IRISH RADIATION RESEARCH SOCIETY

IRRS 2025 Annual Meeting

Imperial Hotel, Dundalk

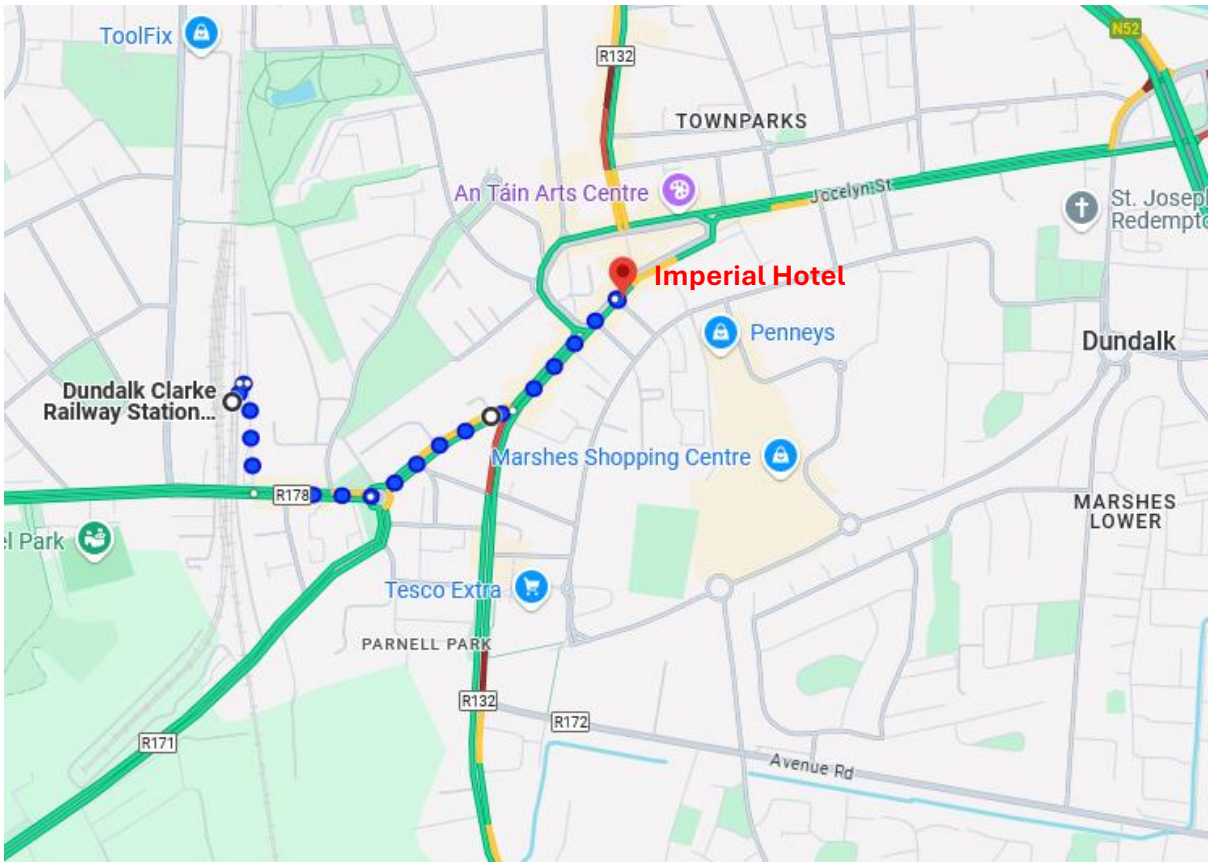
3rd & 4th April 2025

Programme & Abstract Book

Travelling to Imperial Hotel Dundalk

This year the IRRS is taking place in the Imperial Hotel Dundalk (https://www.imhotel.ie/). It is centrally located in Dundalk, a 15-minute walk (1 km) from Dundalk Railway Station. A discounted rate has been agreed with the hotel, which can be availed of by making a phone booking and noting it is for the IRRS annual meeting (subject to availability).

The Imperial Hotel will also host the conference dinner on the night of the 3rd of April, in the Gallery Restaurant. Menu selections are to be made in advance, please see link in e-mail sent to attendees to complete selections if you have not yet done so.



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Programme

Day 1 – Thursday 3rd April 2025

1:00 PM	**Arrival & Lunch**	
2:00 PM	**Welcome**	Stephen McMahon
	Session 1 – Optimising radiotherapy delivery Chair: Stephen McMahon	
2:05 PM	Photon-FLASH: Advancing normal tissue sparing strategies across dose rate, dose and beam structure.	Kathryn Brown
2:20 PM	FLASH irradiation preserves cardiac function in a preclinical mouse model	Mihaela Ghita-Pettigrew
2:35 PM	*In Vitro* Assessment of Photon Radiation Responses in GBM Models at Ultra-high (FLASH) Dose-Rates	Malachy McIvor
2:45 PM	Targeting SETD7 as a potential cardiac radioprotective strategy	Brianna Kerr
2:55 PM	Mechanistic models of radiation response can predict experimental DNA damage yields across radiation qualities	Shannon Thompson
3:10 PM	Assessing the relative contribution of DSB repair pathways as a function of LET in vitro and in silico	Francisco Liberal
3:20 PM	Modelling Intrinsic Radiosensitivity and Relative Biological Effectiveness in Clinical Radiotherapy Plans	Mohammed Dakheel
3:30 PM	**Coffee Break**	
4:00 PM	Gold Sponsor Presentation	RPS Services
	Session 2 – Radiation protection Chair: Simon O'Toole	
4:10 PM	Accurate determination of natural radionuclides in soil samples using mass attenuation coefficients derived from transmission measurements	Cara McKeever
4:25 PM	The National Radiation Dose Report	Kevin Kelleher
4:35 PM	Overview of research to support Ireland's National Radon Control Strategy	Alison Dowdall
4:50 PM	Radionuclide levels present within dredge material collected from Irish ports and assessment of suitability for disposal at sea using criteria derived from IAEA-TECDOC-1759	Angus Collison
5:00 PM	The PIANOFORTE radiation research partnership and the 2026 Integrated Regulatory Review Service Mission to Ireland	Veronica Smith
5:10 PM	Radiobiome: Host-Gut Microbiome Functional Resilience to Radiation	Michaela Walsh
5:30 PM	**Invited Speaker – Anna Fogtman, European Space Agency**	
7:30 PM	**Conference Dinner – Gallery Restaurant**	

Day 2 – Friday 4th April 2025

8:30 AM	**Arrival & Refreshments**	
9:00 AM	**PPI Speaker – John Joyce**	
	Session 3 – Radio-sensitisation techniques Chair: Niamh Lynam-Lennon	
9:30 AM	Metabolic effect induced by mannose and related metabolic gene mediated radiosensitisation of HPV negative head and neck Squamous Cell Carcinoma (HNSCC)	Tongchuan Wang
9:45 AM	Inhibiting the CXCR4/CXCL12 signalling axis with targeted gold nanoparticles sensitises prostate cancer to radiotherapy	Xinyi Liu
10:00 AM	Impact of a novel gold nanoparticle formulation on local and systemic radiation-induced effects in an in vivo model of head and neck cancer	Lydia McQuoid
10:10 AM	Radio Sensitisation using Molecular Targeted Gold Nanoparticles in Prostate Cancer: Multiparameter Immune Profiling in a Syngeneic Tumour Model	Sarah Chambers
10:20 AM	Countering the proteinase activated receptor 1 (PAR-1) pro-tumour phenotype using a novel nanotherapeutic approach	Oscar Pooley
10:30 AM	**Coffee Break**	
	Session 4 – Novel therapeutic and experimental approaches Chair: Francisco Liberal	
11:00 PM	Direct post-irradiation single-cell whole-genome DNA sequencing to elucidate radiation-induced mutations	John Kildea
11:15 PM	MicroRNA-31 Enhances Radiosensitivity in Pancreatic Ductal Adenocarcinoma by Targeting ATOX1 and GPx8 Antioxidant Activity	David Hackett
11:30 PM	Enhancing Cancer Radiotherapy Efficacy Using NanOx, a Novel Oxygenating Nanoemulsion that Reverses Tumour Hypoxia	Maitiú Ó Murchú
11:40 PM	Effects of oxygen-loaded Perfluorocarbons (PFC) nanoemulsions on hypoxia reversal and radiosensitization in pancreatic cells	Xuehua Lin
11:50 PM	Comparison of 2D and 3D in vitro cancer cell line models in SABR	Fiona O'Neill
12:00 PM	**Lunch & IRRS AGM**	
1:00 PM	Close	

Abstracts

Session 1 - Medical Physics & Modelling

Photon-FLASH: Advancing normal tissue sparing strategies across dose rate, dose and beam structure.

