structure information when modelling targeted radionuclide therapy. By moving beyond dose based metrics, mechanistic descriptors of DNA damage offer a more transferable framework for predicting radiobiological outcomes across different cellular morphologies.

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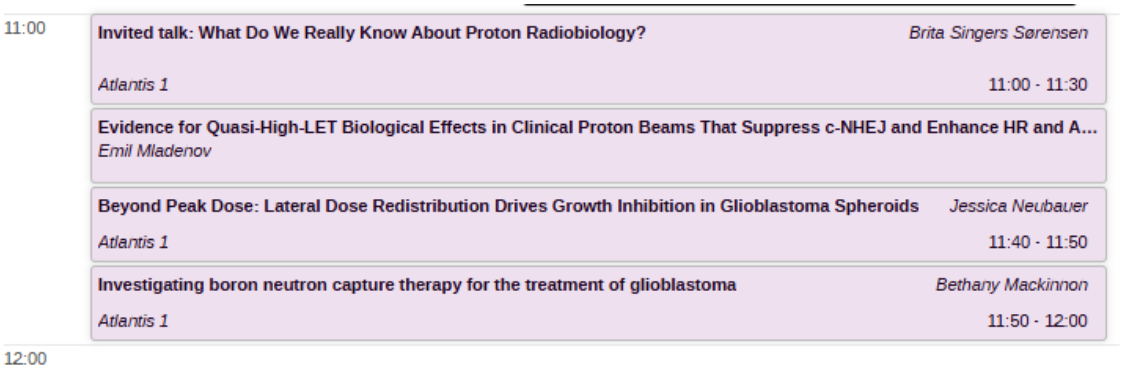
61. FORRCE project: Development of a Neural Network-Driven computational framework for the simulation of the biological effects of radiopharmaceuticals at the cell population scale in Target Radionuclide Therapy (TRNT)

Author: Omar Rodrigo Garcia Garcia[1]
Co-authors: Ana Vaniqui [2]; Carmen Villagrasa [1]; Drouet Guillaume [1]; Estelle Davesne [1]; María Luz Olaguibe [2]; Perrot Yann [1]
[1] ASNR; [2] SCKCEN

Background: Treatments based on radiopharmaceuticals like Ac-225 or Lu-177 have shown promising results for PSMA-positive metastatic castration-resistant prostate cancer1,2. However, predictive dose-response models are still lacking in Target Radionuclide Therapy (TRNT) since experimental measurements of short-ranged emitter's dose deposits are very challenging at cellular level. Predictive Monte Carlo based simulations offer a powerful tool for overcoming these constraints. In particular, the combination of Track Structure (MCTS) codes, such as TOols for PArticle Simulation (TOPAS), for handling radiation transport, with Agent Based Modeling (ABM) simulator, like CompuCell3D (CC3D), for the biological response of a cell population, has successfully reproduced survival curves for single X-ray irradiation of prostate cancer cells3.
One of FORRCE (Framework for Outcomes of Radionuclide Radiation on Cellular Environments) project aims, funded by PIANOFORTE partnership, is to develop a Neural Network-Driven computational framework that bridges physical energy deposition with biological outcomes across multiple spatial-temporal scales.
Materials and Methods: Constant communication and data transfer between MCTS and ABM codes is ensured, while calculation of early radiation DNA damage is assessed from the interpolation of large precalculated databases, such as MINAS TIRITH4.
This framework will be adapted for continuous alpha irradiation simulation on both 2D and 3D cell culture models and its biological response.
Results: The development of an initial prototype for a planar cell culture, in which CC3D drives the computing chain, highlighting three important characteristics:
1) For each CC3D time step of 1min, TOPAS performs the irradiation using a volumetric alpha particle source representing a solution of Ac-225 under secular equilibrium located in the extracellular environment.
2) The incident alpha particle's energy is registered on spherical phase-spaces situated on the cell's center of mass, representing their nuclei, and used to cumulatively sample a DSB induction probability function, constructed from the cubical spline interpolation of MINAS TIRITH database.
3) Deposited dose is tracked using a voxelated TOPAS component, matching the CC3D lattice, and will be used for other biological endpoints.
Conclusion: The prototype accounts for the specific characteristics of TNRT, combining various calculation methods. The proposed framework enables the simulation of
biological effects over a relevant time period, thereby providing a better understanding of radionuclides effect on a cell population. For the next step, the FORRCE project will produce experimental data to validate, fine-tuning and development support of this platform with diverse biological endpoints, encompassing survival curves and fluorescence microscopy time lapses of γH2AFX foci and other specific proteins such as the ones related to the cell cycle regulation and p53 controlled apoptosis.
1 DOI: 10.2967/jnumed.116.178673;[2] DOI: 10.1093/neuonc/noae192;
[3] DOI: 10.3390/ijms251810061; [4] DOI: 10.1088/1361-6560/acb196

Session 6A: Biology of Advanced Radiation Modalities

Chairs: Kristoffer Petersson and Vilde Skingen
Room: Atlantis 1



Invited talk: What Do We Really Know About Proton Radiobiology?

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Proton therapy offers superior dose distributions compared with conventional photon radiotherapy and has become an established treatment modality. However, despite decades of research, many of the fundamental questions regarding the biological effects of proton irradiation remain unresolved. While a constant relative biological effectiveness (RBE) of 1.1 is currently applied in clinical proton therapy, there is increasing recogni-tion that this simplification does not adequately reflect the complexity of the biological response.

Much of what is commonly accepted as knowledge in proton radiobiology is based on in vitro observations, whereas the amount of in vivo evidence remains surprisingly limited. Much of our current understanding of proton radiobiology derives from cell-based studies investigating survival, DNA damage, repair mechanisms, and molecular signalling pathways. These studies have provided important mechanistic insights and have clearly demonstrated that proton irradiation can induce biological responses that differ from those of photon irradiation. Nevertheless, the translation of these findings into clinically relevant biological effects remains challenging. In vitro systems inherently lack the tissue architecture, vascular response, immune interactions, and temporal dynamics that shape radiation response in vivo.

This presentation will critically examine the evidence supporting our current understanding of proton radio-biology and discuss the strengths and limitations of the experimental models that have shaped the field. Par-ticular emphasis will be placed on the distinction between mechanistic observations and biologically relevant endpoints, and on how this distinction influences our interpretation of proton RBE. The presentation will focus on in vivo studies investigating normal tissue toxicity, fractionation effects, and LET-dependent biological re-sponses, highlighting where experimental evidence supports current assumptions and where important uncer-tainties remain.

107. Evidence for Quasi-High-LET Biological Effects in Clinical Proton Beams That Suppress c-NHEJ and Enhance HR and Alt-EJ

Author: Emil Mladenov[1]
Co-authors: Mina Pressler; Veronika Mladenova; Aashish Soni; Fanghua Li; Feline Heinzelmann; Johannes-Niklas Esser; Razan Hessenow; Eleni Gkika; Verena Jendrossek; Beate Timmermann; Martin Stuschke; George Iliakis
[1] *UME-Essen*

Background: Protons are conventionally regarded as a low-linear energy transfer (low-LET) radia-tion modality with a relative biological effectiveness (RBE) of 1.1, suggesting direct mechanistic simi-larity to X-rays in the underpinning biological effects. However, exposure to spread-out Bragg peak (SOBP) protons reveals instructive deviations from this assumption. Indeed, proton beams have a maximum LET of ˜5 keV/µm but display reduced reliance on classical non-homologous end joining (c-NHEJ) as well as an increased dependence on homologous recombination (HR) and alternative end joining (alt-EJ). These features are well described in cells exposed to high-LET radiation and typically manifest be-tween 100 and150 keV/µm. We hypothesized that this apparent discrepancy reflects biological consequences of proton beam properties that remain uncharacterized.
Materials and Methods: In the present study, we outline experiments aiming to uncover specific interactions between DSB repair mechanisms after proton radiation. We utilized indirect immunofluorescent analysis to investigate the dose-dependent engagement of HR for both entrance and SOBP protons as we previously showed for X-rays. We visualized the formation of γH2AX foci as a marker for DSB formation and RAD51 foci as indicator for ongoing HR, together with the accumula-tion of RPA protein to the single-stranded DNA regions indicating active DNA end resection. More-over, we investigated the activation of G2-checkpoint at selected conditions after exposure of cells to X-rays or protons, and performed analysis that follow the formation of structural chromosomal abnormalities (SCAs).
Results: Consistent with our previous findings with X-rays, HR engagement after exposure to either entrance or SOBP proton beams declined with increasing the radiation dose, from ˜80% at 0.2 Gy to less than 20% at higher doses. RAD51/γH2AX foci ratios, reflecting HR engagement, were modestly higher following SOBP proton irradiation, in line with increased HR utilization. G2-checkpoint activa-tion, previously linked to HR, was also stronger after exposure to SOBP protons, as well as the DNA end resection. Moreover, exposure to SOBP protons resulted in increased formation of SCAs indi-cating the engagement of error-prone DSB repair pathways.
Conclusions: Collectively, our results suggest quasi-high-LET biological characteristics for SOBP pro-tons and raise the question as to the physical proton properties that underpin them. We also speculate that the commonly employed definition of LET may be insufficient to provide rational explanation of the SOBP proton effects observed at a cellular level.

Work supported by grants from BMFTR (02NUK037B, 02NUK043B, 02NUK054B, 02NUK082A, 50WB1836, and 50WB2118), German Research Foundation (DFG-IL51.10, IL51.11, GRK1739, and GRK2762/1), DAAD Project #57515880.

4. Beyond Peak Dose: Lateral Dose Redistribution Drives Growth Inhibition in Glioblastoma Spheroids

Author: Jessica Neubauer[1]
Co-authors: Aikaterini Rousseti [1]; Nikolaos Mallousis [1]; Laura Quintero Velasquez[1]; René Heller [2]; Judith Reindl[1,3]
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Proton minibeam radiotherapy (pMBT) is a promising approach to improve normal-tissue sparing while maintaining antitumor efficacy, but the dose metrics that best predict biological response in spatially fractionated fields remain unclear. Here, we use glioblastoma spheroids to quantify how lateral dose distributions, beyond peak dose or particle number, shape growth inhibition in a controlled 3D tumor model.

LN229 spheroids were irradiated at the Millipede (Millimeter-Sized Particle Exposure for Dedicated Biological Experiments) beamline of the Ion Beam Center, Helmholtz Center Dresden-Rossendorf with 10 MeV protons using a dedicated self-designed irradiation setup for 3D cell culture samples and online microscopy for proper positioning. We compared a homogeneous reference field with two spatially fractionated modalities producing distinct lateral profiles: a collimated (broader/flattened) pattern (σ=80 µm) and a magnetically focused pattern (σ=73 µm). Irradiation conditions were planned using dose values at three isodose reference radii (0.50r, 0.75r, and 1.00r) to enable controlled comparisons between modalities at matched isodose reference radii and similar proton numbers. Post-irradiation growth was monitored by brightfield imaging, enabling quantitative extraction of time-resolved growth curves and growth-delay phenotypes.

Homogeneous irradiation reproduced the expected dose–response, ranging from growth delay at intermediate doses to strong growth suppression at higher doses. In contrast, spatial fractionation revealed that matching isodose reference radii doses do not necessarily yield matching biological outcomes. Across comparable particle numbers and isodose reference radii doses, collimated patterns consistently induced stronger growth delay than focused patterns. This indicates that the spatial redistribution of dose within the spheroid, rather than peak dose alone, can dominate the net growth response.

Our analyses point to the proliferative outer rim as a key driver: growth inhibition tracked more closely with the effective dose delivered to peripheral regions (approximately 0.75–1.00r) than with peak dose or global mean dose. The data support a volume- and structure-dependent mechanism in which the fraction of proliferating cells receiving sublethal-to-moderate doses and steep lateral dose gradients modulate overall spheroid control.

Together, these results extend our previous preliminary observations obtained with a substantially wider focused beam and demonstrate that beam shaping at fixed proton numbers can meaningfully alter tumor-model response. This 3D platform enables systematic optimization of pMBT parameters and provides mechanistic guidance for selecting biologically relevant dosimetric predictors for spatially fractionated proton therapies.

50. Investigating boron neutron capture therapy for the treatment of glioblastoma

Author: Bethany Mackinnon[1]
Co-author: Jason Parsons [1]
[1] *University of Birmingham*

Background: Glioblastoma (GBM) is the most commonly diagnosed malignant brain tumour in adults. Despite an average survival of ˜15 months, few advancements have been made to the standard of care for these patients for decades. This is largely due to the significant inter- and intra-tumoural heterogeneity and infiltrative nature of GBM in addition to the challenges associated with crossing the blood brain barrier. Treatment usually involves maximal safe surgical resection followed by concurrent X-ray radiotherapy and temozolomide (TMZ) chemotherapy. However, TMZ has limited efficacy where tumours lack MGMT promoter hypermethylation which represents ˜50% of the GBM patient population. Additionally, no standard of care has been established for recurrent disease which occurs in over 90% of cases. This, paired with the incredibly poor prognosis, highlights the significant unmet need for the improvement in available treatments for these patients.

Boron neutron capture therapy (BNCT), whilst not a new concept, has recently gained traction for its ability to precisely target tumour cells, sparing healthy tissue in the process. Due to advances in small accelerator-based neutron source designs, and the continued development of improved boron delivery agents, this densely ionising (high-LET) form of radiotherapy offers a unique, non-invasive, and highly effective treatment for GBM. BNCT is a bimodal therapy that utilises tumour-targeted boron compounds in combination with thermal energy neutrons. Once the boron has accumulated within the tumour cells, the tumour area is irradiated with neutrons, triggering a neutron capture reaction. This fission reaction results in the release of alpha particles (4He) and lithium nuclei (7Li), both of which deposit their energy over ˜5-9 μm, less than the diameter of a cell. These high LET charged particles cause irreparable DNA damage, resulting in tumour cell death. As the epithermal neutrons alone do not damage the tissue, the surrounding tissue is spared. Clinically, boronophenylalanine (BPA) is used as the boron source, which is believed to enter cells via LAT1 – an amino acid transporter frequently overexpressed by many cancer types. Despite this, additional preclinical research for BNCT in GBM models is needed.

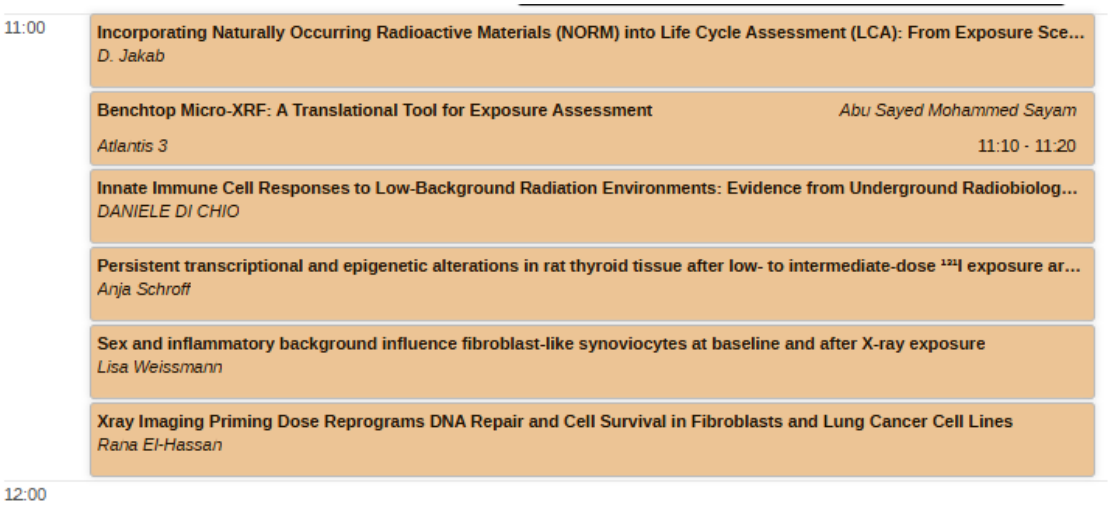
Materials and Methods: MTT, colony formation, and spheroid growth assays were employed to assess the toxicity and radiosensitising capacity of several boron compounds with X-rays on a range of GBM cells. The University of Birmingham's high flux accelerator-driven neutron facility was used to assess the efficacy of BNCT in this disease model. ICP-MS was used to evaluate the uptake of the boron compounds, and LAT1 expression was modulated through siRNA and plasmid transfection.

Results: We found the boron compounds to be non-toxic and ineffective following X-rays, as expected. However, following neutrons in BNCT experiments, these compounds elicited significant GBM cell killing and spheroid growth inhibition which was significantly greater than X-rays (RBE>5). Boron uptake was confirmed using ICP-MS. LAT1 modulation experiments are currently underway.

Conclusions: This research has highlighted BNCT as a more effective treatment for GBM than X-rays and established a strong foundation for future work aiming to understand the DNA damage and repair kinetics activated post-BNCT.

Session 6B: Mechanistic Low-Dose Responses Exposure Assessment

Chairs: Siamak Hagdoost and Inigo Martinez **Room:** Atlantis 3



65. Incorporating Naturally Occurring Radioactive Materials (NORM) into Life Cycle Assessment (LCA): From Exposure Scenarios to Integrated Environmental Impact Assessment

Authors: B. Michalik[1]; J. Mrdakovic Popic[2]
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Assessing environmental impacts in different and complex industrial systems increasingly requires approaches which may account for the combined effects of multiple stressors. In the case of Naturally Occurring Radioactive Materials (NORM), radiological hazards commonly co-exist with other chemical (and physical) contaminants, yet these interactions remain insufficiently addressed within assessment frameworks regarding overall adverse impact related to industrial activities. While Life Cycle Assessment (LCA) is widely used to evaluate environmental impacts of a product or process throughout its entire life cycle, it still does not fully describe the cumulative, potentially interacting effects of radionuclides alongside other stressors, limiting its relevance for comprehensive environmental protection.

The EU-funded PIANOFORTE LCA4NORM project advances a framework designed to overcome this limitation by explicitly integrating radiation impacts of NORM into multi-stressor environmental assessments at all lifecycle stages. Building on updated datasets and reviewed exposure scenarios, this work shifts the focus from simply identifying exposure situations to characterizing how radiological and non-radiological stressors jointly influence human health and ecosystems. This perspective reflects the growing need to connect radiation protection with broader environmental and public health objectives and aligns with evolving European policy priorities, including the Circular Economy Plan.

A central feature of the approach is the development of Unit Exposure Scenarios (UES), which decompose complex industrial activities into modular units that enable consistent representation of combined exposures within Life Cycle Inventory (LCI) databases. This structure supports the analysis of synergistic and antagonistic effects between NORM, heavy metals, and other co-contaminants, facilitating a more realistic characterization of environmental stressors.

Furthermore, the project contributes to bridging disciplinary boundaries by embedding radiological considerations within existing impact categories related to human health, ecosystem quality, and ecosystem services. Generally, by adopting a One Health perspective and aligning with the objectives of the United Nations Sustainable Development Goals, LCA4NORM promotes a more integrated understanding of environmental risks. In doing so, it supports the development of assessment tools capable of informing policies that simultaneously address radiation protection and overall environmental sustainability, while still acknowledging the role of resource recovery strategies within a broader systems context.

The project overview, including introduction conceptual framework and methodology as well as first results from making inventory data basis and evaluation of existing examples of LCA, will be presented in a more comprehensive way.

84. Benchtop Micro-XRF: A Translational Tool for Exposure Assessment

Author: Abu Sayed Mohammed Sayam[1]
Co-authors: Sarah Hardin [1]; Jennifer Dziedzic [1]; Tomás Guilarte [1]; Aaron Specht [2]
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Background: Laboratory-based micro-X-ray fluorescence (micro-XRF) is an analytical tool to assess elemental exposure and profile in micrometer-scale resolution with minimum sample preparation. It can provide an assessment of metals and other emerging contaminants that are increasingly linked to chronic low-dose health effects. However, effective quantitative and qualitative interpretation is needed to realize its potential.

Materials and methods: Bone phantoms were prepared using plaster-of-Paris as constituent compounds and were doped with various concentrations of copper (Cu), iron (Fe), lead(Pb), and zinc (Zn). All the phantoms were measured for 50 kV and 1 mA current across titanium and molybdenum filter setups with the goal to compare between standard-based intensity calibration and reference-free fundamental parameter method (FPM). For the qualitative study, we took rat brain slices prepared in i) paraformaldehyde (PFA)-perfused 40 µm sections (mounted, no coverslip, not dehydrated), and ii) phosphate-buffered saline (PBS)-perfused 20 µm sections (formalin-fixed, paraffin-embedded). These rats underwent four Pb-treatments (control, low, medium, high) before being euthanized. The whole sections were scanned at 50 kV and 1 mA current without any beam filtration with the goal to see Pb effects on Zn profile in the hippocampal brain and how it varies across sample preparation techniques. Mean intensity(cps), coefficient of variation (CV), and high-end tail contrast (99th percentile/mean) were computed. Pb/Zn ratio profiles were used to reduce sensitivity to absolute intensity scaling and preparation-dependent effects.

Results: Bone phantom study showed that although the reference-free approach followed a similar trend in Cu, Pb, and Zn, it performed worse (r2 = 0.9) compared to the standard-based approach (r2 = 0.99) in Fe. The performance of a specific element also depends on the beam filtration as well. For rat brain slices, we found that the mean Pb intensity in hippocampal ROIs was higher in PBS-prepared sections (1265 ± 25 cps) than in PFA-prepared sections (930 ± 36 cps), with a similar trend observed for Zn. In contrast, the mean Pb/Zn ratio was lower. PBS-prepared sections exhibited reduced heterogeneity, with lower CV values for Pb, Zn, and Pb/Zn profiles. High-end tail contrast for Pb was slightly higher in PBS-prepared sections, while Zn and Pb/Zn tail contrast were higher in PFA-prepared sections.

Conclusion: Benchtop micro-XRF enables multi-elemental profiling and quantitative analysis in biological tissues and other types of matrices. This allows us to investigate exposure pathways as well as potential physiological outcomes in an accessible manner. Additionally, reference-free approaches can give us relative quantitative merits in unknown matrices when appropriate phantoms are unavailable. In the future, we aim to develop application-specific, optimized, and portable XRF setups leveraging the robust capability of the benchtop micro-XRF system.

89. Innate Immune Cell Responses to Low-Background Radiation Environments: Evidence from Underground Radiobiology Experiments at LNGS

Author: Daniele Di Chio[1]
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[1] *Università degli studi Roma3, dipartimento di Biologia;* [2] *Centro Nazionale Intelligenza Artificiale e Tecnologie Innovative per la Salute, Istituto Superiore di Sanità e INFN, Sezione di Roma1, Italia*

The biological effects of low-dose radiation remain a subject of ongoing debate, with increasing interest in how environmental background radiation influences molecular and cellular responses in biological systems. The completed INFN-DISCOVER22 project, together with the ongoing ECLIPSE project, aims to investigate the impact of reduced background radiation environments on the innate immune response.

The study was conducted at the INFN Gran Sasso National Laboratories, which provides a unique experimental setting for investigating cellular responses under conditions of strongly reduced environmental radiation exposure. Its geological composition, together with the advanced ventilation system, significantly shields cosmic radiation and neutron flux, enabling controlled low-background radiation experimental conditions.

In this framework, in vitro cultures of human HL60 cells were maintained in parallel at an external reference laboratory and in the underground LNGS facility, with biweekly passaging over a period of up to 30 days. At defined adaptation time points (7, 15, and 30 days), the ability of HL60 cells to differentiate into innate immune-like cells (i.e. monocytes and neutrophils) was evaluated by CD's expression. The functional properties of the resulting differentiated cells were assessed using both chemical stimulation and bacterial challenge in order to better reproduce in vivo–like conditions.

Exposure to Escherichia coli allowed the evaluation of cellular responses to a pathogenic stimulus, both in terms of activation of immune-related pathways and the capacity of differentiated cells to inhibit bacterial growth and suppress infection-like processes.

Cellular responses were analysed by immunofluorescence microscopy, reactive oxygen species (ROS) production assays based on dichlorodihydrofluorescein diacetate (DCFH-DA), and colony-forming unit (CFU) counting in experiments with E. coli ATCC 25922 strain.

Cellular responses were analyzed by immunofluorescence microscopy, dichlorohydrofluorescein diacetate (DCFH-DA)-based reactive oxygen species (ROS) production assays, and colony-forming unit (CFU) counting after an in vitro neutrophil-bacteria challenge with E. coli strain ATCC 25922.

A more pronounced functional response was observed at 15 days in immune cells derived from HL60 cultured in the underground environment. This enhanced response appeared to decrease at 30 days, suggesting the occurrence of a time-dependent cellular adaptation process to reduced background radiation conditions.

These findings support the hypothesis that environmental background radiation contributes to the modulation of key molecular and cellular immune functions. Ongoing and future studies will further investigate and characterize the kinetics of this adaptation and its underlying mechanisms, with particular emphasis on temporal dynamics and pathway-level regulations.

105. Persistent transcriptional and epigenetic alterations in rat thyroid tissue after low- to intermediate-dose ^{131}I exposure are age-dependent

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Background: 131I is a byproduct of nuclear fission that is released into the environment during nuclear accidents. Following the Chernobyl accident, there was a significant increase in thyroid cancer among children in regions affected by fallout, highlighting age at exposure as an important risk factor. However, the molecular mechanisms underlying radiation-induced thyroid carcinogenesis following low-dose 131I exposure remain unclear. Epigenetic dysregulation, such as DNA methylation, has been proposed as a response to ionising radiation, in addition to DNA damage and its repair. Because of its dynamic influence on gene expression and its heritability, DNA methylation is considered an important candidate for mediating long-term tissue responses and a potential factor contributing to carcinogenesis.

Materials and Methods: To determine how age at exposure influences DNA methylation and gene expression after low- to intermediate-dose 131I exposure, male Sprague–Dawley rats were internally exposed to 0, 0.5, 5, 50, or 500 kBq 131I at 5 weeks (young) or 17 weeks (adult) of age and euthanised one year after exposure. DNA and RNA were extracted from thyroid tissue and analysed using reduced representation bisulfite sequencing (RRBS) and RNA sequencing, respectively. Analyses of DNA methylation and differential gene expression were performed, followed by Gene Ontology term enrichment.

Results: A clear age-dependent transcriptional response was observed, with young rats exhibiting a significantly higher number of differentially expressed genes (DEGs) compared to adult animals. Eight genes (*Bmp2, Btbd3, Clstn2, Hgf, Itk, Klhdc7a, Lhx3, Pla2g4a*) were consistently upregulated in young animals at all dose levels, suggesting a sustained response independent of dose. No comparable pattern was observed in adult animals, and only *Herpud1* and *Hspa5* were downregulated at three of the four doses. Across all doses and age groups, several differentially methylated cytosines were identified. In contrast, differentially methylated regions were rare, and only a limited number were located in gene promoter regions. Enrichment analyses further supported the age-related differences, with young rats showing signatures of mitochondrial and metabolic stress at higher doses and activation of signaling pathways (e.g., MAPK, RAS) at lower doses, whereas adults exhibited consistent enrichment of signaling pathways for endoplasmic reticulum stress and the unfolded protein response.

Conclusion: These findings indicate that age at exposure is a key factor influencing the thyroidal transcriptional response following low- to intermediate-dose ^{131}I exposure. Further studies are needed to better understand the implications of the stronger response observed in young animals and its potential impact on thyroid health and carcinogenesis.

115. Sex and inflammatory background influence fibroblast-like synoviocytes at baseline and after X-ray exposure

Authors: Ann-Sophie Flohr[1]; Teresa Irianto[1]; Margaux Piltz[1]; Michael Rückert[1]; Laura Ruspeckhofer[1]; Lena Winterling[1]; Tom Unterleiter[1]; Rainer Fietkau[2]; Stefanie Corradini[2]; Udo S. Gaipl[1]; Lisa Weissmann[1]

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While sex and inflammatory background are well-established determinants of immune responses, their influence on responses to radiation exposure remains poorly understood. Both factors are also highly relevant in low-dose radiotherapy (LD-RT), as degenerative musculoskeletal diseases often show marked sex-specific differences in incidence and are associated with varying degrees of inflammation. Fibroblast-like synoviocytes (FLS) are the principal structural cell type of the synovial membrane lining the joint capsule. In healthy joints, they contribute to the maintenance of joint homeostasis, under pathological conditions they can acquire an invasive phenotype, promote synovial thickening, and secrete a broad range of pro-inflammatory mediators, further driving inflammation and cartilage degeneration. We thus aimed to investigate FLS of both sexes in an inflammatory (human TNFα transgenic, hTNFα tg) and a healthy (C57Bl/6 littermates, wt) murine model

FLS from male and female mice with either a healthy or inflammatory background were isolated from the hind paws, expanded to P4, and phenotyped by multicolor flow cytometry. At P5, cells were treated ex vivo with low doses of X-rays (0, 0.1, 0.5, 1.0, and 2.0 Gy) and analyzed 48h and 96h after irradiation with regard to cell growth, cell death, cell cycle distribution, PD-L1 expression, and cytokine release using flow cytometry and ELISA.

At baseline, male-derived hTNFα tg FLS showed significantly higher secretion of IL-6 and CXCL1, whereas in female-derived cells, CXCL1 levels were significantly higher in wt than in hTNFα tg derived cells. No sex-specific differences in basal growth were observed in wt FLS, whereas female derived hTNFα tg cells showed significantly higher cell numbers than male derived cells. After irradiation, healthy male FLS exhibited more pronounced growth effects than female FLS (0.5-2 Gy vs. 0.1 and 2 Gy, 96h). In contrast, inflammatory female-derived cells initially showed stronger growth-reducing effects than male-derived cells (48h: 0.1-2 Gy vs. 2 Gy), whereas at 96h both sexes showed reduced growth at 1 and 2 Gy. In wt cells, female-derived FLS showed higher apoptotic rates than male-derived FLS at 96h (0.5-2 Gy vs. 2 Gy), whereas the opposite pattern was observed in inflammatory cells, with increased apoptosis in males (0.5-2 Gy) and no effect in females. PD-L1 surface expression at 96h was significantly increased in inflammatory female FLS at 2 Gy, whereas inflammatory male FLS showed a significant decrease at 0.1 and 0.5 Gy. No significant differences in cell cycle distribution were detected in either group.

Sex and inflammatory background influence the basal phenotype and radiation response of murine FLS. Opposing sex-specific effects in hTNFα tg and wt FLS suggest that both factors may modulate (LD)RT responses. Further studies are needed to place these observations into a broader biological and clinical context.

Acknowledgement: Supported by the BMFTR (TOGETHER, 02NUK073).

10. Xray Imaging Priming Dose Reprograms DNA Repair and Cell Survival in Fibroblasts and Lung Cancer Cell Lines

Author: Rana El-Hassan[1]
Co-authors: Aya Zebiane; Leen Katmawi Sabbagh; Charbel Feghaly; Hanine Bou Hadir; Rafka Challita; Wassim Abou-Kheir; Larry Bodgi
[1] *American University of Beirut*

Background: Conventional radiotherapy (RT) typically relies on limited pre-treatment imaging, whereas adaptive radiotherapy (ART) integrates daily computed tomography (CT) with immediate treatment plan modification prior to irradiation. Although an individual diagnostic CT delivers a low biological dose, repeated imaging immediately preceding therapeutic fractions may influence DNA damage signaling pathways. The impact of such closely timed sequential exposures remains incompletely characterized. In this study, we investigate how the time interval between a CT-like low dose and a subsequent therapeutic high dose affects DNA damage signaling dynamics, repair kinetics, and survival outcomes under ART-relevant conditions.
Materials & Methods: Two NSCLC cell lines (H1792 and A549) and one normal human fibroblast line (GM03652) were subjected to:

1. Single low-dose CT-like irradiation (0.1 or 0.2 Gy)
2. Single high-dose RT (2 Gy);
3. Sequential exposure consisting of CT $\rightarrow$ Δt (1, 5, 10, or 20 min) $\rightarrow$ RT; and
4. Repeated exposure comprising CT $\rightarrow$ 10 min $\rightarrow$ CT $\rightarrow$ 1 min $\rightarrow$ RT.

