THE ARR

The Association for Radiation Research

Association for Radiation Research (ARR) 2024 Conference

Abstract Book

UNIVERSITY OF BIRMINGHAM

Edgbaston Park Hotel, University of Birmingham, UK

Monday 24th - Wednesday 26th June 2024

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WELCOME TO ARR CONFERENCE 2024

On behalf of the Association for Radiation Research (ARR), I am delighted to welcome you to Birmingham for the ARR 2024 Conference.

Radiotherapy is a vital treatment used in approximately half of all cancer patients. Despite recent developments in radiotherapy delivery and treatment, advances are still needed to improve our understanding of the biological responses of different forms of radiotherapy (photons and particle ions), and to devise strategies leading to improve the efficacy of the radiotherapy treatment whilst minimising toxic side effects for cancer patients.

We are pleased to provide an engaging and diverse programme focussed on radiation research covering a range of different topics, highlighting some key advancements but also opportunities for development. We have exciting talks from both national and international experts in the field, and importantly from early career researchers.
We hope that this will provide a fantastic platform for interdisciplinary learning, networking and development of new collaborations. We therefore look forward to your involvement and a stimulating meeting.

Jason Parsons
Chair of ARR 2024
Vice-Chair of ARR

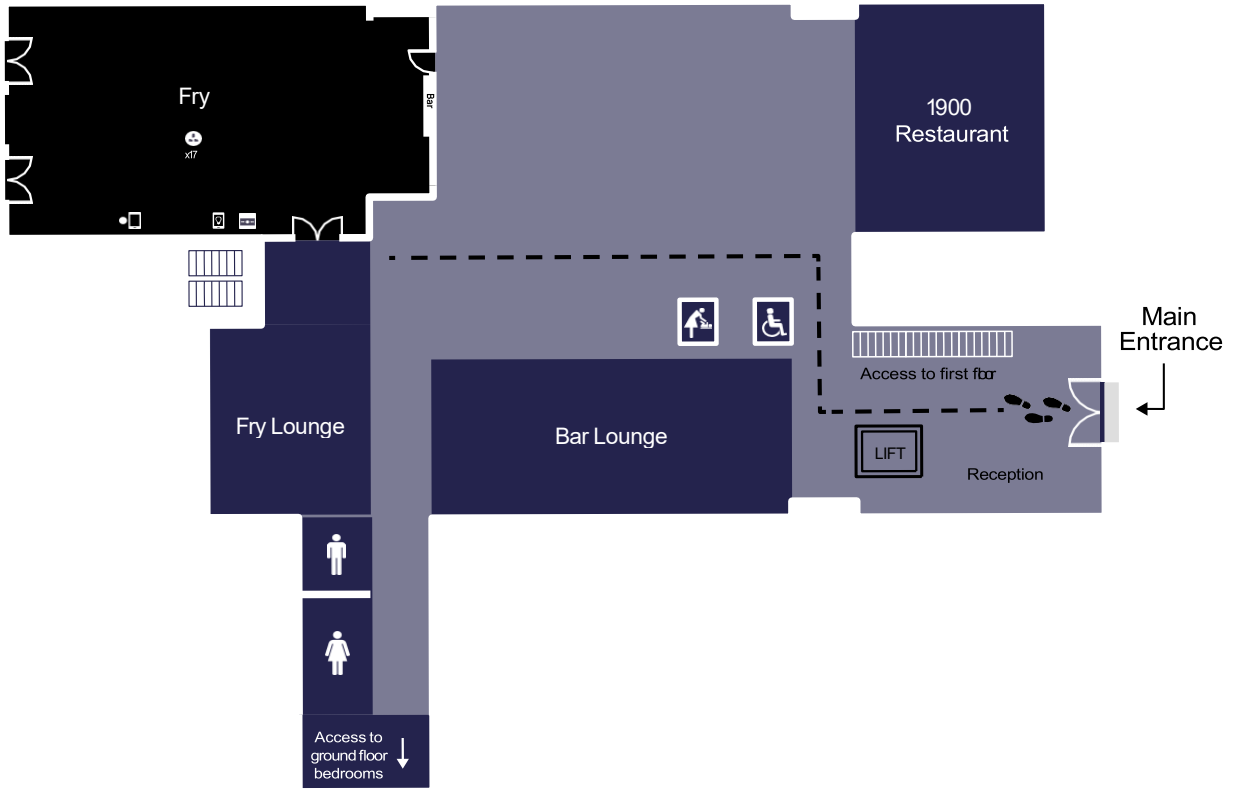
Platinum Sponsor

Silver Sponsors

Edgbaston Park Hotel

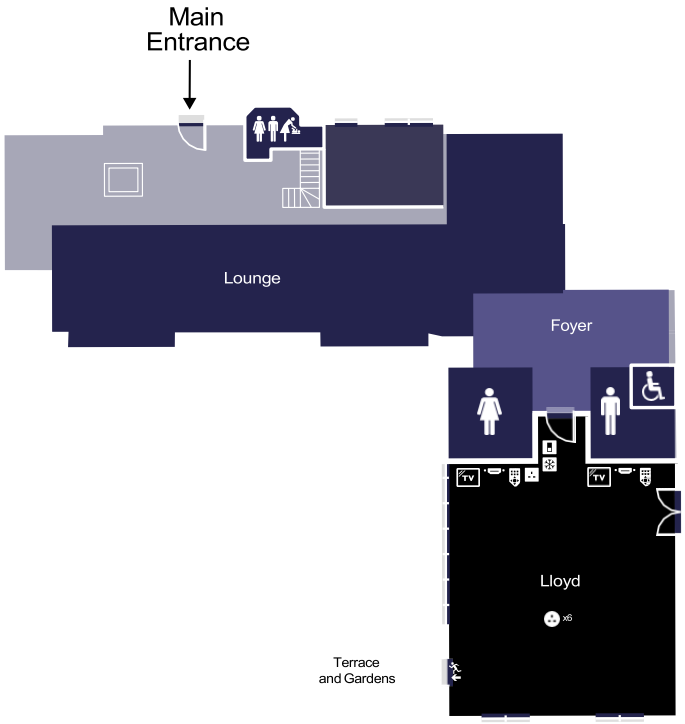
Venue Plan

Edgbaston Park Hotel | Fry Room
Meeting room
Dinner



Hornton Grange | Lloyd Suite

Registration
Exhibition, posters,
lunch BBQ



SCIENTIFIC COMMITTEE

- Professor Jason Parsons (Chair, University of Birmingham)
- Professor Grant Stewart (University of Birmingham)
- Professor Stuart Green (University Hospitals Birmingham)
- Professor Chris Talbot (University of Leicester)
- Dr Helen Bryant (University of Sheffield)
- Professor Anthony Chalmers (University of Glasgow)
- Professor Geoff Higgins (University of Oxford)
- Dr Liz Ainsbury (UK Health Security Agency)

KEY INFORMATION

The Registrations Desk will be situated in the Lloyd Suite, shown on the venue plan on page 4. Please come here on your arrival to register and collect your conference badge.