Kathryn H. Brown[1*], Mihaela Pettigrew[1], Owen McLaughlin[1,2], Malachy McIvor[1], Daniel Sforza[3], John Wong[3], Mohammad Rezaee[3], Conor K. McGarry[2], Stephen J. McMahon[1], Karl T. Butterworth[1]

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[2] Northern Ireland Cancer Centre, Belfast Health & Social Care Trust, Northern Ireland, UK.
[3] Department of Radiation Oncology and Molecular Radiation Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Introduction:
Ultra-high dose rate radiotherapy (RT) or FLASH is an emerging technique delivering RT in extremely short pulses. A growing body of evidence has shown that RT delivered at >40 Gy/s spares normal tissues due to a radiobiological phenomenon known as the FLASH effect. FLASH sparing has been extensively reported using electron and proton sources yet there is little evidence for the FLASH effect following ultra-high dose rate photon irradiations.

Methods:
In this study we assessed radiation-induced skin toxicity across conventional (2.7 Gy/min) and FLASH (72 Gy/s) dose rates, doses (18.1, 21.3 & 25.8 Gy) and pulsed beam exposures (average dose rate 2.8 – 72 Gy/s). For all set-ups, a custom 3-D printed positioning tool was used for reproducible irradiation of the skin on the hind leg of C57BL6 mice using the FLASH-SARRP or SARRP (Xstrahl). Skin toxicity was visually scored and histopathological analysis completed at 8 or 12 weeks. Tumour growth delay was also assessed using a melanoma (B16F10) xenograft model irradiated with 16 Gy at FLASH and conventional dose rates.

Results:
Skin toxicity was delayed and within a shorter timeframe for FLASH (21.3 Gy) in comparison to conventional RT (20.2 Gy). Tissue analysis showed signs of hyperplasia in conventional mice and significant fibrosis deposition ($p < 0.0001$) compared to FLASH. As doses were sub-lethal, signs of tissue recovery were observed for both dose rates from 8 weeks post RT. A dose dependent relationship for FLASH sparing was observed with tissue damage reduced at lower doses (18.1 & 21.3 Gy) and increased for higher doses (25.8 Gy). A pulsed FLASH beam exposure reduced the average dose rate to 2.8 Gy/s and resulted in loss of FLASH sparing. FLASH was found to be equally effective for tumour control with no significant differences in tumour growth delay compared to conventional exposures ($p = 0.998$).

Conclusion:
These results demonstrate the feasibility to deliver photon FLASH exposures using the FLASH-SARRP with results consistent with observations from previous studies using proton and electron beams. Our data show the potential benefit of using photon FLASH to spare normal tissues whilst maintaining tumour control.

FLASH irradiation preserves cardiac function in a preclinical mouse model

Mihaela Ghita-Pettigrew [1]*, Kathryn H. Brown[1], Brianna Kerr[1], Gerard Walls[1], Stephen McMahon[1], Karl Butterworth[1]

[1] Patrick G. Johnston Centre for Cancer Research, Queens University Belfast, Belfast, Northern Ireland, United Kingdom.

Introduction: FLASH radiotherapy (RT) is an emerging technique involving the delivery of radiation at ultra-high dose rates (> 40 Gy/s). FLASH-RT has been shown to reduce radiation-induced toxicities in multiple normal tissue models without compromising the tumour control. The is currently no evidence supporting the cardioprotective benefits of FLASH-RT compared to conventional RT using FLASH photons.

Methods and Materials: C57Bl/6 female mice were irradiated with 20 Gy delivered to the whole heart using FLASH-SARRP platform (Xstrahl, Life Sciences) using a single pulse of 80 Gy/s (FLASH) or 2 consecutive pulses with 10 seconds pause between pulses (non-FLASH) . Conventional irradiation was delivered using SARRP (Xstrahl, Life Sciences) heart with a dose rate of 3.6 ± 0.18 Gy/min. Transthoracic Echocardiography was used to assess the functional and conduction changes at 10 weeks after irradiation.

Results: Radiation induced cardiotoxicity was observed as a significant decrease in the Global Longitudinal Strain (GLS) in the conventional irradiated group as early as 10 weeks irradiation to -10.1%±0.87% when compared to age-matched controls with a GLS of -18.21%±0.53 ($p=0.002$). While the FLASH irradiated arm showed a modest GLS decrease to -16.6 % ± 0.66%, this was significantly higher than the conventional irradiated arm ($p=0.008$). The non-FLASH irradiated arm showed a significant decrease to -11.65% ± 1.38%, significantly lower than the FLASH irradiated arm ($p=0.02$).

A significant shortening of the QRS interval to 8.58 ms ±0.49 ms and 10.9 ms ±1.59 ms for the conventional irradiated arm and non-FLASH irradiated arm respectively, compared to age-matched controls of 13.46 ms ±0.53 ms ($p<0.009$). Flash irradiated arm showed no decrease in the QRS interval or any other conduction changes at 10 weeks post irradiation.

Conclusions: This study presents the first evidence of photon FLASH sparing of cardiac strain and conduction abnormalities in the heart.

In Vitro Assessment of Photon Radiation Responses in GBM Models at Ultra-high (FLASH) Dose-Rates

Malachy P McIvor[1*], Mihaela Pettigrew[1], Bríanna M Kerr[1], Kathryn H Brown[1], Kevin M Prise[1], Stephen J McMahon[1], Karl T Butterworth[1]

[1] Patrick G. Johnston Centre for Cancer Research, Queen's University Belfast, Northern Ireland, UK

Introduction

Glioblastoma (GBM) is the most prevalent primary brain tumour in adults with 5-year survival rates of ~ 5%. Radiotherapy plays a key role in GBM management but results in collateral damage to normal brain tissue. FLASH radiotherapy (FLASH-RT) is emerging as a promising technique due to its potential to spare normal tissues whilst effectively controlling tumours. Preclinical studies have demonstrated FLASH sparing in the brain, yet most studies have been conducted using protons or electrons. Our group has commissioned the FLASH-SARRP to assess the impact of radiobiological variables (dose, dose-rate and hypoxia) on the responses of GBM cells to photon FLASH-RT.

Materials and methods

Clonogenic survival assays were performed using human (U87-WT, U251) and murine (GL261) GBM cells which were exposed to conventional (0.04 Gy/s), "non-FLASH" (5.5-19.9 Gy/s) and FLASH dose-rates (39.8 – 99.1 Gy/s). Studies were undertaken under oxic and hypoxic conditions (<0.3% O_2). Proteomics analysis was performed using Human Cytokine Arrays (ab133998, Abcam) to characterise the protein expression profiles of 80 targets.