DNA damage signaling and double-strand break formation were assessed by immunofluorescent quantification of pATM and γH2AX foci, together with micronucleus formation. Clonogenic assays and sphere formation assays were performed in cancer cell lines to evaluate reproductive survival and self-renewal capacity.
Results: In H1792 cells, delivery of 0.1 Gy 10 min before 2 Gy significantly reduced pATM activation and decreased surviving fraction (SF) and sphere formation unit (SFU), consistent with attenuated DDR signaling and diminished clonogenic potential. At a 1 min interval, residual γH2AX foci remained elevated despite reduced pATM, indicating persistence of DNA damage without effective repair.
Similarly, in A549 cells, short intervals (1-10 min) between a CT-like dose of 0.1 Gy and RT increased residual γH2AX, suppressed pATM activation, and reduced SF, supporting a radiosensitizing phenotype rather than adaptive protection. Increasing the CT-like dose to 0.2 Gy led to detectable modulation of DDR markers but did not translate into significant alterations in clonogenic survival or sphere formation in cancer cells.
Normal fibroblasts exhibited a distinct interval-dependent response. While short intervals (1-10 min) increased residual γH2AX across cell types, extending the interval to 20 min following 0.2 Gy pre-exposure resulted in radioprotection selectively in fibroblasts.
In the repeated sequence (CT$\rightarrow$10 min$\rightarrow$CT$\rightarrow$1 min$\rightarrow$RT), dual CT exposures produced a marked accumulation of residual γH2AX foci compared with either single CT$\rightarrow\Delta$t$\rightarrow$RT or RT alone. pATM responses varied by cell line but were generally reduced or unchanged, indicating enhanced residual damage without compensatory ATM activation.
Conclusion: CT-like imaging doses alter ATM-dependent DNA damage signaling in a manner that depends on dose, timing, and cell type. Short inter-exposure intervals preferentially enhance radiosensitivity in lung cancer cells, whereas longer intervals with 0.2 Gy confer selective radioprotection in normal fibroblasts. Repeated CT exposure further increases residual DNA damage without increasing ATM activation, underscoring differential DDR thresholds between malignant and normal tissues within ART-like workflows.

Keynotesession 2

Chair: Eirik Malinen
Room: Atlantis 1

The Speaker

Andrea Mairani

Andrea Mairani is the Scientific Director of the Heidelberg Ion Beam Therapy Center (HIT). His research spans biophysical modeling, micro- and nanodosimetry, and the development of advanced treatment-planning approaches for proton and heavy-ion therapy. Prof. Mairani has played a central role in integrating emerging ion species—such as helium and oxygen—into clinical and preclinical research frameworks. His work is characterized by close collaboration between physics, radiobiology, and clinical practice, with a consistent focus on improving the predictive accuracy and therapeutic effectiveness of particle-based radiotherapy.

Biophysics-Informed Innovation: Pushing the Boundaries of Particle-Based Cancer Treatment

Author: Andrea Mairani[1]

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Particle therapy has entered an era in which physical precision alone is no longer sufficient. The next major advances will come from integrating accurate particle transport, mechanistic radiobiology, tumour microenvironment information and adaptive clinical workflows into treatment design. This plenary lecture will discuss how biophysics-informed innovation can push the boundaries of particle-based cancer treatment, moving from geometrically conformal dose delivery toward biologically guided therapy.

Charged particles offer distinct physical and biological advantages through the Bragg peak, reduced lateral scattering for heavier ions, and increased linear energy transfer (LET). Yet, in current clinical practice, many of these advantages remain only partially exploited. Biological optimisation is still largely based on simplified relative biological effectiveness models, while clinically relevant modifiers such as hypoxia, genetic background, stemness, immune status, tissue-specific radiosensitivity and interpatient heterogeneity are insufficiently represented. To fully exploit protons, helium, carbon and emerging ion species such as oxygen, treatment planning must evolve from dose-based optimisation to multi-dimensional optimisation of dose, LET, radiation quality, time structure and biological effect.

I will present a translational vision in which fast Monte Carlo engines, advanced biophysical models and experimental biological readouts become enabling technologies for next-generation particle therapy. This includes high-LET tumour targeting, LET boosting, heavy-ion arc therapy, multi-ion concepts, helium ion therapy, ion FLASH, mini-beam approaches and adaptive MRI-guided ion therapy. These developments aim to intensify treatment in resistant tumour subvolumes while maintaining or improving normal-tissue sparing.

A further frontier is the explicit modelling of dynamic and distributed organs-at-risk, including circulating lymphocytes and the immune system. By combining spatiotemporal beam delivery, blood-flow modelling and cell-population-specific radiosensitivity data, particle therapy may be optimised not only to control tumours but also to preserve immune competence and reduce radiation-induced lymphopenia.

Together, these examples illustrate a broader strategy: particle therapy should become predictive, adaptive and biologically individualised. The future of the field will depend on rigorous physical modelling, mechanistic radiobiology, clinically realistic computation, and close integration between research platforms and treatment rooms. Biophysics-informed innovation can transform ion beams from precise physical tools into personalised biological interventions for patients with cancer.

Bacq & Alexander Award Session

Room: Atlantis 1

The Laureat

Klaus-Rüdiger Trott

Klaus-Rüdiger Trott (born 16 July 1940) is a German radiation biologist whose scientific career has made significant contributions to radiation biology, radiotherapy, and radiation protection.
He studied medicine at Ludwig Maximilian University of Munich and began his career in radiation research in the late 1960s, working with radiation biologist Otto Hug. He subsequently joined the Gesellschaft für Strahlenforschung (Society for Radiation Research, today Helmholtz Munich), where his early research focused on the biological effects of ionising radiation on cells and tissues. During the 1970s, his work increasingly addressed questions at the interface of radiation biology and cancer therapy.
From 1975 to 1981, Trott served as a member of the German Commission on Radiological Protection (Strahlenschutzkommission). Following the Chernobyl nuclear accident in 1986, he became further involved in radiation protection and the assessment of radiation risks.
In the late 1980s, Trott was appointed Professor of Radiation Biology at St Bartholomew's Hospital in London, where he established and led his own research laboratory. He also had a lasting impact through the education and mentorship of scientists who later took leading roles in radiation research and radiation protection across Europe.
After his retirement, Trott remained dedicated to education, continuing his teaching activities at the Technical University of Munich from 2016. Since 2001, he has been a member of the German National Academy of Sciences Leopoldina in recognition of his contributions to radiation biology.
Through decades of research, teaching, mentorship, and service, Klaus-Rüdiger Trott has helped shape radiation biology in Europe and has had a lasting influence on generations of scientists in the field.

My personal history of radiation biology research - Radiation protection of the unborn child in utero

Author: Klaus-Rüdiger Trott

The embryo and the fetus in utero are generally perceived as being particularly radiosensitive. Major experimental studies, most of them carried out by European radiobiologists, demonstrated disturbances of normal development at low radiation doses, doses potentially encountered even in diagnostic radiology. The principal risk that radiologists explain to concerned pregnant women is that radiation could impair fetal brain development, potentially causing severe mental retardation if the dose exceeds a scientifically established threshold.

The scientific basis for this claim is an epidemiological study of roughly 1,600 Japanese women who were pregnant when exposed to radiation from the atomic bombs at Hiroshima and Nagasaki. These women gave birth to apparently healthy children, but 25 of them were later diagnosed with "severe mental retardation." From this data, a safe threshold dose of 0.3 Gy was derived. Crucially, each woman's exposure was estimated primarily from her distance from the hypocenter, not from any direct dosimetric measurement.

This is where the argument becomes problematic. Most people killed in Hiroshima and Nagasaki did not die from radiation exposure but from physical trauma, extensive burns, shock, and infection. Those who survived within 1–2 km of the hypocenter were, in effect, the lucky ones among a catastrophic scene of destruction. Trauma, burns, shock, and infection are all independently known to impair normal fetal development, and all of them correlate just as strongly with distance from the hypocenter as radiation dose does, and thus with the incidence of mental retardation. There is, in short, no scientific evidence that radiation was the sole or even the principal cause of mental retardation in these children. It is therefore unsound to base radiation protection recommendations for pregnant women on this dataset.

By contrast, the Oxford Survey of Childhood Cancer (OSCC), a UK case-control study, provides a scientifically solid foundation on which such regulations can be built. I recommend that clinical risk–benefit evaluations for medical radiation exposure of pregnant women, whether diagnostic or therapeutic, focus on the risk of radiation-induced childhood cancer. Since it is generally agreed that there is no dose threshold for radiation-induced carcinogenesis, adopting this basis would also put an end to the long-running, ultimately unproductive debate over threshold doses for fetal harm.

Wednesday, 09.09.2026

Session 7A: Targeted Radionuclide Therapy BNCT

Chairs: Asta Juzeniene and Sandra Kristiansen
Room: Atlantis 1

09:00

Invited Talk: Advancing Targeted Radionuclide Therapy: Radiobiological Insights from Bench to Bedside *Julie Nonnekens*
Atlantis 1 09:00 - 09:30

In vitro evaluation of ATR inhibition with AZD6738 in combination with the PSMA-targeted radiopharmaceutical [212Pb]...
Victoria Dunne

Impact of PSMA Density and Intracellular Trafficking on [212Pb]Pb-AB001 Binding and Retention in Prostate Cancer Mo...
Ms Rugile Liukaityte

Hyperfractionated ^{177}Lu-octreotate improves therapeutic efficacy in neuroendocrine tumor models with low and high so...
Nishte Rassol

10:00

Gold Nanorods for Nuclear Medicine Theranostics: Enhanced Radiation-Induced DNA Double-Strand Breaks in Glioblas...
Ludovica Binelli

In silico radiobiological modeling of BNCT using GEANT4-DNA: from track structure to cell survival
ALESSANDRO SOMENZI

Boron Neutron Capture Therapy (BNCT): Disentangling the Biological Effects of Mixed Radiation Fields *emilia formicola*
Atlantis 1 10:20 - 10:30

Invited Talk: Advancing Targeted Radionuclide Therapy: Radiobiological Insights from Bench to Bedside

Author: Julie Nonnekens[1]
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Targeted radionuclide therapy (TRT) is an increasingly important modality for the treatment of disseminated cancer, yet its optimization is hampered by an incomplete understanding of its distinct radiobiology. In contrast to external beam radiotherapy, TRT delivers low dose rates and highly heterogeneous radiation at the cellular and subcellular level, requiring dedicated radiobiological frameworks.

This lecture highlights mechanistic insights into the DNA damage response induced by clinically relevant radionuclides, with emphasis on how radiation quality, dose rate, and subcellular localization shape DNA lesion complexity and repair. Particular attention is given to the role of specific DNA repair pathways as determinants of therapeutic response and potential targets for radiosensitization. In addition, the limitations of conventional dosimetry for TRT are addressed, underscoring the need to integrate microdosimetric and biological parameters.

These experimental findings are placed in a translational context, illustrating how radiobiological understanding can inform treatment optimization, combination strategies, and patient stratification. Embedding radiobiology into the development and clinical implementation of TRT is essential to advance precision radionuclide therapy and improve patient outcomes.

44. In vitro evaluation of ATR inhibition with AZD6738 in combination with the PSMA-targeted radiopharmaceutical (212Pb)Pb-AB001 in prostate cancer models

Authors: Andrius Kleinauskas[1]; Francisco D. C. Guerra Liberal[2]; Joe M. O'Sullivan[3]; Kevin M. Prise[2]; Li-Wei Ma[1]; Marie-Catherine Drigeard Desgarnier[4]; Marina Vietri[4]; Petras Juzenas[1]; Rugile Liukaityte[1]; Victoria Dunne[2]; Vilde Yuli. Stenberg[1]; Asta Juzeniene[1]

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Background: Prostate-specific membrane antigen (PSMA)-targeted radiopharmaceutical therapy (TRT) has demonstrated clinical benefit in metastatic castration-resistant prostate cancer (mCRPC), including treatment with β-emitting [177Lu]Lu-PSMA-617 (Pluvicto®).

Lead-212 generates the α-emitting daughters 212Bi and 212Po and is being investigated as an alternative to β-emitters due to high LET (50-230 keV/μm) and short tissue range (< 90 μm). Despite this, intrinsic and acquired resistance to TRT remains a challenge in a subset of patients. ATR (ataxia-telangiectasia-mutated (ATM) and Rad3-related protein kinase) is a key regulator of the DNA damage response. Inhibition of ATR has been proposed to enhance the cytotoxic effects of radiation. Therefore, we investigated whether the ATR inhibitor AZD6738 (ceralasertib) enhances the in vitro cytotoxic effects of the PSMA-targeted radioligand [212Pb]Pb-AB001.

Methods: PSMA-expressing prostate cancer models (C4-2, LNCaP, and PC-3 PIP) were evaluated in monolayer cultures and 3D multicellular tumour spheroids. Endpoints included clonogenic survival, ATP-based viability, γH2AX immunofluorescence, apoptosis, necrosis, and cell cycle distribution by flow cytometry. Sensitizer enhancement ratios and combination indices were calculated.

Results AZD6738 enhanced the cytotoxic effect of [212Pb]Pb-AB001 in all studied models. Sensitisation enhancement ratios were 1.6 ± 0.09 in C4-2 cells, 2.2±0.06 in PC-3 PIP cells, and 1.5±0.1 in LNCaP cells.

In 3D spheroids, combination treatment with [212Pb]Pb-AB001 (1 kBq/mL for LNCaP; 5 kBq/mL for C4-2) and AZD6738 significantly prolonged spheroid growth compared to [212Pb]Pb-AB001 monotherapy. Doubling time increased from 6.1±0.3 to 6.5±0.4 days for LNCaP and from 4.8±0.2 to 5.6±0.1 days for C4-2 after [212Pb]Pb-AB001 monotherapy, and to 13.3±0.3 and 12.4±1.1 days, respectively, with combination treatment (day 21 post treatment). AZD6738 monotherapy had no significant effect. Combination indices of 0.3±0.1 for LNCaP and 0.3±0.4 for C4-2 spheroids indicate synergistic interaction in vitro.

Flow cytometry revealed activity-dependent G2 phase accumulation following [212Pb]Pb-AB001 treatment in both models. In PC-3 PIP cells at 24 h, the G2 fraction increased from ˜27% to 84–87% at 3–6 kBq/mL; combination treatment reduced G2 to 26–27%, with a corresponding increase in G1. In C4-2 cells, 12.5 kBq/mL increased G2 from 15% to 21%, which was further reduced to 7% with AZD6738 co-treatment.

Conclusions: AZD6738 enhances the in vitro cytotoxic effects of [212Pb]Pb-AB001 and demonstrates synergistic interaction in PSMA-expressing prostate cancer models. These findings are limited to in vitro systems, and in vivo studies are required to determine translational relevance.

94. Impact of PSMA Density and Intracellular Trafficking on (212Pb)Pb-AB001 Binding and Retention in Prostate Cancer Models

Author: Rugile Liukaityte[1]
Co-authors: Asta Juzeniene [1]; Rina Wangen-Riise [1]
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PSMA-targeted radioligand therapy using 212Pb, which emits a single alpha particle via its decay chain, represents a promising strategy for patients with metastatic castration-resistant prostate cancer. However, the reported intratumoral and interpatient heterogeneity in PSMA expression can influence the efficacy of targeted alpha therapies, which underscores the need to evaluate novel ligands across multiple model systems reflecting a broad range of receptor densities, endogenous internalization and receptor trafficking patterns. Such differences may affect binding, retention, and, hence, therapeutic efficacy. In this study, saturation levels, binding kinetics, and retention of the PSMA-targeting ligand [212Pb]Pb-AB001 were assessed in three prostate cancer cell lines: PC-3 PIP (transduced, high PSMA), C4-2 (moderate, endogenous PSMA), and 22Rv1 (low, endogenous PSMA).

PSMA expression was quantified by flow cytometry using Quantum Simply Cellular anti-Human IgG quantification beads. Receptor saturation was assessed 60 min post addition of 1.6–200 kBq/mL [212Pb]Pb-AB001. Association kinetics were determined by adding 50 kBq/mL [212Pb]Pb-AB001 and measuring binding (μBq/cell) immediately and at 5, 15, 30, 45 and 60 min post-addition. Retention was evaluated by first incubating the cells with 50 kBq/mL [212Pb]Pb-AB001 for 60 min, removing the radioligand and measuring binding immediately, 2 and 4 hours after radioligand removal.

PC-3 PIP cells showed the highest PSMA expression (˜600 000 sites/cell), followed by C4-2 (˜100 000 sites/cell) and 22Rv1 (˜15 000 sites/cell). This trend corresponded to the level of activity bound to the cells and the activities required for saturation, with PC-3 PIP and C4-2 reaching higher Bmax values (105026 μBq/cell and 5459 μBq/cell, respectively) compared to 22Rv1 (153 μBq/cell). The percentage of internalized activity did not differ significantly across the added activities but varied between cell lines: C4-2 exhibited the highest internalization (41±2%), followed by 22Rv1 (32±4%), and PC-3 PIP (12 ±1%). Binding dynamics experiments showed that surface bound activity remained relatively constant across timepoints in all three cell lines, whereas the internalized fraction increased between 1 and 60min from 6% to 12% in PC-3 PIP, 11% to 40% in C4-2, and 18% to 34% in 22Rv1. The retention assays showed that in PC-3 PIP cells, total activity declined by 27% during the first 4hours after radioligand removal, with the reduced fraction observed mainly from the surface while the internalized fraction remained stable, indicating intracellular retention. In contrast, C4-2 and 22Rv1 cells showed a decrease of both internalized and surface bound activity, resulting in a 72–73% reduction of the initial total cell-associated activity, indicating that cell line-specific differences in internalization-recycling balance and intracellular trafficking may be important for ligand retention.

In conclusion, these findings demonstrate that [212Pb]Pb-AB001 binding, internalization, and retention are influenced by PSMA density and cell line-specific ligand trafficking patterns. The data emphasizes the importance of considering both receptor expression and internalization patterns when selecting and optimizing novel targeted radioligand therapies.

120. Hyperfractionated 177Lu-octreotate improves therapeutic efficacy in neuroendocrine tumor models with low and high somatostatin receptor expression

Authors: Emman Shubbar[1]; Eva Forssell-Aronsson[1]; Johan Spetz[1]; Mikael Elvborn[1]; Nishte Rassol[1]

[1] *University of Gothenburg*

Background: Targeted radionuclide therapy (TRT) with 177Lu-octreotate is used for somatostatin receptor (SSTR)-expressing neuroendocrine tumors (NETs), but treatment response varies due to heterogeneity in SSTR expression. Gastroenteropancreatic NETs typically respond well, whereas tumors with lower SSTR expression, such as medullary thyroid carcinoma (MTC), show limited benefit. Hyperfractionated administration may enhance tumor uptake by reducing receptor saturation, allowing receptor recycling and increasing receptor expression. In this study, we compared single and hyperfractionated administration of 177Lu-octreotate to assess effects on tumor uptake, absorbed dose, and therapeutic efficacy in tumor models with high and low SSTR expression.

Materials and Methods: Two human xenograft models were studied: GOT1 (high SSTR expression) and GOT2 (low SSTR expression). GOT1 mice received ¹Lu-octreotate as single or fractionated administrations (30–120 MBq), and biodistribution and dosimetric estimations were performed for 1 × 120 MBq versus 3 × 40 MBq. GOT2 mice received either a single administration of 50 MBq or hyperfractionated treatment (5 × 10 MBq) at 6- or 12-hour intervals. The injected activity was selected to induce receptor saturation after single administration in each tumor model. Tumor growth and survival were monitored.

Results: In GOT1 mice, hyperfractionation increased tumor uptake and absorbed dose, and improved tumor-to-normal-tissue ratios. This resulted in greater tumor reduction, delayed regrowth, and prolonged survival, particularly at lower activities. In GOT2 mice, single administration showed minimal effect, whereas hyperfractionation with 12-hour intervals reduced tumor growth and prolonged survival. No clear benefit was observed with 6-hour intervals.

Conclusions: Hyperfractionated administration of 177Lu-octreotate improves therapeutic efficacy in high- and low-SSTR-expressing human NET xenograft models. The results suggest that, in addition to receptor expression level, treatment response may depend on peptide amount and fractionation interval. Further studies will focus on quantitative dosimetry to define dose–response relationships and clarify how hyperfractionation affects tumor uptake and treatment response in tumors with varying SSTR expression levels.

82. Gold Nanorods for Nuclear Medicine Theranostics: Enhanced Radiation-Induced DNA Double-Strand Breaks in Glioblastoma Cells

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The development of targeted radiotherapy strategies requires a deep understanding of radiation-induced DNA damage mechanisms at the cellular level. Gold nanorods (AuNRs) functionalized with gamma-emitting radiopharmaceuticals, such as technetium-99m (Tc-99m), represent a promising theranostic platform capable of enhancing localized cytotoxic effects through physicochemical mechanisms, including the photoelectric-mediated generation of reactive oxygen species and local dose enhancement at the nanoscale 1.

The aim of this work is to investigate the enhancement of DNA damage in T98G glioblastoma cells treated with AuNRs and exposed to gamma radiation. To simulate Tc-99m decay (gamma rays and Auger electrons), cells were irradiated using an external cesium-137 source. DNA damage was assessed through γH2AX immunofluorescence analysis, quantifying foci formation at different time points post-irradiation.

Preliminary results demonstrate a significant increase in γH2AX foci in cells treated with the combined AuNRs + gamma radiation compared to radiation alone, suggesting that AuNRs effectively amplify DSB induction. The kinetics of foci resolution also indicate a potential impairment of DNA repair mechanisms in the presence of AuNRs. These findings support the hypothesis that AuNR-mediated Auger electron emission enhances localized DNA damage, providing a rationale for the development of nuclear-targeted nanosystems for improved radiotherapeutic efficacy.

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97. In silico radiobiological modeling of BNCT using GEANT4-DNA: from track structure to cell survival

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Background and Aims: Boron Neutron Capture Therapy (BNCT) is a binary form of particle therapy that enables highly selective dose deposition at the cellular level. Its selectivity depends on the boron microdistribution and cellular uptake of boron compounds, leading to strong intercellular dose heterogeneity. Moreover, the radiation field includes multiple particle types with different LET values. In this complex context, Monte Carlo transport codes are essential for accurate dosimetry in both preclinical and clinical settings. Track-structure simulations also provide insights into DNA damage patterns and contributions from individual dose components.
Material and Methods: We used the GEANT4-DNA track-structure code [1–5] to simulate BNCT in vitro radiobiology experiments previously conducted on the PANC-1 pancreatic adenocarcinoma cell line at the TRIGA reactor of the University of Pavia [6]. A realistic PANC-1 nucleus was modeled using DNAFabric [7], based on its hypertriploid genome, measured nuclear size, and chromatin compaction extracted from literature. The "dsbandrepair" example [8] was used to simulate the number and the complexity of double strand breaks (DSBs) produced during the different stages of irradiation: physical, physico-chemical, and chemical stages. Each BNCT field component was modeled using a phase space derived from prior MCNP6 and GEANT4 simulations. For low-energy lithium ions, recently developed GEANT4-DNA cross-section models by ASNR (France) were applied [9]. The alpha particles and lithium ions were uniformly generated inside the cytoplasm, assuming no boron internalization into the nucleus. DNA damage kinetics models [10] were used to simulate the time evolution of radiation-induced foci (RIF), and the Two Lesion Kinetics (TLK) model was used to simulate survival curves. The TLK cell line - dependent parameters used to simulate fibroblasts survival [8] were adjusted in this work to the PANC-1 cancer cell line to match the survival curves we previously obtained in the in vitro BNCT and x-rays experiments [6].
Results: DSB yields per dose were evaluated for each radiation component. A linear correlation was found between absorbed dose and DSB yield in the 0–13 Gy range. Protons (from 14N neutron capture reactions), lithium ions, and alpha particles (from 10B neutron capture reactions) caused higher DSB yields and higher variability than n–p scattering and gamma components. Interestingly the DSB yield obtained using the new lithium ions cross-section models (51 DSB/Gy) were slightly lower than using the default Geant4 models for ions (67 DSB/Gy) and the ratio direct/indirect strand breaks obtained in the former case was 3-fold higher.
Simulated survival curves showed good agreement with experimental data, for BNCT (both with and without 10B incubation) and x-rays irradiations. Results from different cell nucleus and source configurations will be discussed.
Conclusions: Track-structure Monte Carlo codes are a valuable tool to model complex therapies like BNCT, allowing detailed exploration of radiation-induced DNA damage and survival. In this work, thanks also to the implementation of new Li-ions cross-sections models [9], a first Geant4-DNA application to simulated BNCT in vitro experiments is presented. Biological mechanisms involved in the various BNCT dose-components, both separately and in a unique combined source, have been investigated. This work lays the groundwork for future in silico studies of combined treatments, such as carbon-ion radiotherapy integrated with BNCT.

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100. Boron Neutron Capture Therapy (BNCT): Disentangling the Biological Effects of Mixed Radiation Fields

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Background Boron-Neutron Capture Therapy (BNCT) is a tumor-selective radiotherapy (RT) that combines nuclear physics and radiobiology. When localized in 10B-enriched cancer cells, the 10B(n,α)7Li reaction, triggered by epithermal neutrons, yields α-particles and 7Li nuclei 1. These reaction products possess high Linear Energy Transfer (LET), resulting in a Relative Biological Effectiveness (RBE) greater than that of conventional photon-based RT [2]. Although BNCT potential to kill cancer cells is clinically recognized[3], a biologically optimized dosimetry is still lacking. This requires the experimental determination of the RBE of the various components making up the mixed radiation field inherent to BNCT, which comprises neutrons, low-energy protons, gamma rays, Lithium and alpha nuclei.
Building on our previous work, linking the BNCT enhanced effectiveness to the complexity of clustered DNA damage [4], the present study therefore aims to disentangle such specific biological contributions as a part of the ANTHEM (AdvaNced Technologies for Human-centrEd Medicine) project for the implementation of novel accelerator-based (AB)-BNCT.
Materials and Methods: U87 glioblastoma and WM266 melanoma cell lines were irradiated at three facilities: neutron irradiation for BNCT proper was conducted at the LENA facility (University of Pavia) using a TRIGA Mark II reactor; photon irradiation, used as a reference, at IRCCS – Fondazione Pascale (Naples); finally, exposure to monoenergetic protons and 7Li ions was carried out at the CIRCE Laboratory (University of Campania, Caserta), where a dedicated dosimetric campaign was performed, including beam calibration and absolute dose determination based on an accurate characterization of energy loss and dose deposition under the specific experimental conditions [5]. Clonogenic survival, DNA foci kinetics, and cancer cell invasion were measured.
Results: The clonogenic assay showed RBE values for neutrons of 3.1 and 2.3 for WM266 and U87 cells, respectively, while lithium ions yielded RBE values of 2.8 and 3.7. Survival data regarding boron-enhanced neutron irradiation and low energy protons, as well as foci and invasion assays, are currently under analysis. Further experiments with α -particle beams are planned.
Conclusions: These findings will provide a more radiobiologically robust dosimetry for personalized AB-BNCT treatment planning.
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Session 7B: Preclinical Models, Normal Tissue Experimental Platforms

Chairs: Peter Sminia and Mehran Hariri
Room: Atlantis 3

09:00

First implementation of synchrotron Microbeam Radiation Therapy (MRT) for spontaneous canine osteosarcoma
Olga Martin

Radiation-Induced Microenvironmental Modulation of Granule Cell Precursor Migration and Implications for Medullobla...
Tünde Szatmári

Impact of the Cerebellar Microenvironment on Radiation-Induced Tumorigenesis in a Ptch1$^{+/-}$ Medulloblastoma Model: I...
Arianna Casciati

Assessing the impact of novel tissue-engineered bioactive dressings on wound healing of irradiated murine skin
Ms Fanny Brunard

Proton irradiation of murine salivary glands induces sustained proliferative activity and DNA damage signaling indepen...
Dr Inga Juvkam

10:00

Radiobiological studies with laser-driven protons at ELIMAIA-ELIMED: from fibroblasts to 3D spheroid models
Pavel Blaha

Potential Implication of Biodosimetry Methodology for Predicting Radiotherapy Normal Tissue Toxicity: Current Eviden
Oleg Belyakov

Proton Irradiation Preserves Metabolic Homeostasis and Protects Normal Stem Cell Niches Compared with X-ray or Car...
Dr Kave Moloudi

148. First implementation of synchrotron Microbeam Radiation Therapy (MRT) for spontaneous canine osteosarcoma

Authors: Elette Engels[1]; Olga Martin[2]; Michael Lerch[2]; Elisabeth Schultke[3]; Stewart Ryan[4]; Valentin Djonov[5]
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Background: Curative radiation therapy (RT) is limited by damage to normal tissues. Submillimetre spatially fractionated RT (SFRT), such as mini- and micro-beams, use heterogenous radiation fields that distribute dose into high (peak) and low (valley) regions. This dose gradient varies by 5-50 times, allowing more preservation of healthy tissue function than conventional RT. Tumour control is maintained by high peak doses (˜20-800 Gy) guaranteeing tumour death in the beam path. Destruction of immature tumour vasculature and immunogenic tumour cell death triggering both local and systemic anti-tumour immune responses add for further cancer control. Microbeam radiation therapy (MRT) is a preclinical SFRT with the highest degree of normal tissue sparing. In translating MRT to the clinic, the response to large, spontaneous cancers that are similar in humans must be considered. This study is the first to investigate MRT for spontaneous osteosarcoma in companion dogs. We aimed to assess the feasibility, safety and response of these tumors to X-ray microbeams followed by standard-of-care chemotherapy (carboplatin).
Materials and Methods: Osteosarcomas in the distal limb in four canine patients were partially irradiated with MRT at the Imaging and Medical Beam Line (IMBL) of the ANSTO Australian Synchrotron. Dogs' limbs were anchored to a movable stage and exposed to an average of peak and valley doses of130 Gy and 4.1 Gy respectively, in a 20x20-mm radiation field (17 to 40% of gross tumor volume). Dogs were imaged with CT and dynamic contrast-enhanced MRI (DCE-MRI) prior to treatment and post-treatment to investigate changes in bone density, tumor volume, tumor activity and permeability. The affected limbs of two dogs were amputated within 1-2 weeks post-MRT, consistent with normal treatment, but two dogs opted-out of amputation were observed for up to 6 months. Tumor samples from in-field and out-of-field areas were collected for histopathology and local immune response comparison. Serial blood and urine samples were collected for proteomic and biochemical analysis.
Results: DCE-MRI showed that MRT caused partial disruption in osteosarcoma vasculature and increased vascular trans-permeability facilitating the accumulation of carboplatin. Although partially irradiated, MRT/carboplatin treatment induced tumour ablation or tumour shrinkage also out of irradiation field, due to the destruction of tumour vasculature and boosting of anti-tumour immune responses. According to CT, histology and biochemical markers, the receding tumour mass was replaced by osteogenesis resulting in rapid bone recovery. The animals started to load the legs on second day after treatment. Multiplexed proteomics analysed over 7,000 plasma proteins and revealed the greatest number of differentially expressed proteins post-treatment compared to pre-treatment. Gene Ontology enrichment analysis of differentially expressed proteins revealed time points associated with bone remodelling and the activation of the adaptive immune response. In addition, no skin toxicity and hair loss for up to 6 months post-treatment has been observed.
Conclusion: These promising outcomes indicate MRT can safely and efficiently treat large, spontaneous osteosarcoma similar to human clinical cases. We will continue with the curative intent Phase 2 osteosarcoma veterinary trial where the entire tumor will be irradiated.