Day	**Opening Times**
Monday 24th June	08.30-22.00
Tuesday 25th June	08.30-22.00
Wednesday 26th June	08.30-15.00

Conference Social Events

Event	**Date/Time**	**Venue**
Welcome Reception	Monday 24th June 17.50-19.15	The Lloyds Suite at Edgbaston Park Hotel
BBQ	Monday 24th June 19.15-22.00	The outdoor area outside of the Lloyds Suite at Edgbaston Park Hotel
Conference Dinner	Tuesday 26th June 19.00-22.00	The Fry Suite at Edgbaston Park Hotel

CONFERENCE PROGRAMME

Monday 24th June

08.30-10.00	**Registration and poster setup – Lloyd Suite**
10.00-10.20	**Welcome – Fry Suite**
10.20-12.00	**Session 1 – Molecular Responses to Radiation** Chair – **Grant Stewart** (University of Birmingham) Co-Chair - **Lydia Gardner** (Queen's University Belfast) **Invited Speakers** **Amanda Chaplin** (University of Leicester) - *Redefining Non-Homologous End Joining DNA-repair using Cryo-electron Microscopy.* **Tom Clarke** (Boston, USA) – *ZNF280A links DNA double-strand break repair to human 22q11.2 distal deletion syndrome* **Oral Presenters** **Emma Melia** (University of Birmingham) - *Chk1 and Wee1 inhibitors increase radiosensitivity of head and neck cancers to X-ray and proton irradiation* **Lydia Gardner** (Queen's University Belfast) - *Characterising the role of SSB repair pathways in response to low and high LET radiation*
12.00-13.00	**Lunch – Lloyd Suite**
12.00-13.00	ARR Committee meeting (for ARR committee only)
13.00-14.00	**Keynote Speaker - Grant Stewart** (University of Birmingham**) -** *ACTIN on a hunch when it comes to identifying novel DNA double-strand break repair disorders*
14.00-15.40	**Session 2 – Radiotherapy and the Immune Response** Chair – **Anna Wilkins** (Institute of Cancer Research) Co-Chair - **Irene Fischetti** (Fondazione IRCSS Istituto Nazionale dei Tumori, Milan) **Invited Speakers** **Rita Pedrosa** (Queen Mary University of London) - *Targeting radiation induced vascular endothelial-cell dysfunction to modulate response to therapy* **Hala Estephan** (University of Oxford) - *Hypoxia inhibits MHC I expression and antigen presentation to escape immune surveillance*

	Oral Presenters **Irene Fischetti** (Fondazione Irccs Istituto Nazionale Dei Tumori) - *Radiotherapy combined with TLR stimulation elicits adaptive immune response against prostate cancer* **Jiamei Fu** (Tongji University) - *Bilateral DAD contributes to the fatal toxicity of pre-existing ILD mice after partial thoracic irradiation*
15.40-16.10	**Tea/coffee break – Lloyd Suite**
16.10-17.50	**Session 3 – Radiation Physics and Mathematical Modelling** Chair – **Jamie Dean** (UCL) Co-Chair - **Shannon Thompson** (Queen's University Belfast) **Invited Speakers** **Stephen McMahon** (Queen's University Belfast) - *Modelling intrinsic radiosensitivity - How far does DNA repair get us?* **Andreas Kyprianou** (Warwick) - *Proton beam de-energisation and the Bragg Peak for cancer therapy via jump stochastic differential equations* **Oral Presenters** **Charlotte Heaven** (University of Manchester) – *Modelling reveals the impact of LET on the cell cycle and release of DNA fragments* **Fred Currell** (University of Manchester) - *Manchester Inhomogenous Radiation Chemistry by Linear Expansions (MIRaCLE): A Radiation Chemistry Toolkit*
17.50-19.00	**Complimentary Welcome Drinks & Poster Session – Lloyd Suite**
19.00-22.00	**BBQ**

Tuesday 25th June

08.30-09.00	**Registration and poster setup – Lloyd Suite**
09.00-10.40	**Session 4 – Protons and high-LET radiation** Chair – **Jason Parsons** (Birmingham) Co-Chair - **Nathalie Lovgren** (Oxford) **Invited Speakers** **Richard Amos** (University College London) - *Development of clinical and pre-clinical light-ion beam therapy in the UK* **Mark Hill** (Oxford) – *Radiation track structure: how does their spatial and temporal properties drive the radiobiological response.* **Oral Presenters** **Maria Fabbrizi** (University of Birmingham) - *OGG1 and PARG play critical roles in the biological response to protons of increasing LET* **Victoria Dunne** (Queen's University Belfast) - *Development of next generation PSMA-targeted radionuclides 212Pb-AB001 and 225Ac-PSMA-617 in preclinical prostate cancer models*
10.40-11.10	**Tea/coffee break – Lloyd Suite**
11.10-12.10	**Keynote Speaker** – **Yolanda Prezado** (Institut Curie) - *Spatially fractionated radiation therapy: current status of clinical and preclinical studies and knowledge gaps*
12.10-13.10	**Lunch/ARR AGM (Fry room)**
13.10-14.50	**Session 5 – Advanced radiotherapy technologies** Chair – **Kristoffer Petersson** (Oxford) Co-chair - **Alice Ormrod** (Birmingham) **Invited Speakers** **Marianne Azner** (University of Manchester) **Uwe Oelfke** (Institute of Cancer Research) **Oral Presenters** **Jia-Ling Ruan** (University of Oxford) - *Effect of FLASH radiotherapy on muscle-invasive bladder cancer* **Jonathan Hughes** (University of Birmingham) - *Utilising ultra-high dose rate proton radiation to observe the FLASH effect in HNSCC*

14.50-15.20	**Tea/coffee break – Lloyd Suite**
15.20-17.00	**Session 6 – Biology and Epidemiology for Radiobiological Protection** Chair – **Christophe Badie** (UKHSA) Co-Chair - **Jordan Elliot** (University of Manchester) **Invited Speakers** **Christophe Badie** (UKHSA) – *New insights in radiation leukaemogenesis* **Amy Berrington** (Institute of Cancer Research) – *Cancer risks from paediatric CT scans* **Oral Presenters** **Jordan Elliot** (University of Manchester) - *Investigating RNA Damage Induced by Ionizing Radiation in Various Radioprotective Environments* **Gerard Walls** (Queen's University Belfast) - *Entresto as a novel radioprotectant in a partial heart irradiation mouse model*
17.00-18.00	**Drinks and poster session – Lloyd Suite**
19.00-	**Conference Dinner**