Results

FLASH sparing was observed in all cells irradiated at FLASH (99.1 Gy/s) compared to CONV dose-rates under oxic conditions (FLASH Sparing: U87 = 1.19±0.31; U251 = 1.23±0.21; GL261= 1.37± 0.26, $p < 0.05$). U87 and U251 cells both showed increased FLASH sparing under hypoxia (OER_{FLASH} vs OER_{CONV}: U87 =1.67 vs 1.4, U251 = 1.80 vs 1.61 $p < 0.001$). Clonogenic survival following dose-rate modulation from 5.5 Gy/s – 99.1 Gy/s reveal an increase in cell survival above a threshold of 19.9 Gy/s. Proteomics analysis of U251 cells irradiated under FLASH and CONV dose-rates produced significant differences in cytokine expression under oxic and hypoxic conditions.

Discussion

These data represent one of the first reports of *in vitro* responses to photon FLASH-RT. The FLASH effect was displayed under oxic conditions but is more significant under hypoxia, suggesting that FLASH sparing partially oxygen dependent. Further studies to understand the mechanistic principles of photon FLASH are required.

Targeting SETD7 as a potential cardiac radioprotective strategy

Brianna Kerr [1], Issy McGrugan [1], Johan Linderoth [2], Karl Butterworth [1].

[1]Patrick G. Johnston Centre for Cancer Research, Queen's University Belfast, Belfast, United Kingdom.
[2] Lund University Cancer Centre, Lund University, Lund, Sweden.

Introduction: Radiotherapy is a central modality in the management of thoracic cancers and also has a role in the mediastinal irradiation of Hodgkin's lymphoma (HL). Around 50% of patients with HL patients develop cardiotoxicity which has recently been linked to alterations in the epigenetic regulator gene, *SETD7*. However, the underpinning mechanisms through which SETD7 mediates radiation response remain unclear. In this study, we examined the potential radio-cardioprotective effect of perturbing SETD7 activity in a thoracic-related context *in vitro*.
Methods: Clonogenic survival assays were performed in AC16 (cardiomyocytes), MRC5 (lung fibroblast) and H460 (NSCLC) cell lines. Cells were treated with *(R)*-PFI-2 (2-10 nM) for 24 h prior to irradiation with 2-8 Gy Xrays. DNA double-strand breaks (DSBs) were quantified by immunofluorescent staining in response to 10 nM *(R)*-PFI-2 and 0 Gy or 2 Gy irradiation. The expression of 80 human cytokines was quantified in cardiomyocytes 72 h after 0 Gy or 8 Gy irradiation, with and without 10 nM *(R)*-PFI-2.
Results: *(R)*-PFI-2 increased the clonogenic survival of cardiomyocytes and NSCLC cells in a dose-dependent manner, with minimal cytotoxicity observed. This radio-cardioprotection was independent of residual DSBs and cell cycle progression. Cytokine expression analysis revealed the strong anti-inflammatory effect of *(R)*-PFI-2.
Discussion: We have shown SETD7 inhibition by *(R)*-PFI-2 confers survival benefits to irradiated cardiomyocytes *in vitro*. Pathway enrichment analysis revealed the negative regulation of apoptosis as an underpinning biological mechanism of *(R)*-PFI-2-mediated cardioprotection. Thus, SETD7 inhibition is a potential novel radio-cardioprotective strategy in the context of thoracic irradiation *in vitro*. Yet, further investigations are required to fully understand the multifaceted role of SETD7 in cardiotoxicity.

Mechanistic models of radiation response can predict experimental DNA damage yields across radiation qualities

Shannon Thompson*, Matthew Duffy, Kevin Prise, Stephen McMahon

Patrick G. Johnston Centre for Cancer Research, Queens University Belfast, Belfast, Northern Ireland, United Kingdom.

Introduction: Beside preferential tumour targeting, therapeutic ions have an increased relative biological effectiveness (RBE) compared to X-rays. However, the exact magnitude of this increase remains uncertain with further quantification needed to exploit the clinical benefits of ions. Here, computational models simulating DNA damage and repair after irradiation have been optimised and validated against experimental results to advance their use in radiation research.
Methods: Radiation interactions and double strand break (DSB) damage was simulated in a fibroblast nucleus using the TOPAS-nBio toolkit. Following this, DSB repair, misrepair, and formation of lethal chromosome aberrations was simulated using the Medras biological response model. Experimental data for each damage type was gathered from the literature and compared to simulated results. A subset of the experimental data was used to optimise default model parameters, improving the quality of damage predictions with the experimental results. Additional simulations were then completed using the optimised model predictions and validated against the remaining experimental data.
Results: Models predicted quantifiable differences in RBE for different radiation qualities. DSB and chromosome aberration predictions were higher than measured yields, although with similar LET trends. Model predictions were improved by modelling damage foci rather than DSBs, the exact yield of which cannot be accurately measured *in vitro*. This was achieved by considering an approximated 1 μm damage resolution, similar to immunofluorescence staining limits. For chromosome aberrations, the misrepair rate between DSB ends was optimised improving predictions with the experimental data, but with some differences remaining due to experimental variation and simulation uncertainties.
Conclusion: Mechanistic models predicted clear differences in damage between radiation qualities and were further optimised to provide better agreement with the current experimental data. This highlights how mechanistic models, with the guidance of more robust experimental data, can be used to further explore RBE.

Assessing the relative contribution of DSB repair pathways as a function of LET in vitro and in silico

Francisco D.C. Guerra Liberal[1], Lydia Gardner[1], Kevin Tabury[2], François Chevalier[3], Stephen J. McMahon[1]

1 - The Patrick G Johnston Centre for Cancer Research, Queen's University Belfast
2 - Belgian Nuclear Research Centre (SCK CEN), Radiobiology Unit, Nuclear Medical Application Institute, Mol, Belgium
3- CIMAP, Université de Caen Normandie

Particle therapy is gaining popularity due to its dosimetric benefits which offer superior tumour conformation. Additionally, particle radiation also has a higher LET than X-rays, leading to more complex DNA damage and a higher RBE. While potentially beneficial, there remains significant uncertainty in how RBE depends on genetic features of cells. Understanding how cells respond to and repair these damages is crucial for optimising radiotherapy.
This study evaluates how loss of different DSB repair genes impact on radiosensitivity. CRISPR-Cas9 was used to generate defects in different genes directly involved in DSB repair, including ATM, BRCA1, DCLRE1C, LIG4, PRKDC, TP53 and FANCD2, in RPE-1 cells. Cells were exposed to 6 different LETs using X-rays, protons, carbon ions and alpha particles. Experimental data was then compared with predictions from a mechanistic model of radiation response (Medras).
Data revealed that cells lacking ATM and NHEJ repair genes were particularly radiosensitive, even at high LET. While RBE increased with LET for all knockout lines, RBE increased more slowly for cells that were more sensitive to X-rays. Data showed no significant difference in DNA repair pathway dependence as a function of LET. Medras modelled responses were in agreement with both the genetic background and LET dependencies of radiosensitivity, without including any assumption of a change in repair pathway dependence with LET.
This research further highlights the importance of DSB repair pathways, particularly NHEJ, in determining cellular sensitivity to different radiation qualities, but suggests in this system that there is little difference in repair pathway dependence between X-rays and high-LET radiation. Mechanistic approaches like Medras offer a promising approach to predict radiation responses, to support more personalised and effective cancer treatments based on genetic profiles.

Modelling Intrinsic Radiosensitivity and Relative Biological Effectiveness in Clinical Radiotherapy Plans

Mohammed Dakheel*, Kevin Prise, Stephen McMahon

Patrick G. Johnston Centre for Cancer Research, Queens University Belfast, Belfast, Northern Ireland, United Kingdom.