45. Radiation-Induced Microenvironmental Modulation of Granule Cell Precursor Migration and Implications for Medulloblastoma

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Introduction: Radiation carcinogenesis has traditionally been linked to misrepaired DNA damage, but growing evidence shows that ionizing radiation also alters the tissue microenvironment and induces epigenetic changes that may contribute to tumor development. The interplay between direct DNA damage, microenvironmental remodeling and altered cellular behavior remains incompletely understood.

Mice heterozygous for Patched1 (Ptch1/) spontaneously develop medulloblastoma, a cerebellar tumor and are highly sensitive to radiation-induced tumorigenesis. Granule cell precursors (GCPs), the cells of origin of medulloblastoma, proliferate in the external granular layer (EGL) before migrating to the internal granular layer (IGL) to differentiate. Disruption of this regulated migration may prolong residence in a proliferative niche and increase transformation risk. As GCPs interact with microenvironmental (MEV) cells such as microglia and astrocytes, we investigated whether radiation-induced MEV changes affect GCP migration.

Materials and Methods: Migration of GCPs isolated from Ptch1/ cerebellum was assessed using transwell assays. Cells were irradiated (0, 0.1, 2 Gy), cultured for 72 h, then seeded in upper chambers. Lower chambers contained complete medium, conditioned medium (CM) or extracellular vesicles (EVs) from microglia or astrocytes. Migrated cells were quantified after 4–24 h.

For indirect MEV effects, C8B4 microglia and C8D1A astrocytes were irradiated, cultured for 72 h, and their CM or EVs applied to non-irradiated GCPs. Migration-related gene expression was analyzed by RT-PCR.

Results: Direct irradiation significantly reduced GCP migration in a sustained manner, with low-dose (0.1 Gy) effects comparable to 2 Gy.

Under basal conditions, microglia-derived EVs enhanced GCP migration, confirming their modulatory role. However, irradiation impaired this effect: CM and EVs from irradiated microglia significantly reduced migration, with EVs alone sufficient to transmit inhibitory signals. Astrocyte-derived effects were weaker and only significant at specific time points. RT-PCR revealed deregulation of genes involved in migratory signaling.

Conclusion: Ionizing radiation impairs GCP migration both directly and indirectly through MEV alterations, particularly via irradiated microglia and their EVs. EV-mediated signaling emerges as a key mechanism by which radiation reshapes precursor cell behavior. Impaired migration may prolong GCP residence in the proliferative EGL, increasing susceptibility to transformation and contributing to medulloblastoma development. These findings support a model in which radiation-induced carcinogenesis extends beyond DNA damage to include altered microenvironmental EV signaling and intercellular communication.

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74. Impact of the Cerebellar Microenvironment on Radiation-Induced Tumorigenesis in a Ptch1/ Medulloblastoma Model: In Vitro and Ex Vivo Approaches

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Background: Medulloblastoma (MB), the most common malignant pediatric brain tumor, comprises four major molecular subtypes, including one driven by aberrant activation of the Sonic hedgehog (Shh) signaling pathway. Under physiological conditions, Shh is secreted by Purkinje cells and regulates the expansion of granule cell precursors (GCPs), which subsequently differentiate and migrate to form the mature cerebellum. Shh-dependent MB arises from transformed GCPs, a transient progenitor population highly sensitive to oncogenic insults. This study aims to elucidate the mechanisms underlying radiation-induced brain tumorigenesis using the Patched1 heterozygous (*Ptch1/*) mouse model, which is predisposed to MB and exhibits markedly increased tumor incidence following neonatal irradiation. This model provides a powerful system to investigate the interplay between genetic susceptibility and ionizing radiation exposure.
Materials and Methods: A large cohort of *Ptch1/* mice was exposed postnatally (P2) to low (0.1 Gy) or high (2 Gy) doses of 60Co gamma radiation. High-dose irradiation significantly increased tumor incidence and reduced latency, whereas low-dose exposure elevated tumor risk without affecting onset. To investigate microenvironmental contributions, we employed two complementary in vitro co-culture systems using GCPs isolated from postnatal day 2 Ptch1/ cerebella: (i) conventional co-cultures with astrocytes or microglia, and (ii) advanced co-cultures with organotypic cerebellar slices preserving native cytoarchitecture. In both models, GCPs and their microenvironmental counterparts were exposed to 0.1 or 2 Gy γ-radiation in defined combinations of irradiated and non-irradiated cells to distinguish cell-autonomous from non-cell-autonomous effects. Carcinogenesis-related endpoints and inflammatory pathway activation were evaluated 72 hours post-irradiation.
Results: In vivo analyses supported a dose-dependent model of radiation-induced tumorigenesis in which high and low dose MBs are characterized by distinct transcriptional profiles, that may suggest a contribution of microenvironmental modulation. In vitro experiments confirmed that the microenvironment plays a critical role in shaping radiation responses. Co-culture with irradiated astrocytes or microglia significantly enhanced radiation-induced pro-tumorigenic changes in GCPs. Specifically, microenvironment-derived inflammatory signals promote increased stemness and self-renewal capacity, indicating that inflammation-driven pathways amplify tumorigenic processes following irradiation. These effects were more pronounced at 2 Gy, further supporting a dose-dependent response.
Conclusions: These findings highlight the importance of microenvironmental interactions in radiation-induced carcinogenesis and demonstrate the value of in vitro co-culture systems for modeling non-targeted radiation effects. This work also provides new insights into the mechanisms driving medulloblastoma development.

64. Assessing the impact of novel tissue-engineered bioactive dressings on wound healing of irradiated murine skin

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Background: During radiotherapy, large doses of ionizing radiation passing through the skin can cause lesions (radiodermatitis) that affect patients' quality of life. To better understand the adverse effects of radiotherapy and potential treatments, we developed a murine model of acute and chronic radiodermatitis. Irradiated skin showed dose-dependent damage but despite macroscopic healing, retained wound healing impairment. Current treatments provide temporary pain relief but aren't curative. To address this clinical need, a biological dressing produced by tissue engineering has been developed using adipose-derived stem/stromal cells, which help to accelerate skin healing through the secretion of soluble bioactive factors. In our previous work, we showed that applying these cellularised dressings to wounds created in irradiated murine skin significantly improved global wound closure, reaching 77.0 ± 15.7% compared with 61.3 ± 11.0% in untreated irradiated skin. We hypothesize that repeated topical application of the dressings not only promotes faster closure but also improves the quality of the healed tissues.
Materials and methods: The dressings were produced using the self-assembly method using a serum-free medium. Full-thickness excisional wounds were created on both sides of the dorsal skin of mice (n=12), with the left side previously exposed to a dose of 45 Grays (1 cm2). The biological dressings were applied weekly to provide optimal bioactive factor delivery and match clinical feasibility. At day 35, the end of treatment, scar tissues were harvested for histological and immunohistochemical analyses. In parallel, media conditioned by the dressings for 72 hours were collected to quantify the secretion of pro-angiogenic factors using enzyme-linked immunosorbent assays.
Results: Histological assessment using a defined scoring system revealed improved healing outcomes in irradiated skin treated with the dressings. Untreated irradiated tissues had a low healing score of 2/12, whereas treated irradiated wounds reached a score of 6/12, approaching the quality observed for wounds in non-irradiated skin (9/12). Treatment improved the architecture of the neoepidermis and promoted the formation of a more uniform neodermis with better extracellular matrix organization. A 2.3-fold increase in microvascular density was measured in treated irradiated granulation tissue compared with untreated tissue by quantification of anti-CD31 labelling. These structural improvements correlated with an elevated secretion of major pro-angiogenic mediators in conditioned media, including VEGF, Ang-1, PAI-1, and HGF, known to stimulate vascular network formation and tissue remodelling.
Conclusions: Our preliminary results indicate that the application of cellularised dressings seemed to improve the quality of cutaneous wound healing in irradiated skin. This improvement could be attributed in part to the secretion of pro-angiogenic factors that stimulate angiogenesis.

87. Proton irradiation of murine salivary glands induces sustained proliferative activity and DNA damage signaling independent of delivery mode

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Background: Proton therapy is a tissue-sparing cancer treatment that exploits the Bragg peak (BP) for localized dose deposition. Low-energy protons at the distal end of the BP have increased linear energy transfer (LET), and in vitro studies demonstrate increased cell lethality and more clustered DNA damage at this position compared to the proximal BP. However, in vivo, LET gradients are intrinsically coupled to spatial dose distribution and irradiated volume, complicating direct translation of these findings to normal tissue responses. To investigate this in a clinically relevant setting, we established a murine head irradiation model targeting the major salivary glands, comparing a high-LET, low-volume (HLLV) delivery, in which protons stop within the animal, to a low-LET, high-volume (LLHV) transmission setup. The aim of this study was to determine whether these distinct proton delivery modes result in different proliferative activity or DNA damage in murine salivary glands.
Methods: Female C57BL/6 mice underwent fractionated irradiation with protons to the maximally tolerated acute level. Two different delivery modes were used: (1) HLLV, with the BP approximately at the mouse head midline (25 MeV protons) or (2) LLHV, with a "shoot-through" approach (60 MeV protons). While LLHV gave a homogenous fraction dose and LET distribution across the mouse head (around 6.5 Gy and 1.4 keV/mm, respectively), HLLV gave doses and LETs in the range of 0-10 Gy and 2-20 keV/mm, respectively. The left and right submandibular (SMG) and parotid glands (PG) were collected 100 days after irradiation. Cellular proliferation and DNA damage were assessed by immunofluorescent staining on the SMG and PG using the proliferation marker Ki-67 and DNA damage marker γH2AX.
Results: When both proton delivery modes were analyzed together, irradiation increased Ki-67 and γH2AX levels in SMGs and to a lesser extent in PGs, compared to controls. When the delivery modes were assessed, Ki-67 expression in SMGs and PGs increased significantly more after LLHV than HLLV, while the effect on γH2AX was comparable. Right SMGs displayed higher Ki-67 levels than left for both delivery modes. This was less evident for PGs, although LLHV generally induced higher Ki-67 levels than HLLV.
Conclusion: At 100 days after irradiation, murine salivary glands exhibited increased proliferative activity and persistent DNA damage, indicating long-term alterations in tissue homeostasis following proton irradiation. For HLLV, giving higher dose to the right salivary glands, a corresponding increase in proliferative activity was found. However, right–left asymmetry was also observed under homogeneous LLHV irradiation. Together, these findings indicate that proliferative activity and DNA damage are not solely determined by proton delivery mode but may instead reflect tissue-intrinsic factors emerging during long-term gland remodeling.

104. Radiobiological studies with laser-driven protons at ELIMAIA-ELIMED: from fibroblasts to 3D spheroid models

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Background: Laser-driven proton (LDP) beams offer a distinct time structure, combining ns-scale bunch duration with very high instantaneous dose rates. This makes them attractive for radiobiological comparison with conventionally accelerated protons. At ELI Beamlines, the ELIMAIA-ELIMED beamline, powered by the L3 HAPLS 1 PW laser, allows controlled multi-shot irradiation of biological samples with intermediate-energy LDP and supports systematic in vitro radiobiology studies.
Materials and Methods: Pilot experiments used AG01522 normal human fibroblasts irradiated with multi-shot LDP of mean energy ˜ 23.5 MeV, doses of ˜ 0.4-1.5 Gy, and bunch duration of ˜ 5 ns; DNA double-strand breaks were quantified as 53BP1 foci. Samples irradiated with LDP at ELIMAIA-ELIMED were benchmarked against LDP in the single-shot regime as well as conventionally accelerated protons. Subsequent campaigns extended the work to HT29 and LLC1 tumor models grown as 3D spheroids, together with corresponding 2D conformations. Endpoints included survival, spheroid growth kinetics, PD-L1, heat-shock proteins, hypoxia-related factors, and bulk RNA sequencing.
Results: In AG01522 fibroblasts, multi-shot LDP irradiation produced a clear dose-dependent increase in 53BP1 foci. The observed response was comparable to single-shot LDP and conventional proton data when interpreted in the context of the much longer multi-shot delivery time, suggesting that DNA repair during exposure contributed to the lower foci yield measured 30 min after irradiation. The newer spheroid campaigns extend this work toward physiologically more relevant tumor models and allow comparison of LDP and conventional proton responses across the multiple complementary biological endpoints. Bulk RNA sequencing has been performed and is under evaluation, while protein assays are ongoing.
Conclusions: Together, these studies show that ELIMAIA-ELIMED has transitioned into an actively used, user-ready platform for radiobiological research with laser-driven protons. The combination of published fibroblast data and ongoing 2D/3D tumor studies provides a basis for investigating how the temporal structure of LDP delivery influences biological response. Practical specifics and future opportunities associated with laser-driven beams in ultrafast radiobiology are highlighted and discussed as well.

55. Potential Implication of Biodosimetry Methodology for Predicting Radiotherapy Normal Tissue Toxicity: Current Evidence, and Future Directions

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Biodosimetry biomarkers have emerged as important tools for evaluating cellular responses to ionizing radiation and for understanding inter-individual variability in radiotherapy outcomes. In radiation oncology, predicting patient-specific normal tissue toxicity (NTT) remains a major challenge, as individuals receiving comparable dose–fractionation schedules often exhibit markedly different acute and late tissue reactions. This variability limits safe dose escalation and constrains the ability to optimize tumor control while preserving normal tissue function. Consequently, considerable research has focused on identifying biological markers capable of predicting individual radiosensitivity prior to or during radiotherapy. Among the most extensively investigated approaches are cytogenetic biodosimetry assays, which quantify radiation-induced chromosomal aberrations in peripheral blood lymphocytes. Classical biomarkers such as dicentric chromosomes, micronuclei, and stable chromosome exchanges detected by fluorescence in situ hybridization (FISH) represent well-established indicators of DNA damage resulting from misrepaired double-strand breaks. Because these aberrations are closely linked to genomic instability and mitotic cell death, they provide a mechanistic connection between molecular radiation damage and clinically observable tissue injury. Several studies have therefore explored the use of ex vivo radiation-induced cytogenetic damage as a surrogate marker for predicting radiosensitivity and the risk of radiotherapy-related toxicity. In addition to classical cytogenetic endpoints, emerging biomarkers related to the DNA damage response, including γ-H2AX foci formation and G2-phase chromosomal radiosensitivity assays, have been proposed as complementary indicators of radiation sensitivity. Recent technological advances can further expand the biodosimetry field through the integration of high-throughput molecular approaches, including proteomics, gene expression profiling, and next-generation sequencing (NGS). These approaches enable the identification of radiation-responsive proteins, transcriptional signatures, and genetic variants associated with radiosensitivity, offering new opportunities for developing predictive biomarker panels. This review critically evaluates the current evidence regarding the use of biodosimetry-based biomarkers for predicting normal tissue toxicity in radiotherapy. We discuss the biological basis, methodological considerations, and clinical relevance of classical cytogenetic assays and emerging molecular biomarkers. Finally, we highlight future directions for biodosimetry research, emphasizing the potential of multi-omics integration, sequencing technologies, and machine learning–assisted analysis to improve the predictive accuracy of radiosensitivity biomarkers and support the development of personalized radiotherapy strategies.

60. Proton Irradiation Preserves Metabolic Homeostasis and Protects Normal Stem Cell Niches Compared with X-ray or Carbon-ion Radiation

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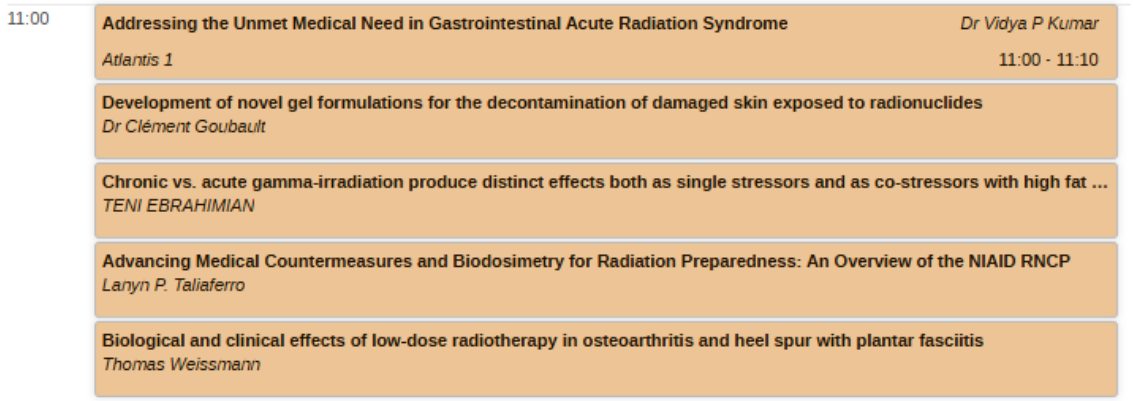
Background and aims: Proton therapy plays a distinctive role in modern radiotherapy due to its ability to deliver highly localized radiation doses to tumors while sparing surrounding healthy tissues. Minimizing treatment-related side effects is particularly important in pediatric patients, who often have long-term survival prospects. This study evaluated and compared protein expression changes induced by proton irradiation with those caused by X-ray and carbon-ion radiation in human adipose-derived stem cells (ADSCs). We also examined metabolic responses to understand how radiation quality influences cellular metabolism, stress adaptation, and stem cell function.
Methods: A quantitative proteomic approach based on LC–MS/MS was employed to characterize protein expression changes and identify affected biological pathways in ADSCs following irradiation. Cells were exposed to a dose of 2 Gy using three different radiation including X-rays, protons and carbon ions. Total cellular proteins were extracted 48 hours after irradiation to capture early molecular responses and analyzed by high-resolution LC–MS/MS to obtain a comprehensive proteomic profile. After protein identification and quantification, biostatistical analyses identified significantly differentially expressed proteins various groups. KEGG pathway enrichment analysis was then performed to reveal biological processes affected by each radiation modality, with significantly altered pathways further examined to understand the underlying molecular and metabolic mechanisms.
Results: Heatmap analysis indicated that approximately 2,500 proteins were detected per sample. Pathway enrichment analysis identified 229 biological pathways, of which 57 were associated with metabolic processes. Proton irradiation specifically resulted in the downregulation of 51 metabolic pathways (KEGG p-value = 0.0022–0.1797), whereas exposure to X-rays or carbon ions affected only 6 metabolic pathways. In addition, proteins involved in multiple metabolic pathways showed a more pronounced reduction following proton irradiation compared with X-ray and carbon-ion radiation.
Conclusions: Quantitative proteomic analysis shows that different radiation modalities induce distinct molecular and metabolic responses in human adipose-derived stem cells. Although all irradiation types altered protein expression, proton irradiation caused stronger modulation of metabolic pathways than X-ray and carbon-ion radiation. Notably, proton exposure promoted balanced regulation of key metabolic networks such as glycolysis, the tricarboxylic acid cycle and oxidative phosphorylation that supporting ATP production and redox homeostasis. This metabolic stability may help preserve normal stem cell function after irradiation. These findings provide new insights into the biological effects of proton radiation and suggest potential advantages in protecting normal tissues, particularly by maintaining cellular homeostasis and limiting metabolic disruption which may reduce long-term adverse effects. Overall, the results support the clinical relevance of proton therapy and highlight the need for further studies on its radiobiological mechanisms and long-term impact on normal tissue preservation.

Session 8A: Translational Radiation Protection Medical Countermeasures

Chairs: Radia Tamarat and Carmen Rios
Room: Atlantis 1



3. Addressing the Unmet Medical Need in Gastrointestinal Acute Radiation Syndrome

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Exposure to ionizing radiation, whether from nuclear incidents or radiological accidents, frequently results in significant and detrimental effects on human health. The extent and location of damage can vary depending on the geographical scope of the exposure, potentially affecting single or multiple organs to different degrees. Damage to the gastrointestinal (GI) system typically manifests within days and generally requires a higher radiation dose compared to the dose needed to impact the hematopoietic system. The U.S. Food and Drug Administration (FDA) has approved nine medical countermeasures for hematopoietic Acute Radiation Syndrome (H-ARS), including Neupogen, Neulasta, Leukine, NPLATE, Stimufend, Ziextenzo, Nypozi, Zarxio, and Udenyca. However, there are currently no FDA-approved treatments for gastrointestinal Acute Radiation Syndrome (GI-ARS), highlighting a critical unmet medical need. To effectively pursue the development of such a countermeasure, a robust and accurate preclinical model is essential. This model should closely mimic the radiation-induced GI injury and possess the necessary throughput for preclinical drug evaluation.

We have developed a novel partial body irradiation (PBI) model in mice, utilizing a clinical linear accelerator, that uniquely replicates GI injury and the subsequent progression of damage to multiple organs (Radiat Res. 2024). The prophylactic and mitigating effects of YK-4-250, a proprietary angiotensin receptor 1 blocker conjugated to the catalytic antioxidant developed by Trocar Pharma, demonstrated statistically significant improvements in the survival of lethally irradiated C57BL/6 mice. Mechanistically, this survival benefit correlated with the restoration of jejunal crypt cells, increased cellular proliferation, and reduced DNA damage and increase in DNA repair. Furthermore, YK-4-250 treatment enhanced gut barrier integrity, thereby mitigating bacterial translocation to adjacent organs. Our findings suggest that YK-4-250 mitigates the GI damage via a four-prong mechanism of reducing and repairing DNA, disrupting angiotensin II/ AT1 binding, reducing ROS and upregulating the cytoprotective ACE2 enzyme. Consequently, we propose that YK-4-250 holds significant promise as a countermeasure for GI-ARS.

The views expressed here are those of the authors and do not reflect the official policy of AFRRI, USUHS, DoD or the US Government. Neither I nor my family members have a financial interest in the product, service, or organization providing financial support for this research. Funding for this work was from NIAID-IAA and AFRRI Intramural funds to Dr. Vidya P. Kumar.

9. Development of novel gel formulations for the decontamination of damaged skin exposed to radionuclides

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The project RESILIENCE-R-2023, funded by the European Defense Fund (EDF), aims at developing novel countermeasures against Chemical, Biological, Radiological and Nuclear (CBRN) threats. In particular, the sub-project INDECRAD targets the design of human decontaminating agents for RN threats. Based on previous work [1, 2], we developed innovative hydroxypyridinone (HOPO)-loaded and calixarene-loaded formulations in gel forms for the decontamination of damaged skin exposed to radionuclides such as Uranium (U), transuranic actinides (Plutonium (Pu) and Americium (Am)) or Cobalt (Co). Biocompatible cellulose derivatives and a thermosensitive gelling agent were used to impart thermo-gelling properties to the formulation and ensure gelation at skin temperature once applied, as well as to confer optimal rheological properties and adherence to the skin. The efficacy of HOPO and calixarene gels for the decontamination of skin exposed to radionuclides was evaluated using an ex vivo dynamic transdermic diffusion system (Franz cells), for which a damaged skin model has been developed. Lacerated rat or human skin explants were used to mimic wounds. The decontaminating efficacy of the two novel formulations was also compared to DTPA-loaded gel.

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49. Chronic vs. acute gamma-irradiation produce distinct effects both as single stressors and as co-stressors with high fat diet on the brain and heart microcirculation in mice

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Association between low-to-moderate doses of ionizing radiation and cardiovascular and cerebrovascular diseases has been suggested, with exposure mode playing a role. Microvascular alterations are involved in cardiovascular diseases, with obesity being a strong risk factor. The aim of this study was to study the effects of different modes of exposure to moderate doses of gamma radiation (137Cs) on endothelial dysfunction in cardiac and cerebral microcirculation, to determine the dose rate effect and to examine their potential interaction with high fat diet (HFD).
C57BL/6J mice were fed with normal or HFD, and acutely (300mGy or 1Gy) or chronically (cumulative 300mGy) exposed to total-body gamma-irradiation using an external beam or internal 137Cs at 500kBq/L in drinking water, respectively. Analyses were conducted at 6 months post-acute irradiation or immediately upon cessation of chronic exposure. Gene expression for oxidative stress (heart and hippocampus), inflammation, endothelial dysfunction and vascular remodeling were assessed. Hippocampus microvessels were identified using CD31 immunostaining. Heart function was assessed using echocardiography.
Chronic exposure (300mGy) caused a more pronounced endothelial and heart dysfunction compared to acute 300mGy exposure using a variety of readouts. While a 300mGy acute dose did not induce adverse alterations, 1 Gy triggered a significant response of the genes involved in brain oxidative stress and inflammation, suggesting detriment. HFD partially alter the response to 300mGy, but significantly potentiated detriment from 1 Gy.
Our results demonstrate a mode of exposure effect on brain endothelial and heart functions and additivity of adverse effects between HFD and 1Gy acute dose.

166. Advancing Medical Countermeasures and Biodosimetry for Radiation Preparedness: An Overview of the NIAID RNCP

Author: Lanyn P. Taliaferro

The National Institute of Allergy and Infectious Diseases (NIAID) Radiation and Nuclear Countermeasures Program (RNCP), established in 2004, plays a critical role in strengthening U.S. preparedness against radiation-induced public health emergencies. This presentation details the program's strategic initiatives to accelerate the research, development, and deployment of medical countermeasures (MCMs) and biodosimetry devices designed to mitigate acute and delayed radiation injuries resulting from nuclear detonations and radiological incidents.

Key programmatic priorities include the advancement of targeted therapeutics for hematopoietic, gastrointestinal, pulmonary, and cutaneous sub-syndromes, alongside innovative treatments for internal radionuclide contamination. Highlighting recent translational successes, the discussion covers the development of orally administered decorporation agents (e.g., HOPO 14-1, currently in Phase I clinical trials), the repurposing of established pharmaceuticals (e.g., lisinopril for radiation-induced lung fibrosis), and the critical application of the FDA Animal Rule to facilitate MCM approval when human efficacy trials are not feasible.

Furthermore, the presentation outlines ongoing efforts to advance rapid, non-invasive biodosimetry diagnostics for effective clinical triage and severity prediction. Through the utilization of specialized animal models—including a nonhuman primate survivor colony to study both acute and delayed radiation effects—and the support of over 1,350 research products, the RNCP continues to drive innovation to address critical unmet needs in radiological and nuclear incident response.

122. Biological and clinical effects of low-dose radiotherapy in osteoarthritis and heel spur with plantar fasciitis

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Low-dose radiotherapy (LDRT) is an established treatment option for painful degenerative musculoskeletal disorders such as osteoarthritis (OA) and heel spurs with plantar fasciitis. However, the underlying mechanisms, structural tissue alterations, clinical and immunomodulatory effects remain insufficiently characterized. In OA, alongside inflammation and clinical assessment by MRI, particular emphasis is placed on cartilage tissue, which is essential for long-term joint function but has been neglected in the context of LDRT. In heel spur, pain outcomes have already been evaluated in several studies, whereas evidence for objectively measurable functional improvement remains limited. Therefore, gait analyses, orthopedic examinations, MRI, and inflammatory parameters are being assessed. This prospective work aims to evaluate the clinical, imaging, and biological effects of LDRT in order to better understand its therapeutic efficacy and improve radiation protection and patient safety.

Patients with OA (n=36) or clinically diagnosed heel spurs with plantar fasciitis (n=35) were prospectively enrolled and treated with LDRT (single dose 0.5 Gy, total dose up to 6.0 Gy per series, max. of two series). At defined time points before (T0) and after therapy (T1-T3), clinical data were collected by medical history and standardized questionnaires. Patients also underwent specialized non-contrast MRI, routine and extended laboratory analyses, and immunological profiling. For plantar fasciitis, functional gait analysis and shearwave elastography were additionally performed. Interim comparative analyses between T0 and T1 (15 weeks after therapy start) are currently available for subsets of the planned cohorts (OA n=21; heel spur n=12).

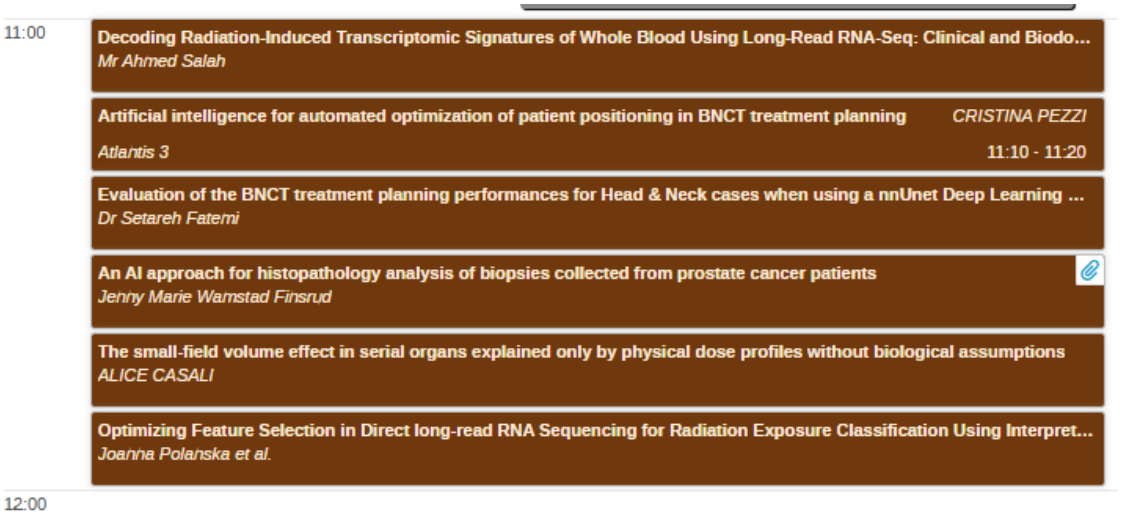
In OA, pain decreased significantly from T0 to T1 according to the Visual Analog Scale, while quality of life improved. In addition, 25% of patients were able to reduce their pain medication. In patients with osteoarthritis of the fingers and wrists (n=9), the AUSCAN score showed significant improvement during hand compression and torsional movements. T cells and CD4+ T cells were significantly reduced. This was accompanied by a decrease in mean platelet volume, suggesting reduced inflammatory activity. In heel spur patients, MRI revealed soft-tissue-related changes, such as muscle or tendon lesions in the area of the plantar fascia, in a significant proportion of patients. Moreover, 9/12 patients showed significant clinical improvement. Regarding immunological parameters, a significant increase in CD8+ T cells, NK cells, and monocytes was observed.

These interim results support the clinical effectiveness of LDRT and suggest that symptom improvement is accompanied by measurable immunological and imaging-detectable changes. Multimodal evaluation may help characterize treatment response and underlying mechanisms of LDRT. ELISA analyses are currently ongoing.

Supported by BMFTR (TOGETHER, 02NUK073) and Deutsche Arthrose-Hilfe e.V.