Wednesday 26th June

08.30-09.00	**Registration – Lloyd Suite**
09.00-10.00	**Weiss Medal Lecture** – Kevin Prise (Belfast) – *The Radiobiology of Advanced Radiotherapy: A brief journey through space and time*
10.00-10.30	**Tea/coffee break– Lloyd Suite**
10.30-12.10	**Session 7 – Tumour Microenvironment and Hypoxia Signalling** Chair –Isabel Pires (University of Manchester) Co-Chair - Lydia McQuoid – Queens University Belfast **Invited Speakers** **Ananya Choudhury** (University of Manchester) – *Translating hypoxia basic science into clinical practice* **Monica Olcina** (University of Oxford) – *Improving radiotherapy in immunosuppressive microenvironments* **Oral Presenters** **Conrado Guerrero Quiles** (University of Manchester) - *Multi-omic analysis of the hypoxic extracellular matrix identifies a gene signature that predicts radiotherapy benefit* **Tatsuya Suwa** (University of Oxford) - *ER stress-induced intracellular C5aR1 increases cancer cell survival in hypoxia*
12.10-13.10	**Lunch – Lloyd Suite**
13.10-14.50	**Session 8 – Radiopharmaceuticals** Chair – **Geoff Higgins** (Oxford) Co-Chair - **Kel Tan** (Invicro) **Invited Speakers** **Sam Terry** (Kings College London) – *Preclinical efforts in molecular radionuclide therapy* **Jon Wadsley** (Sheffield Teaching Hospitals NHS Trust) – *Molecular radiotherapy: Current status and unanswered questions* **Oral Presenters** **Chun Ying Chan** (University of Oxford) - *Development of Novel PARP-Targeted Radiotheranostic for Cancer Imaging and Therapy* **Volkan Yasakci** (University of Manchester) - *A Novel Design of 64/67Cu-based Nano-Radiopharmaceuticals*
14.50-15.00	**Closing remarks**

KEYNOTE SPEAKERS' ABSTRACTS

Yolanda Prezado

Yolanda Prezado is a research professor at the Centre for Research in Molecular Medicina and Chronic Diseases (CiMUS), in Spain and a senior researcher at the Institut Curie (France). She is a research director at the French National Center for Scientific Research (CNRS) (on leave) and head of the interdisciplinary team New Approaches in Radiotherapy (NARA). She has a multidisciplinary background. She received her Ph.D. in Physics from the University of Santiago de Compostela, Spain, in 2003. She is a Medical Physics expert (board certified in Spain and France). She did her Medical Physics residency at Hospital of Salamanca (Spain, 2004-2007), and later worked at Hospital of Pamplona until she was recruited as a beamline scientist at the Biomedical Beamline at the European Synchrotron Radiation Facility.

Since 2011 she has been a permanent scientist at CNRS, her main interests are innovative radiotherapy techniques, combined radio-immunotherapies, radiobiology, and small field dosimetry. Her main research focus are spatially fractionated radiation therapy and proton therapy. One of their main projects is proton minibeam radiation therapy, funded by the European Union via an ERC consolidator grant. She has been the chair of the scientific committee of the European Federation of Medical Physicists from 2019 to 2021 and is the deputy spokesperson of the International Biophysics Collaboration. She has served on many committees and working groups. Her work in proton therapy has been rewarded with the Mr et Mme Peyre prize of the French Academy of Sciences in 2021.

Abstract:
Yolanda Prezado
Institut Curie

Spatially fractionated radiation therapy: current status of clinical and preclinical studies and knowledge gaps

Spatially fractionated radiation therapy (SFRT) is an unconventional therapeutic approach contradicting the classical paradigms of conventional radiation therapy (1). The highly heterogeneous dose distributions employed result in distinct radiobiological mechanisms which lead to a remarkable increase in normal tissue tolerances. The more reported 800 patients treated with SFRT along numerous preclinical experiments suggest that SFRT has the potential to increase the therapeutic index, especially in bulky and radioresistant tumors. This lecture will provide a critical and holistic review of SFRT, discussing not only the main clinical and preclinical findings but also analyzing the main knowledge gaps.

Kevine Prize

Professor of Radiation Biology, at the Patrick G Johnston Centre for Cancer Research at Queen's University Belfast since 2007. Prior to this, he was Head of the Cell and Molecular Radiation Biology Group at the Gray Cancer Institute in Northwood, London.
He received his PhD in Cell Biology and Biochemistry, from the University of Aberdeen, on the mechanisms of action of the chemotherapeutic methotrexate.
He has developed wide-ranging interests in radiation biology including research on low dose radiation risk, radiation quality, drug-RT combinations including nanoparticles, cell and tissue signalling mechanisms. His recent work is developing new biological based models for optimising the temporal and spatial aspects of advanced radiotherapies. A current focus is on the radiobiology of new laser-based approaches to probe extreme ultra-high dose-rate regimes.

He is a Past-President of the Radiation Research Society, a previous RRS Michael Fry award recipient and Friedrich Dessauer awardee of the German Radiation Research Societies. He was the 2018 Douglas Lea Lecturer, (Institute of Physics and Engineering in Medicine) and the 2018 Bacq and Alexander awardee from the European Radiation Research Society. He has supervised 54 PhD students and has published over 380 papers (h=68), with over 15,500 citations.

Abstract:

Kevine Prize
Queen's University Belfast

Weiss Medal Award Lecture: The Radiobiology of Advanced Radiotherapy: A brief journey through space and time

Radiotherapy remains the mainstay of cancer therapy and its utility is continuing to rapidly expand. In recent years, it has gone through a series of technical revolutions which have allowed more and more precise targeting of dose to smaller and smaller "targets" with a range of different types of radiation. With the expansion of molecular (radionuclide) radiotherapy, its application is encompassing the whole gamut of the cancer therapy space from primary tumour to systemic disease and continuing to provide palliation, focussed on quality of life. These technical advances have delivered significant changes in the spatial and temporal way that radiation is delivered into the human body. From a radiobiological perspective, we have incorporated these developments into assuring that pre-clinical studies fully mimic the clinical scenario and can rapidly feedback through reverse translation to the clinical interface. The spatial distribution of radiation exposure is now known to have important consequences, not only in targeted cells and tissues but in bystander cells and out-of-field abscopal effects each of which can have potential clinical impact.

Our understanding of radiation biology has previously been based on the timescale of its actions and the interrelationships between the physical ($< 10^{-12}$ s), chemical ($< 10^{-1}$ s) and biological responses ($> 10^{-1}$ s). Recent studies ($>10^{2}$ Gy/s) have shown that increasing dose-rate may play a critical role in these interactions with clinical potential, particularly with reducing normal tissue complications leading to improved therapeutic ratios. As well as conventional electron, photon and ion sources, laser-based technologies are now allowing the exploration of extreme dose-rate responses ($>10^{10}$ Gy/s) opening up new opportunities to understand fundamental radiation mechanisms.