Background
Proton therapy's clinical advantage over conventional radiotherapy hinges on its physical precision and elevated RBE. However, the assumption of a constant RBE (1.1) fails to account for biological variability, dose fractionation, and linear energy transfer (LET). This study evaluates phenomenological RBE models to quantify their impact on tumour control and normal tissue toxicity in clinical proton plans.

Methods
Using clinical cases from Massachusetts General Hospital (MGH) (head and neck [H&N], liver, prostate), 13 RBE models were implemented to assess spatial RBE variations. Dose-Volume Histograms (DVHs) and LET distributions were generated via MATLAB and computational environment for radiotherapy research (CERR). RBE-weighted doses were calculated for tumour and organs-at-risk (OARs), incorporating patient-specific α/β ratios. Normal Tissue Complication Probability (NTCP) and Tumour Control Probability (TCP) were compared between constant (1.1) and variable RBE models.

Results
RBE predictions varied significantly across models, with values ranging from 1.03 to 1.23 in prostate, 1.03 to 1.44 in liver, and 0.98 to 1.5 in H&N cancers. High LET regions, such as distal tumour edges, increased RBE by 20–50%, elevating NTCP for nearby OARs, such as the brainstem in H&N cases. Low α/β tissues, particularly late-responding OARs, exhibited amplified RBE effects, with rectal NTCP increasing by 40% in prostate cases. Additionally, dose fractionation influenced RBE variability, where hypofractionation reduced model discrepancies, whereas conventional fractionation heightened disagreements. Clinically, variable RBE models predicted greater toxicity risks, such as a 12% increase in NTCP for parotid glands, and altered TCP estimates compared to constant RBE assumptions.

Conclusions
RBE variability is a critical, underappreciated factor in proton therapy planning. Models accounting for LET, α/β, and dose fractionation reveal significant discrepancies in toxicity and tumour control predictions, challenging the clinical utility of a fixed RBE. This work underscores the need for patient-specific RBE optimisation to balance tumour control and toxicity, particularly for OARs near high-LET zones.

Session 2 – Radiation Protection

Accurate determination of natural radionuclides in soil samples using mass attenuation coefficients derived from transmission measurements

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Terrestrial radiation is produced by naturally occurring radioactive elements contained in the Earth's crust, comprising long-lived radionuclides present since the Earth's formation (e.g ^{40}K, ^{232}Th, ^{235}U, ^{238}U), along with their progenies. External gamma-ray exposure from the decay of these radionuclides makes a significant contribution to the average annual dose received by a member of the Irish population. As the level of exposure at a particular location will depend on underlying bedrock geology, knowledge of the dose rates throughout Ireland is required to accurately assess the contribution of this pathway. This work is part of an EPA-funded research project (GRaDE) to improve present estimates of exposure from terrestrial gamma radiation. Gamma dose rates above ground can be estimated directly through measurements with calibrated dose rate meters or indirectly through the analysis of soils by high-resolution gamma spectrometry. The latter approach allows for an evaluation of the contribution from each radionuclide to the overall terrestrial gamma dose, as well as comparison with the results of airborne gamma spectrometry surveys. Determination of gamma activity concentrations is hampered by the self-attenuation of gamma-rays within the soil matrix, which must be accounted for to obtain accurate results. As part of this project, a robust methodology for the determination of self-attenuation correction factors has been developed, based on transmission experiments conducted with collimated gamma-ray beams using samples of known composition. A calibration curve relating measured transmitted intensities with known linear attenuation coefficient can be employed to determine the linear attenuation coefficients for soil samples of unknown composition. These coefficients are used to determine the self-attenuation correction factors using Monte Carlo methods. Preliminary dose rate estimates obtained from soils collected at ten separate locations in the vicinity of the EPA's National Radiation Monitoring Network show good agreement with ambient gamma dose rates at these sites.

Overview of research to support Ireland's National Radon Control Strategy.

Dowdall, A[1]*, O'Connor, A[1] and McLoughlin P[1].

1. Office of Radiation Protection and Environmental Monitoring, EPA, Dublin

Radon gas causes about 350 cases of lung cancer every year in Ireland. Since 2019, there is a legal requirement for the Government to have an action plan to address this public health issue. The current phase of the National Radon Control Strategy (NRCS) sets out a range of actions aimed at reducing the risk from radon.

To deliver on these actions, the strategy also identifies knowledge gaps where targeted research is required. Progress made to date on addressing gaps will be discussed.

In terms of primary prevention in new buildings, research shows that passive prevention measures are the most cost-effective way of protecting the population from radon. Irish research into the installation of passive radon sumps shows reductions of 65 – 75% in radon levels in dwellings with a fully sealed radon membrane also installed.

In terms of secondary prevention, while levels of radon awareness in Ireland are high, the number of people who take action is low. A key priority of the NRCS is to translate radon awareness into action by testing risk communication messaging and so increase the number of existing homes that are tested and remediated for radon.

Following the technical development of an updated national radon risk map, user testing of radon maps was carried out. The best performing risk communication features from the user testing have now been incorporated into the map.

Research to investigate response rates to behaviourally informed communications on radon testing found that improvements in uptake can be achieved depending on the communication used. The next step is to test response rates to radon remediation.

A key metric to track effectiveness of the overall strategy aim of reducing radon exposure is the national (geographic weighed) average. Research is underway to update this metric which will inform the next phase of the NRCS.

Radionuclide levels present within dredge material collected from Irish ports and assessment of suitability for disposal at sea using criteria derived from IAEA-TECDOC-1759

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[2]Office of Radiation Protection and Environmental Monitoring, Environmental Protection Agency, Clonskeagh Square, Dublin 14.

[3]Earth and Life Sciences, School of Natural Sciences, University of Galway, Galway.

[4]School of Physics, University College Dublin, Dublin 4.

Dredging of ports and harbours is essential to maintain navigable waterways throughout Ireland. The material removed as part of this process is typically disposed of at sea. In compliance with the London Convention and London Protocol, the EPA issues dumping at sea (DAS) licences. Between 2010 and 2024, seventeen different ports/harbours were licensed, with 53 million tonnes of dredge material removed. Prior to granting of a DAS licence, representative material is analysed by the EPA's radioanalytical laboratory to confirm that the material does not contain enhanced levels of natural or artificial radioactivity and is suitable for disposal at sea. To develop a more systematic approach to the radiological assessment of candidate dredge materials, based on the concept of '*de minimis*' levels of radioactivity developed by the International Atomic Energy Agency, permission was sought from the seventeen port/harbour authorities to reanalyse dredge material in storage at the EPA. The activity concentrations of radionuclides were determined in 28 samples collected between 2016 and 2023 using high-resolution gamma spectrometry. Using the radiological assessment procedure developed by the IAEA (2015), the dose received by the dredging crew, the public and reference flora and fauna in the marine environment were estimated on the basis of the measured radionuclide levels at a given dredge site and generic conservative model parameters. Dose estimates were found to be below the reference limits set by the IAEA, confirming the material can be regarded as 'non-radioactive' and may be disposed of at sea. The proposed methodology could be applied in future to assess whether materials may be disposed at sea in the context of these international agreements.

References:

IAEA (2015). Determining the Suitability of Materials for Disposal at Sea under the London Convention 1972 and London Protocol 1996: A Radiological Assessment Procedure. IAEA-TECDOC-1759, International Atomic Energy Agency, Viena.