Session 8B: Computational Radiation Biology and Treatment Modeling

Chairs: Johann Matschke and Lars Svensen
Room: Atlantis 3



5. Decoding Radiation-Induced Transcriptomic Signatures of Whole Blood Using Long-Read RNA-Seq: Clinical and Biodosimetric Implications

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Introduction: Gene expression profiling in radiation-exposed blood is a valuable tool for biodosimetry and clinical research. Evaluating the blood's transcriptomic radiation response provides insight into absorbed dose, hematotoxicities, and immune reactions. However, detailed analysis using long-read RNA sequencing is currently limited in its diffusion, despite the potential additional insights that could be extracted, including novel isoform discovery and on-the-field gene expression studies, owing to its portability.
Materials and Methods: Venous whole blood from 3 healthy donors (2 males and 1 female) was irradiated ex vivo with 4Gy X-rays. Sham-irradiated (0 Gy) controls were maintained under identical conditions in the control room. Long-read RNA sequencing was performed using the PromethION platform 6 h post-irradiation. Differentially expressed genes (DEGs) were identified using DESeq2 (FDR < 0.05). Functional enrichment analysis was conducted using the clusterProfiler R package.
Results: In this study, we utilized Oxford Nanopore Technologies' long-read RNA sequencing on human whole-blood samples from three healthy donors 6 hours after exposure to 4 Gy of X-rays. Compared to sham-irradiated (0 Gy) blood, gene-level differential expression analysis identified 117 upregulated and 66 downregulated genes, including canonical DNA damage repair and inflammatory responses. At the transcript level, 102 transcripts were significantly upregulated, and 17 were downregulated, revealing isoform-specific regulation that was not captured at the gene level. Notably, IL32, which showed no significant change at the gene level, exhibited strong upregulation of two transcript isoforms, while WDR74, ITM2B, AK2, and RPS19 displayed changes in transcript usage following irradiation. Leveraging the power of long-read RNA sequencing, we further identified 26 novel transcript isoforms, expanding the catalog of radiation-responsive transcripts.
Conclusions: This is the first comprehensive study of long-read RNA-seq for transcriptomic profiling of human whole blood following ionizing radiation. These findings highlight the ability of long-read RNA sequencing to provide a more detailed view of radiation-induced transcriptomic alterations, underscoring its potential for biodosimetry and clinical applications.

102. Artificial intelligence for automated optimization of patient positioning in BNCT treatment planning

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Background: Boron Neutron Capture Therapy (BNCT) is an advanced form of radiotherapy based on the selective accumulation of boron-containing compounds in tumor tissues followed by irradiation with a thermal neutron beam. The resulting nuclear reactions produce high-LET particles that deposit energy at the cellular level, inducing complex DNA damage and ultimately cell death. Treatment effectiveness depends on several factors, including accurate patient positioning relative to the neutron beam. Despite its relevance, no standardized protocols are currently available, and clinical practice mainly relies on geometric criteria, minimizing the distance between the tumor and the beam port. However, this approach does not consider the complexity of neutron–tissue interactions and radiobiological effects. Artificial intelligence (AI) offers the possibility to integrate dosimetric information within a more comprehensive optimization framework.
Materials and Methods: A Python-based computational framework was developed to automatically evaluate patient positioning configurations using different optimization algorithms. Spatial configurations are assessed through score functions combining dose distributions with radiobiological metrics. Several optimization strategies were implemented and compared, including Grid Search, Guided Random Walk, Differential Evolution, Cross-Entropy Method, and the gradient-based Adam optimizer. The framework was integrated into the IT_STARTS treatment planning toolkit (project funded by INFN, Pavia) and validated using both geometric phantoms and patient CT datasets to enable realistic analyses.
Results: The proposed AI-driven approach showed promising results, with an optimized target coverage and reduction of dose to healthy tissue. Trials on geometric phantoms highlighted the adaptability of the method to different tumor depths, while tests on CT datasets proved its potential clinical applicability. The comparison among optimization algorithms showed that all methods were able to effectively explore the solution space, although following different search strategies and convergence behaviors, providing consistent reliable results.
Conclusions: This work shows that AI-based optimization can support BNCT treatment planning by integrating dosimetric and radiobiological aspects into patient positioning strategies. The framework allows the use of different score functions (e.g. Tumor Control Probability (TCP) and dose-based metrics) and optimization methods, making it possible to adapt the optimization process to specific clinical goals and treatment scenarios. This enables a more tailored and biologically meaningful evaluation of treatment setups, moving beyond purely geometric criteria. The proposed approach represents a step toward more personalized optimization in BNCT. Future developments will include AI-based dose prediction models to further improve computational efficiency.

103. Evaluation of the BNCT treatment planning performances for Head & Neck cases when using a nnUnet Deep Learning model to automatically segment CT images

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Background: Boron Neutron Capture Therapy (BNCT) clinical improvement and personalization are main objectives in the scientific and medical community. Artificial Intelligence (AI) serves as a pivotal tool in the pursuit of high-precision, individualized treatment protocols. The application of Deep Learning (DL) allows to segment large datasets of medical images improving speed and accuracy. Integrating such models into the clinical workflow allows medical physicists to streamline treatment planning through initial automated contouring, which the radiation oncologist can subsequently refine to ensure gold-standard accuracy for final dosimetric calculations.

Materials & Methods: In this work we focus on CT images of Head and Neck cancer cases obtained from a public images database called The Cancer Imaging Archive. We analyzed the database and selected the images that were suitable for the automatic segmentation. We chose to use a Deep Neural network called nnUnet 1 that showed very good performances in segmenting different regions. The database was divided in training and testing sets and the network was trained and tested using a k-fold cross validation.

Results: To evaluate the clinical utility of the nnU-Net segmentations, a subset of the test cohort was used to simulate BNCT treatment plans. These simulations were performed using the IT_STARTS treatment planning system (TPS) developed by INFN - Unit of Pavia incorporating the photon isoeffective dose model [2]. The treatments were planned to obtain an advantageous dose distribution in the tumour with conservative constraint in the healthy tissue. To ensure clinical relevance, the evaluation of the algorithm performance was assessed through radiobiological figures of merit, namely, the Tumor Control Probability (TCP) and the Normal Tissue Complication Probability (NTCP) [3]. This allows us to interpret the difference between the manual contouring and the automatic one in terms of predicted clinical efficacy and toxicity.

Conclusions: Dosimetry was obtained both using the medical segmentations of the tumour and organs at risks (considered the truth) and the automatic segmentations from the nnUnet. Results of TCP and NTCP comparisons will be shown and conclusion on the suitability of such approach in different cases will be drawn, based on the discrepancy between the figures of merits calculated with the two segmentation methods.

Acknowledgements: This work was partially funded by National Institute of Nuclear Physics (INFN), by the CSN5 grant AI_MIGHT. This work was supported in part by the PNC-PNRR-ANTHEM project, CUP: B53C22006730001.

1 Isensee, F., Jaeger, P. F., Kohl, S. A., Petersen, J., & Maier-Hein, K. H. (2021). nnU-Net: a self-configuring method for deep learning-based biomedical image segmentation. Nature methods, 18(2), pp. 203-211.

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radiation based on dose-response assessment from human and an animal model: clinical application to boron neutron capture therapy for head and neck cancer, Physics in Medicine and Biology, 62(20), pp.7938-7958

70. An AI approach for histopathology analysis of biopsies collected from prostate cancer patients

Author: Jenny Marie Wamstad Finsrud[None]
Co-authors: Heidi Lyng; Kari-Anne Frikstad Valen; Ljiljana Vlatkovic; Sonia Hermoso Duran; Tiril Kopland Trondsen; Vilde Eide Skingen

Background: It is standard practice to evaluate Haematoxylin and Eosin (HE) stained histology slides to predict cancer diagnosis and prognosis. This evaluation is, however, most often descriptive, as quantitative analysis is a labor-intensive task when performed manually. Artificial intelligence (AI) models can aid in rapid and consistent quantitative analysis of HE slides. Since HE-stained slides are routinely produced for all patients, extracting relevant information from this resource is highly advantageous. The tumor microenvironment (TME) composition in prostate cancer remains poorly understood, despite its impact on radiation therapy response. AI models such as HoVer-Net have been developed for automated nuclear segmentation and classification of cells based on HE slides, facilitating research into this field.
Aim: We aimed to evaluate the ability of an augmented version of the previously developed HoVer-Net AI model to perform segmentation and classification of cell types in HE-stained sections from prostate cancer biopsies.
Material & Methods: Tumor biopsies collected from 4 patients were processed for histological evaluation. Tissue sections were stained with HE and digitized. The images were analyzed using the HoVer-Net model. It includes identification of nuclei, which are further classified as tumor, stroma and immune cells with subcategories depending on the dataset used to train the model. Two public datasets were applied for training, one with about 200 000 annotated nuclei from pan-cancer samples including prostate cancer and another with about 500 000 from colon cancer samples, yielding two models that were tested on our HE sections. These HE sections were assessed by a pathologist, delineating and categorizing areas according to their tumor grade. Spatial transcriptomics was performed on the same biopsies and further analyzed with SpaCET to identify regions with malignant cells and stroma. To validate the AI model, the AI results were compared with the pathologist's analysis and spatial transcriptomics data. **Results:** The AI model predicted a high density of tumor cells in the areas of the biopsy with Gleason patterns 3,4, and 5, consistent with the growth of malignant cells in this region. In contrast, a larger fraction of the cells was categorized as stroma in the regions scarce of malignant cells. A significant positive correlation ($P < 0.05$) was found between the number of tumor cells (AI) and the estimate of malignant cells from spatial transcriptomics. This was also the case for the relationship between number of stroma cells by AI and estimate of stroma cells from spatial transcriptomics. These results were valid for both AI models trained on two different datasets.
Conclusion: The HoVer-Net AI model enables reliable quantification of cell populations in prostate cancer biopsies. This encourages further use of the model to explore the importance of cell composition for the radiotherapy response.

81. The small-field volume effect in serial organs explained only by physical dose profiles without biological assumptions

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Background: In radiotherapy, the volume effect refers to the dependence of normal tissue tolerance on the irradiated volume of an organ. This phenomenon, for serial organs, in which damage to even one of its units can compromise the entire organ function, occurs only at small irradiated lengths. To date no existing Normal Tissue Complication Probability (NTCP) model, as a function of dose, can reproduce the volume effect in serial organs irradiated with small fields. This limitation suggests that key mechanisms underlying this phenomenon are not fully captured by current modelling approaches. The aim of this work is to reproduce the small-field volume effect in serial organs using only physical dose distributions, without introducing additional biological assumptions, to achieve a clearer understanding of the mechanisms driving this effect.

Materials and methods: In vivo experimental data on rat spinal cord tolerance to photon and proton irradiation were considered. The NTCP model developed in this work is based on the concept of Functional SubUnit (FSU), defined as the basic unit responsible for preserving organ functionality, and depends on four parameters. The model incorporates the dose delivered outside the nominal irradiation length by accounting for the lateral dose profile along the spinal cord. These lateral dose distributions were simulated by GATE, a GEANT4 toolkit. Three out of the four model parameters were determined using photon irradiation data for a single irradiation length, while the last parameter, corresponding to the length of one FSU and therefore related to the volume effect, was derived from irradiation data obtained at different irradiation lengths.

Results: The best-fit parameters were fixed and used to predict the volume effect in more complex irradiation scenarios. The model, accounting for out-of-target dose, was applied to both "bath and shower" and "split-field" configurations. In the former, a large volume receives a low dose while a smaller superimposed volume is exposed to a higher dose; in the latter, the irradiated region is divided into two segments separated by unirradiated tissue. Our approach successfully described the behaviour of the experimental spinal cord volume-effect data across all these irradiation conditions for proton beams, using parameters with clear physical and biological interpretation.

Conclusions: This work, carried out within the GEANT4INFN and HEARTBEAT projects, enabled the development of an NTCP model capable of explaining the volume effect by accounting exclusively for physical dose profiles, without introducing additional biological assumptions associated with hardly quantifiable parameters, such as cell migration probability. This approach enhances the transparency and interpretability of the volume effect in serial organs, allowing for a clearer understanding of the mechanisms underlying this phenomenon.

118. Optimizing Feature Selection in Direct long-read RNA Sequencing for Radiation Exposure Classification Using Interpretable NLP-based Machine Learning

Authors: Joanna Polanska[1]; Tomasz Strzoda[1]
Co-authors: Christophe Badie [2]; Lourdes Cruz-Garcia [2]; Mustafa Najim [2]
[1] *Department of Data Science and Engineering, Silesian University of Technology;* [2] *Radiation, Chemical, Climate and Environmental Hazards Directorate, UK Health Security Agency*

Background: Developing rapid and accurate biodosimetry methods requires advanced molecular profiling, an area in which direct long-read RNA sequencing offers significant potential to capture radiation-induced transcriptomic signatures. However, identifying robust markers of ionizing radiation exposure within this high-dimensional data requires advanced analytical frameworks. Our study aimed to develop an optimized ML pipeline utilizing "Bag of Words" (BoW) encoding and rigorous feature selection to classify irradiated versus control cells, while ensuring cross-cell-line generalizability.

Materials and Methods: Direct long-read RNA-seq data from human fibrosarcoma cells (HT1080) were analyzed, comparing control samples against those 24h post-irradiation. Data NLP representations were built using a BoW encoding with three dictionary sizes: 1024, 156, and 77. To construct statistically stable samples, we aggregated 1000 Monte Carlo simulations, applied L1 normalization, and scaled the data by the square root of the dimensionality to equalize signal strength across dictionaries. Baseline Subtraction was implemented using control samples for calibration. Three classifiers were evaluated: Logistic Regression (LR), Random Forest (RF), and XGBoost (XGB). Feature reduction for LR utilized Feature Forward Selection guided by a Bayes Factor threshold (>20). For tree-based models, Permutation Importance was applied to ensure a robust and unbiased evaluation of feature significance, followed by Piecewise Linear Regression to determine the optimal top-N feature cutoff. External validation was performed on non-irradiated A549 lung cancer cells to assess model specificity.

Results: The proposed methodologies drastically reduced the feature space while maintaining near-perfect classification metrics. For the 1024-word dictionary, LR reduced features to 49, RF to 25, and XGB to 23, achieving Accuracy, F1, and Sensitivity scores ranged 96-98% (95% CI). Even with the smallest 77-word dictionary, high predictive power was retained using only 13 features. External validation on an independent A549 dataset demonstrated remarkable robustness: models effectively classified the novel cell line as "control," with NPV and Specificity reaching 100%, demonstrating that the selected transcriptomic signatures are not overfit to the HT1080 cell line. Furthermore, analyses revealed a high degree of consensus on critical features across all three distinct mathematical models (e.g., 22 shared words among the 49, 25, and 23 features selected by LR, RF, and XGB, respectively, from the initial 1024-word dictionary).

Conclusions: Combining BoW encoding of long-read data with rigorous, model-tailored feature selection provides a highly accurate and biologically transferable framework for radiation biodosimetry. The synergy of robust baseline calibration and dual feature selection strategies (Feature Forward Selection and Permutation Importance) successfully distilled the transcriptome into a compact, universally validated signature across distinct human cancer cell lines.

FLASH + Poster presentations

All the FLASH + Poster will be presented in the FLASH talks and the corresponding poster session.

Monday, 07.09.2026

FLASH + Postersession 1

Chair: Nina Edin
Room: FLASH Session in Atlantis 1, Postersession in Atlantis 2

12:00

Pharmacological Inhibition of HIF-2α with PT2385 or Belzutifan to Enhance the Response of Glioblastoma to Chemorad
Dr Jolie Bou-Gharios

Combined brachytherapy and genetically modified M1 macrophages enhance suppression of breast tumor progression
Dorota Sprus-Lipka

Alterations in Muscle Regeneration Following Total Body Irradiation in a Combined Radiation Setting *Nicolas Morin*
Atlantis 1 12:07 - 12:08

Surface-Functionalized Iron Oxide Nanoparticles as Novel Radiosensitizers *Eglantine Beauchot*
Atlantis 1 12:08 - 12:09

PARP-1 Binding Affinity to Different DNA Models and Inhibitors: A Microscale Thermophoresis Study Relevant to the Ea
Ms Paula Pryba

Impact of leptin and female sex hormones on radiosensitivity of normal breast and endometrial cells *Kiran Dhakal*
Atlantis 1 12:10 - 12:11

Do advanced in vitro models improve prediction of radiosensitization? A comparison of 2D, 3D, and Organ-on-a-Chip s.
Laura Banaszkiewicz

Use of the novel cell division assay (CDA) to evaluate T cell toxicity in response to lutetium-177–DOTATATE peptide rec...
Shaghayegh Gharaghani

Dissecting Radiation-Induced Tumorigenesis: Single-Cell Insights into Microenvironmental Remodeling in SHH Medullo
Barbara Tanno

Exploring the Metabolic Modulation of Ionizing Radiation-induced DNA Damage Response *Ms Nidhi Mol*
Atlantis 1 12:15 - 12:16

Comparative Radiobiological Responses in 2D and 3D Cellular Models Exposed to X-rays and Ultrashort Pulsed Electro
Dr Elena Kalita

Sequential events of apoptosis in the irradiated zebrafish neuroepithelium *Hongyuan Shen*
Atlantis 1 12:17 - 12:18

The level of gamma-H2AX foci in peripheral blood mononuclear cells of patients undergoing radiotherapy for primary a
Dr Aneta Węgierek-Ciuk

Variation of individual DNA damage response of U2OS cells exposed to photon radiation *Halina Lisowska*
Atlantis 1 12:19 - 12:20

Ascorbate induces cancer cell death without direct DNA double-strand break formation through delayed chromatin deg...
Dr Mehran Hariri

Radiation Enhances the Cellular Connectivity Through Tunneling Nanotubes in Glioblastoma Cells *Judith Reindl*
Atlantis 1 12:21 - 12:22

Spatiotemporal Interaction of DNA Damage for Investigating the Biological Effects of High-LET Particle Radiation
Selina Jasmin Nnedu Onoh

15. Pharmacological Inhibition of HIF-2α with PT2385 or Belzutifan to Enhance the Response of Glioblastoma to Chemoradiotherapy in vitro

Author: Jolie Bou-Gharios[1]
Co-authors: Carole MURA [1]; Elodie CZUBA [1]; Pierre COLIAT [2]; Georges NOEL [3]; Hélène BURCKEL [1]
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Background: Glioblastoma (GBM) remains highly resistant to ionizing radiation (IR) and temozolomide (TMZ), largely attributable to hypoxia-driven adaptive mechanisms. Hypoxia-inducible factor-2α (HIF-2α) is enriched in GBM tissue, correlates with high-grade gliomas, poor prognosis and regulates genes critical for glioma stem cell maintenance. Its limited expression in normal tissue and the availability of selective small-molecule antagonists make HIF-2α an attractive target. We evaluated whether pharmacological HIF-2α inhibition with PT2385 or Belzutifan could sensitize GBM to chemoradiotherapy in hypoxic 2D cultures and 3D spheroid models.
Materials and Methods: U-251 and U-87 cell lines were cultured under hypoxia (1% O) and treated with PT2385 (20 μM) or Belzutifan (10 μM), alone or combined with IR (2 or 6Gy) and/or TMZ (1-10 μM). HIF-2α and downstream targets (GLUT1, VEGFA, EPO) were assessed by ELISA and Western blot. Clonogenic assays, cell cycle analysis and immunofluorescence (pATM, γH2AX, 53BP1, pDNAPKcs, RAD51 and MSH6) quantified survival and DNA damage/repair, whereas migration was evaluated by wound-healing assays. For 3D models, spheroids grown in ultra-low-attachment plates with B27/EGF/bFGF medium were characterized for stem/progenitor and hypoxia markers, then treated with HIF-2α inhibitors ± TMZ ± 2Gy to monitor growth, outgrowth and endpoint viability.
Results: Hypoxia markedly increased HIF-2α expression in both lines, which was significantly reduced by PT2385 or Belzutifan, together with the downregulation of GLUT1 and VEGFA, confirming effective pathway inhibition. In 2D, the triple combination HIF-2α inhibitor+2Gy+TMZ significantly decreased clonogenic survival of U-251 versus IR/TMZ alone, increased G/M accumulation and elevated induced and residual double-strand breaks (DSB), consistent with impaired DNA repair. In U-87, clonogenic reduction and DSB accumulation were present but were modest compared to IR+TMZ. HIF-2α inhibition, particularly with Belzutifan, favored non-homologous end joining in U-251 versus homologous repair in U-87. Neither of the triple combinations significantly altered migration. GBM spheroids exhibited time-dependent enrichment in stem/progenitor and hypoxia markers. The addition of PT2385 or Belzutifan to 2Gy+TMZ significantly decreased spheroid diameter over time, while Belzutifan reduced the end-point viability and spheroid outgrowth on adherent surfaces, indicating improved growth control and invasion-like behavior.
Conclusions: Pharmacological HIF-2α inhibition enhanced GBM sensitivity to IR/TMZ under hypoxia by reducing clonogenic survival and increasing DSB persistence in 2D with differential involvement of DNA repair pathways. In 3D, HIF-2α targeting restricted spheroid growth and invasion-like outgrowth. These data support HIF-2α–based combinations as promising hypoxia-targeted chemoradiosensitization strategies in GBM, warranting in vivo validation and future clinical translation.

21. Combined brachytherapy and genetically modified M1 macrophages enhance suppression of breast tumor progression and modulate the tumor-associated immune response

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Co-authors: Alina Drzyzga [1]; Damian Sojka [1]; Ewelina Pilny [1]; Joanna Ciepla [1]; Justyna Czapla [1]; Karolina Sinek [1]; Magdalena Jarosz-Biej [1]; Marta Szlag [2]; Piotr Wojciaszek [3]; Ryszard Smolarczyk [1]; Sybilla Matuszczak [1]; Tomasz Cichoń [1]; Zbigniew Urbaś [3]
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Background: Macrophages play a crucial role in cancer development. They can polarize into either a pro-inflammatory, anti-cancer M1 phenotype or an anti-inflammatory, pro-tumorigenic M2 phenotype. In the tumor microenvironment (TME), a specific population of macrophages—tumor-associated macrophages (TAMs)—play a crucial role in tumor progression. Due to the significance of macrophages in the TME, there is growing interest in reducing TAM numbers or modifying their functionality, for instance, by reprogramming TAMs from a M2 (pro-tumor) to a M1 (anti-tumor) phenotype. The TME also influences the effectiveness of radiation therapy, as TME-stimulated immunosuppression may lead to radioresistance. Moderate doses of radiation (e.g., 5 Gy) can enhance the release of immune-stimulatory cytokines, promoting tumor infiltration by immune cells. This study aimed to develop cell therapy using M1 macrophages engineered to express IL-12, in combination with brachytherapy. These modified M1 macrophages are designed secrete IL-12 into the TME and reprogram existing pro-tumor TAMs into anti-tumor macrophages. Additionally, the study evaluated the effects of this therapy on lung metastases and systemic immune responses.
Materials and Methods: BALB/c mice with well-developed 4T1 breast cancer tumors were treated with M1/IL-12/RFP macrophages followed by contact radiotherapy (brachytherapy, BT) at a dose of 5 Gy. Tumor growth was monitored throughout the study. Flow cytometry analyses were performed to assess changes within the tumor microenvironment, including immune cell infiltration and vascularization.
Results: The results of the present study demonstrated that three doses of M1/IL-12/RPF and brachytherapy, at a single dose of 5Gy inhibited 4T1 tumors' growth and changed the number of innate and adaptive immune system cells. M1/IL-12/RPF macrophages effectively reduced the accumulation of protumorogenic M2 tumor-associated macrophages and increased the infiltration of cytotoxic CD8+ T lymphocytes. This therapy had an impact on changes in the levels of tumor-infiltrating immune cells and resulted in reduced tumor blood vessel density. Moreover, three doses of M1/IL-12/RPF decrease the number of lung metastases.
Conclusion: These data confirm the influence of combination BT and M1/IL-12/RFP macrophages on TME and constitute a promising strategy in anticancer therapy.
The work was financed by the National Science Center, Poland, UMO-2021/41/B/NZ5/03245

23. Alterations in Muscle Regeneration Following Total Body Irradiation in a Combined Radiation Setting

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The increasing risk of accidental or deliberate exposure to ionising radiation raises concerns regarding victim management. While the systemic effects of total body irradiation (TBI) are relatively well characterized, its impact on specific organs or tissues and their capacity to heal after injury remains poorly understood. Therefore, a deeper understanding of radiocombined injury is essential to improve management in such contexts.

This study was conducted on a rat model combining TBI (8 Gy, Cobalt 60 gamma rays, 0.3 Gy/min), inducing an acute hematopoietic radiation syndrome (H-ARS) with moderate mortality rate, with a localized muscular injury performed by myotoxin injection into the left soleus muscle. Clinical status, hematopoietic syndrome severity and muscle regeneration were assessed over a 63-days follow-up through clinical monitoring and sample collections (blood, bone marrow, spleen, muscle). 8 Gy TBI induced approximately 15% lethality and a severe hematopoietic syndrome, characterized by profound pancytopenia secondary to bone marrow aplasia. Circulating cytokine levels were also found to be profoundly reduced during this critical period. None of these radiation-induced symptoms was exacerbated by an additional muscle injury. However, muscle regeneration was significantly impaired by irradiation, with a significant reduction in muscle mass (20%) at the end of the study (D63). Histological analyses revealed an altered local inflammatory response and the presence of persistent necrotic fibres concurrent with hematopoietic syndrome. Structural alterations were still observed at D63, with a decrease in fibre diameter and localised fibrosis. Moreover, deleterious impact of irradiation on muscle regeneration was also confirmed by altered gene expression profiles of myosin subtypes and myogenesis transcription factors in the radiocombined context.

Our findings demonstrate that, although acute radiation hematopoietic syndrome severity remained similar when combined with muscle injury, irradiation impaired muscle regeneration and induced chronic muscle changes. These results highlight the importance of considering the impact of radiation on multiple organs, particularly injured ones. These patients will likely require prolonged acute follow-up and additional measures to improve long-term management in order to avoid chronic disability.

26. Surface-Functionalized Iron Oxide Nanoparticles as Novel Radiosensitizers

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Background Radiotherapy remains limited by off-target toxicity and insufficient tumor selectivity, highlighting the need for effective radiosensitizers(1). Increasing evidence identifies thioredoxin reductase (TrxR) inhibition as a central determinant of radiosensitization, as disruption of redox homeostasis amplifies radiation-induced oxidative damage(2). Iron oxide nanoparticles (IONPs) exhibit intrinsic radiosensitizing properties but remain insufficient to fully overcome tumor redox defences. Therefore, combining IONPs with metal-based TrxR inhibitors represents a rational strategy to potentiate redox imbalance and enhance therapeutic efficacy(3).

Materials and Methods: IONPs were surface-functionalized with chelating ligands and subsequently loaded with metal ions. Their physicochemical properties were characterized using dynamic light scattering (DLS) and inductively coupled plasma (ICP) analysis. The biological effects of the engineered IONPs were evaluated in vitro using the A549 cell line. Cytotoxicity was assessed by MTT assays, and enzymatic activity was quantified through absorbance-based measurements. Finally, the radiosensitizing potential of the platform was determined using clonogenic survival assays following irradiation with 225 kV X-rays delivered at a dose rate of 2 Gy/min.

Results: The obtained results demonstrate that the synthesized IONPs were stable and monodisperse in size. MTT assays indicated that the nanoparticles were non-cytotoxic up to 50 µg Fe/mL. In vitro, TrxR enzymatic activity was significantly reduced after 48h of incubation with ion-loaded IONPs compared to non-loaded nanoparticles. Finally, the amplification factor (AF) reached 12% for ligand-functionalized IONPs, whereas ion-loaded IONPs exhibited an increased AF of 27%.

Conclusions: The results demonstrate that the developed nanosystems act as effective radiosensitizers by significantly inhibiting TrxR activity and enhancing radiation-induced cytotoxicity in cancer cells.

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27. PARP-1 Binding Affinity to Different DNA Models and Inhibitors: A Microscale Thermophoresis Study Relevant to the Early Radiation-Induced DNA Damage Response

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Background Poly(ADP-ribose) polymerase 1 (PARP-1) is a key nuclear enzyme that functions as an immediate sensor of DNA damage and a central regulator of the cellular response to genotoxic stress. Upon binding to DNA strand breaks, PARP-1 catalyzes the transfer of ADP-ribose units from its natural substrate NAD onto itself and target proteins, forming poly(ADP-ribose) chains that recruit DNA repair machinery. Exposure to ionizing radiation increases PARP-1 activity levels significantly within one minute, reflecting its critical role in the early phase of the DNA damage response. PARP inhibitors are widely used in cancer therapy, particularly in combination with radiotherapy, where their effectiveness depends not only on catalytic inhibition but also on the phenomenon of DNA trapping.
Materials and Methods Binding interactions of PARP-1 were investigated using Microscale Thermophoresis (MST) on a Monolith NanoTemper NT.115 instrument. Dissociation constants (Kd) were determined for PARP-1 complexes with seven different inhibitors (talazoparib, olaparib, rucaparib, veliparib, iniparib, A966492, AZD2461) and with NAD. Additionally, PARP-1 binding was analyzed using various DNA models, including single-strand break (SSB), blunt-ended double strand break (DSB), single strand DNA (ssDNA), and double strand DNA (dsDNA) in a dumbbell configuration as a model of an undamaged DNA. MST was evaluated as an alternative to commonly used surface-based methods such as Surface Plasmon Resonance (SPR) and Bio-Layer Interferometry (BLI).
Results MST enabled reliable quantification of PARP-1 binding affinities across multiple inhibitors and DNA models. Distinct differences in PARP-1 affinity toward various DNA structures were observed, confirming its preferential recognition of strand break–like conformations. Analysis of selected inhibitors highlighted the dominant role of DNA trapping mechanisms. Additionally, we investigated whether the presence of DNA is required for PARP-1 activation and for enabling access of NAD and inhibitors to its catalytic pocket.
Conclusions This study demonstrates that MST is a powerful and versatile method for investigating PARP-1 interactions with their inhibitors and various DNA models. Compared with SPR and BLI, MST avoids surface immobilization effects, requires minimal sample quantities, and enables measurements in complex buffers, although it currently faces limitations in precise kinetic analysis. The findings emphasize that, in the context of ionizing radiation response, rapid PARP-1 activation and inhibitor-induced DNA trapping are critical determinants of therapeutic efficacy. Expanding MST toward improved kinetic resolution may provide new opportunities for studying dynamic protein–DNA interactions central to radiation-induced DNA repair processes.

31. Impact of leptin and female sex hormones on radiosensitivity of normal breast and endometrial cells

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Background Breast cancer development involves complex interactions between genetic, environmental, and lifestyle factors. Established risk factors include prolonged exposure to female sex hormones, obesity, and ionizing radiation. While each factor is independently characterized, their synergistic effects on the radiosensitivity of normal breast epithelium remain unexplored. The aim of this work is to test if leptin (the obesity hormone), in combination with estrogen (female sex hormone), plays a role in the radiosensitivity of normal breast cells.
Methods Normal breast epithelial cells MCF10A were cultured and treated for one week with: Estrogen alone (E2; 10nM), three different doses of leptin (L50ng/ml, L100ng/ml, and L200ng/ml), and a combination of both estrogen and the different leptin doses. (E+L50ng/ml, E+L100ng/ml, and E+L200ng/ml). All the experimental groups were exposed to acute γ-radiation (3.4 Gy) after the treatment, and analysed γH2AX foci, chromosomal aberrations by FISH, and gene expression of selected genes by RT-qPCR as markers of DNA damage and repair.
Results Preliminary data from γH2AX foci suggest that cells treated with solely E2 have higher DNA damage, while those treated with different doses of leptin had reduced foci frequency. A similar recovery effect of leptin was observed for gene expression.
Conclusion Preliminary data indicates the effect of estrogen and leptin alone and in combination with response to radiation. Repeated experiments will give more results which will provide a better understanding.

63. Do advanced in vitro models improve prediction of radiosensitization? A comparison of 2D, 3D, and Organ-on-a-Chip systems

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Background: In vitro cell-based models are considered the gold standard in the early stages of drug and therapeutic development. They are typically based on 2D culture systems, which have limitations, including simplified cell interactions and the lack of physiological gradients. Therefore, more advanced models, including 3D cultures and Organ-on-a-Chip platforms, are being developed to better mimic in vivo conditions. While 3D systems provide greater structural complexity than 2D cultures, Organ-on-a-Chip devices further improve physiological relevance by incorporating dynamic flow and microenvironmental control. These advanced models more accurately reproduce tumor architecture and microenvironmental features, such as oxygen gradients and hypoxia. This is particularly important in cancer research, where realistic in vitro models are essential for evaluating radiosensitizers — agents that increase tumor cell sensitivity to ionizing radiation and enhance the effectiveness of radiotherapy.