Overall, we are entering an exciting era for radiobiology as we start to integrate new knowledge on spatial and temporal effects and understand their potential applications

Grant Stewart

For nearly two decades the Stewart lab has studied how defects in DNA repair and replication contribute to genome stability using a combination of fundamental discovery science and human genetics. This research focus stemmed from the identification that hypomorphic hMRE11A mutations in patients cause an Ataxia-Telangiectasia (A-T)-like disorder, which provided vital evidence that an inability to detect and repair DNA double strand breaks (DSBs) is linked to neurodegeneration. Since this initial finding, the Stewart lab has discovered a multitude of new genome stability maintenance factors including MDC1, RNF168, TRAIP, BOD1L and DONSON. Critically, the identification of these factors has not only helped understand the pathological mechanisms underlying the development of disease, but it has also uncovered novel cellular pathways involved in maintaining cellular health by promoting genome stability e.g. the Mre11-Rad50-Nbs1 complex binds MDC1 at sites of DNA damage, which then helps relocalize RNF168 to trigger a ubiquitin-dependent DSB repair pathway. As a recognition of his work on the role of RNF168 in preventing genome instability and human disease, the Professor Stewart was awarded the Lister Institute Research prize (2009). To date, the Stewart lab has identified 10 new human disease genes, including most recently TONSL, RECQL1, SLF2 and SMC5, which have highlighted how different cellular stress response pathways protect the genome from distinct genotoxic insults and how this is important for maintaining normal foetal growth and development.

Abstract:

Grant Stewart
Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, University of Birmingham

ACTIN on a hunch when it comes to identifying novel DNA double-strand break repair disorders

Genome instability is a genetic trait that is common to all cancer. Abnormal repair of DNA damage is the most frequent underlying cause of genome instability and probably represents the most important event that contributes to, and in some cases initiates the development of cancer. Therefore, cellular pathways that control the repair of damaged DNA as well as those that regulate cell cycle checkpoints and the apoptotic machinery represent an inherent anti-tumour barrier that must be surpassed for a tumour to develop. However, it is becoming evident that defective DNA damage repair is a pathogenic process that contributes to the development of many diseases not just cancer and that this can affect organs and tissues in a variety of different ways.The biochemical pathways involved in responding to damaged DNA are collectively termed the DNA damage response (DDR) and consist of those that regulate DNA damage detection, cell cycle checkpoint activation, DNA repair and apoptosis. Much of our insight about how different proteins are involved in regulating the DDR and the pathological consequences if this fails, has come about from the study of rare inherited human syndromes associated with genome instability and a high prevalence of cancer e.g. Ataxia-Telangiectasia and Nijmegen Breakage Syndrome. Studying these rare human diseases has not only provided a wealth of invaluable information about how defects affecting the DDR contributes to cancer development but it has also provided critical insight into how DNA damage drives neuro-degeneration, abnormal brain development, immune system dysfunction, growth failure and infertility.

Recently, we have identified a novel DNA double strand break repair disorder that sheds light on how the process of repairing DNA breaks is coordinated by nuclear actin. By studying this new disease we uncovered evidence that the actin network is not just a ubiquitous structural framework that dictates the shape and movement of cells but that it can be finely tuned to provide specificity to specific cellular processes.

INVITED SPEAKER ABSTRACTS

Development of clinical and pre-clinical light-ion beam therapy in the UK
Richard A. Amos, Department of Medical Physics and Biomedical Engineering,
University College London

Interest in the application of protons and other light-ions for radiotherapy continues to grow globally due to the favourable dose-deposition characteristics compared to x-ray based techniques. The potential to reduce radiation-related toxicities for cancer patients indicated for radiotherapy drives this interest. In recent years NHS England (NHSE) has developed a national proton beam therapy (PBT) service located at the Christie Hospital in Manchester and at University College Hospital in London. This service provides treatment to those patients indicated for PBT along with participation in clinical trials to test the efficacy of PBT for disease sites not yet indicated.

There is also a growing need to have greater access to facilities for pre-clinical research in the field of light-ion beam therapy. Innovative treatment techniques such as ultra-high dose rate (UHDR) and spatially-fractionated ion-beam delivery have shown some evidence of improved efficacy in early pre-clinical investigations, however the underlying mechanisms are yet to be understood. Furthermore, radiobiological studies of various light-ion species are desirable to ascertain their relative effectiveness. To meet this need there are plans to develop an ion-therapy research facility (ITRF) in the UK. Such an undertaking requires a cost-effective solution to be viable. Research is ongoing to design a laser-hybrid accelerator for radiobiological applications (LhARA), and for this system to be the source for the ITRF.

This presentation will provide an update on the current status of the PBT clinical service and summarize ongoing research for the development of the LhARA and ITRF.

Radiotherapy “big data”: the role of AI and advanced image analysis
Marianne Azner, *University of Manchester*

This presentation will explore the impact of advanced technologies such as deep learning (DL) and large-scale image analysis (e.g. voxel-wise analysis, radiomics) on treatment planning, delivery, and outcomes in radiotherapy. We will review clinical applications, as well as developments for research, e.g. use large cohorts of real-world data to learn from every patient and enhance our understanding of dose-response relationships.

New insights in radiation leukaemogenesis
Christophe Badie, *UKHSA*

To improve health risk estimates and radiological protection for low dose and dose-rate exposures of ionising radiation (IR) encountered in occupational, medical, and public/emergency situations, further research is required. Epidemiological studies provided clear evidence for increased leukaemia incidence following IR exposure even at low doses with acute myeloid leukaemia (AML) being the most prevalent. Animal studies greatly contribute to improve the understanding of radiation-induced AML (rAML). Murine rAML feature both hemizygous chromosome 2 deletions and point mutations (R235) within the haematopoietic regulatory gene Spi1. Analysing 123 rAML, we identified new pathways without Spi1 R235 where the decrease in Spi1 gene expression is negatively correlated with Spi1 promoter DNA methylation at specific CpG sites. Moreover, we generated mouse models to track preleukemic cells in vivo to reveal the sequence of molecular events and identify the cells of origin and we confirmed the ‘’two-hit’’ mechanism using a hemizygous Spi1 R235C point mutation conferring hypersensitivity to rAML. Similar SPI1/PU.1 polymorphisms in humans could lead to IR enhanced susceptibility following medical or environmental exposure. Increased rAML sensitivity and shortened

dose-dependent latency of this model allow to quantify rAML risk at low doses/dose-rates, otherwise prohibited by the high numbers of animals to reach statistical significance. Data generated are being used to assemble biologically based risk projection models to evaluate low dose rAML incidence and the role of hyper-radiosensitivity (HRS) in rAML by altering the probability of Spi1 mutations occurrence/persistence relevant for human populations. Last, we identified an epigenetic signature of IR in therapy-related AML patients.

Cancer Risks from Pediatric CT Scans
Amy Berrington, *Institute of Cancer Research*

CT scans save lives by improving diagnosis, limiting unneeded medical procedures, and enhancing treatment. The rapid growth in use over the last few decades has raised concerns, however, about the associated levels of radiation exposure especially in children. This led to several large-scale epidemiological studies of cancer risks in cohorts of children who underwent CT scans. The results from the UK, Australian and European CT scan cohorts will be presented and compared with a focus on leukemia and brain tumours. Methodological issues will be described and the results put into context with other low-dose epidemiological studies. Finally the implications for radiation protection will be discussed.