The National Radiation Dose Report

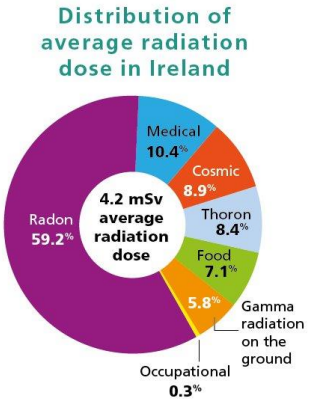
Kilian Smith[1], Lee O'Hora[2], Kevin Kelleher[1]*, Agnella Craig[2]

1: Environmental Protection Agency
2: Health Information and Quality Authority

The Environmental Protection Agency (EPA) and Health Information and Quality Authority (HIQA) conducted an updated assessment of the average radiation doses received by the Irish population. The assessment is an update of a 2014 report and the dose arising from the most significant radiation exposure pathways were calculated i.e. radon and thoron gas, medical exposures, radioactivity in food and drinking water, terrestrial and cosmic radiation, occupational exposures and human-made radioactivity in the environment.
The radiation doses for each of the exposure pathways were determined using the most up to date information available on the levels of radioactivity in the home, in the workplace and in the environment. This information was used in conjunction with the current population of Ireland and other relevant data to determine the average effective dose.
The assessment found that the average effective dose a member of the public receives is 4.2 mSv and, on average:

- nearly 60% of the dose is due to Radon in indoor air, with over 8% coming from Thoron.
- 10 % of the dose comes from medical exposures, mainly from medical imaging.
- 9 % comes from cosmic radiation, of which 2 % is due to exposure received if flying.
- 7 % comes from food and drinking water.
- 6 % comes from terrestrial gamma radiation.

People in Ireland receive a slightly higher average radiation dose than the worldwide average, mainly due to radon exposure in indoor air. Radioactivity from artificial sources, such as discharges from nuclear facilities abroad, fallout from historic nuclear weapons testing and past nuclear accidents make up less than 1 per cent of overall exposure.



The PIANOFORTE radiation research partnership and the 2026 Integrated Regulatory Review Service Mission to Ireland

Veronica Smith

Environmental Protection Agency

The purpose of this presentation is to provide an overview of two topics relevant to radiation protection in Ireland: (i) the Pianoforte radiation research partnership which is a potential funding mechanism for radiation research and (2) the Integrated Regulatory Review Service Mission to Ireland in 2026 in which a team of international experts will review Ireland's regulatory framework for radiation safety.

The PIANOFORTE European Partnership for radiation protection research commenced in 2023. Through a series of open calls, it provides funding for radiation research, training courses and scholarships and travel grants for early career researchers and professionals. Nine projects were successful in the first open call in 2023 and eight projects were selected in the second open call in 2024. Many of these research projects are in the medical field. A brief overview of PIANOFORTE will be provided to raise awareness of it as a potential funding stream.

The Integrated Regulatory Review Service is offered by the International Atomic Energy Agency (IAEA) to strengthen and enhance the effectiveness of national regulatory infrastructure for nuclear safety, radiation safety, radioactive waste and transport safety, and the security of radioactive sources. The Department of the Environment, Climate and Communications invited the IAEA to arrange such a Mission for Ireland and it will take place in January 2026. A key element of the Mission is the self-assessment performed in advance by the host country of its regulatory infrastructure for nuclear and radiation safety against IAEA Safety Standards. The two regulatory bodies in Ireland for radiation safety, the EPA and HIQA, along with their parent departments have begun this process. An overview of the Mission will be provided.

Radiobiome: Host-Gut Microbiome Functional Resilience to Radiation

Michaela Walsh*, Prof. Brendan McClean, Assoc. Prof. Luis Leon-Vintro, Dr. Nicholas Brereton

University College Dublin, St. Luke's Radiation Oncology Network, European Space Agency

The gut microbiome is involved in functions important for human health, and is implicated in cancer therapy effectiveness [1], and side-effects [2]. It is also implicated in astronaut health, with mice displaying gut microbiome dysfunction during spaceflight [3] potentially underlying spaceflight pathology. Radiotherapy patients and astronauts are exposed to harmful levels of radiation, and the effect this has on the gut microbiome is poorly understood.

This study characterises the resilience of the gut microbiome to radiation by investigating the radiosensitivity of exemplar gut bacteria species. Bacterial samples were exposed to radiation doses representative of radiotherapy and spaceflight scenarios using a LINAC with bacterial growth and functionality subsequently characterised. This functional response to radiation will be explored using super-resolution microscopic imaging, a panel of microplate enzymatic assays, and multiomics approaches.

Initial studies using the strain Lactobacillus acidophilus have shown it to be affected by exposure to doses from 2 Gy to 50 Gy. Bacterial growth rate was reduced, and statistically significant decreases (t-test, $p<0.05$) in optical density were observed in treated samples versus untreated samples.

As bacterial growth is compromised by radiation, important functions performed by L. acidophilus may be compromised, providing insight into the functional radiosensitivity of the gut microbiome with health implications for radiotherapy patients and astronauts. Future research will explore this potential functional compromise in detail but radiation exposure will likely compromise growth of this bacterial population, potentially contributing to toxicities in patients and pathology in astronauts. These initial findings and approaches will be expanded to explore radiosensitivity, at different dose levels, in multiple gut bacteria strains involved in metabolic processes critical to health.

This irradiation system will facilitate investigations into how the functional radiosensitivity of the gut microbiome contributes to radiotherapy and spaceflight pathology, with potential to improve health outcomes for radiotherapy patients and astronauts in the future.

References

[1] N. Iida, A. Dzutsev, C. A. Stewart, L. Smith, N. Bouladoux, R. A. Weingarten, D. A. Molina, R. Salcedo and T. Back, "Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment," Science, pp. 967-970, 2013.

[2] A. Mitra, G. W. G. Biegert, A. Y. Delgado, P. Okhuysen, L. E. Colbert and A. H. Klopp, "Microbial Diversity and Composition Is Associated with Patient-Reported Toxicity during

Chemoradiation Therapy for Cervical Cancer," International Journal of Radiation Oncology Biology Physics, vol. 107, no. 1, pp. 163-171, 2020.

[3] N. Brereton, E. Gonzalez, M. Lee and B. Tierney, "Spaceflight alters host-gut microbiota interactions," npj biofilms and microbiomes, 2024.

Session 3 – Radio-sensitisation techniques

Metabolic effect induced by mannose and related metabolic gene mediated radiosensitisation of HPV negative head and neck Squamous Cell Carcinoma (HNSCC)

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Introduction:
Radiotherapy (RT) is a cornerstone of multidisciplinary cancer treatment, particularly for HPV-negative head and neck squamous cell carcinoma (HNSCC), a subtype with poor prognosis. However, the severe adverse effects of RT pose significant clinical challenges. This study investigates the potential of mannose, a safe and natural monosaccharide, to enhance the radiosensitivity of HPV-negative HNSCC by targeting phosphomannose isomerase (PMI), a key enzyme in mannose metabolism.

Methods and Results:
Using CRISPR/Cas9 technology, PMI was knocked out (PMI KO) in HNSCC cells. PMI KO cells exhibited a 20-fold increase in sensitivity to mannose, as shown by cell viability assays. When combined with mannose, PMI KO significantly delayed tumour growth in vivo by 16 days compared to controls. Seahorse assays, ATP measurements, and 13C-glucose labelling LC-MS revealed reduced oxygen consumption rates (OCR), extracellular acidification rate (ECAR) metabolic quiescence, and decreased ATP levels in PMI KO cells treated with mannose. Clonogenic assays demonstrated that PMI KO combined with mannose enhanced radiosensitivity by a sensitizer enhancement ratio (SER) of 1.51 under normoxia (21% O_2) and 1.35 under hypoxia (0.2% O_2). Additionally, mannose increased radiation induced unresolved DNA double-strand breaks (2-fold) and reactive oxygen species (ROS) in PMI KO cells. Under hypoxic conditions, a major contributor to radiation resistance, mannose treatment combined with PMI ablation reduced succinate levels, leading to HIF-1α destabilization and enhanced radiosensitivity. 3D-tumoursphere models were used to verify that mannose-induced metabolic suppression coupled with PMI depletion facilitated oxygen-dependent radiosensitisation, through reduced oxygen consumption.