Materials and Methods: DU-145 and RWPE-1 cell lines were cultured under standard conditions and used to compare responses across in vitro models. Spheroid morphology was confirmed using an Olympus IX73 microscope after staining with calcein-AM and propidium iodide (PI). Cytotoxicity of the tested radiosensitizers was evaluated using the MTT assay in 2D cultures and the Alamar Blue assay in 3D and Organ-on-a-Chip systems. The viability of control cells was set to 100%. Measurements were performed using a Victor Nivo multimode microplate reader (Revvity, USA) and experiments were conducted in triplicate. Clonogenic survival in DU-145 cells was assessed following treatment and exposure to ionizing radiation (0 – 6 Gy). Colonies were formed over 12 days, then fixed, stained, and counted. Survival curves were generated using the linear–quadratic model, and all statistical analysis was performed using GraphPad Prism 7 software.

Results: Spheroid formation was successfully achieved in both 3D culture systems. In conventional 3D plate cultures, well-defined spheroids with a visible necrotic core were formed after approximately 7–8 days, whereas in the Organ-on-a-Chip system, comparable structures were observed after only 2–3 days of culture. Cytotoxicity assays revealed differences in cellular responses depending on the model used, with 3D and Organ-on-a-Chip systems generally showing reduced sensitivity to the tested radiosensitizers compared with 2D cultures. Clonogenic analysis demonstrated significant differences in radiobiological response to ionizing radiation across the tested in vitro models, emphasizing the influence of model complexity.

Conclusions: Advanced in vitro models, including 3D cultures and Organ-on-a-Chip systems, provide a more physiologically relevant platform for studying tumor responses to ionizing radiation. Notably, Organ-on-a-Chip devices enable faster formation of tumor-like structures and offer enhanced microenvironmental control compared to conventional 3D cultures. The observed differences between models highlight the importance of model selection for accurate evaluation of radiosensitizers.

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69. Use of the novel cell division assay (CDA) to evaluate T cell toxicity in response to lutetium-177–DOTATATE peptide receptor radionuclide therapy

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Background: The treatment with lutetium-177–DOTATATE (¹Lu-DOTATATE) improves survival in patients with metastatic neuroendocrine tumors but is limited by bone marrow toxicity. Individualized dose planning is challenging due to uncertainty in absorbed dose estimates and patient sensitivity to radiation. Here, we investigated the in vitro sensitivity of patient T cells to 177Lu-DOTATATE as a tool in individualized dose optimization.
Material and method: Blood samples were collected from healthy individuals. Peripheral blood mononuclear cells were prepared and treated with different concentrations of 177Lu-DOTATATE, corresponding to specific absorbed doses. Cells were also exposed to external beam radiation as a comparison. Cell survival and DNA damage was then analyzed using our in-house developed CDA and γH2AX assays
Results: Our data indicate a dose dependent, but non-linear in vitro T cell sensitivity to 177Lu-DOTATATE at treatment relevant doses. We detected an unexpected hypersensitivity at very low (0.15-0.25) radiation doses. The 177Lu-DOTATATE individual cell sensitivity correlated with EBR sensitivity. However, the kinetic of 177Lu-DOTATATE double-strand break induction in T cells was different to EBR as indicated by γH2AX staining.
Conclusion: The CDA could measure the in vitro sensitivity of T cells to 177Lu-DOTATATE and may be a useful tool for evaluating individualized hematological toxicity.

71. Dissecting Radiation-Induced Tumorigenesis: Single-Cell Insights into Microenvironmental Remodeling in SHH Medulloblastoma

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Background Radiation-induced carcinogenesis has classically been explained by the accumulation of unrepaired or misrepaired DNA damage leading to genomic instability. More recently, however, it has become apparent that ionizing radiation can elicit broader biological responses beyond direct genotoxic effects. These include alterations in the tissue microenvironment, inflammatory and stress responses, and epigenetic modifications that may influence cell behaviour and tissue homeostasis. Such radiation-induced changes are thought to interact with intrinsic cellular susceptibility and developmental context, potentially modulating cancer risk. The relative contributions and interactions among DNA damage, microenvironmental alterations, and epigenetic regulation in radiation-induced tumorigenesis are complex and not yet fully elucidated.

Materials and Methods Ptch1+/ mice, a model recapitulating key molecular and histopathological features of Sonic Hedgehog (SHH)–driven human medulloblastoma (MB), were exposed postnatally (day 2) to low (0.1 Gy) or moderate doses (2 Gy) of 60Co gamma radiation (γ-rays). Following irradiation, cerebella were harvested and processed for single-cell transcriptomic profiling. Tumor incidence and latency were monitored longitudinally.

Results To investigate the impact of radiation-induced microenvironmental alterations on carcinogenesis, we employed Ptch1+/ mice, a well-established model of radiation-induced tumorigenesis. In this model, exposure to a moderate dose significantly increases MB incidence and accelerates tumor onset, whereas low-dose exposure elevates tumor risk without affecting latency. This system therefore provides a robust framework to dissect the interplay between genetic susceptibility and ionizing radiation.

To further elucidate the molecular mechanisms underlying microenvironment-driven tumor promotion, cerebellar tissues were collected one week following postnatal irradiation (P2) with either moderate (2 Gy) or low (0.1 Gy) doses and subjected to single-cell transcriptomic profiling. The analysis identified distinct cellular populations and signaling pathways differentially engaged in a dose-dependent manner. Among the most affected compartments, endothelial cells exhibited a marked expansion across both radiation doses. Microglial populations were significantly increased exclusively following low-dose exposure. In contrast, proliferating granule cell precursors emerged as the most radiosensitive population, showing an approximate 50% reduction after moderate-dose irradiation. **Conclusions** Radiation doses imprint distinct oncogenic trajectories in the developing cerebellum, which modulate MB risk, indicating that radiation induced tumorigenesis may arise through heterogeneous, non–dose dependent mechanisms.

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80. Exploring the Metabolic Modulation of Ionizing Radiation-induced DNA Damage Response

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INTRODUCTION Ionizing radiation (IR) is a major source of physiological and biochemical damage in the human body, which can lead to both deterministic and stochastic effects (Upton, 1977). Since DNA is the key target biomolecule for IR-induced damage, chronic low-dose exposures to these IRs typically induce various simple and complex DNA lesions (International Agency for Research on Cancer, 2000). This engages a multifaceted DNA damage response (DDR) mechanism consisting of damage sensors, repair proteins, and pathways (Wilkinson et al., 2023). While the DDR pathways are extensively investigated, their interaction with coordinating critical physiological processes remains incompletely understood. Evidence shows that cellular metabolism can be a key modulator of DDR responses under IR exposure, which can influence radiosensitivity and radioresistance (Cucchi et al., 2021). Specifically, mTOR-mediated modulation of IR-induced DDR is an increasingly recognized metabolic-DDR connection (Chen et al., 2010). Although this interplay is well-understood in cancer cells, there are limited insights into the mechanistic and genetic underpinnings, especially in healthy cells or under different kinds of radiation exposure.
METHODOLOGY: The study establishes sub-lethal doses of fractionated X-rays (0-10 Gy) and non-cytotoxic doses of pharmacological mTOR modulators (metformin and rapamycin) in normal as well as cancer cell types (such as hTERT RPE1 and A549 cells). Cell survival is assessed using clonogenic assays, while DNA damage and repair kinetics were quantified via γH2AX immunofluorescence. mTOR pathway involvement and activity are assessed through western blotting of the downstream proteins. Importantly, a pooled CRISPR-Cas9 knockout screen is conducted under different experiment conditions using a customized library of sgRNAs targeting around 1137 genes involved in DDR. Following the selection period, sgRNA abundance is quantified using next-generation sequencing, which will be further bioinformatically analysed for gene essentiality, enrichment, and depletion across conditions.
RESULTS AND CONCLUSION Results include the comparison of differential DNA damage foci and cell survival with different doses of drugs and radiation, in different experimental groups. CRISPR screens will identify key DDR genes that determine radiosensitivity and radioresistance, as well as those whose function is dependent or independent of mTOR signalling. Different cell types will reveal context-dependent observations under radiation exposure. In conclusion, this study provides a systematic and mechanistic framework to map the mTOR axis and DDR genes interaction under clinically relevant fractionated X-rays. These findings can be applied to the improvement of precision radiotherapy through radiosensitization strategies, in radioprotection for human spaceflight, and drug repurposing to modulate radiation outcomes. References:

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83. Comparative Radiobiological Responses in 2D and 3D Cellular Models Exposed to X-rays and Ultrashort Pulsed Electron Beams

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Background: Despite the growing transition in radiobiological research toward more physiologically relevant 3D models, emerging irradiation modalities such as ultrashort pulsed electron beams (UPEB), currently being developed as promising alternatives to conventional radiation sources, remain poorly investigated in 3D systems. This study aimed to compare DNA damage, oxidative stress, and cell survival in malignant and non-malignant lung cell models cultured under 2D and 3D conditions following exposure to X-rays and ultrashort pulsed electron beams (UPEB).
Materials and Methods: A549 (malignant, ATCC CCL-185) and MRC-5 (non-malignant, ATCC CCL-171) cells were maintained under standard culture conditions. Three-dimensional spheroids were generated by the hanging drop method and cultured for 7 days before irradiation. Samples were exposed to 1 Gy of either X-rays (MultiRad 350 kV system, Precision) or UPEB (AREAL linear accelerator, CANDLE SRI, 3.6 MeV, 0.45 ps, UHPDR 10^{10} Gy/s). DNA damage (comet assay, 4 and 24 h), clonogenic survival, and intracellular ROS levels (DCFDA-based two-photon microscopy, 4 and 24 h) were assessed for comparative analysis.
Results: After 1 Gy X-ray exposure, clonogenic survival remained high in both lines (SF˜0.6-0.75), with moderate 2D-3D differences. In contrast, UPEB markedly reduced reproductive survival, with complete loss in MRC-5 3D spheroids, while A549 spheroids remained relatively resistant (SF0.65). In 3D A549 spheroids, both X-ray and UPEB induced early DNA damage that decreased by 24 h, suggesting efficient repair. In contrast, 3D MRC-5 spheroids showed persistent DNA damage up to 24 h, particularly after UPEB exposure. In 2D cultures, DNA damage was more pronounced in A549, whereas MRC-5 showed no change after X-ray exposure but increased damage following UPEB irradiation. Both X-ray and UPEB irradiation induced ROS accumulation in 2D cultures of both cell types, with a weaker effect observed after UPEB, while 3D spheroid models showed no oxidative response.
Conclusions: The results highlight the importance of 3D models in evaluating emerging irradiation modalities, revealing effects not evident in 2D cultures. The greater sensitivity of non-malignant MRC-5 spheroids compared with malignant A549 spheroids may reflect a less cohesive 3D organization and a reduced capacity to repair radiation-induced damage and maintain clonogenic potential after UPEB exposure. Notably, UPEB induced pronounced effects in 3D spheroids despite minimal ROS response, suggesting the involvement of ROS-independent mechanisms. Further studies are needed to clarify the mechanisms underlying the differential effects of UPEB and X-ray irradiation in 3D systems, including the possible contribution of irradiation pulse structure.

88. Sequential events of apoptosis in the irradiated zebrafish neuroepithelium

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The embryonic neuroepithelium harbours proliferating neural progenitor cells that populate the future brain. These cells are highly sensitive to ionizing radiation and succumb to apoptosis within hours after exposure. Because apoptosis is a dynamic process taking place in ordered sequences, delineating its stages *in situ* will elucidate how apoptosis progresses in the irradiated neuroepithelium. In this study, we perform 2 Gy total-body irradiation on the apoptosis-reporting *secA5* transgenic zebrafish embryos and analyze apoptosis in the forebrain neuroepithelium at 2, 6, 10, 14 and 20 h postirradiation. We find that Caspase-3 activation precedes phosphatidylserine exposure on the neuroepithelial cell membrane, which is then followed by chromatin condensation and cell protrusion. By classifying apoptotic neuroepithelial cells into initial, middle and latent stages, we show that apoptosis initiates around 6 h postirradiation and peaks around 10 h, while its entire progression extends until 20 h and beyond. These results reveal the timing and progression of apoptosis in the irradiated neuroepithelium and underscore the promise of secA5 transgenic zebrafish as model for studying radiation-induced cell death under natural physiology.

99. The level of gamma-H2AX foci in peripheral blood mononuclear cells of patients undergoing radiotherapy for primary and second primary cancer

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Background: The level of γ-H2AX foci in peripheral blood mononuclear cells (PBMC) has emerged as a sensitive biomarker for assessing DNA double-strand breaks induced by ionising radiation. It is also a promising biomarker for predicting response to radiotherapy (RT) and individual radiosensitivity in cancer patients. In this context, our study aims to evaluate and compare the level of γ-H2AX foci in PBMC from patients with first primary cancers (FPC) and second primary cancers (SPC) following radiotherapy.

Materials and Methods: Peripheral blood samples (PB) were collected from 232 patients treated with external-beam radiotherapy for FPC of the breast, brain, or lymphatic tissue, and from 96 unrelated patients who developed various SPC. PB samples from patients with FPC were collected before the first radiotherapy fraction and at two timepoints (1 and 4 weeks) after the last radiotherapy fraction. PB samples from patients with SPC were collected upon hospital admission and 4 weeks after the last radiotherapy fraction. Peripheral blood mononuclear cells (PBMC) were isolated and analysed for γ-H2AX foci fluorescence in vivo. In addition, the response of PBMC to an in vitro irradiation with a dose of 2 Gy was analysed at the level of γ-H2AX foci fluorescence.

Results: Our results revealed no significant differences in the level of γ-H2AX foci fluorescence in PBMC in vivo between patients with FPC and SPC following radiotherapy. However, the in vitro results showed significant differences in the efficiency of γ-H2AX foci fluorescence disappearance (DNA repair) in PBMC from patients with FPC and SPC after in vitro irradiation. The most efficient DNA repair in vitro was observed in PBMC from breast cancer patients. The least efficient DNA repair in vitro was observed in PBMCs from patients with lymphoma and SPC.

Conclusions: The findings suggest that PBMC from patients who develop SPC following RT are not characterised by enhanced radiosensitivity, as measured by the level of γ-H2AX foci in vivo and in vitro.

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106. Variation of individual DNA damage response of U2OS cells exposed to photon radiation

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Background: Individual cells of the same type and in the same position in the cell cycle show stochastic cell-to-cell variability (noise) in gene and protein expression, even under the same environmental conditions. The aim of the study was to test if the DNA damage response of cells shows noise, on top of the randomly distributed DNA damage.

Materials and Methods: This was tested by comparing the frequency of DNA repair foci in sister nuclei of binucleated cells (intra BNC variability) and nuclei of two BNC (inter BNC variability). Inter cell variability was also measured for mononucleated cells (MNC). Binucleation was induced in U2OS cells by cytochalasin B. Cells were exposed to 2 Gy of photons, 53BP1 and γH2AX foci were visualised and counted in nuclei of binucleated and mononucleated cells 30, 60, 120 and 180 min post exposure. Coefficients of variation were calculated as indicators of variability.

Results: For both types of foci, the frequencies observed in sister nuclei within BNC were positively correlated and exhibited significantly lower variation compared to the variability measured between single nuclei from randomly paired BNC. The contribution of noise to the total variability was 28 % for 53BP1 foci and 50 % for γH2AX foci.

Conclusions: The results demonstrate that the variability of focus frequency observed in nuclei of BNC results from a combination of noise and random damage variability.

111. Ascorbate induces cancer cell death without direct DNA double-strand break formation through delayed chromatin degradation and necrotic response

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Background DNA double-strand breaks (DSBs) are widely considered the primary lesions underlying the effectiveness of radiotherapy and many chemotherapeutics. Pharmacological ascorbate has shown promising anticancer effects, largely attributed to oxidative stress–induced DNA damage. However, the role of direct DSB formation and repair pathways, particularly non-homologous end joining (NHEJ), in ascorbate-induced cytotoxicity remains unclear.
Materials and Methods We investigated the effects of pharmacological ascorbate using wild-type and NHEJ-deficient (XRCC4 and DNA-PKcs knockout) cancer cell models. Cell survival and viability were assessed in both 2D and 3D systems. DNA damage was evaluated using pulsed-field gel electrophoresis (PFGE) and immunofluorescence analysis of 53BP1 and γH2AX. Cell cycle distribution and cell death modalities (apoptosis and necrosis) were analyzed by flow cytometry.
Results Ascorbate reduced cell survival and viability in a concentration-dependent manner across all cell models, with increased sensitivity observed in XRCC4-deficient cells. PFGE analysis demonstrated that, unlike ionizing radiation, ascorbate did not induce prompt DSBs. Consistently, early time-point analysis showed no significant increase in 53BP1 foci. However, 24 hours post-treatment, increased 53BP1 foci and pronounced pan-nuclear γH2AX signals were observed, suggesting chromatin degradation and delayed DNA damage formation. Ascorbate induced G2/M cell cycle arrest, particularly in XRCC4-deficient cells, and triggered a strong necrotic response without activation of apoptosis at higher concentrations.
Conclusions Pharmacological ascorbate induces cancer cell death independently of direct DSB formation, instead promoting delayed DNA damage associated with chromatin degradation and necrosis. These findings challenge the paradigm that direct DSB induction is the primary driver of therapy-induced cytotoxicity and highlight alternative mechanisms that may be exploited for cancer treatment. These findings suggest that delayed-DSB-driven mechanisms contribute to therapy response and warrant further investigation of DNA repair pathway engagement under oxidative stress.

126. Radiation Enhances the Cellular Connectivity Through Tunneling Nanotubes in Glioblastoma Cells

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Background: Developing therapy resistance and exhibiting high invasiveness are significant challenges in treating aggressive cancers, such as glioblastoma, where intercellular communication plays a crucial role in cellular organization, survival, and resistance to treatment. Tunneling nanotubes (TNTs), nanometer-sized membranous channels that connect distant cells, have emerged as an efficient form of intercellular communication that may enable cancer cells to evade therapeutic interventions.

Methods: In this study, we investigated the responses of TNT networks to low linear energy transfer (low-LET) X-ray irradiation in two established glioblastoma cell lines, U87 MG and LN229. Initially, we assessed radiosensitivity using colony formation assays to measure cell survival. Then, we used confocal live-cell microscopy to monitor TNT network dynamics over a 24-hour period following irradiation and performed co-staining experiments to identify cargoes transported through TNTs.

Results: We observed a significant increase in TNT-mediated cellular connectivity 6 to 10 hours after 1.8 Gy X-ray irradiation in both cell lines. In contrast, cells treated with a higher radiation dose (3.9 Gy) exhibited reduced TNT connectivity; however, it remained slightly elevated compared to sham-irradiated controls. The co-staining experiments revealed the presence of calcium and mitochondria within TNTs. These cargoes are known to facilitate cancer cell migration and survival, potentially contributing to treatment resistance.

Conclusions: Taken together, these results strongly suggest that TNT-mediated intercellular communication may be a critical mechanism that supports glioblastoma resistance to radiotherapy.

130. Spatiotemporal Interaction of DNA Damage for Investigating the Biological Effects of High-LET Particle Radiation

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Co-authors: Daniela Schilling [1]; Giuseppe Bevilacqua [2]; Jessica Neubauer [3]; Judith Reindl [3,5]; Laura Quitero Velasquez [3]; Nikolaos Mallousis [3]; Paulina Hromekova [1]; René Heller [4]; Robert Reisdorf [4]; Stephanie E. Combs [1]; Thomas Friedrich [2]; Thomas Schmid [1]

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Research Question: The biological effectiveness of ionizing radiation is strongly influenced by the spatiotemporal organization of induced DNA damage. While single- and double-strand breaks form within milliseconds, their interactions may persist over minutes to hours. This is particularly relevant for high linear energy transfer (LET) radiation, which induces complex, clustered DNA damage on submicrometer scales. However, the contribution of such delayed interactions to the overall cellular radiation response remains insufficiently understood.
Methods: Clonogenic survival was assessed using a colony formation assay optimized for low cell numbers and small irradiation fields. U2OS cells stably expressing GFP-tagged MDC1 were employed to visualize DNA damage response dynamics following irradiation with photons, protons, and carbon ions. The spatiotemporal recruitment of MDC1 to DNA double-strand breaks was monitored using epifluorescence live-cell imaging with high temporal and spatial resolution.
Results: The optimized colony formation assay enabled reproducible measurement of clonogenic survival despite limited irradiated areas and low cell numbers. Stable expression of MDC1-GFP in U2OS cells provided robust visualization of radiation-induced DNA damage. Following broadbeam irradiation with protons and carbon ions, rapid MDC1 recruitment to damage sites was observed. The integration of fluorescence microscopy with single-cell analysis allowed precise spatial localization of DNA lesions and detailed temporal tracking of repair dynamics.
Conclusion: The established experimental platform enables highly resolved spatiotemporal analysis of DNA repair following irradiation. These methods provide a foundation for systematic investigations of temporally structured irradiation schemes and contribute to a deeper understanding of LET-dependent biological effects of ionizing radiation.
Funded by the Federal Ministry for Research, Technology and Space (BMFTR), collaborative project “BIOMICRO” (02NUK095C).

Tuesday, 08.09.2026

FLASH + Postersession 2

Chair: Nina Edin
Room: FLASH Session in Atlantis 1, Postersession in Atlantis 2

12:00

High-throughput flow cytometry screening to identify inhibitors of proton radiation–induced DNA repair in glioblastoma
Linn Helena Steive

Physical Characterization of Preclinical Conventional and FLASH High-Energy X-ray Irradiations using a Rhodotron®
Dr Jolie BOU-GHARIOS

Evaluation of brain electrical activity and different blood parameters as biomarkers of ionising radiation exposure
Nicolas Morin

A framework for experiments and simulations of LET and RBE in proton irradiation *Britt Haanen*
Atlantis 1 12:04 - 12:05

Hydrogels as new materials for space radiation shielding applications *Robin Vanhoeijen*
Atlantis 1 12:05 - 12:06

SOBP proton irradiation of CHO cells for Dirty Dose Kill model parameterization *Ms Matilde Helgesen*
Atlantis 1 12:06 - 12:07

Minibeam Radiotherapy Improves the Efficacy of Checkpoint Blockade in Syngeneic Head and Neck Tumors
Parmeshwar Katare

Enhancing Radiotherapy via Cherenkov Light Photoactivation in Cancer Treatment *Dr Justyna Czapla*
Atlantis 1 12:09 - 12:10

Towards Silicon Carbide Dosimeters for Radiation Monitoring in Space and Harsh Environments *Daniel Radmanovac*
Atlantis 1 12:11 - 12:12

Investigating the Use of Helium Ions in Spatial Fractionated Radiotherapy: A Simulation and In Vitro Study
Isabella Hestholm

Engineering Radiation Resistance in Human Cells Through the Tardigrade-Derived DNA Repair Mechanism, the Dsup
Natassha Shanmugam

Preclinical FLASH and minibeam experiments at the Dresden proton therapy facility *Dr Joerg Pawelke*
Atlantis 1 12:15 - 12:16

39. High-throughput flow cytometry screening to identify inhibitors of proton radiation–induced DNA repair in glioblastoma

Author: Linn Helena Steive[1]
Co-authors: Alexandra Gade; Johannes Landskron; Randi Syljuåsen; Tiril Hillestad; Tord Hompland
[1] *Oslo University Hospital*

Background: Glioblastoma (GBM) is a primary brain malignancy with significant unmet clinical need and poor prognosis which is partially attributable to its pronounced radioresistance. Proton radiotherapy is considered promising for GBM due to its favourable dose distribution and precision of delivery. Protons induce higher ionisation density compared with X rays, generating more clustered and complex DNA damage. This is thought to activate distinct DNA repair mechanisms and cell signalling pathways resulting in differing biological responses. We hypothesise that this can be exploited to identify compounds that preferentially radiosensitise GBM cells to proton irradiation.

Materials and Methods: We previously established a high throughput, flow cytometry–based screening platform using Reh suspension cells in 384 well plates[1]. The assay quantifies radiation induced DNA damage repair (γH2AX), G2/M checkpoint abrogation (phospho histone H3), and DNA content (Hoechst). In the current work, we optimised the protocol for direct comparison of drug effects in proton versus X ray irradiated cells. Samples from four parallel plates, treated with or without 6 Gy proton or X-ray radiation, were barcoded using differential AlexaFluor 647 labelling and pooled before antibody staining and analysis, enabling simultaneous multiparameter screening of ˜10,000 cells per condition. We developed a spread-out bragg peak (SOBP) proton radiation protocol to deliver a uniform dose across the entire medium volume in a 384-well plate format. The SOBP protocol utilises four differentially weighted energy layers (69MeV (0.481), 72MeV (0.226), 74.9MeV (0.162), 77.5MeV (0.132)) to deliver a uniform dose of 6 Gy with linear energy transfer ranging from 4.6 to 7.6 keV/µm across the SOBP.

Results: Previous validation demonstrated that the platform robustly identifies DNA damage response–targeting radiosensitisers across multiple cell lines treated with X-rays[1]. In the newly optimised setup, parallel proton and X ray screens can be performed in a single run, allowing sensitive comparison of drug induced effects on DNA repair and G2 checkpoint regulation. This enables systematic identification of compounds with differential radiosensitising potential between proton and X ray irradiation.

Conclusions: We have optimised a high throughput flow cytometry screening approach to identify compounds that inhibit DNA repair and/or abrogate the G2 checkpoint following proton versus X ray irradiation. Screening of >2,000 compounds is currently underway, with results expected in the next few months. This work will identify candidate DNA repair inhibitors for further testing in GBM models and provide insight into the distinct biological responses induced by proton irradiation.

References: 1 Naucke, C., Rødland, G. E., Eek Mariampillai, A., Hauge, S., Steive, L. H., Bjerke, I. A., Lindbergsengen, L., Grøsvik, A. S. G., Siggerud, V., Kongsrud, K., Savu, D., Stokke, T. & Syljuåsen, R. G. A flow cytometry-based screening platform identifies candidate radiosensitizers targeting the DNA damage response [Manuscript submitted for publication, 2026].

42. Physical Characterization of Preclinical Conventional and FLASH High-Energy X-ray Irradiations using a Rhodotron®

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Background: Ultra-high dose-rate (UHDR) "FLASH" radiotherapy is a promising approach to spare normal tissues without compromising tumor control. Yet, most FLASH preclinical studies rely on electrons, low-energy photons or protons and data with high-energy X-rays remain scarce. Clinically, megavoltage (MV) X-rays are widely used to treat tumors, making the development of a robust high-energy X-ray FLASH platform essential. We performed physical and dosimetric characterization of a 10 MV X-ray beam generated by an industrial Rhodotron® at conventional (CONV) and UHDR to qualify a dedicated setup for future FLASH studies on mouse brain irradiations.

Materials and Methods: A TT300 Rhodotron® with a 10 MeV electron beamline and a tungsten target to generate 10 MV X-rays was used. Beam characterization included technical optimization of the 10 MV line and detailed dosimetry at CONV and UHDR. Depth-dose curves, dose profiles and dose-rate measurements were assessed in RW3 solid water plates using PinPoint 3D (TM31016) ionization chamber (IC, PTW), Gafchromic EBT4 films and alanine dosimeters over a beam current range of 0.025–5 mA. Alanine was used for confident dosimetric reference at CONV and UHDR, along with the IC at CONV dose rates, while EBT4 films were used for relative profiling, 2D mapping and comparative dosimetry under collimator/shielding unit. Monte Carlo simulations guided the design of a dedicated collimator and body shielding unit for mouse brain irradiations. Reproducibility of dose rate and spatial dose distribution were evaluated in mouse phantoms for CONV and UHDR protocols.

Results: Depth-dose curves showed a dose maximum at 1.3 cm and a PDD, of 0.49,comparable with a clinical 6 MV FFF beam and were comparable between CONV (0.05 mA) and UHDR (5 mA), indicating unchanged beam quality with dose-rate. Dose-rate versus current was linear for all detectors within the investigated range. A homogeneous field of 2.26 × 1.4 cm was considered suitable for mouse brain irradiation. Technical improvements, including a Monte Carlo–designed collimator and optimized beamline settings, enabled stable UHDR delivery and reproducible setup. Dose-rates >100 Gy/s were reached in mouse head phantoms and dose variability at the target position remained low (2% for CONV and FLASH) across repeated irradiations, confirming good reproducibility.

Conclusions: We validated a 10 MV X-ray beamline on an industrial Rhodotron® capable of delivering both CONV and UHDR FLASH irradiations with preserved beam homogeneity and quality, relevant depth-dose characteristics and highly reproducible dosimetry in mouse head phantoms. This platform provides a solid physical basis for upcoming in vivo studies on the FLASH effect of high-energy X-rays on normal brain and intracranial tumors, which represents a key step toward future clinical translation of X-ray UHDR at megavoltage energies.

43. Evaluation of brain electrical activity and different blood parameters as biomarkers of ionising radiation exposure

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In the context of increasing geopolitical instability, nuclear or radiological incidents — accidental or intentional — represent a credible threat that could lead to a massive influx of victims who could develop acute radiation syndrome. Once the medical and surgical emergency phase is over, rapid identification and triage of irradiated individuals is essential for effective medical treatment for those victims most likely to develop early and life-threatening symptoms. Current biological dosimetry techniques exist but are insufficient for mass events triage: blood cell counts lack irradiation specificity, and while cytogenetics is accurate, it is too slow for large-scale emergencies. Furthermore, despite being an important information, absorbed dose does not take into account inter-individual variability.

The objective of this study is to evaluate the potential of cerebral electrical activity, assessed through electroencephalography (EEG), as an early biomarker for radiation exposure and compare with haematological and biochemical biomarkers. Indeed, previous evidence exists of early radiation-induced central nervous system changes1–5, even at non-lethal doses. The purpose is to identify exposed subjects and anticipate the severity of symptoms following irradiation, in order to improve medical management in large-scale radiological emergencies.

The methodological approach is based on a rat irradiation model. Animals were subjected to whole-body irradiation with 8.7 Gy (LD) of Cobalt-60 gamma radiation delivered at a dose rate of 0.3 Gy/min. A subset of animals was implanted with EEG transmitters prior to irradiation, while the remaining animals underwent longitudinal haematological and biochemical blood sampling.

Our preliminary results reveal that exposed animals show an increased prevalence of sleep stages associated with light slow-wave sleep (less restorative) at the expense of deep slow-wave sleep. Furthermore, sleep quality is impaired after exposure compared to non-irradiated rats due to greater instability in the transitional and paradoxical slow-wave sleep stages.

Alongside this, on non-implanted animals, initial analyses confirm a decrease in the number of lymphocytes and thrombocytes, and certain blood markers appear to be disrupted after irradiation. Flt3-ligand is particularly affected by irradiation, with an increase leading to a concentration almost twice that of non-irradiated rats.

Cerebral electrical activity appears to be a promising biomarker of radiation exposure, complementing the information provided by blood compound analysis in triage aimed at distinguishing between irradiated and non-irradiated subjects. We are continuing our experiments to evaluate the prognostic potential of these markers in order to anticipate the severity of acute radiation syndrome, taking into account inter-individual variability.