Redefining Non-Homologous End Joining DNA-repair using Cryo-electron Microscopy
Amanda Chaplin, *University of Leicester*

Cellular DNA is exposed to multiple sources of damaging agents, including endogenous sources such as oxidation and exogenous sources such as radiation. DNA repair mechanisms are vital as DNA double-strand breaks (DSBs) can cause cell death and eventually cancer if left unrepaired. Non-homologous end joining (NHEJ) is one of the two mechanisms required for DSB repair. NHEJ is dependent on several canonical proteins, namely DNA-PKcs, Ku70/80, DNA Ligase IV, XRCC4 and XLF, in addition to several regulatory proteins. Traditionally, NHEJ was thought to consist of three simple linear steps. However, recent cryo-EM data has provided an unexpected glimpse of alternate complex protein arrangements, leading us to propose that the mechanism of NHEJ is more complicated that originally believed. We have identified two alternate long-range DNA-PK dimers, one mediated by Ku80 and the other by XLF. These dimers are essential for efficient DNA repair. We have also recently shown that the accessory protein, PAXX can stabilise specifically the Ku80 DNA-PK dimer and how this has overlapping roles with XLF. Furthermore, we have used cryo-EM to visualise small molecules such as IP6 binding and DNA-PKcs inhibitors, which will aid in future therapeutic development.

Translating hypoxia basic science into clinical practice
Ananya Choudhury, *University of Manchester*

Scientists have been aware of the importance of hypoxia for decades culminating in the Nobel prize for Physiology in 2019. Hypoxic cancers are more likely to metastasise, be treatment resistant and have a poor prognosis. This talk will bridge the gap between laboratory discoveries and patient care. I will discuss discoveries and interventions that allow targeting hypoxia to improve patient outcomes in the clinic.

ZNF280A links DNA double-strand break repair to human 22q11.2 distal deletion syndrome.
Tom Clarke, *Boston, USA*

DNA double-strand breaks (DSB) are one of the most deleterious forms of DNA damage, and if unresolved result in DNA mutations and chromosomal aberrations that can cause disease, including cancer. Repair of DSBs by homologous recombination (HR) requires extensive nucleolytic digestion of DNA ends in a process known as DNA end-resection. In recent years, progress has been made in understanding how this process is initiated,

however the later stages of this process – long range DNA end-resection, is not well understood. Indeed, many questions remain as to how the DNA helicases and endonucleases that catalyze this process are regulated, a key step to avoid spurious activity in the absence of breaks. The importance of DNA end-resection in human disease is highlighted by several human genetic syndromes which are caused by mutations or deficiencies in key proteins involved in this process. In this study, using high throughput microscopy (HTM) coupled with a cDNA "chromORFeome" library, we have identified ZNF280A as a novel chromatin factor that is essential for DNA double-strand break repair. Mechanistically, we demonstrate that ZNF280A promotes long-range DNA end resection by facilitating the recruitment of the BLM-DNA2 helicase-nuclease complex to DNA double-strand break sites, enhancing efficiency of the enzymatic activity of this complex at DNA damage sites. ZNF280A is therefore a key accessory factor for DNA end-resection and DNA repair by homologous recombination. Importantly, ZNF280A is hemizygously deleted in a human genetic condition, 22q11.2 distal deletion syndrome. Features of this condition include congenital heart disease, microcephaly, immune deficiency, developmental delay, and cognitive deficits – features that are associated with other human syndromes caused by defects in genes involved in DNA repair. Remarkably, cells from individuals with a 22q11.2 distal deletion have defects in homologous recombination and increased incidence of genome instability, providing the first evidence of defective DNA repair as a potential mechanistic explanation for several clinical features associated with this human condition.

Hypoxia inhibits MHC I expression and antigen presentation to escape immune surveillance
Hala Estephan, *University of Oxford*

Hypoxia is a common feature of solid tumors that has previously been linked to resistance to radiotherapy and chemotherapy, and more recently to immunotherapy. Hypoxic tumors exclude T cells and inhibit their activity, suggesting that tumor cells acquire a mechanism to evade T cell recognition and killing. Using an unbiased proteomic approach to determine what mechanisms contribute to tumor immune evasion by hypoxia, we found that hypoxia inhibited MHC I at the protein level and in consequence induces a significant change in antigen presentation. Hypoxia decreases MHC I expression in an oxygen-dependent manner, mediated by the activation of autophagy through the PERK arm of the unfolded protein response. Furthermore, using an immunopeptidomics-based LC-MS approach, we found a significant reduction in presented antigens under hypoxia. Inhibition of autophagy under hypoxia rescued MHC I expression and enhanced antigen presentation. In experimental tumors, reducing mitochondrial metabolism through a complex I inhibitor increases tumor oxygenation and both MHC I levels as well as the immunopeptidome. These data provide the molecular mechanism governing tumor immune evasion in hypoxic conditions, offering novel insights for therapeutic interventions targeting hypoxia-induced alterations in antigen presentation.

Radiation track structure: how does their spatial and temporal properties drive the radiobiological response.
Mark Hill, *Oxford*

Ionising radiation is far more biologically effective than might be expected from the limited amount of energy deposited or the comparatively small amount of DNA damage induced, compared to the vast amount of endogenous damage arising from normal metabolism of the cell. This is due to the unique way energy is deposited along highly structured tracks of ionisation and excitation events, resulting in the correlation of DNA damage sites from the nanometre to the micrometre scale. Correlation of these events along the track on the nanometre scale results in clustered damage, which not only includes DNA double-strand breaks (DSB) and the more difficult to repair complex DSB (which includes additional damage within a few base pairs) but also non-DSB clusters. Track structure varies significantly with radiation quality and the increase in relative biological effectiveness (RBE) observed with increasing linear energy transfer (LET) in part corresponds to an increase in the probability and complexity of clustered DNA damage produced. Likewise, with increasing LET there is an increase probability of correlation over larger scales, associated with packing of DNA and associated

chromosomes within the cell nucleus. This can also have a major impact on biological response, with difference becoming more pronounced with low doses associated with radiation protection exposures. The proximity of the correlated damage along the track increases the probability of miss-repair through pairwise interactions resulting in an increase in probability and complexity of DNA fragments/deletions, mutations and chromosomal rearrangements. The temporal properties radiation can also have a major impact on the resulting biological effectiveness.

Understanding the mechanisms underlying the biological effectiveness of ionising radiation can provide an important insight into the resulting radiation biology, improving the efficacy of radiotherapy, as well as the risks associated with exposure. This requires a multi-scale approach for modelling, considering the physics of the track structure from the millimetre to the nanometre scale, temporal aspects of exposure, the structural packing of the DNA within the nucleus, the resulting chemistry, along with the subsequent biological response. In addition to an overview of the link between physical interactions, associated chemistry and biological response, the presentation will also highlight some of the common misconceptions.