Discussion and Conclusion:
These findings highlight the radiosensitising potential of mannose and uncover several novel mechanisms, including metabolic reprogramming and HIF-1α downregulation leading to increased radiation sensitivity. Mannose shows promise as a clinically significant adjuvant to radiotherapy for HPV-negative HNSCC, addressing a critical need for effective, less toxic treatments.

Inhibiting the CXCR4/CXCL12 signalling axis with targeted gold nanoparticles sensitises prostate cancer to radiotherapy

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Introduction: Gold nanoparticles (AuNPs) are effective radiosensitisers due to their high X-ray absorption and unique physicochemical properties. However, clinical translation is limited by the need for large treatment concentrations and passive accumulation. To overcome this, an antagonistic CXCR4-targeting ligand was conjugated to the AuNP surface, enabling target specificity. Activation of the CXCR4/CXCL12 axis is linked to radiotherapy (RT) resistance via increased cell proliferation and migration. Our novel nanoparticle, AuXR4, antagonises CXCR4 to counter resistance mechanisms while promoting targeted internalisation of radiosensitising AuNPs.

Methods: AuXR4 was synthesised using the Turkevich method by reducing gold chloride with sodium citrate. Dynamic light scattering (DLS) and UV-vis spectroscopy characterised physicochemical properties, while atomic absorption spectroscopy (AAS) and dark field/hyperspectral microscopy confirmed internalisation. Radiosensitising potential and radiation-induced DNA double-strand breaks (DSBs) were evaluated via clonogenic assays and immunostaining. Changes in pAKT/AKT and cell migration were analysed using western blotting, transwell assays, and xCELLigence migration/invasion analysis.

Results: AuXR4 nanoparticles, with a hydrodynamic size of 38 nm, exhibit excellent colloidal stability under physiological ionic stress. Pre-treatment of CXCR4-expressing prostate cancer cells with AuXR4 resulted in efficient internalisation, achieving 39 and 30 pg Au/cell in PC3 and DU145 cell lines, respectively. AuXR4 increased radiation sensitivity by 20% on average across cell lines, supported by a significant (~2-fold, $p<0.01$) increase in DNA DSBs 2 hours post-irradiation. At the molecular level, AuXR4 suppressed CXCR4/CXCL12 activation, reducing pAKT/AKT levels by 67% and 72% in PC3 and DU145 cells, outperforming untargeted AuNPs. Additionally, AuXR4 significantly attenuated tumour cell migration and invasion toward chemo-attractants, such as CXCL12, TGF-β, and FBS, with molecular analysis revealing suppression of epithelial-to-mesenchymal transition (EMT) proteins.

Conclusions: The optimised nanoparticle AuXR4 targets the CXCR4/CXCL12 pathway, enhancing tumour cell uptake, increasing DNA DSB damage, and reducing clonogenic survival. Notably, AuXR4 also inhibits tumour cell migration toward CXCL12, potentially reducing metastatic risk, which will be explored further in future studies.

Impact of a novel gold nanoparticle formulation on local and systemic radiation-induced effects in an *in vivo* model of head and neck cancer.

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Introduction
Ionising radiation is a commonly used treatment modality against many forms of cancer focusing specifically on solid tumours to induce DNA damage and ultimately promote disease control[1]. Increasing evidence suggests that radiotherapy in combination with immunotherapy can promote an immune response capable of reducing the growth of untreated secondary tumours – the radiation induced abscopal effect (RIAE)[2-4]. Our lead gold nanoparticle formulation, termed AuX2R, is a proven potent radiosensitiser in various *in vitro* / *in vivo* models of cancer. Here we investigate the impact of ionising radiation (IR) combined with AuX2R and anti-PD1 on the growth of primary (targeted) and secondary (abscopal) tumours using a murine model of head and neck cancer (HNSCC).

Methods
MOC1 cells (mouse oral carcinoma cell line) were subcutaneously implanted ($0.5x10^6$ cells) into counter-opposing sites of C57BL/6 mice, representing primary and secondary tumours. One dose of AuX2R treatment was administered directly to primary tumours 24 h prior to radiation treatment (x2 8 Gy fractions), with anti-PD1 administered twice weekly for 4 weeks.

Results
Initial observations from an ongoing study indicate that radiation treatment delayed the growth of MOC1 primary tumours, an effect enhanced with the combination of either anti-PD1 or AuX2R. At present, it appears that the combination of all three treatments will most significantly impair tumour growth. Encouragingly, animals treated with IR, anti-PD1, and AuX2R in combination also appear to be delaying growth of the secondary tumours.

Discussion
While this study is clearly at an early stage, the encouraging preliminary data are pointing toward a potential role for AuX2R to act as a stimulant to anti-PD1 treatment, promoting RIAE. To strengthen this dataset, tumours will be resected post-necropsy for immune analysis using an established flow cytometry panel (see Dr Chambers abstract). Radiation alone cannot induce abscopal effects due to the ability of cancer cells to prohibit immune activation, however coupled with anti-PD1 treatment, activation of the immune cycle is promoted[3,4]. At this point, our emerging data appear to indicate that AuX2R augments this response suggesting a potential role for the addition of radiosensitisers with existing radiotherapy/immunotherapy combination regimes in the clinic. This study will be completed with full quantitative analysis prior to the IRRS meeting.

[1]Wang, J.S. *et al*. Biological effects of radiation on cancer cells. *Military Medical Research* **5**, (2018). [2]Rodriguez-Ruiz, M.E. *et al*. Immunological mechanisms responsible for Radiation-Induced Abscopal Effect. *Trends Immunol*, **39**, 644-655, (2018). [3]Hu, Y. *el al*. Radiation therapy enhanced by NBTXR3 nanoparticles overcomes anti-PD1 resistance and evokes abscopal effects. *Int J Radiation Oncol Biol Phys*, **111**, 647-657, (2021). [4]Morisada, M. *et al*. PD-1 blockade reverses adaptive immune resistance induced by high-dose hypofractionated but not low-dose daily fractionated radiation. *Oncoimmunology,* **7**, (2018).

Radio Sensitisation using Molecular Targeted Gold Nanoparticles in Prostate Cancer: Multiparameter Immune Profiling in a Syngeneic Tumour Model

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Introduction: Gold nanoparticles are solid colloidal particles that are effective radiosensitisers through physical and chemical interactions with radiation, increasing the tumour cell killing effect of radiotherapy. We have formulated a molecular targeted AuNP for specific uptake by tumour cells.
Methods: To study the necessary pre-clinical safety / toxicity of our targeted AuNP, we developed a multiparameter flow cytometry panel to characterise immune cells within tumour tissue, peripheral blood, and splenocytes isolated from a syngeneic tumour model of prostate cancer (DVL3 cells). Treatment groups include SARRP irradiation (IR) (n=10), AuNP treatment (n=10), and a combination of both IR + AuNP (n=11), compared to untreated tumours (n=7). Samples were harvested at 24 h and 7 days post treatment. Lipopolysaccharide (LPS) was used as a positive control stimulant of the immune system (n=4). A gating strategy was used to exclude debris, select viable cells, exclude doublets, and select for CD45+ Leukocytes.
Results: An average viability of 68% in tumours, 88% in blood, and 86% in spleen was achieved. Specifically, we quantified innate immune cells (neutrophils (CD11b+CD11c-Ly6G+), macrophages (CD11b+F4/80+) expressing either MHC II (M1) or CD206 (M2), dendritic cells (CD11c+MHCII+), and natural killer cells (CD3-NKp46+)) and adaptive immune cells (helper T cells (CD3+CD4+), cytotoxic T cells (CD3+CD8+), and B cells (CD3-CD19+)).
Conclusion: Overall, we observed no significant activation of immune cells treated with AuNP alone or combined with IR, compared to LPS. This data confirms the biocompatibility of our targeted AuNP as safe and non-toxic, which forms an important foundation for its further development towards the clinic.