51. A framework for experiments and simulations of LET and RBE in proton irradiation

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Background: Besides radiation dose, biological response from proton irradiation depends on the linear energy transfer (LET). Previous research suggests variable Relative Biological Effectiveness (RBE) of protons, with increased RBE at the distal end of the Bragg Peak (BP) due to high LET at this location. This study aims to establish and demonstrate an integrated framework for investigating LET and RBE in proton irradiation, using a model system of U2OS sarcoma cells. Physical and biological endpoints are consistently linked using dosimetry and simulations to support and verify the irradiation setup.
Methods: Irradiations were performed with 69 MeV protons and 180º gantry angle at a Varian ProBeam360 system, at the proximal and distal part of the BP. The dose delivery and associated uncertainties at both positions were quantified using ion chamber dosimetry. A sensitivity analysis was performed to assess the impact of setup parameters on dose at both depths. This provided an uncertainty budget in an established setup for cell irradiations.
The setup was simulated in m-RayStation for a cell layer placed at either of the two BP locations. Using Raystation's in-built Monte Carlo (MC) the dose and LET in the cell layer was estimated.
Cells were irradiated at the two BP positions with 0, 2, 5 and 8 Gy. Reference irradiation was done with 225 kV X-rays. A linear-quadratic (LQ) cell survival model including a LET-dependent term (Rørvik et al) was fitted to the survival data, which further established a relationship between RBE and LET. Results: Both simulation and dosimetry demonstrated increased dose sensitivity when moving from the front to the back of the BP. For the latter, ±2.5% dose variation was found for different cell flask bottoms while ±1.8% variation was found across the plate supporting the flasks (for 180º gantry angle). In front of the BP, these variations were less than 0.5%. This uncertainty is also reflected in the LET values for the distal BP position, giving 16.0 ±1.0 keV/µm. The LET at the proximal BP position was 1.3 keV/µm.
Cell survival at the different dose levels was similar for protons with low LET (front of BP) and X-rays, while lower survival was found for protons with higher LET. Fitting the LQ model with LET term (Rørvik et al) to the survival data gave a reasonable result with a Pearson's R2 around 0.9. The data supports the idea of a variable RBE with increasing LET, exhibiting RBE=1.0-3.0 ±0.18, with RBE of 1.5±0.18 and upwards for LET=16.0 keV/µm
Conclusion: A framework for estimating RBE in preclinical proton irradiations, based on dosimetry, simulations and experiments, was successfully established. Dosimetry for the cell irradiation setup has led to improvements to reduce uncertainty in the proton dose delivery. For the U2OS cell line, our analysis indicates an RBE at the distal edge of 1.5-3.0 ±0.18. Our framework is expected to provide more knowledge of RBE as a function of LET, and support more sophisticated RBE modelling.

57. Hydrogels as new materials for space radiation shielding applications

Author: Robin Vanhoeijen[1]
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Background: With growing interest in deep space exploration, more effective protection against ionizing cosmic radiation is essential. As current shielding materials are barely capable to shield against the highly energetic particles, novel shielding materials have to be developed. Since low-Z materials, such as hydrogen, have superior shielding capabilities against particulate radiation, water is considered a promising candidate. However, since water in its conventional form is impractical in electronics-rich and microgravity environments, hydrogels are considered a more compelling alternative. Hydrogels are chemically tunable, 3D polymeric networks capable of retaining large amounts of water. In this research, different innovative hydrogel shielding materials are developed and their shielding effectiveness will later be compared via the quantification of dsDNA breaks in a tissue-engineered skin model. Moreover, by integrating metal-based fillers, we aim to mitigate the effects of secondary photons and neutrons produced inside the shielding material as well, by using elements having a high-Z and neutron absorption coefficient.
Materials & Methods: Tunable hydrophilic polymers were synthesized, that can be crosslinked into a 3D network by UV-light into hydrogels of varying thicknesses. These hydrogels were doped with different high-Z compounds, to improve the radiation shielding as well as increase the mechanical properties of the resulting materials. The physicochemical properties, as well as the behavior upon irradiation with X-rays of the doped materials were tested with different assays (tensile testing, swelling, gel fraction, TGA, FT-IR, DSC). Moreover, a tissue-engineered human skin model was set-up to assess DNA damage upon irradiation with X-rays. Lastly, GEANT4 Monte Carlo simulations on the different developed materials are currently being performed to assess the shielding capability of the different hydrogels against high energy protons.
Results: The developed hydrogels are absorb 3 to 16 times their own weight in water, and maintain their mechanical integrity upon irradiation with X-rays, while maintaining a gel fraction of over 95%. Moreover, we have successfully shown that a metal oxide can be chemically modified into a UV-reactive monomer (methacrylate-containing metal) so it can be covalently coupled inside the polymer network, making it possible to tune the resulting mechanical properties of the hydrogels (Youngs moduli ranging from 75 kPa to 2.3 MPa) at 0 to 7 wt.% loading. Finally, we have also shown that γH2AX foci can be visualized in our tissue-engineered skin model upon irradiation with X-rays at doses from 0.2 to 5 Gy.
Conclusions: Our innovative hydrogels based on the combination of low-Z/high-Z elements hold promise for radiation shielding applications in space. Their mechanical tunability and ease of handling support integration into spacesuits or habitat structures, in either single-layer or multilayer configurations.

68. SOBP proton irradiation of CHO cells for Dirty Dose Kill model parameterization

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[1] *University of Oslo;* [2] *RaySearch Laboratories AB*[1] *University of the Bundeswehr Munich, Munich, Germany* Background: Accurate modelling of the Relative Biological Effectiveness (RBE) is essential for optimizing proton therapy. The Dirty Dose Kill (DDK) model provides a framework separating dose into "clean" (low LET) and "dirty" (high LET) components, introducing a cell-specific parameter (ξ). However, extraction of ξ from experimental data is often ill-conditioned without high-fidelity dosimetry. This study aims to investigate the effects of SOBP proton irradiation on CHO cell survival using a high-control experimental setup.
Materials and Methods: Irradiations will be performed using SOBP proton fields. A novel setup has been developed in which cell culture flasks are submerged directly in a water phantom during irradiation. Custom 3D-printed holders ensure reproducible positioning within the beam. This configuration enables detailed dosimetric characterization at each cell location, allowing direct comparison between biological response and corresponding dose and LET distributions. Clonogenic survival will be assessed at multiple depths within the SOBP, corresponding to varying dirty dose fractions, defined using an LET threshold.
Results:Clonogenic survival will be quantified across multiple positions within the SOBP, enabling analysis of depth-dependent variations in biological response. These measurements are expected to provide the basis for extracting the DDK model parameter ξ and evaluating its sensitivity to local LET and dose composition. The proposed setup is anticipated to improve dosimetric consistency, particularly in regions with steep gradients in dose and LET.
Conclusions: This project aims to presents a refined experimental methodology for radiobiological studies in proton beams. The integration of 3D-printed holders within a water phantom enables precise alignment between dosimetry and biological measurements. The resulting data will support parameterization of the DDK model and contribute to improved RBE modeling in proton therapy.

72. Minibeam Radiotherapy Improves the Efficacy of Checkpoint Blockade in Syngeneic Head and Neck Tumors

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Combining radiation therapy (RT) with immune checkpoint blockage (ICB) offers a promising avenue to improve tumor control beyond either treatment alone by leveraging both local and systemic antitumor effects. However, conventional broadbeam radiotherapy (BBRT), which delivers a uniform dose across the tumor volume, may inadvertently damage radiosensitive immune cells within the tumor microenvironment, thereby impairing the immune response that is essential for effective synergy with immunotherapy. Minibeam radiotherapy (MBRT) is an innovative spatially fractionated RT technique that delivers radiation through an array of parallel, narrow beamlets instead of a uniform broad field. By sparing portions of both tumor and surrounding normal tissue, MBRT may reduce radiation induced toxicity, preserve vascular and stromal integrity, and, importantly, maintain viable immune cell populations within and around the tumor.
The purpose of this study was to compare therapeutic responses to BBRT and MBRT, delivered alone or in combination with PDL-1 ICB, in two syngeneic head and neck cancer models with differing immunogenicity. MOC1 (highly immunogenic) and MOC2 (less immunogenic) tumor cells were implanted subcutaneously in the hind legs of C57BL/6J mice. Animals received a single fraction of BBRT (15 Gy), MBRT (15 Gy), or no irradiation. Anti PDL-1 antibody (10 mg/kg) was administered intraperitoneally on days 2, 5, and 8 post irradiation. Tumor growth was measured longitudinally to assess treatment efficacy. In the MOC1 model, anti PDL-1 monotherapy induced a modest reduction in tumor volume (˜15–20%), whereas both BBRT and MBRT led to significant tumor regression (˜50–54%). Combining PDL-1 blockade with either RT modality further improved tumor control, indicating additive or synergistic effects. In the less immunogenic MOC2 model, PDL-1 monotherapy alone did not affect tumor growth. BBRT and MBRT each produced moderate tumor reduction (˜35–45%), and the addition of PDL-1 blockade led to further suppression of tumor progression. These findings demonstrate that MBRT provides tumor control comparable to BBRT while potentially offering immunological advantages. Importantly, the enhanced response to combination therapy was observed in both immunogenic and less immunogenic tumors, underscoring the broad therapeutic potential of this strategy. Collectively, our results highlight MBRT as a promising RT modality for integration with ICB in head and neck cancer. By reducing toxicity and preserving immune functionality, MBRT could serve as a more immune compatible backbone for future radioimmunotherapy regimens.

86. Enhancing Radiotherapy via Cherenkov Light Photoactivation in Cancer Treatment

Authors: Justyna Czapla[1]; Wiktoria Papuga-Zemlik[1]; Krzysztof Szczepanik[2]; Alina Drzyzga[3]; Artur Beberok[4]; Zuzanna Rzepka[4]; Dorota Wrześniok[4]; Krzysztof Ślosarek[5]; Ryszard Smolarczyk[1]
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Background: Radiotherapy (RT) remains a standard treatment modality for more than half of all cancer patients. Despite advances in radiotherapy enabling more precise delivery of higher radiation doses, locoregional recurrence is still a leading cause of cancer-related mortality. Therefore, the development and clinical implementation of strategies that enhance radiotherapy efficacy are urgently needed. One such approach is radiotherapy enhanced with Cherenkov light photoactivation (RECA), which exploits Cherenkov light generated during external beam radiation therapy (EBRT) to activate photodynamic agents accumulated in tumor tissue, thereby enhancing the overall effectiveness of the treatment.

The aim of this study was to assess whether drugs with established phototoxic properties could be effectively repurposed for use in RECA-based therapy and to optimize EBRT conditions to achieve maximal therapeutic efficacy.

Materials and Methods: The phototoxic potential of lomefloxacin, and doxycycline was evaluated following EBRT delivered at 6, 15, and 20 MV (dose rate: 100 MU/min), using either a single dose (1 × 10 Gy) or a fractionated regimen (3 × 2.5 Gy). 8-methoxypsoralen was used as a reference photodynamic drug. The photosensitizing effects of these repurposed drugs were assessed in the human head and neck cancer cell lines. In vitro phototoxicity was determined by measuring tumor cell viability (Annexin V/7-AAD), proliferation, apoptosis (caspase-3/7 activity and/or TUNEL assay), cell cycle disruption, mitochondrial membrane potential, and DNA double-strand breaks (γH2AX).

Results and Conclusions: These endpoints collectively enabled mechanistic evaluation of RECA, particularly in terms of induction of DNA damage and drug-mediated phototoxicity. The obtained results indicate that EBRT photoactivates photodynamic agents, thus enhancing tumor cell killing efficacy. However, no significant differences were observed between the radiation energies applied, indicating that 6MV energy is suitable for the RECA strategy. Importantly, the fractionated dose regimen demonstrated superior efficacy within the RECA framework, highlighting the potential benefits of clinically relevant fractionation schedules and energies, and further supporting RECA as a promising radiosensitization approach.

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98. Towards Silicon Carbide Dosimeters for Radiation Monitoring in Space and Harsh Environments

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Space radiation and the associated health risks remain a critical challenge in long term human space exploration. In deep space, galactic cosmic rays produce a mixed field of protons, helium, and high-LET heavy ions, creating conditions that are exceptionally harsh and unlike terrestrial environments. Reliable monitoring and dosimetry are essential to quantify, manage, and mitigate the human exposure to ionizing radiation in space. Current dosimetry solutions, while mature for ground-based use, are often constrained in space by limited sensitivity ranges or power consumption. Further limitations apply when operating in environments with largetemperature fluctuations.
Silicon, despite being widely used in radiation detectors, suffers from significant Degradation in harsh radiation environments due to displacement damage and charge trapping. Its relatively narrow bandgap makes it highly sensitive to temperature, leading to increased leakage currents and a strong temperature dependent performance. As damage accumulates, stable Operation without active cooling becomes increasingly difficult and limits the use of silicon as a detector material in harsh conditions.
Silicon carbide (SiC) offers a compelling alternative: As a wide-bandgap semiconductor, it exhibits excellent thermal stability from very low temperatures up to hundreds of ∘C and maintains low leakage current even after receiving high doses of photon or ion radiation. These properties make SiC a promising detector material, well-suited for space-borne instrumentation and hostile environments.
We present the development of a low-footprint SiC-based dosimeter for mixed radiation fields as an alternative to silicon systems. The design targets a broad dynamic range to resolve both the low- and high-LET components of galactic cosmic radiation, with emphasis on achieving stable operation at high bias voltages while minimizing power consumption and maximizing battery life. Prototype construction is ongoing and first system tests are planned for mid-2026 with accelerated protons and heavier ions from a synchrotron. Beyond personal dosimetry, further applications in radiation and temperature extreme environments are discussed.

101. Investigating the Use of Helium Ions in Spatial Fractionated Radiotherapy: A Simulation and In Vitro Study

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Background Spatially fractionated radiotherapy (SFRT) has emerged as a promising approach to enhance the therapeutic ratio by delivering highly heterogeneous dose distributions that can spare normal tissues while effectively targeting tumors. While most SFRT research has focused on photon and proton beams, helium ions offer unique physical and biological advantages, including reduced lateral scattering, sharper Bragg peaks, and potentially higher relative biological effectiveness (RBE). However, their role in SFRT remains largely unexplored.

Method Monte Carlo simulations are being implemented using the TOPAS platform to model helium ion SFRT dose distributions and predict the corresponding biological response within the MONAS framework. In parallel, in vitro clonogenic assays with X-ray irradiation have been conducted to establish baseline cell survival. Pre-plating and post-plating methodologies were systematically implemented and compared across multiple dose levels to assess their impact on clonogenic survival outcomes. Helium ion irradiation experiments are currently in preparation.

Results Initial X-ray experiments demonstrate clear dose-dependent decreases in cell survival. Differences between pre- and post-plating approaches were observed, indicating that experimental methodology can influence measured clonogenic survival. Ongoing simulation work is expected to characterize helium ion SFRT dose distributions and their potential biological impact.

Conclusions This work represents an ongoing effort to combine simulation and experimental approaches for the evaluation of helium ions in SFRT. Preliminary results highlight the importance of experimental design in clonogenic assays and provide a foundation for upcoming helium irradiation studies. The integration of these approaches will contribute to the validation of helium ions as a potential modality for advanced radiotherapy.

121. Engineering Radiation Resistance in Human Cells Through the Tardigrade-Derived DNA Repair Mechanism, the Dsup

Authors: Natassha Shanmugam[1]; Ozdemirhan Sercin[1]
[1] *DLR*

Background Ionising radiation remains one of the most significant challenges for long-duration deep space missions due to its deleterious genomic effects, which can lead to carcinogenesis and degenerative diseases in astronauts. While physical shielding is limited by mass constraints, gene therapy has emerged as a potential mitigation strategy. A promising candidate is the Damage Suppressor (Dsup) gene from tardigrades, organisms capable of surviving radiation doses up to 5000 Gy.

Methods This project, conducted at the Radiation Biology Department of the German Aerospace Center, aimed to integrate the Dsup gene into multiple mammalian cell lines (HEK293T, RPE1, and A549 with varying p53 status). The study assessed both the intrinsic toxicity of Dsup and its potential radioprotective effects on cell viability. Gene delivery was performed using chemical lipofection. Additional experiments investigated the effects of hypoxic conditions and antioxidant treatments (N-acetylcysteine [NAC] and nicotinamide [NAM]) on irradiated cells.

Results Dsup was successfully cloned and expressed in HEK293T cells. However, its expression appeared to be cytotoxic in RPE1 and A549 cell lines. Overall results remain inconclusive due to low transfection efficiency using chemical lipofection. Independently, both hypoxia and antioxidant treatments demonstrated a protective "rescue effect" in cells exposed to X-ray irradiation.

Discussion The limited transfection efficiency suggests that alternative delivery methods, such as lentiviral systems, are necessary to properly evaluate Dsup's radioprotective potential. Future studies will explore combined treatments (Dsup with antioxidants and/or hypoxia) to determine whether effects are synergistic, additive, or redundant. Additionally, Dsup may be investigated for its ability to compensate for the loss of DNA damage repair proteins using CRISPR/Cas9-mediated knockouts, further elucidating its potential role in radioprotection.transfection efficiency of these cells via chemical lipofection was poor, therefore a repeat should be conducted using a lentiviral delivery system. In addition to this, this internship also conducted studies on the treatment of cells in hypoxic conditions and with NAC/NAM (seperately); both of which demonstrated a "rescue effect" when irradiated with X-rays. Future experiments are to be conducted to merge Dsup + Antioxidants, and Dsup + Hypoxia to investigate if there is a synergistic, additive or redundant effect of protectiveness against ionising radiation. Moreover, Dsup can also be tested to see if it cancompensate the activity of a DNA Damage Repair protein if it is knocked out using the CRISPR/Cas9 system.

123. Preclinical FLASH and minibeam experiments at the Dresden proton therapy facility

Author: Joerg Pawelke[1]
Co-authors: E. Bodenstein [1]; Elke Beureuther [1]; F. Horst [1]
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Background: Proton therapy (PT) is an advanced form of external beam radiotherapy, but it continues to be shaped by innovation. Translational research into emerging developments such as image-guided and online adaptive PT, as well as ultra-high dose rate (FLASH) and spatially fractionated PT, will benefit from a suitable infrastructure for experimental research located in close proximity to clinical practice. We report on the experimental research opportunities at the proton therapy facility in Dresden, Germany, with focus on in-vivo FLASH and minibeam experiments.
Materials and Methods: The Dresden proton therapy facility is equipped with a ProteusPLUS therapy system (IBA) based on an isochronous cyclotron providing beam currents up to 500 nA. It has one treatment room equipped with a rotating gantry where patients are treated and an experimental room equipped with two horizontal beamlines 1. The first beamline delivers static pencil beams with energies in the range 70 – 226.7 MeV controlled by dedicated hard- and software developed in-house. The second beamline is equipped with a pencil beam scanning nozzle providing clinical-like fields and beam parameters.
A large pool of beam diagnostic and dosimetry equipment such as different types of ionization chambers, diamond and scintillation detectors as well as radiochromic films is available. Measurements with these detectors can be performed in air or water, using various types of phantoms. For radiobiological experiments, laboratories with standard equipment for cell culture, including genetically modified cells, and histology are available in the same building. An small animal facility on the same floor, equipped with an image-guided X-ray radiotherapy system and a combined PET/MRI scanner, can be used for pre-treatment imaging and post-treatment monitoring of irradiated mice. For reference irradiation, several 200 kV X-ray tubes and MV photons from a clinical linear accelerator are available in the same building.
Results: Experiments using the proton beam are possible in parallel with the clinical operation. Therefore, experiments can be carried out during the day on weekdays, with high beam availability guaranteed for the experiments whilst patients are being prepared and during set-up changes. Approved setups and workflows allow a large variety of radiobiological experiments. In-vivo experiments using mice and zebrafish embryos are performed with static pencil beams in different settings, e.g., partial irradiation of mouse brains and homogeneous treatment of subcutaneous tumors on the legs and ears of mice. The high beam current, combined with the controlled delivery of short pulses, enables FLASH PT experiments irradiating in the plateau and spread-out Bragg peak , with dose rates up to 600 Gy/s. A collimation setup [2] is used to irradiate orthotopic tumors in mice with 250 µm wide proton minibeams.
Conclusions: The Dresden facility has unique features, providing two complementary research beamlines and the necessary infrastructure for in-vitro and in-vivo small animal experiments which are also available for external researchers from academia and industry.
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[2] M. Ahmed et al. (2024) Front. Oncol. 14:1473625

129. Single-shot Bragg peak reconstruction in clinical carbon-ion beams with PRAGUE, a silicon carbide multilayer detector

Author: Mariacristina Guarrera[1]
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Background: Accurate, fast, and dose-rate-independent measurements of Percentage Depth-Dose (PDD) distributions are essential for next-generation Quality Assurance (QA) and online range verification in ion therapy, particularly under Ultra-High Dose Rate (UHDR) conditions relevant to FLASH radiotherapy (FLASH-RT). Under these conditions, conventional water-scanning ionization chambers are not suitable for true single-shot operation. Moreover, their dosimetric characterization is challenging and requires the development of innovative protocols complementary to those used in conventional radiotherapy. Silicon carbide (SiC) is an attractive detector material due to its high radiation hardness, fast charge collection, wide dynamic range, and stable response over a broad range of irradiation conditions. Within the PRAGUE (Proton RAnGe measure Using silicon carbidE) project, a multilayer SiC detection system has been developed for depth-dose reconstruction under both conventional (˜10 pps) and high (˜10^1 pps) intensity conditions without the need for mechanical scanning. The integration of a dedicated holder for the irradiation of biological samples will enable direct preclinical studies, allowing shot-to-shot dose measurements inside the biological target with high accuracy (3%).
Methods: In this work, the complete PRAGUE system, consisting of a 50-layer stack of SiC p–n junction detectors—each featuring a 10 μm active layer and a sensitive area of 15 × 15 mm²—coupled to a dedicated multichannel acquisition unit, was characterized at CNAO using clinical 115 MeV/u carbon-ion beams. The aim was to reconstruct the Bragg peak profile in a single irradiation, providing a fast and efficient alternative to conventional scanning procedures. The reconstructed PDD was benchmarked against measurements performed with EBT4 radiochromic films and a commercial Bragg peak monitoring system based on the PTW Bragg Peak Chamber Type 34080.
Results: The PRAGUE system enabled single-shot PDD reconstruction across 50 sampling layers, showing good agreement with both reference techniques in terms of Bragg peak position and overall profile shape. The detector response was stable and reproducible under high Linear Energy Transfer (high-LET) carbon-ion irradiation, confirming the suitability of SiC technology for relative dosimetry in therapeutic ion beams. Combined with the intrinsic fast response of SiC and the multichannel readout architecture, these features enable beam monitoring without moving components and with reduced experimental complexity.
Conclusion : PRAGUE represents the first solid-state online detector for PDD reconstruction of conventional and ultra-high dose rate beams, intended for clinical and FLASH applications. The system, complete with a multichannel acquisition unit, represents an absolute novelty in relative dosimetry, supporting the safe and reliable clinical implementation of FLASH treatments.

FLASH + Postersession 3

Chair: Nina Edin
Room: FLASH Session in Atlantis 1, Postersession in Atlantis 2

15:00

Characterizing the impact of hypofractionated radiation therapy on the genomic landscape of murine soft tissue sarcon
Max Labrecque

The Use of Circulating miRNAs for the Detection of Glioblastoma CNS WHO Grade 4 Recurrence *Razan Muhtadi*
Atlantis 1 15:31 - 15:32

Evaluation of the therapeutic effect of combined low-dose radiation and adipose tissue stem cells on wound healing in
Ewelina Pilny

Detecting Early Radiation-Induced Submandibular Gland Alterations via Foundation Model and MRI *Dr Manish Kakar*
Atlantis 1 15:33 - 15:34

Obesity, hormones, and their influence on the immune microenvironment in radiation-associated female cancer
Lea Di Maria

Establishment of a functioning triage in case of a radiation accident using cytogenetic testing in human peripheral bloo
Lama Ramadan

Gene expression regulation within hypoxic and radiotherapy resistant regions of prostate cancer
Sonia Hermoso Duran

Development of a combined therapy (extracellular vesicles derived from mesenchymal stem cells and interleukin-2 cytc
Philippine Scoliège

Multiparametric MRI Characterization of FDG-PET–Defined Dose-Escalation Subvolumes in Head and Neck Cancer
Lars Svensen

Molecular Stratification of Prostate Cancer Patients Using Senescence Signatures for Optimized Radiation Therapy wit.
ke deng

Defining redox-dependent DNA damage response vulnerabilities after ionizing radiation using arrayed combination CRI...
Zoe Hughes

Base-excision repair increases DNA double-strand break clustering within heavy-ion tracks and modulates repair at δ-e
Laura Schwan

α-Radiation-Induced Multi-Omics Remodeling of Osteoblastic Bone Marrow Niche Cells: Implications for Bone Marrow .
Satoru Monzen

Transcriptomic dysregulation during spaceflight suggests organ-specific role in systemic responses *Aidan Meade*
Atlantis 1 15:55 - 15:56

Microbeam radiotherapy shows promise for metastatic triple negative breast cancer: insights from a dose-escalation ch
Olga Martin

The Radiation and Nuclear Countermeasures Program (RNCP) at the National Institute of Allergy and Infectious Disease...
Carmen I. Rios

12. Characterizing the impact of hypofractionated radiation therapy on the genomic landscape of murine soft tissue sarcoma

Author: Max Labrecque[1]
Co-author: Michael Monument [1]
[1] *University of Calgary*

Background: Soft tissue sarcoma (STS) is a rare form of cancer that is disproportionately common among children & young adults. Despite a decade of advancements in cancer immunotherapy, outcomes for patients with advanced STS have not improved, and treatment options remain limited. However, recent clinical trials have yielded promising results by combining immune checkpoint blockade with hypofractionated radiation therapy. Improving our understanding of high-dose radiation therapy as a mechanism by which immune-cold sarcomas may be sensitized to other therapies is necessary to expand treatment options for patients with advanced disease.
Materials & Methods: Our project examined the impact of multiple high-dose radiation treatment courses on a well-known mouse model of undifferentiated pleomorphic sarcoma, a common sarcoma subtype (KrasG12D/p53-/-). These treatments used 6Gy and 8Gy fractions with the goal of remodelling the tumour microenvironment. Whole-exome sequencing was performed on tumours after radiation treatment and compared to genomic data from spontaneous and engrafted forms of untreated KrasG12D/p53-/- tumours, as well as another known murine STS model (Pten-/-/p53-/-) and a novel cre-recombinase driven STS model (KrasG12D/Pten-/-/p53-/-).
Results: In our irradiated tumours, we describe distinct genomic profiles of passenger mutations and copy-number changes in genes related to cancer. The impact of radiation therapy on the tumour genomic landscape is detectable after a single high-dose fraction, and varies based on total dosage delivered & length of the treatment course. Pathway analysis of genes with copy-number changes reveals functional shifts in the tumour genotype, suggesting genomic mechanisms by which tumours may be "primed" for combination therapies.
Conclusions: Hypofractionated radiation treatment significantly and consistently alters the genomic landscape of soft-tissue sarcoma, suggesting genomic mechanisms for tumour immune sensitization. We also observe distinct genomic profiles in novel murine mouse models of sarcoma based on driver mutation and induction method, expanding our arsenal of tools and informing future investigations of new combination therapies using hypofractionated radiation therapy.

20. The Use of Circulating miRNAs for the Detection of Glioblastoma CNS WHO Grade 4 Recurrence

Author: Razan Muhtadi[None]
Co-authors: Abend; Barsegian; Bernhard; Bernhardt; Combs; Diehl; Eder; Fischer; Gempt; Hönikl; Krieg; Lindemann; Multhoff; Ostheim; Schmid; Stewart

Glioblastoma CNS WHO Grade 4 (GBM) is associated with poor prognosis and high recurrence rates despite the current therapeutic approaches. Conventional imaging, such as CT and MRI, has a limited ability to detect tumor recurrence and distinguish it from tissue necrosis and postoperative scar tissue. This study aims to investigate the potential of tumor-origin miRNA species released into whole blood to support early detection of tumor recurrence through a molecular biological approach.

Whole-blood samples (n = 33) from seven patients and tumor biopsies were used to identify biomarkers. Corresponding to tumor surgery and tumor recurrence, we examined miRNAs expressed in pre-surgical tumor and blood samples, showing downregulation post-surgery, consistent with reduced tumor burden, and returning to pre-surgery levels at the time of tumor recurrence. Using NGS small RNA sequencing, we identified 1–19 miRNA species across seven patients who showed this pattern. The 45 most promising miRNAs were selected for validation using qRT-PCR. In addition, miR-21 was included as a promising candidate reported in the literature. To address challenges in miRNA normalization, five normalization strategies and unnormalized raw Ct values were compared using a predefined plausibility analysis. Two of these approaches (median ΔCt normalization and unnormalized raw Ct values) showed no systematic bias, but the other recommended normalization approaches systematically shifted all 46 examined miRNAs, e.g., 2-6-fold up or down relative to the pre-surgery samples (used as a reference), which is considered an artifact. Depending on the normalization method, the agreement between NGS and qRT-PCR ranged from 23.6 to 27.9%.

Although the concordance between NGS and qRT-PCR was low at the individual-patient level, qRT-PCR identified a common set of miRNAs that were differentially regulated across patients. Notably, miR-424-3p and miR-362-5p were shared by five of seven patients, followed by miR-1307-3p and miR-22-5p, each shared by four patients. Interestingly, miR-21 did not show the predefined recurrence-associated pattern in any patient. Blood-based miRNA profiling may complement imaging in longitudinal monitoring of GBM patients and warrants further validation in larger cohorts.

22. Evaluation of the therapeutic effect of combined low-dose radiation and adipose tissue stem cells on wound healing in mice

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Background: Chronic non-healing wounds are an increasing public health challenge due to aging populations and the rising prevalence of metabolic disorders. Cell-based therapies, particularly adipose tissue–derived mesenchymal stromal cells (ADSCs), show promise because of their immunomodulatory, pro-angiogenic, and regenerative properties. Low-dose radiation therapy (1 Gy; LDRT) is a safe, anti-inflammatory approach that modulates immune cell activity and cytokine secretion, and has been used for decades in inflammatory and degenerative conditions. Given these complementary mechanisms, we investigated the therapeutic potential of combining ADSCs with LDRT in a mouse model of wound healing.
Material and Methods: In vitro: ADSCs were irradiated with single doses (0.1, 0.5, 1 Gy) using a 6 MV photon beam from a TrueBeam accelerator at 10cm depth. Effects on cell survival, ROS levels, cell cycle, and mitochondrial membrane potential were analyzed at 24 and 72hours post-irradiation. In vivo: Full-thickness 8mm wounds were created on mouse backs. Animals were randomly assigned to four groups: (1) LDRT (0.5 or 1Gy), (2) perilesional injection of 1×10 ADSCs, (3) combination of LDRT and ADSCs, and (4) untreated controls. Wound size was photographed and measured, and blood samples were collected for hematological analysis.
Results: Low-dose irradiation did not exert any significant effects on ADSCs under in vitro conditions. On day 5, wound areas were reduced by 15% in the 1Gy group and 8% in the 0.5Gy group compared with controls. Hematological analysis revealed a marked increase in eosinophil percentages on day 5 in irradiated mice, with 4-fold and 3-fold elevations in the 0.5Gy and 1Gy groups, respectively. No significant differences were observed following the combined application of ADSCs and low-dose radiotherapy.
Conslusions: The obtained results suggest that the combination of low-dose radiotherapy may contribute to accelerated wound healing, which may be used to develop new, effective therapies in the future.
This work was funded under the internal project SN/MGW8/2024.