Proton beam de-energisation and the Bragg Peak for cancer therapy via jump stochastic differential equations.
Andreas Kyprianou's, *University of Warwick*

Proton beam therapy is an approach to treating certain cancers, that has been in operation in the UK for less than a decade. It consists of firing a high energy protons beam towards cancerous tissue. The physics of proton deceleration ensures that the beam energy can be focused into unhealthy tissue. The Bragg Peak describes the rate of energy loss per unit length along the beam and is used as a calibration tool for treatment preparation. Bortfeld (1997) proposed a parametric family of curves that can be accurately calibrated to data. The Bortfeld curve is strictly a one-dimensional profile and there are currently no known mathematical models in higher dimensions. We build from first principles the first mathematical model for the de-energisation of protons using stochastic differential equations. Our approach affords us the luxury defining the natural analogue of the Bragg Peak curve in two or three dimensions. This work is purely theoretical and a first step providing the foundations for future work in which we will develop comparative studies with existing methods.

Modelling intrinsic radiosensitivity - How far does DNA repair get us?
Stephen McMahon, *Queen's University Belfast*

It is well-established that genetic differences between cancers significantly affect their radiosensitivity, which in turn plays a major role in determining treatment response in clinical settings. However, despite this knowledge, there has been limited application of radiosensitivity models to personalise radiotherapy doses based on these differences, due to challenges in building robust predictions of responses across different biological systems.

The role of DNA repair role in radiation response is well-established, with decades of literature supporting its critical influence on cell fate. Extensive research has been undertaken to understand these processes, both to better characterise the response of different cells to radiation, and to identify potential targets for radiosensitisation.

This talk will review our work on modelling DNA repair in response to radiation-induced damage. This includes simulating the initial distributions of damage, its interaction and (mis)repair, and its subsequent consequences for cell fate. Importantly, this approach also considers the function of different genetic pathways in these systems, and the impact that dysregulation of key DNA repair processes has on radiosensitivity.

This modelling approach effectively captures numerous aspects of biological responses, including initial DNA damage and its repair over time, as well as biological consequences such as mutation and chromosome aberration formation, and overall clonogenic survival. This is applicable across different radiation qualities, and in cells of different DNA repair capacities, validated in both cell line data and CRISPR-Cas9 knockout screens.

However, while this work highlights the critical impact of DNA repair dysregulation on radiosensitivity, it also offers the opportunity to explore the prevalence of such factors in clinical cohorts. Analysis of both cell line and patient population databases shows that only a small fraction of samples – on the order of a few percent – exhibit mutational profiles associated with DNA repair defects which materially affect radiosensitivity.

This suggests that while these defects can serve as a significant radiosensitivity marker when present, they cannot explain the entire range of radiosensitivity observed in patient populations. This highlights the need for exploration of other regulators of response. Some possible pathways which may be driving these effects will be discussed, to highlight areas to underpin future radiobiological modelling.

SFRT/FLASH irradiators for pre-clinical research: Microbeams, FLASH SARRP and LFXT
Uwe Oelfke, *Institute of Cancer Research*

Reviving the paradigms of spatially fractionated radiotherapy (SFRT) and dose delivery at Ultra-High dose rates (> 40 -100 Gy/s, FLASH) has inspired a wealth of pre-clinical research in the community of radiation physics and biology for the last decade.

The main aim of these studies was i) to elucidate the biological mechanisms of the observed dose sparing effects in normal tissues and ii) to study the tumoricidal properties of SFRT and FLASH. While these efforts led to several exciting hypotheses and potential explanations of the collective 'in-vivo' set of data, we are still lacking a satisfying understanding of their underpinning radiobiological mechanisms.

One severe bottleneck for rapid progress of the respective research is the lack of suitable experimental irradiation facilities. For FLASH RT, the most common irradiation modality are electron beams, either generated by dedicated LINACs or modified clinical accelerators with energies ranging from 6 - 10 MeV. Another prominent modality for FLASH RT are high energy protons. For photons at pre-clinical energies of 150 – 300 kV, a dedicated irradiator – the FLASH SARRP – has recently been introduced.

For SFRT, utilizing spatial beam widths of microbeams (< 250 microns), minibeams (1-5 mm) and larger grids of radiation (> 5mm), there is a scarce spectrum of facilities available. The most pronounced bottleneck is the availability of pre-clinical microbeam irradiators, especially since the ESRF in Grenoble has recently decided to put the microbeam-mode of operation of beamline ID17 on hold.

We will report on the development and commissioning of our micro-beam and FLASH irradiators at the Centre for Cancer imaging and will also cover the development of the line-focussed X-ray source (LFXT), currently ongoing at the Technical University of Munich. The concept of the LFXT, originating from work completed at ICR in 2017, promises to deliver microbeams at flash dose rates of up to 100 Gy/s with an unprecedented geometrical accuracy.

Our adaptation of a conventional SARRP platform for the delivery of microbeams is based on the integration of an electronically controlled slit collimator, allowing varying beam widths ranging from 50 - 170 microns. We will describe the process of dosimetric commissioning, report on the achievable dose patterns and describe the developed workflow for the irradiation of several 'in-vivo' tumour models including a set of initial results.

This section will be followed by a brief report on the initial dosimetric calibration of the FLASH SARRP platform, which consists of two rotatable x-ray tubes operating at a maximal output at 150 kV and a current of 630 mA resulting in dose rates between 80 and 90 Gy/s. Finally, the talk will introduce the concept of the LFXT and its technical realisation with its first prototype at TUM.

Improving radiotherapy in immunosuppressive microenvironments.
Monica Olcina*, University of Oxford*

Colorectal cancer subtypes with the worst prognostic outcome are stromal-rich, have poor CD8+ T-cell infiltration and high complement gene expression levels. The impact of high complement expression on treatment outcome in these tumours, however, is still unclear. When grown subcutaneously, tumour organoids originally derived from villinCreER Apcfl/fl KrasG12D/+ Trp53fl/fl TrgfbrIfl/fl mice, display features resembling those of colorectal cancers associated with poor outcome; including stromal-rich regions and poor CD8+ T-cell infiltration. Following RNA-sequencing of these tumours we have found that the complement system is the most significantly upregulated immune system pathway to be upregulated shortly following radiotherapy. In this model, we also find that C5aR1 is robustly expressed on malignant colorectal epithelial cells, suggesting tumour-cell specific functions. Indeed, targeting C5aR1 results in increased radiation-induced cell death specifically in tumours but not normal tissues and this results in improved tumour control following radiotherapy. Collectively, these data suggest that upregulation of complement genes may be part of the stress response mounted by irradiated tumours and that targeting C5aR1 could be targeted to improve tumour radiation response without increased toxicity in the normal tissue.