Countering the proteinase activated receptor 1 (PAR-1) pro-tumour phenotype using a novel nanotherapeutic approach

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Background: Pancreatic cancer (PDAC) caused 432,000 deaths in 2018, with tumour resection as the only curative option.[1] Over 80% of patients are diagnosed with unresectable tumours, while others with borderline resectable disease undergo neoadjuvant chemotherapy or chemoradiotherapy (CRT) to improve surgical success.[2] The efficacy of radiotherapy is limited by the proximity of the pancreas to sensitive organs and high intrinsic radioresistance.[3] A targeted radiosensitiser, such as a gold nanoparticles (AuNPs), could enhance the impact of radiotherapy while minimising damage to surrounding tissues. Proteinase-activated receptor 1 (PAR-1), often overexpressed in PDAC, promotes tumour growth, migration, EMT, and survival, making it a promising therapeutic target.[4]
This project investigates the molecular impact of PAR-1 signaling in PDAC comparing PAR-1 antagonistic peptides against the small molecule inhibitor vorapaxar, assess the impact of antagonism on key oncogenic and pro-survival traits, in addition to any alteration of the radiation response. In parallel, we have developed a PAR-1 targeting, antagonistic AuNP, designed to increase the radiation sensitivity of PDAC tumour models.
Results: Alamar blue cytotoxicity studies indicate no direct impact on PDCA survival following PAR-1 inhibition, either with vorapaxar or PAR-1 targeting pepducins ($p<0.05$). Similarly, PAR-1 inhibition alone had no direct impact on modulating radiation sensitivity. Irrespective, overexpression of the PAR-1 receptor in PDAC tumour models, provide a means of promoting targeted AuNP internalisation. AuNP-PAR1 was synthesised using the Turkevich method, with the final nanoparticle possessing a hydrodynamic size of 38 nm, charge of 15.53 mV and a polydispersity index of 0.15. Importantly, the formulation proved stable both over time, and under conditions of stress, with no significant increase in size under salt or serum protein stress. AuNP-PAR1 was avidly internalised resulting within 24 h, reaching 31.57 and 59.29 pg/cell in BXPC and PANC-1 cells respectively. Importantly, target specificity was confirmed by pre-blocking the PAR-1 pepducin binding site, attenuating AuNP-PAR-1 uptake by 47%.
Conclusions: PAR-1 specific, pepducin-functionalised AuNPs exhibit good stability under physiological stress, with the ability to be avidly internalised into *in vitro* cell models of PDAC. Importantly, we have demonstrated specificity for the PAR-1 receptor, a result that will help minimise uptake in low PAR-1 expressing normal cells. PAR-1 inhibition alone confers no intrinsic radiosensiting properties, however, clonogenic assays underway to establish the radiation dose modifying potential of the fully functionalised PAR-1 AuNP.

1. Brunner M, *et al*. Int J Mol Sci. 2019 Sep 13;20(18):4543. **2.** Schorn S, *et al*. Cancer Treat Rev. 2017 Apr;55:96-106. **3.** Seshacharyulu P *et al*. Biochim Biophys Acta Rev Cancer. 2017 Aug;1868(1):69-92. **4.** Macfarlane SR, *et al*. Pharmacol Rev. 2001 Jun;53(2):245-82.

Session 4 - Novel therapeutic and experimental approaches

Direct post-irradiation single-cell whole-genome DNA sequencing to elucidate radiation-induced mutations

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Introduction: Our understanding of radiation-induced DNA damage has advanced significantly in recent decades. It has led to improvements in cancer prevention and treatment. However, studying how radiation causes somatic mutations—which are the result of both DNA damage and subsequent repair—remains a challenge. Part of this challenge is the due to the stochastic nature of radiation. Each cell in an irradiated sample experiences the same radiation field uniquely. To study the mechanism(s) of radiation action effectively, we need to examine the mutations induced in individual irradiated cells. Here we report on our experimental and computational efforts to undertake and improve direct post-irradiation single-cell whole-genome DNA sequencing (direct piSCS).

Methods: We irradiated four clonally-expanded B-lymphoblastoid cell samples with 6 MV x-rays (0.5 Gy, 1.5 Gy and 3.0 Gy, and sham), individually sequenced about 500 cells from each sample using the Chromium CNV kit (10X Genomics), and compared the reconstructed genomes against the genome of the donor. The experiment was conducted twice. To computationally model the irradiation and sequencing, we developed an open-source sequencing simulation toolkit called RadiSeq that uses as input a radiation-damaged genome in the Standard DNA Damage format.

Results: In our first experiment, we observed an increase in CNVs with dose. However, no dose dependence was observed in the second experiment. By modelling the conditions of both experiments in RadiSeq, we were able to explain the difference in results as due to a difference in the GC bias of the sequencing.

Discussion: Direct piSCS is challenging but necessary in order to efficiently study how radiation causes mutations. Our group was the first to attempt direct piSCS but obtained discordant results from two experiments. Using RadiSeq we are able to explain our experimental results and suggest ways to improve future attempts at direct piSCS.

MicroRNA-31 Enhances Radiosensitivity in Pancreatic Ductal Adenocarcinoma by Targeting ATOX1 and GPx8 Antioxidant Activity

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Introduction:
Radioresistance remains a significant challenge in treating pancreatic ductal adenocarcinoma (PDAC), with radiotherapy being beneficial in only 30% of patients. MicroRNAs (miRNAs) are small non-coding RNA molecules that may play an essential role in regulating radioresistance by regulating oxidative stress and antioxidant activity. In this study, we investigated the role and potential mechanisms linking miR-31 to PDAC radioresistance.

Methods:
A pCMV-miR vector containing a miR-31 mimic was stably expressed into a miR-31-deficient PDAC cell line, BxPC-3. Additionally, a pmiRZip lentivector suppressing miR-31 was stably expressed in a miR-31-abundant PDAC cell line, Panc-1. Clonogenic assays were conducted to explore the role of miR-31 manipulation and miR-31 targets on radiosensitivity. Fluorometric ROS assays were performed to quantify ROS levels. The expression of potential miR-31 targets was measured by Western blot and ELISA.

Results & Discussion:
It was found that the manipulation of miR-31 altered the radiosensitivity in PDAC cells. MiR-31 overexpression in BxPC-3 cells significantly enhanced radiosensitivity. Reciprocally, miR-31 suppression in Panc-1 cells significantly enhanced radioresistance. Manipulation of miR-31 promoted alterations in the DNA damage response, radiation-induced apoptosis and ROS levels. Using online bioinformatics tools, we identified the 3'UTR of *ATOX1* and *GPx8*, both of which are involved in the antioxidant defence system, as predicted targets of miR-31. Overexpression of miR-31 resulted in significantly reduced levels of GPx8 and ATOX1 in BxPC-3 cells. Moreover, miR-31 independent silencing of ATOX1 and GPx8 in BxPC-3 cells resulted in significantly enhanced radiosensitivity. GPx8 expression conferred protection against radiation-induced DNA damage via ROS detoxification, resulting in enhanced cell survival.