28. Detecting Early Radiation-Induced Submandibular Gland Alterations via Foundation Model and MRI

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Background: Emerging evidence suggests that early radiation-induced damage in murine models is localized to specific regions within the submandibular (SMG) and sublingual glands1. The subtle nature of these changes in SMG makes manual visual inspection unreliable for detecting early post-radiation alterations. In this study, we leveraged foundation model (SAM) [2] and attention-based vision classifiers (ViT Base 16) [3] to develop an explainable AI (XAI) framework[4] for identifying early regional alterations induced by radiation therapy (RT) in SMG via MRI analysis. By detecting these early post-radiotherapy changes, this approach holds the potential for personalized follow-up to mitigate long-term side effects, ultimately enhancing patients' health-related quality of life.
Materials and Method: In this investigation, C57BL/6J mice (n=29) received 66 Gy of fractionated radiotherapy targeting the oral cavity, swallowing muscles, and salivary glands. The dose was delivered over five or ten days in one or two daily fractions, respectively—a schedule known to induce substantial early and late toxicity[5]. T2-weighted MRI scans were acquired 3–5 days post-irradiation. We employed SAM model to segment the SMG, followed by ViT base 16 model to classify the segmented images into control and irradiated groups. Attention maps were then cross-referenced with histological sections graded for acinar atrophy in the SMG.
Results: Using raw MRI data, a ViT Base 16 model[3] classified control and irradiated SMGs with 72% average accuracy. Cross-referencing XAI attention maps with histopathological grades for acinar atrophy revealed statistically significant post-radiotherapy alterations in both the cranial and caudal regions of the SMG. These findings highlight the model's potential for identifying early radiation-induced alterations in the SMGs in a pre-clinical model.
Conclusion: Attention based XAI maps can identify early RT-induced alterations in murine SMG both in cranial and caudal parts of SMG glands. While this preclinical study shows promise, clinical validation is necessary before it can be integrated into clinics.
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2022, doi: 10.1093/jrr/rrac066.

37. Obesity, hormones, and their influence on the immune microenvironment in radiation-associated female cancer

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Background: Epidemiological evidence shows that early age at menarche is associated with higher susceptibility to radiation-induced cancers in women, with up to 24% and 31% higher risk for breast and endometrial cancers, respectively. Childhood obesity, linked to lower age at menarche, further amplifies this vulnerability, although the underlying mechanisms remain unclear. Immune dysfunction is also a recognized risk factor in the development of cancer. The interplay between metabolic, hormonal, and immunological factors remains unclear in the context of radiation-induced tumorigenesis.
Objectives: We hypothesize that early menarche increases vulnerability to radiation-induced cancers through the combined effects of obesity- and hormonal-driven alterations on the immune status. Thus, the aim of this study is to address the mechanisms linking obesity and sex hormones to radiation-induced breast and endometrial cancer susceptibility, with a particular focus on how these factors modulate the immune microenvironment.
Materials and Methods: To test this, in vitro models using breast and endometrial cell lines will be employed. Cells will be treated with leptin, to mimic obesity, and a combination of sex hormones. They will be irradiated according to different scenarios. We will assess transcriptional changes in inflammatory, DNA damage response, and stress-related genes by RT-qPCR. Measurement of cytokines secretion will be performed, by ELISA and flow cytometry, to evaluate how hormonal and metabolic factors influence breast and endometrial cells immune signalling. Bystander effects on neighbouring non-irradiated cells will also be examined, including effects on cell growth and immune cell activation, and polarization.
Results: Experiments are ongoing; the first results will be presented during the meeting.
Conclusion: By integrating hormonal, metabolic, and immunological perspectives, this work aims to provide mechanistic insight into how early-life obesity and hormonal status influence susceptibility to radiation-induced breast and endometrial cancers and how immune modulation shapes the tissue microenvironment in this context.

40. Establishment of a functioning triage in case of a radiation accident using cytogenetic testing in human peripheral blood samples

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Background: Effective response to large-scale radiological or nuclear incidents requires rapid and robust triage to support clinical management of acute radiation syndrome (ARS). Within the EDF RESILIENCE project, our study aims to establish an ARS-severity prediction-based triage based on the individual exposure level using two cytogenetic assays: Dicentric chromosome aberration (DCA) assay and cytokinesis-block micronucleus (CBMN) assay. The objective is to enable a correct diagnosis and to discriminate between unexposed individuals and those who will later develop a mild ARS, and high ARS severity degree. Besides, we compared biodosimetry and physical dosimetry estimates.
Materials and Methods: Peripheral blood samples were collected from three participant groups: healthy volunteers (unexposed), breast cancer patients undergoing partial body irradiation (PBI) representing the mild-ARS group, and leukemic patients undergoing total body irradiation (TBI) representing the high-ARS group. Blood samples of cancer patients were obtained before, during, and after radiotherapy (RT) treatment when applicable. Blood samples were cryopreserved and shipped to the collaborating laboratories within the RESILIENCE project. Chromosomal damage was assessed in lymphocytes using DCA and CBMN assays. Whole-body dose estimations were calculated with Biodose Tools software based on a laboratory-specific calibration curve. In parallel, the HEDOS tool was used to estimate a whole-body equivalent physical radiation dose delivered to a circulating fraction of irradiated blood cells during RT. This estimation is based on clinical data obtained from the patient treatment plan.
Results: Preliminary experiments conducted on healthy participant and two breast cancer patients showed highly consistent biological dose estimates between different cytogenetic laboratories and also showed alignment with the physical dose estimate derived from the HEDOS tool. TBI samples have presented challenges due to pre-radiotherapy low lymphocyte counts, which hinder the establishment of successful cultures. Further investigation is necessary to identify suitable patient profiles with adequate lymphocyte levels to ensure reliable assay performance in this subgroup.
Conclusions: Our first findings suggest that DCA and CBMN assays appear promising in discriminating between unexposed individuals and those exhibiting mild-ARS supporting their potential utility for predicting acute clinical outcomes and supporting triage decisions during large-scale

radiation emergencies.
Disclaimer: Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the granting authority can be held responsible for them (RESILIENCE- R- 2023), project No. 101168024.

54. Gene expression regulation within hypoxic and radiotherapy resistant regions of prostate cancer

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Background: Tumor hypoxia is a major cause of radiotherapy resistance and poor outcome in prostate cancer. Gene expression programs associated with hypoxia reflect disease aggressiveness. Knowledge of their key regulators may facilitate the development of new treatment strategies. Research in this field has been limited by the lack of technologies enabling genome-wide identification of genes expressed within hypoxic regions. Spatially resolved genomics allow characterization of gene expression within defined hypoxic tumor regions.

Here, we developed an integrative framework combining digital histopathology and spatial transcriptomics in tumor biopsies to perform such analyses and to characterize distinct hypoxia-associated gene expression patterns.

Materials and Methods: Biopsies from 95 patients with intermediate- to high-risk prostate cancer who received the hypoxia marker pimonidazole prior to surgery were analyzed. Digital hypoxia imaging revealed heterogeneous spatial distributions, including regions with hypoxia gradients from mild to severe levels, and regions with stable hypoxia. Digital hypoxia images were spatially mapped with gene expression data obtained from consecutive 10x Genomics Visium sections of 4 patients, allowing co-localization measurement of genes and hypoxia in microregions of 71.5 μm2. Differential expression analysis comparing histologically defined hypoxic and non-hypoxic regions was performed to define a hypoxic gene expression program. Bulk transcriptomic genes were used to validate gene expression patterns and to investigate their prognosis.

Results: Within the hypoxic gene expression program, two distinct patterns were identified: (A) a gradient-dependent pattern, comprising genes whose expression correlated with hypoxia levels, and (B) a stable pattern, including genes consistently up-regulated in hypoxic regions without any changes in hypoxia level. This approach thus allowed us to identify genes that apparently are regulated by hypoxia, while the other genes are most likely regulated by other mechanisms such as gene amplification.

A total of 365 genes exhibited a gradient-dependent pattern, while 935 genes followed a stable pattern. Gene set enrichment analysis revealed distinct biological processes underlying these patterns. Glycolytic metabolism was enriched in the gradient-dependent group, whereas cell proliferation pathways were enriched in the stable group. The proliferative pathway was associated with tumor aggressiveness and poor outcome.

Conclusions: The integrative framework permitted the identification of two mechanisms that regulate the hypoxic gene expression program in tumor biopsies of prostate cancer. The gradient-dependent pattern was regulated by hypoxia, and the stable pattern was characterized within hypoxic regions but was not directly driven by hypoxia. Its specific association with proliferative pathways and poor outcomes highlights their potential relevance as therapeutic targets.

78. Development of a combined therapy (extracellular vesicles derived from mesenchymal stem cells and interleukin-2 cytokine) for musculo-cutaneous radiation burn

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Background: Human exposure to high radiation doses causes acute localized radiation syndrome, characterized by chronic inflammation, vascular damage, and cachexia. Current therapeutic strategies based on bone marrow-derived mesenchymal stem cells (BM-MSCs) are limited by lengthy production times and autologous constraints. This study demonstrates whether a sequential therapy combining human MSC-derived small extracellular vesicles (HuMSC-EVs) and low-dose interleukin-2 (IL-2) promotes neovascularization, immunomodulation, and muscular regeneration in a murine model of musculo-cutaneous radiation injury.
Materials and methods: HuMSC-EVs (100 µg) are locally administered in the hindlimb of TgPax7-GFP mice 14 days after a single 80-Gy X-ray irradiation. Low-dose IL-2 (1 µg) is subsequently administered on days 30, 31, and 32 to selectively expand regulatory T cells (Tregs). Animals are euthanized at day 35 or day 40. Tissue neovascularization is assessed by microangiography, immune cell populations are characterized by spectral flow cytometry, and satellite cell numbers are quantified by immunohistochemistry.
Results: HuMSC-EV administration improves neovascularization of irradiated tissue, with an increased angiographic score of 1.7-fold at day 40 and 1.3-fold at day 60 ($p < 0,01$) respectively. The sequential combination with IL-2 administration stimulates the wound healing process and induces an increase in Treg numbers of 11-fold in the spleen ($p < 0.001$) and 2-fold in the muscle ($p < 0.05$), alongside a 1.7-fold increase in Ly6C(low) monocytes in the muscle compared to untreated animals ($p < 0.05$). More importantly, neutrophil counts decrease 1.6-fold in the spleen and 3-fold in the blood compared to untreated animals ($p < 0.05$). Finally, this anti-inflammatory microenvironment correlates with an increase of satellite cell numbers by 4.5-fold compared to untreated animals ($p < 0.05$).
Conclusion: This study demonstrates that the combined administration of HuMSC-EVs and low-dose IL-2 shifts the immune profile toward an anti-inflammatory state, promoting satellite cell-mediated muscle regeneration. These findings support HuMSC-EVs combined with IL-2-driven Treg expansion as an interesting therapeutic strategy for high number of victims in case of an emergency radiological context.

91. Multiparametric MRI Characterization of FDG-PET–Defined Dose-Escalation Subvolumes in Head and Neck Cancer

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Background Locoregional recurrence after definitive chemoradiotherapy for head and neck squamous cell carcinoma (HNSCC) is frequently attributed to biologically aggressive and radioresistant tumor subregions. FDG-PET–guided dose painting aims to escalate radiation dose to metabolically active areas; however, the biological characteristics of these PET-defined subvolumes remain incompletely understood.
Purpose To characterize FDG-PET–based dose-escalation regions using multiparametric MRI (mpMRI) and evaluate whether PET-defined boost volumes demonstrate imaging features consistent with aggressive tumor biology.
Materials and Methods In this prospective imaging study, 57 patients with newly diagnosed, locally advanced HNSCC underwent pre-treatment FDG PET/CT as part of routine radiotherapy planning and study-specific mpMRI including diffusion-weighted MRI (DW-MRI) and dynamic contrast-enhanced MRI (DCE-MRI). FDG-PET–defined gross tumor volumes (GTVs) and two relative uptake-based boost subvolumes (BOOST-33 and BOOST-66) were delineated. MRI-derived parametric maps included apparent diffusion coefficient (ADC), fractional blood volume (fBV), volume transfer constant (Ktrans), and extravascular extracellular volume fraction (ve). Following multi-step deformable image registration into a common DW-MRI space, voxel-wise analyses were performed to compare MRI parameter distributions across tumor regions.
Results FDG-PET–defined boost regions demonstrated significantly lower ADC and ve values compared with the overall GTV, consistent with increased cellular density. Boost regions also exhibited reduced fBV and elevated Ktrans, suggesting decreased vascular density combined with increased vascular permeability. The highest uptake boost subvolume (BOOST-66) showed the most pronounced deviations in diffusion and perfusion parameters. These findings indicate that PET-defined subregions correspond to densely cellular, poorly vascularized, and structurally abnormal tumor tissue.
Conclusion FDG-PET–based dose-escalation volumes in HNSCC exhibit distinct multiparametric MRI characteristics consistent with aggressive and potentially radioresistant tumor biology. These results provide biological support for PET-guided dose painting and suggest that integration of PET and mpMRI biomarkers may further refine individualized radiotherapy strategies.

93. Molecular Stratification of Prostate Cancer Patients Using Senescence Signatures for Optimized Radiation Therapy with Radiosensitizers

Authors: ke deng[1]; Antti Kiviaho[2]; Matti Nykter[3]; Marcus Ruscetti[4]; Alfonso Urbanucci[1]
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De novo metastatic prostate cancer (PC) patients receiving radiation therapy for primary disease invariably experience relapse. However, the determinants of relapse risk remain poorly defined. Senescence induced radiation in tumor cells is a well-known phenomenon, but the extent to which it can be thought as a treatment escape mechanism is not well investigated. By analysing data from early-onset prostate cancer (EOPC) and late-onset prostate cancer (LOPC) cohorts, we identified that senescence-associated gene signatures can stratify patients based on progression free survival (PFS). Metastatic patients with low senescence scores could be candidates for intensification therapy with so-called radiosensitizers. To explore therapeutic possibilities, we evaluated the potential of using radiosensitizers and identified bromodomain inhibitors as promising candidates. We observed enhanced senescent markers upon treatment with combination of radiation with bromodomain inhibitors, which seems mediated through impaired DNA damage repair. We propose implementing senescence profiling using biopsy samples would stratify metastatic PC patients and guide the use of radiosensitizers in the intensification therapy.

128. Defining redox-dependent DNA damage response vulnerabilities after ionizing radiation using arrayed combination CRISPR screening and oxidative lesion imaging.

Author: Zoe Hughes[1]
Co-author: Ozdemirhan Sercin [1]
[1] *DLR*

Background Ionizing radiation threatens genomic integrity during long-duration space missions by inducing complex DNA damage through direct ionization and secondary reactive oxygen species. Internal biological countermeasures are needed, as physical shielding is limited by spacecraft mass constraints.
Materials & Methods This study proposes the "Shield and Repair" model, where cellular redox pathways ("shield") and DNA damage response pathways ("repair axis") function redundantly to maintain genomic stability. Previously published gene screens were evaluated to establish predicted synthetic lethality pairs between redox and DNA repair pathways. High-throughput screening using machine learning further profiled genes via ionizing radiation and hydrogen peroxide sensitivity. A fluorescent reporter plasmid was engineered utilizing the SB100x transposon system to enable stable integration and real-time monitoring of Base Excision Repair dynamics. Functional validation employed an arrayed combinatorial CRISPR-Cas9 knockout matrix in hTERT-RPE1p53KO cells. Knockouts were processed, verified, and tested.
Results The screening strategy for identifying synthetically lethal gene pairs was established and yielded numerous redox and DNA damage response targets. Initial investigation involved knockout generation, exposure to 2 Gy x-ray radiation, and ATP-based luminescence assays to assess survivability as a measure of possible synthetic lethal interactions. Thus far, pairs show decreased survivability but no definitive synthetic lethal interactions. However, immunofluorescence analysis of γ-H2AX foci suggests altered double-strand break signaling in double knockout backgrounds. Generation of the fluorescent reporter plasmid was successful and will allow improved visualization of altered repair dynamics. Further verification of CRISPR knockout screens with radiation is needed.
Conclusion This work provides a framework for understanding synergy between metabolic detoxification and DNA repair, highlighting molecular vulnerabilities that could guide biological countermeasures for deep-space missions and precision strategies for cancer radiotherapy.

131. Base-excision repair increases DNA double-strand break clustering within heavy-ion tracks and modulates repair at δ-electron-induced breaks

Author: Laura Schwan
Co-authors: Nicole Averbeck; Marco Durante; Burkhard Jakob

Background: High energetic heavy ions, which represent a small fraction of galactic cosmic rays contribute significantly to the overall radiation dose of space explorers and thus, the harmful effect of space radiation of which DNA damage is a crucial factor. Traversing a cell nucleus, swift heavy ions induce clustered complex DNA damage along their path (in-track) while δ-electrons can damage DNA distant to ion tracks (off-track), with less complex lesions similar to sparsely ionizing radiation.

In-track, difficult to repair DNA double-strand break (DSB) clustering is characteristic for high-LET radiation. We aimed to find out whether an indirect induction of DSBs via base excision repair (BER) at in-track clustered non-DSB lesions adds to in-track DSB clustering. We further sought to elucidate whether the overall damage complexity induced by high-energetic ions influences the choice of DSB-repair pathways within traversed cells. In this context, we focused on off-track DSBs whose complexity is similar to X-ray-induced lesions.

Materials and Methods: Confluent human fibroblasts (AG1522D) and osteosarcoma cells (U2OS) were irradiated horizontally with iron ions (350 MeV/u) or X-rays. To study the impact of BER on in-track DSB clustering, cells were treated with inhibitors against BER factors OGG1 and APE1 prior to irradiation. At certain time points after irradiation, cells were fixed and immunofluorescence-stained for several DNA-damage-relevant markers (γH2AX, 53BP1, RAD51, RPA, OGG1) and imaged using confocal microscopy. Live-cell imaging directly at the beam line was performed with U2OS cells that express GFP-tagged DSB marker NBS1.

Results: Cells treated with BER inhibitors showed less DSBs along heavy-ion tracks, suggesting that BER processes induce additional in-track DSBs. Furthermore, we revealed that besides in-track damage, also simpler off-track DSBs co-localize more frequently with resection (RPA) and HR markers (RAD51) in cells directly hit by iron ions than in cells exposed to X-rays. In addition, we found that G1-phase fibroblasts show faster repair of δ-electron-induced DSBs than X-ray-induced DSBs.

Conclusions: BER processes induce additional in-track DSBs in cells exposed to heavy ions, indicating BER's impact on in-track DSB clustering. Clustered DNA damage is processed more frequently via resection-dependent repair. The increased frequency of DSB resection or HR use at δ-electron-induced DSBs in cells directly hit by ions suggests that the high load of complex DNA damage influences the processing of off-track DSBs whose damage quality resembles X-ray damage. The observation that G1 fibroblasts repair off-track DSBs faster than X-ray-induced DSBs further suggests a shift in the nuclear-wide DNA damage response in ion-traversed nuclei, presumably due to the high damage load of a single ion traversal.

132. α-Radiation-Induced Multi-Omics Remodeling of Osteoblastic Bone Marrow Niche Cells: Implications for Bone Marrow Toxicity in Targeted Radionuclide Therapy

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Background: Targeted α-emitting radionuclide therapy, including radium-223 dichloride, is clinically effective for bone metastases but may cause unpredictable haematological toxicity. Osteoblastic cells form a regulatory bone marrow niche that supports haematopoietic stem and progenitor cells through cytokine and metabolite secretion. We hypothesised that α-radiation induces multi-layered molecular changes in osteoblastic niche cells, thereby contributing to bone marrow radiosensitivity and individual variation in treatment response.
Materials and Methods: Primary murine bone marrow cells were isolated and differentiated into osteoblastic cells in vitro. Cells were exposed to 0, 0.5, or 1.0 Gy α-radiation using a ˆ241Am source. Haematopoietic clonogenic survival was evaluated by colony-forming cell assays. Osteoblastic differentiation was confirmed by flow cytometric analysis of CD45, RUNX2, and bone alkaline phosphatase. Radiation-responsive molecular alterations were assessed using LC-MS/MS-based proteomics, targeted lipidomics, and miRNA microarray analysis. Pathway enrichment and integrated network analyses were performed to explore potential functional links among altered proteins, lipid species, and miRNA target genes. In the extended analysis, additional validation of selected radiation-responsive candidates was performed to refine the molecular signature associated with α-radiation-induced osteoblastic niche damage.
Results: α-radiation markedly reduced haematopoietic clonogenic survival, with a higher biological effectiveness than X-rays. Osteoblastic differentiation was confirmed after culture induction, and irradiated osteoblastic cells exhibited distinct molecular alterations. Proteomic analysis identified radiation-responsive proteins including PDIA3, PDIA6, ATP5B, RPL4, ITIH3, and SERPINC1. Lipidomic profiling showed significant changes in phosphatidylcholine and lysophosphatidylcholine species, suggesting membrane remodelling and altered glycerophospholipid metabolism after α-radiation exposure. miRNA analysis revealed marked downregulation of several miRNAs, including miR-1895, miR-370-3p, and miR-188-5p. Integrated network analysis suggested functional associations between altered miRNA target genes, mitochondrial activity, endoplasmic reticulum stress, transcriptional regulation, apoptosis, cytokine signalling, and lipid metabolism. The extended dataset further supported the reproducibility of selected protein/lipid/miRNA candidates as components of an α-radiation-responsive osteoblastic niche signature.
Conclusions: α-radiation induces coordinated proteomic, lipidomic, and miRNA alterations in osteoblastic bone marrow niche cells. These changes may reflect radiation-induced niche damage and may contribute to the biological basis of bone marrow toxicity during α-emitting radionuclide therapy. The identified molecular signatures provide candidate biomarkers and mechanistic targets for predicting individual susceptibility to haematological adverse effects in targeted radionuclide therapy.

133. Transcriptomic dysregulation during spaceflight suggests organ-specific role in systemic responses

Authors: Aidan Meade[1]; Alonso Sanchez Izquierdo[1]; Neda Totilaite[1]; Nicholas Brereton[2]
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Human spaceflight exposes astronauts to space radiation and microgravity, driving physiological health impacts including DNA damage, oxidative stress, immune dysregulation and metabolic alterations. Although previous work has highlighted the role of mitochondrial dysfunction, inflammatory modulation and shifts in lipid metabolism with the hallmarks of responses to spaceflight, the extent to which coordinated organ-specific responses are involved remains poorly understood. This study aimed to elucidate this role in the differential gene expression (DGE) observed within the spleen, liver and thymus under spaceflight.

RNA-Seq datasets from C57BL/6J mice flown on NASA's rodent research (RR) missions RR9 and RR23 were obtained from GeneLab. The consensus bioinformatics pipeline (QC, trimming, STAR alignment, featureCounts quantification) was implemented in Galaxy, followed by DESeq2-based DGE analysis with Benjamin-Hochberg correction. Over-representation analysis was performed using Gene Ontology (GO) and EGG databases to identify enriched biological pathways.

A set of significant DEGs were identified across each organ. Those in the spleen included markers of immune response, including cytokine-cytokine receptor interactions, peptidase inhibition and membrane transporter activity. Key features were the observation of downregulation of chemokines (e.g., Cxcl3) and cytokine regulators (Il1rn, Il36g), suggesting suppressed inflammatory signalling, contrasted by upregulation of Epor, potentially indicating compensatory erythropoietic adaptation. In the liver strong evidence of alterations to lipid and fatty acid metabolism were observed, particularly associated with PPAR signalling, arachidonic acid and retinol metabolism, driven by the upregulation of genes of the Cyp4a family and genes associated with lipid transport (Apoa4, Slc27a1). Between these organs a shared downregulation of acute-phase proteins (Saa1/2) suggests the systemic modulation of inflammatory processes. Within the thymus strong features of changes to DNA conformation, binding and bending were observed.

While these findings align with those observed previously, including a reported spaceflight-induction of mitochondrial stress, alteration of immune balance and hepatic immune dysregulation, the study extends those findings to suggest a clearer organ-specific role in these responses, where, in the spleen, immune suppression dominates, while metabolic reprogramming does so in the liver. An interesting observation is that altered erythropoiesis and acute-phase suppression may occur at the systemic level, and that previous observations of elevated cytokines in astronauts contrast with our observations of cytokine downregulation, suggesting a species-dependent response. This work confirms that spaceflight alters multiple physiological signalling mechanisms and supports a concept of coordinated organ-specific responses, with implications for health risk during long-duration space missions.

149. Microbeam radiotherapy shows promise for metastatic triple negative breast cancer: insights from a dose-escalation chick embryo study

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Co-authors: Alan Toh [4]; Paolo Pellicioli [1]; Cristian Fernandez-Palomo [1]; Ismail Sanchez-Gonzalez [1]; Maximilian Jaquerod [1]; Prateek Arora [1]; Ilaria Di Manici [5]; David Reparaz Pernaut [1]; Chloe Prunier [6]; Emilien Dosda [6]; Michael MacManus [7]; Michael Krisch [5]; Erik Thompson [4]
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Background: Microbeam Radiation Therapy (MRT) is a form of sub-millimetre spatially fractionated radiotherapy that has demonstrated an excellent therapeutic ratio against cancer in preclinical studies. However, its therapeutic effect on human triple-negative breast cancer (TNBC), and its potential to control the metastatic spread of surviving cancer cells after treatment, remain unclear. Following conventional radiotherapy (RT) for curative purposes in cancer treatment, distant metastasis commonly occurs. This project aimed to assess whether MRT could control TNBC and whether it might suppress tumour cell spread.
Materials and Methods: Human MDA-MB-231 triple negative breast cancer xenografts were implanted in-ovo onto the chorioallantoic membrane (CAM) of the chick embryos. Tumors were treated with escalating doses of MRT (2, 4, 6, 8, and 10 Gy valley doses with corresponding 152, 304, 457, 609, and 761 Gy peak doses) at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. Primary tumor endpoints were assessed as well as metastatic burden in the lower CAM, liver, lung, and brain of the chick embryos. Irradiated and control tumors underwent RNAseq analysis.
Results: Analysis of survival at experimental endpoint revealed a 100% survival rate for the groups treated with 2/4/6-Gy valley MRT and unirradiated controls. A small rate of death embryos was found at higher doses in the 8 and 10-Gy valley dose groups. Quantification of proliferation of tumour cells in the MDA-MB-231 tumors revealed a dose-dependent decrease of proliferation, statistically significant in the MRT 8-Gy and 10-Gy valley dose. The presence of metastatic cells in the lower CAM, liver, lung, and brain of the chick embryos was determined by GFP immunostaining, expressed by the breast cancer cell lines. The metastatic quantification by qPCR revealed a general decrease in metastasis formation in treated embryos versus unirradiated CTR, with a more marked effect in liver and brain. Bioinformatic analysis reveals a dose-dependent activation of genes related to cancer cell death as well as modulation of genes associated with pathways known to be involved in the Epithelial-Mesenchymal Transition.
Conclusions: This study presents, for the first time, an investigation into the relationship between MRT and metastatic potential employing a chick embryo model, highlighting the importance of understanding this association. MRT shows promise not only in controlling primary TNBC tumours, but also in controlling the spread of metastatic disease, which is the main cause of death in TNBC patients.

167. The Radiation and Nuclear Countermeasures Program (RNCP) at the National Institute of Allergy and Infectious Diseases (NIAID)

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The Radiation and Nuclear Countermeasures Program (RNCP) at the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), is dedicated to developing medical countermeasures (MCMs) for radiological public health emergencies. Key components of the RNCP include comprehensive drug development and biodosimetry approaches, as well as regulatory coordination with federal partners such as the US Food and Drug Administration (FDA).

Drug development activities with the RNCP span basic to advanced countermeasure studies that may include safety/toxicity and efficacy studies, PK/PD, and drug dose optimization in well-established animal models. A recent advancement includes a Phase 1 human clinical trial to test human safety and to bridge data from animal studies to humans in accordance with the FDA Animal Rule.

Our approach to biodosimetry includes development and validation of tools that assess biological outputs of radiation injury and measure absorbed radiation dose. As of yet, there are no FDA-cleared biodosimetry devices; thus, this is an area of great need. Accurate, reliable, and user-friendly biodosimetry devices are needed to ensure adequate triage and to guide medical treatment decisions during a radiation emergency.

The RNCP also facilitates interactions between product sponsors and the FDA through our Office of Regulatory Affairs to streamline the approval process. RNCP success includes the approval of several drugs for the hematopoietic effects of the acute radiation syndrome through the FDA Animal Rule and regularly transitions projects to our Biomedical Advanced Research and Development Authority (BARDA) partners for further advanced development.

The RNCP's extensive portfolio and collaborative efforts with grantees, contractors, and other stakeholders are crucial in advancing candidate MCMs and biodosimetry tools for eventual approval/clearance – a requirement for products to be included in the Strategic National Stockpile and for use during a radiological public health emergency.

Late breaking abstracts

These are the late breaking abstracts which will be presented together with the other posters during the poster sessions.

Monday, 07.09.2026

Postersession 1

Room: Atlantis 2

151. European Defence Fund (EDF) Resilience 2023: First results regarding the radiation pillar as an example of a new funding resource for radiobiological research within Europe

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In 2017, the European Union (EU) enlarged their action plan to enhance preparedness against chemical, biological, radiological and nuclear (CBRN) security risks. A total budget of €100 million was allocated over four years (2023-2026), at €25 million per year, for CBRN research. As an example, we report on first results of EDF Resilience R 2023 related to early and late radiation-induced health effects:
(1) A lab on the chip (microfluidic card) prototype for point-of-care RNA isolation purposes was build and validated to work on the next generation of this microfluidic card. The second point-of-care diagnosis microfluidic card represents an integrated workflow including RNA isolation from whole blood, conversion into cDNA and qRT-PCR. It comprises a four gene set being predictive of the later manifesting hematologic acute radiation syndrome (H-ARS) severity categories. A first prototype will be presented.
(2) The dicentric (DCA) and micronucleus (CBMN) cytogenetic assays are successfully used to discriminate three clinically relevant groups, namely, unexposed, low- and high-H-ARS severity groups. No dose estimation is required. Details will be presented by other teams (Moreno et al., Ramadan et al.) at this conference.
(3) Several hundred Ukrainian Chernobyl radiation victims are examined for still detectable radiation-induced genes (radiation-to-gene associations) with impact on e.g., cardiovascular diseases (gene-to-effect associations). First mRNA seq NGS results will be shown.
Following EDF Resilience, a new program (Resilience II) currently develops. An actual status regarding that will be provided.
Acknowledgments: Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the granting authority can be held responsible for them (RESILIENCE- R- 2023), project No. 101168024.

157. Synergistic Effects of Stress and Ionizing Radiation on Metabolism

Author: Jana Cizkova[1]
Co-authors: Alzbeta Filipova [2]; Lenka Andrejsova [1]; Anna Carrillo [1]; Ondrej Jan Dolezal [1]; Zuzana Sinkorova [1]
[1] Department of Radiobiology, Military Medical Faculty, University of Defence; [2] Department of Radiobiology, Military Faculty of Medicine, University of Defence in Brno, Trebesska 1575, 500 05 Hradec Kralove, Czech Republic

Background: Ionizing radiation exposure frequently co-occurs with psychological stress, yet their biological interaction remains poorly characterized. We examined longitudinal changes in hematological parameters, immune cell subpopulations (flow cytometry), and neuroendocrine/inflammatory markers (ELISA) following combined radiation-stress exposure in female BALB/c mice.
Methods: Mice received total body irradiation (5.4 Gy, 60Co), social isolation stress (individual housing post-treatment), or their combination, with or without vitamin C or amifostine pretreatment. Peripheral blood was analyzed at Days 1, 2, 7, 14, 21, and 30 post-exposure for hematological parameters, seven immune cell populations (B, T, Th, Tc, Treg, NK, DP cells), Th/Tc ratio, and plasma ACTH, corticosterone, and IL-6 levels.
Results: Radiation induced profound lymphopenia (92-95% reduction on Day 7), with B cells most severely affected. Social isolation stress markedly exacerbated immunosuppression and impaired recovery (B cells 15% vs. 96% recovery at Day 30 in combined vs. radiation-alone groups). Th/Tc ratio analysis revealed pronounced biphasic dysregulation, characterized by transient Th skewing (ratios 10-19) followed by severe Tc-dominance (ratios 0.02-0.14 at Day 30), indicating sustained Th cell deficiency. ELISA analysis demonstrated robust HPA axis activation, with corticosterone reaching 8.3-fold elevations (250 ng/mL, Days 2-7), temporally correlating with immune nadir. Amifostine was associated with enhanced stress-related HPA activation (250 vs. 100 ng/mL), whereas vitamin C facilitated faster HPA normalization (100 vs. 250 ng/mL Day 14) and superior preservation of immune cell populations.
Conclusion: These findings identify glucocorticoid-mediated immunosuppression as a central mechanism underlying stress–radiation synergy and demonstrate that vitamin C more effectively preserves immune function than amifostine under stress-modulated radiation exposure.