Targeting radiation induced vascular endothelial-cell dysfunction to modulate response to therapy
Rita Pedrosa, Queen Mary University of London

30% of all cancer patients, over 90,000 new cancer cases, will receive radiotherapy as part of their curative treatment in the UK. However, resistance to radiotherapy is still a major challenge especially in non-small cell lung cancer (NSCLC) patients. Tumour vasculature-derived angiocrine signals (chemokines, cytokines, and growth factors secreted by vascular endothelial cells) have important roles in modulating responses to DNA-damaging agents, however their role in resistance to RT remains unexplored. In this study, we investigate how RT-induced vascular inflammation/dysfunction and derived angiocrine signals mediate resistance to RT.

Using a published scRNA seq dataset (Nolan et al, Nature Cancer, 2022), we have uncovered an enrichment upon radiation of a subset of vascular endothelial cells in the lung that possess immune modulatory, antigen presenting functions and unique expression of PD-L1, named iMECs (immune modulatory endothelial cells). This subcluster of ECs presents upregulation of inflammatory associated signatures such as TNF-a/NF-Kb and Jak/STAT3, among others. Furthermore, the existence of this iMEC subcluster was confirmed in human scRNA seq datasets and at protein level using image mass cytometry of early-stage lung cancer samples. Moreover, using both immunofluorescence and western blot nuclear fractionations, we have confirmed that RT induces activation of the canonical inflammatory pathway- NF-KB in mouse lung endothelial cells in vitro. We have also analysed the secretory phenotype (angiocrine signalling) of human pulmonary microvascular endothelial cells upon radiation in vitro, confirming secretion of several cytokines and chemokines associated with immune cell regulation.

Using 2D co-culture models, we have also demonstrated that vascular endothelial cells provide radioprotective signals to some tumour cell lines, and this effect seems to be, at least partially, contact-independent, as demonstrated with transwell experiments.

Initial in vivo data, using multifocal adenoviral-cre induced KP (KrasG12D p53LOF) NSCLC mouse model, suggests that tumour growth is resistant to hemithorax RT of 5 fractions of 2Gy, without major changes in blood vessel numbers or vascular growth patterns. Furthermore, immune characterisation of this model by flow cytometry revealed a more immunosuppressive response. Unifocal orthotopic NSCLC mouse models were also used to interrogate targeted RT (small animal radiation research platform, SARRP)-derived vascular responses and their effects on the TME. Using KP cells and CMT cells unifocal lung tumours, has revealed distinct responses to RT, with KP cells model being less sensitive to treatment compared with CMT. Interestingly, distinct vascular remodelling responses are observed within the two models.

Future studies will investigate the differential RT-derived vascular responses (and proportions of iMECs in the tumour vasculature) and associated immune infiltrate profiles comparing resistant (KP multifocal and unifocal models) vs sensitive (KL- KrasG12D Lkb1fl/fl; CMT167) mouse models, and how these might correlate with responses to RT. Additional in vitro studies will aim to address the ability of endothelial cells upon RT to recruit and possibly change immune cell phenotypes.

In conclusion, we have shown that RT induces NF-KB dependent vascular endothelial-cell inflammation, and the derived angiocrine signals contribute to protection of adjacent TC, while also possibly regulating immune cell responses. Therefore, modulating vascular EC responses to radiation might prove beneficial in improving responses to treatment of RT resistant NSCLC.

Preclinical efforts in molecular radiotherapy
Samantha Terry, *Kings College London*

Molecular radiotherapy (MRT), where primary tumour and metastases are irradiated, is an exciting research area for radiobiologists to apply their expertise from X-ray radiation to. With increased numbers of radiopharmaceuticals being tested preclinically and becoming available in the clinic, and a great investment of pharmaceutical companies in this area, there is now a need to better understand the biological effects of radionuclides. In this presentation, we will describe where the field is at preclinically and where future efforts are best placed to truly make the most of the potential of these radiopharmaceuticals.

Molecular radiotherapy- current status and unanswered questions
Jon Wadsley, *Sheffield Teaching Hospitals NHS Trust*

Molecular radiotherapy (MRT) refers to the delivery of radiation to malignant tissue via the interaction of a radiopharmaceutical with molecular sites. This may be administered orally, intravenously or more directly, for example by infusion into the hepatic artery. Whilst historically this treatment modality has been restricted to a small number of rare tumour sites, more recently a rapidly growing number of new therapies covering a wider range of cancers have emerged.

In this session we will review the current clinical indications for MRT and the evidence supporting these. We will consider where the major gaps are in our knowledge, and what further research is required to allow us to optimise treatments for each individual patient. We will review recent clinical trials which have attempted to address some of these issues and the lessons learned from these.
Finally we will consider current initiatives seeking to promote MRT research in the UK and further afield.

ORAL PRESENTER ABSTRACTS

O1: Development of Novel PARP-Targeted Radiotheranostic for Cancer Imaging and Therapy

Dr Chung Ying Chan, Dr Zijun Chen[2], Dr Mathew Veal[1], Mr Michael Mosley[1], Prof Véronique Gouverneur[2], Associate Prof Bart Cornelissen[1,3]

[1]MRC Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, Oxford, United Kingdom, [2]Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Oxford, United Kingdom, [3]Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Purpose:

Theranostic is a concept in medicine that unites diagnosis with cancer therapy, in which radiotheranotic has been widely adopted and integrated clinically in Targeted-Radionuclide Therapy (TRT). TRT is a precision-oriented therapeutic approach that leverages specific radiopharmaceuticals designed to selectively deliver therapeutic doses of radiation to cancer cells while minimising damage to healthy tissues. Targeting PARP (poly(ADP-ribose)polymerase) represents a promising approach for TRT development for cancer, due to its aberrant expression in many cancer types and critical role in repairing damaged DNA. Therefore, combining therapeutic radionuclide (e.g. ^{123}I, an Auger electron emitter) and a PARP inhibitor, a radiolabelled-PARP inhibitor, can fulfill the role of PARP-TRT to precisely deliver cytotoxic-radiation to PARP-expressing cancers, enabling their destruction while minimising radiotoxicity to healthy cells.

To this end, PARP-targeting radiotheranostic, ^{123}I-CC1 has been developed, which can emit Auger electron and bind to PARP enzyme in the cancer cells, offering therapeutic potentials for PARP-expressing cancers. Furthermore, ^{123}I-CC1 allows whole-body visualisation of PARP-expressing lesions via SPECT imaging, potentially enabling clinicians to select patients for PARP-TRT based on imaging (which can be described as 'see what you treat'). The goal of this project is to comprehensively explore the therapeutic potential of ^{123}I-CC1 in different types of PARP-expressing cancers, particularly those hard-to-treat cancers such as pancreatic cancers and glioblastoma.

Method:

Radiosynthesis of ^{123}I-CC1 was achieved via copper-mediated ^{123}I-iododeboronation of a boronic pinacol ester precursor. The level and specificity of cell uptake and the therapeutic efficacy of ^{123}I-CC1 were determined in PARP-expressing human cancer cells: breast carcinoma, pancreatic adenocarcinoma, and glioblastoma. Tumour uptake and tumour growth inhibition of ^{123}I-CC1 were assessed in mice bearing human cancer xenografts (MDA-MB-231, PSN1, and U87MG).