Conclusion:
Our study demonstrates, for the first time, that manipulating miR-31 regulates ATOX1 and GPx8 expression, modulating ROS detoxification and promoting either a radioresistant or radiosensitive phenotype. Thus, the miR-31-ATOX1-GPx8 radiosensitivity axis represents a promising therapeutic target in PDAC to boost radiation response.

Enhancing Cancer Radiotherapy Efficacy Using NanOx, a Novel Oxygenating Nanoemulsion that Reverses Tumour Hypoxia.

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Introduction:

Radiotherapy is used to treat over 50% of cancer patients and is used in combination with surgery, chemotherapy, and immunotherapy, for cancers of the breast, lung, and oesophagus [1-3]. Ionising radiation exerts its anti-cancer effect through direct DNA damage and indirectly through reactive oxygen species production via water radiolysis. This DNA damage is made permanent in the presence of molecular oxygen; however, it is reversible under hypoxia [4]. Therefore, hypoxia confers significant radiotherapy resistance. Given that it is a common feature of solid tumours it offers a unique vulnerability to exploit to improve radiotherapy efficacy. To address this, we have developed a biocompatible, oxygenating perfluorocarbon nanoemulsion with imaging capacity (NanOx) that has potential to increase the radiosensitivity of hypoxic oesophageal adenocarcinoma (OAC) cells with acquired radioresistance.

Methodology:

NanOx biocompatibility was assessed *in vitro* using 2D and 3D HepG2 cells, and *in vivo* using zebrafish embryos. NanOx oxygen delivery kinetics were assessed using Seahorse bioanalyzer and via HIF-1α expression by Western immunoblot. Radiosensitisation efficacy studies were conducted using an isogenic model of OAC acquired radioresistance (colony forming assays, DNA damage and repair via 53BP1 foci immunofluorescence, cell cycle kinetics, and apoptosis).

Results:

NanOx was biocompatible across *in vitro* and *in vivo* models, significantly increased supernatant oxygen levels, and significantly reduced HIF-1α expression. NanOx, in combination with radiotherapy, significantly reduced the surviving fraction of radioresistant, hypoxic OAC cells with a sensitiser enhancement ratio of 2.22. Combining NanOx with radiotherapy did not alter the level of DNA damage but significantly impaired DNA damage repair through increased 53BP1 foci persistence. NanOx combined with radiotherapy significantly increased the number of radioresistant OAC cells in G_2/M and apoptotic cells, in comparison with either treatment alone.

Conclusion:

We invented a novel oxygenating perfluorocarbon nanoemulsion with imaging potential which can significantly improve response to radiotherapy in radioresistant, hypoxic OAC cells.

Acknowledgements:
Thank you to Breakthrough Cancer Research for funding this PhD project *(PhD Scholarship, 2021)*.

References:
1. Delaney, G., et al., *The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines.* Cancer: Interdisciplinary International Journal of the American Cancer Society, 2005. **104**(6): p. 1129-1137.
2. Begg, A.C., F.A. Stewart, and C. Vens, *Strategies to improve radiotherapy with targeted drugs.* Nat Rev Cancer, 2011. **11**(4): p. 239-53.
3. Baskar, R., et al., *Cancer and radiation therapy: current advances and future directions.* Int J Med Sci, 2012. **9**(3): p. 193-9.
4. Robert Grimes, D. and M. Partridge, *A mechanistic investigation of the oxygen fixation hypothesis and oxygen enhancement ratio.* Biomedical Physics & Engineering Express, 2015. **1**(4): p. 045209.

Effects of oxygen-loaded Perfluorocarbons (PFC) nanoemulsions on hypoxia reversal and radiosensitization in pancreatic cells

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Introduction
Hypoxia is a predicator of poor clinical outcome to radiotherapy. Oxygen is a potent chemical radiosensitizer which stops repair of DNA strand break and therefore overcomes radiation resistance. Pancreatic ductal adenocarcinoma (PDAC) is one of the most hypoxic cancer types. Methods to oxygenate PDAC and to enhance radiation efficacy are desirable.

Therefore we developed a PFC nanoemulsion which is able to load significant amount of oxygen, to boost radiation response by reversing hypoxic microenvironment in PDAC.

Methods
PFC nanoemulsions (NE) was formulated via sonication. Oxygen loading was performed by bubbling oxygen through NE, called oxyNE. In hypoxic conditions we studied the effectiveness of oxyNE in monolayer BxPC3 cells. Clonogenic assay was performed to determine cell survival. The role of oxyNE in modulating hypoxia was evaluated using HIF-1α-probed western blot. Immunofluorescent microscopy of 53BP1 was used to analyze DNA damage/repair. Also, we grew BxPC3 spheroids in GelMA, and Live/Dead staining was used to determine viability of hypoxic spheroids after treatment.

Results
In monolayer BxPC3 cell model, NE and oxyNE both significantly resulted in HIF-1α degradation in hypoxic conditions. However only oxyNE sensitized BxPC3 cells to 4Gy radiation, causing a significant reduction in colony formation in clonogenic assay. Upon treatment with NE and oxyNE followed by radiation, formation of 53BP1 foci was increased at 1 hour post radiation, while only oxyNE maintained a high number of 53BP1 foci at 24 hour post radiation, indicating that oxyNE stabilized DNA damage induced by radiation. Using Live/dead staining we observed that oxyNE in combination with radiation impeded spheroids growth, with average diameter of spheroids decreased compared to untreated spheroids.

Discussion
oxyNE is a promising oxygen carrier to alleviate cellular hypoxia and enhance radiation efficacy. Animal study will be conducted to validate the ability of oxyNE to reverse hypoxia of PDAC, after intra-tumoural administration.

Comparison of 2D and 3D *in vitro* cancer cell line models in SABR.

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Conventional radiotherapy and the mechanisms which underpin it are well characterised. However, the same cannot be said for more recent treatment approaches, such as stereotactic ablative radiotherapy (SABR). It has been demonstrated that radiobiological differences exist between conventionally fractionated radiotherapy and hypo-fractionated ablative regimens, with the *in-vitro* implications of hypo-fractionated dose delivery remaining under-examined. The central dogma of radiobiology is that of the 5 Rs: reoxygenation, DNA repair, radiosensitivity, redistribution in the cell cycle and repopulation. However, the effects of SABR cannot be fully explained through these principles and further analysis is required.

Typical *in vitro* cell culture is done in 2D, however this method does not take into account the relevant environment that cells normally grow in and as such unable to model cell behaviour accurately. There are a number of different methods that can be utilised to culture cells in 3D, with this study utilising Matrigel.

This pilot study is seeking to examine the response of different cell lines grown in 2D and in 3D to different radation regimes. All cell irradiations were performed on a clinical medical linac accelerator, using radiochromic film for dosimetric measurements together with manual calculations to verify the dose delivered to the cell lines.

Utilising a panel of cell lines from lung, pancreas and prostate cancer, cells have been grown in 3D culture using Matrigel as well as in 2D. Following treatment with a representative conventional fractionated dose of 3 x 2.75Gy and a representative SABR fraction dose of 1 x 8.25Gy, cells were analysed both phenotypically and using transcriptomic methods. Colony forming efficiency with bystander effect helped to evaluate the impact of the different regimes on the growth of the cells, while PCR methods were applied to elucidate the gene expression changes.

Preliminary data has suggested that changes to cells grown in 2D show different colony forming efficiency in response to the differing doses but additional experiments are required to evaluate the response to cells grown in 3D along with exploring the transcriptomic signatures that are driving this response.