158. Radioprotective Effects of Amifostine- Modified Hyaluronic Acid Nanoparticles

Author: Ondrej Jan Dolezal[1]
Co-authors: Anna Carrillo [1]; Jana Cizkova [1]; Nikola Plistilova; Zuzana Sinkorova [1]
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Background: Ionizing radiation induces severe damage to biological systems through oxidative stress and DNA injury. Radioprotective agents reduce radiation-induced damage by scavenging reactive oxygen species and supporting cellular recovery. Hyaluronic acid nanoparticles (HANPs) may improve the delivery and efficacy of these compounds. This study evaluated the radioprotective potential of HANPs and HANPs modified with amifostine (WR1065 for in vitro and WR2721 for in vivo experiments).
Materials and Methods: Mice primary T and B cell lines were pretreated with HANPs, HANPs-WR1065, free WR1065, or their combinations before exposure to ˆ60Co gamma irradiation (2 Gy). Cell viability was determined 24, 48, and 72 hours post-irradiation using the MTS assay, and cell-cycle distribution was analyzed by flow cytometry. All experiments were performed in triplicate. For the in vivo study, female C57BL/6 mice received HANPs, WR2721-HANPs, or free WR2721 by intraperitoneal or intratracheal administration 30 - 60 min before whole-body irradiation with 8 Gy ˆ60Co gamma rays. Irradiated, untreated, and saline-treated mice served as controls. Survival, body weight, and clinical status were monitored for 30 days.
Results: Radioprotective efficacy in vitro was expressed as the relative viability of treated irradiated cells compared with irradiated controls. Reduced G2/M arrest was considered indicative of decreased radiation-induced DNA damage. HANPs exhibited a modest radioprotective effect, which was enhanced by amifostine modification. These findings indicate that HANPs reduce radiation-induced cytotoxicity and may modulate the cellular response to DNA damage while maintaining excellent biocompatibility. In the in vivo study, intraperitoneal administration did not improve survival. In contrast, mice treated intratracheally with WR2721-HANPs showed 100% survival throughout the 30-day observation period, demonstrating a significant radioprotective effect.
Conclusions: Modified hyaluronic acid nanoparticles, particularly WR2721-functionalized HANPs administered intratracheally, showed promising radioprotective activity. These findings support further development of nanoparticle-based radioprotective strategies.

160. EFFICIENCY OF NANOPARTICLES IN THE PROCESS OF FIBROSIS

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Background: Ionizing radiation induces both early and late normal tissue injury. While early radiation effects are characterized by inflammation and cellular damage, late effects are associated with progressive tissue remodeling that may lead to radiation-induced organ fibrosis (RIOF). The lungs are among the most radiosensitive organs and are therefore particularly susceptible to the development of fibrosis. Epithelial–mesenchymal plasticity (EMP) is considered a key mechanism underlying radiation-induced fibrogenesis, characterized by the loss of epithelial markers, such as E-cadherin, and the acquisition of mesenchymal markers, including vimentin. The present study aimed to characterize radiation-induced changes in selected EMP markers using human and murine lung cell models and determine the effect of hyaluronic acid nanoparticles (HANPs) on RIOF and EMP.
Materials and Methods: Human lung adenocarcinoma (A549) and murine lung epithelial (MLE-12) cell lines were used as in vitro models to investigate radiation-induced EMP. Cells were divided into control and irradiated (IR) groups. HANPs were added before 1 h of irradiation. Irradiation was performed using an (_ˆ60)"Co" gamma irradiator at doses of 15 and 30 Gy for A549 cells, and 5 and 10 Gy for MLE-12 cells. Real-time monitoring of cell proliferation and cytotoxicity was performed using the xCELLigence system. Cell viability and proliferative activity were further assessed using the EdU incorporation assay. EMP-associated phenotypic changes were evaluated by immunofluorescence staining and flow cytometry of E-cadherin and vimentin, and RT-PCR.
Results: Ionizing radiation induced changes consistent with epithelial–mesenchymal plasticity in both A549 (15, 30 Gy) and MLE-12 (5, 10 Gy) cell lines. Immunofluorescence analysis revealed decreased E-cadherin expression accompanied by increased vimentin expression, indicating a shift towards a mesenchymal-like phenotype. Real-time monitoring using the xCELLigence RTCA system (Agilent), together with the EdU assay, demonstrated significant radiation-induced alterations in cell behavior, including reduced proliferation, decreased viability, and increased cytotoxicity compared with the control groups. HANPs successfully alternated the changes of radiation-induced changes in both cell lines.
Conclusions: Ionizing radiation promotes phenotypic changes associated with epithelial–mesenchymal plasticity in human and murine lung cell models. The established in vitro model provides a suitable platform for investigating the molecular mechanisms of radiation-induced fibrosis. HANPs provided a suitable approach to alleviating EMP.

Tuesday, 08.09.2026

Postersession 2

Room: Atlantis 2

153. The Role of Surface Functionalization in Platinum Nanoparticle-Mediated Radiosensitization for Proton Beam Therapy in Glioblastoma

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Co-authors: Razan Hessenow [2]; Christian Möllers [3]; Christian Bäumer [4]; Lars Krenz [5]; Stephan Barcikowski [5]; Beate Timmermann [6]; Anzhela Galstyan [7]
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Proton beam therapy (PBT) represents a cutting-edge cancer treatment that leverages unique physical properties to deliver radiation directly to tumors while minimizing exposure to surrounding healthy tissue. Metal nanoparticles (NPs) have emerged as promising radiosensitizers capable of amplifying PBT efficacy by boosting radiation-induced DNA damage, with reactive oxygen species (ROS) generation likely representing one contributing pathway. In addition, the influence of NP surface functionalization remains insufficiently explored. Here, we investigate the synergistic effects of methoxy polyethylene glycol (mPEG)- and bovine serum albumin (BSA)-functionalized platinum nanoparticles (PtNPs) on the enhancement of PBT efficacy.

Functionalized PtNPs were tested across multiple concentrations in glioblastoma (GBM) cells. Radiosensitization effects were assessed through short-term viability assays and long-term clonogenic survival assays. In addition, several functional analyses were performed, including ROS production via H2DCFDA assay, DNA damage using γ-H2AX analyses, and cellular uptake and subcellular localization by transmission electron microscopy.

Interestingly, both functionalized PtNP formulations exhibited measurable cytotoxicity in GBM cells even in the absence of irradiation. However, only limited radiosensitization was observed under the investigated conditions. One possible explanation is that intracellular trafficking of the NPs leads predominantly to lysosomal/endosomal accumulation, thereby restricting their access to critical cellular targets and reducing their ability to enhance radiation-induced damage. Ongoing studies comparing PtNPs bearing different surface functionalities are expected to provide further insight into how surface coatings and intracellular localization influence NP-mediated radiosensitization.

These findings highlight the critical importance of surface functionalization in nanoparticle-mediated radiosensitization, underscoring the need for further mechanistic investigation to optimize PtNP design for clinical proton therapy applications.

154. Eliminating Therapy-Induced Senescence Following (212Pb)Pb-AB001 Treatment in PC-3 PIP Cell Line

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Background: Targeted alpha therapy is a promising treatment strategy for metastatic castration-resistant prostate cancer. The PSMA-targeting radioligand [212Pb]Pb-AB001 delivers highly cytotoxic alpha radiation to tumor cells, inducing DNA damage and growth inhibition. However, previous studies have suggested that treatment may cause therapy-induced senescence in PC-3 PIP cell line.

Although senescent cells no longer proliferate, they remain metabolically active and secrete factors that can promote tumor progression, treatment resistance, and disease recurrence. ABT-263 (navitoclax) may selectively eliminate these cells, by inhibiting anti-apoptotic BCL-2 family proteins that are frequently upregulated in senescent cells. This study investigated whether ABT-263 can enhance the efficacy of [212Pb]Pb-AB001 by targeting therapy-induced senescence.

Methods: PSMA-expressing PC-3 PIP cells were treated with [212Pb]Pb-AB001 (0 - 200 kBq/mL, 1h) or ABT-263 (0 - 64 μM, 72h) as monotherapies. For combination treatment, cells received 50 kBq/mL [212Pb]Pb-AB001, followed by 72h incubation period, and subsequent treatment with ABT-263 (0 – 64 μM) for another 72h.

Clonogenic assay was performed to assess long-term proliferative capacity, while CellTiter-Glo assay was used to evaluate ATP-based metabolic activity.

Results: Treatment with [212Pb]Pb-AB001 resulted in a dose-dependent reduction in clonogenic survival, demonstrating a loss of proliferative capacity. While clonogenic survival decreased in a dose-dependent manner, metabolic activity persisted seven days after treatment, indicating the presence of metabolically active but non-proliferating cells consistent with a senescent phenotype. Combination treatment with ABT-263 and [212Pb]Pb-AB001 produced greater reductions in metabolic activity than both ABT-263 and [212Pb]Pb-AB001 monotherapies across all tested concentrations.

Conclusion: [212Pb]Pb-AB001 effectively suppresses the proliferative capacity of PC-3 PIP prostate cancer cells and may induce therapy-induced senescence. Combining radioligand treatment with senolytic drug ABT-263 enhanced cytotoxicity and reduced the population of metabolically active cells, suggesting successful targeting of senescent cells. These findings support further investigation of combination strategies, to improve the therapeutic efficacy of PSMA-targeted alpha therapy in prostate cancer.

161. Radiosensitising Effect of a Novel Quinazoline Derivative in the A431 Cancer Cell Line: An In Vitro Study

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Background: Quinazoline derivatives are anticancer agents that inhibit the Epidermal Growth Factor Receptor (EGFR), a key regulator of tumor cell survival and proliferation. Nowadays, several quinazoline-based drugs are widely used in the targeted therapy of patients with tumors overexpressing this receptor. In addition to their direct antitumor effects, some of these compounds have demonstrated radiosensitising properties, enhancing the efficacy of radiotherapy, therefore highlighting their potential in combination treatment strategies. The aim of this study was to investigate the in vitro anticancer and radiosensitising activity of two novel quinazoline compounds (C1 and C2).
Materials and Methods: The two novel quinazoline derivatives were synthesized based on the (3-bromophenyl)quinazoline-4,6-diamine scaffold. Their biological evaluation was performed using the EGFR-overexpressing A431 cancer cell line. Their anticancer activity was tested using the MTT assay and the Western blotting technique. In order to examine whether the compounds exhibited radiosensitising activity, the clonogenic assay was performed. Furthermore, in order to elucidate the mechanisms underlying the radiosensitising effect, flow cytometry and cytogenetic analyses, including the G2 chromosomal radiosensitivity assay, were performed.
Results: Both compounds C1 and C2 exhibited potent cytotoxic activity against A431 cells, with IC50 values 2.9 ± 0.3 μM and 1.5 ± 0.7 μM respectively. They also both targeted effectively EGFR. However, only C2 demonstrated radiosensitising activity. In addition, C2 induced G0/G1 cell cycle arrest. The results of the G2 chromosomal radiosensitivity assay showed that the number of chromatid aberrations per cell following irradiation in the presence of C2 (4.66 ± 0.06) was markedly lower than that observed following treatment with an ATM/ATR inhibitor (8.62 ± 0.88). This indicates that the radiosensitising effect of C2 is not mediated through G2/M checkpoint abrogation and does not impair the G2-phase DNA repair pathways, where homologous recombination plays a major role. In contrast, cytogenetic analyses revealed that the frequency of excess acentric fragments per cell in G1-irradiated cells was 25.9% higher in the presence of C2 compared with irradiation alone, suggesting that C2 interferes with the DNA repair pathways during the G1 phase, potentially involving the non-homologous end joining repair mechanism (NHEJ).
Conclusions: Further studies are required to better clarify the conditions that promote radiosensitisation and to elucidate the molecular pathways involved in this response. The promising in vitro results obtained with C2 provide a strong rationale for subsequent in vivo studies.

162. Ferroptosis-guided BET/HDAC co-targeting potentiates conventional and FLASH radiotherapy in high-risk neuroblastoma

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Background: High-risk neuroblastoma (HR-NB) remains limited by radioresistance and relapse, while radiotherapy dose escalation is constrained by late toxicity in survivors. Because radiation-induced tumor control relies in part on oxidative damage and regulated cell death, we tested whether ferroptosis-guided epigenetic sensitization can act as a force multiplier for conventional and FLASH radiotherapy.
Materials and Methods: A ferroptosis-focused epigenetic screening platform identified BET and HDAC inhibition as complementary sensitizing axes in HR-NB. BET/HDAC co-targeting with S3.1/T10 was evaluated for ferroptotic contribution using lipid peroxidation and pharmacological rescue assays. Radiosensitization was quantified by cell-death and clonogenic survival assays after conventional and FLASH electron irradiation. In vivo efficacy was tested in SH-SY5Y xenografts treated with fractionated radiotherapy (20 Gy; 2 Gy × 10) alone or combined with S3.1/T10. Cross-modality relevance was explored in additional refractory cancer models and after non-thermal plasma exposure.
Results: BET/HDAC co-targeting lowered the threshold for radiation-induced cell death, with evidence of ferroptotic contribution, and potentiated both conventional and FLASH radiotherapy. Individual sensitizers achieved sensitizer enhancement ratios at 10% survival of 1.21 and 1.54. In vivo, S3.1/T10 alone slowed tumor growth, while combination with fractionated radiotherapy induced faster and deeper tumor regression than radiotherapy alone, including in established tumors approaching 500 mm³. No relapse has been observed to date in the combination arm during ongoing follow-up, whereas relapse occurred after radiotherapy alone, with manageable tolerability. The same sensitization logic extended to breast, colorectal, non-small-cell lung and pancreatic cancer models, and increased susceptibility to non-thermal plasma-induced cell death.
Conclusions: Ferroptosis-guided BET/HDAC co-targeting provides a mechanistically grounded and clinically actionable strategy to strengthen radiotherapy response in HR-NB. By converting resistant tumor cells into a death-responsive state, this approach directly fits combined-modality radiobiology, including conventional fractionation and FLASH, while supporting extension to other refractory cancers and emerging redox-based therapies such as non-thermal plasma.

Postersession 3

Room: Atlantis 2

152. A Time-Dependent Ac-225 Decay Chain Source Model for Cellular Dosimetry Including Recoil Nuclei: A FORRCE Project Contribution

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Background: Targeted radionuclide therapy (TRNT) delivers radiation internally through radiopharmaceuticals that selectively bind to cancer cells, among them, Ac-225 is a particularly promising radionuclide, as its decay chain releases multiple α-particles, enabling highly localized energy deposition and complex DNA damage.
However, current dosimetry models typically assume a time-independent decay spectrum in secular equilibrium, neglecting temporal evolution and recoil nucleus contributions. This work, developed within the FORRCE project (Framework for Outcomes of Radionuclide Radiation on Cellular Environment), funded by the PIANOFORTE European initiative, aims to advance the understanding of dose-response relationships in TRNT through a modular, mechanistic platform bridging physical energy deposition with biological outcomes across multiple spatial and temporal scales.
In this context, we present a time-dependent Ac-225 source model that accounts for both α-emission and recoil nuclei across multiple cellular geometries, providing a more realistic dosimetry scenario for subsequent scoring of DNA damage distribution and subcellular dose estimation.
Methods: Monte Carlo Track Structure simulations were performed using a custom version of OpenTOPAS. The source extension was developed to model the temporal evolution of the Ac-225 decay chain using the Bateman equations. Two cellular geometries were investigated (spherical and ellipsoid) embedded in three irradiation media geometries: Cubical, spherical, and spheroidal. A phase space scorer recorded the α-particle energy spectrum and absorbed dose to the cell and nucleus. All cases were simulated under equal activity and volume conditions to enable direct comparison.
Results: For a spherical cell, a spherical emission medium yielded a 10% higher absorbed dose to the cell compared to a cubical medium, alongside an increased number of recoil nuclei reaching the cellular surface, consistent with the spherical geometry providing more uniform exposure to the source.
When comparing cell shapes using conformal emission shells of equal volume and activity, the ellipsoidal cell received 54% higher absorbed dose than the spherical cell, despite approximately 50% fewer α-particles and recoil nuclei reaching its surface. This is explained by two compounding geometric effects. First, because the shell thickness was uniform across all three semi-axes, it represented a much larger fraction of the short semi-axis than of the long one, producing a non-isotropic source distribution relative to the flattened cell geometry; many emitted trajectories were therefore geometrically unlikely to intersect the cell at all, reducing the total number of particles reaching the surface. Second, among the particles that did reach the cell, the mean deposited energy was higher in the ellipsoidal case: trajectories intersecting the flattened cell preferentially traversed its longer semi-axis, increasing the probability that the full α-particle range (including the Bragg peak) was contained within the cellular volume, rather than escaping through the opposite surface

as occurs more frequently in the spherical geometry. The net effect is a smaller number of particles each depositing a larger share of their energy, yielding a higher total absorbed dose
Recoil nuclei contributed a small fraction of the total absorbed dose: 0.08% for the sphere and 0.11% for the ellipsoid. The temporal evolution of the α-energy spectrum was also characterized, showing the growing contribution of decay chain daughters over time.
Conclusions: Both emission medium geometry and cellular shape significantly influence absorbed dose, with the ellipsoidal cell configuration yielding the highest dose deposition despite receiving fewer incident particles at its surface, as a result of geometric self-shielding effects within the flattened cell volume. While medium geometry was varied here to characterize these effects systematically, in realistic exposure scenarios cells are typically embedded in much larger volumes and exposed to particles from a wide range of distances and directions (e.g., adherent to a culture surface or embedded in a 3D matrix); the surrounding medium shape itself is therefore expected to be of secondary importance compared to the local source distribution and cell geometry. The low recoil nucleus contribution at the cellular surface is consistent with a non-internalized radiopharmaceutical distribution.
Future work will extend the framework by incorporating a radiopharmaceutical concentration gradient model, investigating cell internalization scenarios, and expanding to more realistic cellular geometries.

155. Influence of Uranium Speciation on Nephrotoxicity in Human Renal Proximal Tubule Cells

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Uranium is a naturally occurring actinide with both chemical and radiological toxicity, with the kidney as its primary target organ. While high-dose toxicity is well characterized, the effects of environmentally relevant low-dose exposure remain poorly understood. A key factor influencing uranium behavior in biological systems is its aqueous speciation, which governs bioavailability, cellular uptake, and toxicity. However, most in vitro studies do not adequately account for physiologically relevant uranium speciation.

This study investigated how ligand-controlled uranium speciation influences cytotoxicity in human renal proximal tubule (HK-2) cells under physiologically relevant conditions. Cells were exposed for 24 h to uranium (U(VI)) prepared as uranyl carbonate complexes in media with controlled bicarbonate and calcium concentrations. Cytotoxicity was assessed using a resazurin-based viability assay, alongside evaluation of cell morphology and uranium uptake.

Preliminary results show that uranium uptake and cytotoxicity are strongly influenced by solution chemistry. Interactions between uranium and media constituents promote the formation of distinct soluble and insoluble complexes, leading to variability in cellular responses. Notably, cytotoxicity observed under physiologically relevant conditions differs from previously reported values, suggesting that conventional in vitro models may not accurately reflect uranium bioavailability in biological systems.

These findings highlight the critical role of uranium speciation in modulating nephrotoxicity and emphasize the need to incorporate physiologically relevant chemistry into in vitro models. This work improves mechanistic understanding of uranium-induced kidney toxicity and supports more accurate interpretation of toxicological data for environmental risk assessment and drinking water guideline development.

156. Evaluation of Piperazine-Based Radioprotective Compounds

Authors: Alžběta Filipová[1]; Natálie Živná[2]; Vojtěch Chmil[1]; Darina Muthná[3]; Martina Řezáčová[3]; Radim Havelek[3]; Marcela Milanová[1]; Lukáš Prchal[2]; Rafael Doležal[2]; Vít Řeháček[4]; Jaroslav Pejchal[5]; Jana Žďárová Karasová[5]; Jan Marek[2]; Aleš Tichý[1]

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Background: The growing risk of nuclear incidents, together with the expanding use of ionizing radiation in medical diagnostics and radiotherapy, highlights the urgent need for effective radiation countermeasures to protect healthy tissues and mitigate radiation-induced damage. Despite the discovery of numerous radioprotectors and radiation mitigators, their clinical use is often limited. Therefore, the development of safe and effective radioprotective substances remains a priority. The present study focuses on the development and evaluation of novel piperazine-based compounds with radioprotective potential.

Materials and methods: Novel piperazine-derived compounds were synthesized and analytically characterized, followed by cytotoxicity and radioprotective evaluation in vitro and in vivo.

Results and conclusion: Selected compounds showed variable radioprotective effects in whole-body irradiated BALB/c mice. The most effective compound improved 30-day survival to approximately 50%, compared with 20% in the irradiated control group, while other compounds showed moderate or no protective effects. One compound was associated with reduced survival. The reference compound amifostine demonstrated the highest efficacy, reaching approximately 90% survival, but its use is limited by dose-dependent toxicity. The synthesized piperazine derivatives demonstrate promising radioprotective properties and encouraging biological activity, warranting further optimization and evaluation.

Acknowledgements: This work was supported by project No: NU23-08-00256 and project No: DZRO-FVZ22-ZHN II.

163. Are Broad Beam, Minibeam RT, and Microbeam RT dose-equivalent? A controlled cross-modality comparison in mouse melanoma.

Authors: Jakub Korzeniowski[1]; Paolo Pellicioli[2]; David Reparaz Pernaut[2]; Aymen Ladjimi[2]; Verdiana Trappetti[2]; Veljko Grilj[3]; Michael K Fix[4]; Valentin Djonov[2]; Cristian Fernandez-Palomo[2]
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Background Sub-millimeter spatially fractionated radiation therapy (smSFRT), including Minibeam (MBRT) and Microbeam (MRT) RT, delivers heterogeneous doses to tumors, yet dose equivalences between broad beam (BB), MBRT, and MRT remain unknown. Using a reverse approach, we irradiated one tumor model with all three techniques across a dose range to find iso-equivalences.

Materials and Methods B6F10 melanoma in the mouse pinna was treated, ten days after tumor implantation, with one of eight conditions: BB (7, 15, 18, or 22Gy); synchrotron MRT (400/7Gy peak/valley, ˜105Gy mean); MBRT alone (36/0.3Gy, ˜9.2Gy mean); MBRT plus a 7 Gy BB boost (29/0.2Gy + 7 Gy BB, ˜14.4Gy mean); or unirradiated controls (beam geometries in figure). MBRT+BB matched MRT's valley at an MBRT-like peak.

Results Median survival was highest for MRT (28 days), then MBRT+BB (24) and 18Gy BB (17). Tumor growth delay (TGD) ranked: 18 days for both MRT and 18Gy BB; 15 days for 15Gy BB; 10 days for MBRT+BB; 7 days for 7Gy BB; and 3 days for MBRT alone. MBRT alone ranked last despite a 36Gy peak and a mean dose (˜9.2Gy) exceeding 7Gy BB. Complete ablation occurred exclusively in 4 MRT and 1 of 15Gy BB animals.

Conclusion The answer to our title is negative: clean cross-modality equivalence does not exist. 18Gy BB matched MRT's growth delay yet mice reached the termination point sooner than MBRT+BB (which had worse TGD), indicating toxicity limited survival at 18Gy, with clear toxicity at 22Gy. While valley and mean dose governed growth delay, ablation required a high peak dose that MRT reached but MBRT at 36Gy did not, leaving room to escalate MBRT's peak dose. These results suggest that effective prescription requires both a valley dose floor and a peak dose threshold; parameters that currently only smSFRT can simultaneously deliver while preventing normal tissue toxicity.

164. Tracing tumor-material uptake by antigen-presenting cells after submillimetric spatially fractionated radiotherapy

Author: Ismael Sanchez Gonzalez[1]
Co-authors: Paolo Pellicioli; Valentin Djonov [1]
[1] *University of Bern*

Background: Submillimetric spatially fractionated radiotherapy (smSFRT) delivers radiation through arrays of submillimetric beamlets, generating high-dose peaks separated by low-dose valleys. This spatial dose modulation permits high peak doses while limiting normal-tissue toxicity compared with homogeneous conventional irradiation. In preclinical melanoma models, the antitumor efficacy of smSFRT has been shown to depend on CD8 T cells, highlighting the importance of antigen-presenting cells (APCs) as potential upstream mediators of this immune response. However, measuring APC abundance within an irradiated tumor does not reveal which subsets have internalized tumor-derived material, acquired an immunostimulatory phenotype, and can subsequently activate tumor-specific CD8 T cells. This study describes the ongoing establishment of a tumor-material tracing model to investigate these sequential events after smSFRT.
Materials and Methods: B16-F10 melanoma cells were engineered to express cytoplasmic ZsGreen and ovalbumin (OVA) with its N-terminal signal sequence deleted. OVA served as a defined surrogate intracellular tumor antigen, while ZsGreen traced tumor-derived material. Expression and localization were validated by flow cytometry and confocal microscopy. For the in vivo feasibility study, subcutaneous flank tumors were established in C57BL/6 mice by injecting 1×10 cells. At a tumor volume of approximately 100 mm^3, tumors received smSFRT using 200-µm-wide beamlets with 800-µm center-to-center spacing and a 50-Gy peak dose, or no irradiation. Tumor growth was monitored in an exploratory cohort (n=3/group). In a separate feasibility cohort, tumors were collected three days after treatment, dissociated, and analyzed by imaging spectral flow cytometry using a BD FACSDiscover™ A8 Cell Analyzer. Tumor-associated myeloid APCs were identified by sequential gating of CD45.2 leukocytes, exclusion of CD3ε T cells, CD19 B cells, and NK1.1 NK cells, separation of Ly6C monocytes, and identification of CD11bI-A/I-E cells within the remaining non-monocytic myeloid compartment. The ZsGreen Max Intensity image feature was used to quantify concentrated fluorescent signal within gated APCs. Representative single-cell images were additionally reviewed to confirm the punctate intracellular localization of ZsGreen-containing tumor-derived material.
Results: smSFRT delayed tumor growth compared with no irradiation, confirming biological activity of the selected regimen. Three days after treatment, smSFRT-treated tumors showed a higher frequency of tumor-associated myeloid APCs. A larger ZsGreen-high APC fraction was also observed after smSFRT; in representative tumors, 51.3% of APCs were ZsGreen-high after smSFRT versus 21.7% without irradiation. Single-cell images confirmed bright, punctate intracellular tumor-derived material. These findings are consistent with increased APC uptake of tumor material after smSFRT. Whether this reflects greater availability of phagocytosable debris, enhanced APC phagocytic activity or altered APC composition remains unresolved.
Conclusions and Outlook: A major strength of this OVA/ZsGreen-expressing model is its ability to connect smSFRT-induced tumor damage with antigen-specific immunity. ZsGreen identifies APCs that have internalized tumor-derived material, whereas OVA serves as a defined surrogate intracellular tumor antigen that can be followed through antigen presentation to CD8 T-cell recognition. Ongoing high-dimensional phenotyping will identify the ZsGreen APC subpopulations involved, including M1- and M2-like tumor-associated macrophages and cDC1 and cDC2, and characterize their immunostimulatory or regulatory state using markers such as CD80 and PD-L1. H-2K–SIINFEKL tetramers combined with activation and effector markers will determine whether smSFRT induces an active OVA-specific CD8 T-cell response rather than nonspecific T-cell infiltration. Sorted ZsGreen APC subsets will subsequently be tested for their ability to present

OVA-derived antigen and activate antigen-specific CD8 T cells. This model may reveal how the spatially heterogeneous tumor damage produced by smSFRT is converted into antigen-specific CD8 T-cell immunity and help identify treatment conditions and APC populations that support an effective antitumor response.

165. Early metabolic changes in bank voles following low-dose radiation exposure at the contaminated areas of the Chornobyl exclusion zone: Preliminary findings.

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Background. Understanding physiological responses to low-dose ionizing radiation is important for improving radiation risk assessment. This study investigates preliminary liver metabolomic changes in bank voles to identify early physiological adaptations following radiation exposure.
Materials and Methods. Adult male bank voles (Clethrionomys glareolus) implanted with RFID tags were introduced into the Red Forest of the Chornobyl Exclusion Zone for 16 days. The estimated mean absorbed individual doses were 0.0674 ± 0.007 mGy (control) and 58.68 ± 23.36 mGy (exposed). Following exposure, peripheral blood glucose was measured using a OneTouch Select Simple glucometer (n = 12 control, n = 22 exposed). Liver tissue was subsequently harvested and stored on dry ice. Untargeted bulk LC-MS metabolomic profiling was performed at Bioplatforms Australia using liver tissue samples from control (n = 5) and exposed (n = 4) animals, identifying 204 metabolites. Data were normalized and analyzed using MetaboAnalyst 6.0 and R. Given the limited sample size, metabolomic findings represent preliminary trends.
Results. Unsupervised PCA revealed clear metabolic separation between exposed and control groups (41.9% total variance), which was statistically validated by PERMANOVA ($F = 4.44$, $R2 = 0.388$, $p = 0.038$). Multivariate sPLS-DA identified Rhamnose, 4-Hydroxyhippuric acid, Phenylacetylglycine, Cytidine, Behenic acid, 2-Hydroxy-3-methylbutyric acid, Citicoline, Cinnamic acid, and Cortisol as primary discriminatory features. Univariate Limma testing confirmed significantly lower levels in exposed animals for Cinnamic acid (FDR = 0.009), Cytidine (FDR = 0.038), and 4-Hydroxyhippuric acid (FDR = 0.038), while elevated for Phenylacetylglycine (FDR = 0.026, dose-dependent). Dose-dependent trends also included increases in Cortisol and Citicoline, and a decrease in UDP-glucose ($p < 0.05$). Conversely, elevations in Cortisone, 2-Hydroxy-3-methylbutyrate, and Rhamnose, coupled with reduced Behenic acid, showed significant shifts ($p < 0.05$) without clear dose dependence. Blood basal glucose was significantly elevated in the exposed group compared to control (median ± SE: 6.80 ± 0.31 vs. 5.60 ± 0.33 mmol/L, $p = 0.013$).
Conclusions. Elevation of both cortisone and cortisol suggests HPA axis activation in response to a stressor and increased energetic demands, promoting catabolism and hepatic gluconeogenesis, resulting in intracellular UDP-glucose depletion, elevated blood glucose, and increased 2-hydroxy-3-methylbutyrate, a marker of amino acid catabolism. Reductions in cytidine and behenic acid could indicate accelerated pyrimidine salvage and membrane lipid remodeling to support repair of ROS-induced DNA and membrane damage. Altered rhamnose and phenylacetylglycine (PAGly) levels suggest changes in host-microbiome interactions. Simultaneously, reduction in microbially derived cinnamic acid and 4-hydroxyhippurate may reflect gut dysbiosis together with increased utilization of these aromatic metabolites for their antioxidant properties. Overall, the observed metabolic trends illustrate early physiological adaptations to radiation exposure, particularly in energy metabolism, oxidative stress responses, and gut microbial metabolism.

Participant and Speaker Index

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