Results:

In vitro and in vivo studies showed selective uptake of ^{123}I-CC1 in all models. Significantly reduced clonogenicity was observed in vitro after treatment with as
little as 10 Bq of ^{123}I-CC1. Biodistribution at 1 h after intravenous administration showed tumour uptake of 0.96 ± 0.06, 0.46 ± 0.01 and 0.19 ± 0.01 %ID/g of PSN1, U87MG and MDA-MB-231 xenografts, respectively, correlating their PARP expressions. Intravenous administration of a relatively low amount of ^{123}I-CC1 (3 MBq) was able to significantly inhibit tumour growth of PSN1 and U87MG xenografts tumour growth but was less effective in MDA-MB-231 xenografts, potentially due to less tumour uptake. Importantly, treatment of ^{123}I-CC1 did not cause significant toxicity to normal tissues.

Conclusion: Taken together, these results show the
potential of ^{123}I-CC1 as a radiotheranostic for TRT in treating PARP-expressing cancers.

O2: Manchester Inhomogenous Radiation Chemistry by Linear Expansions (MIRaCLE): A Radiation Chemistry Toolkit

Prof Fred Currell[1], Dr. Marcus Webb[2]
[1]Dalton Cumbrian Facility, University Of Mancehster, Westlakes Science & Technology Park, Moor Row, UK, [2]Department of Mathematics, University of Manchester, UK

Purpose/Objective: The Manchester Inhomogenous Radiation Chemistry by Linear Expansions (MIRaCLE) toolkit will be introduced. This toolkit offers fast and user-friendly means to solve radiation chemistry problems. It solves the reaction-diffusion equation for user-specified chemical reactions, accounting for how chemical species' inhomogenous spatial distributions change over time. The toolkit is applicable to problems occurring in many areas of radiation-science, including radiobiology, radiation therapy, nuclear medicine, nanoparticle dose enhancement, nuclear waste management and handling of special nuclear materials. Its ability to include interactions at solid-fluid boundaries offers the possibility of solving many important problems in these problem domains.

Materials/Methods: In our method, the reaction-diffusion equation is efficiently solved using spectral methods, i.e. representing the concentrations of species using a linear expansion in terms of spectral functions. The time-evolution is split into sequential diffusion and reaction steps with the diffusion steps being solved exactly. The reaction steps are solved numerically using a method which is accurate to second order in the timestep. A second order splitting method is also used to alternate between the diffusion and reaction steps. The resulting method is fast, stable, and accurate. Furthermore, through the correct selection of the spectral functions used, one can naturally include effects of interactions with bounding surfaces (i.e. at solid-fluid interfaces) in a manner which incurs no performance penalty.

Although the software can handle three-dimensional spatial problems, the software architecture and underlying mathematics are such that spherical, cylindrical or translational symmetries can be exploited to provide more compact representations of the problem being solved along with increased performance. The entire software toolkit can be run using Jupyter notebooks in a simple and intuitive manner. This feature will be valuable once the toolkit is released for more widespread use – it is our intention to completely open-source the toolkit once we have gone through a beta-testing phase with a small number of collaborators with new features becoming available to this collaborator-group ahead of the wider community in a rolling fashion.

Results: An earlier version of the MIRaCLE toolkit has already demonstrated its value to the nuclear industry having solved 600 different versions of a radiolysis problem relevant to the safe storage of plutonium [1]. These solutions, representing different conditions and hence exploring the problem's parameter space, were calculated in a single day on a commercial laptop whereas previous approaches would have required several days of super-computer time for even a single set of conditions and would not have been able to consider the reactions taking place at the Pu2O3-water interface.

Solution of more sophisticated Pu2O3-water interface radiolysis problems will be presented. Other studies to be presented will include radiolysis effects along therapeutically relevant proton, helium and carbon ion beams and a study into the role of second-order chemical reactions in competition with surface effects near irradiated surfaces, pertinent to nanoparticle dose enhancement.

Conclusion: The MIRaCLE toolkit will be presented with a view to gaining feedback on features wanted in future releases and to more towards its more widespread use, initially through a close collaborator community.

O3: Development of next generation PSMA-targeted radionuclides 212Pb-AB001 and 225Ac-PSMA-617 in preclinical prostate cancer models

Dr Victoria Dunne[1], Dr Vilde Yuli Stenberg[2,3,4], Mrs Anna Julie Kjøl Tornes Tornes[2,3,4], Miss Rugile Liukaityte[4], Miss Mona-Elisabeth Revheim[2,6], Mr Li-Wei Ma[2], Mr Andrius Kleinauskas[7], Dr Petras Juzenas, Professor Joe M. O'Sullivan[1,8], Professor Kevin M. Prise1[1], Dr Asta Juzeniene[2,5]

[1]Queen's University Belfast, Belfast, United Kingdom, [2]Department of Radiation Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, [3]Institute for Clinical Medicine, University of Oslo, Oslo, Norway, [4]ARTBIO AS, Oslo, Norway, [5]Department of Physics, University of Oslo, Oslo, Norway, [6]The Intervention Center, Division of Technology and Innovation, Oslo University Hospital, Oslo, Norway, [7]Department of Pathology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, [8]Northern Ireland Cancer Centre, Belfast Health and Social Care Trust, Belfast, United Kingdom

Introduction

The Targeted radionuclide therapies (TRT), Radium-223 (223Ra) and lutetium-177-labelled PSMA-617 (177LuPSMA), are currently the only radiopharmaceutical treatments that prolong survival for patients with metastatic-castration resistant prostate cancer (mCRPC). Despite the clinical utility of these TRT, mCRPC remains an aggressive disease with a 5-year survival rate of 30%, therefore, there is an urgent need for novel treatments that can improve patient survival.

Recently, TRT utilising actinium-225 (225Ac), has gained attention as an attractive therapeutic alternative to β-particle emitters due to its high LET (80 keV/µm) and short tissue penetration (40-100 µm), inducing more ionizations per track within the tumour and less normal tissue damage when coupled to a specific targeting molecule. However, the limited supply of 225Ac is unable to meet the demand for large scale clinical trials or widespread hospital implementation. An alternative to 225Ac is lead-212 (212Pb), which acts as an in vivo generator for the high LET α-emitting daughter nuclides bismuth-212 (212Bi) (half-life = 60.6 min) and polonium-212 (212Po) (half-life = 0.3 microseconds). Moreover, the characteristic short half-life of 212Pb maximises energy deposition in cancer cells and in comparison, to α- and β-emitters with longer half-lives (225Ac and 177Lu), the use of 212Pb eliminates the potential problem of storing radioactive waste (83).

This study aimed to examine the efficacy of PSMA-targeting radioligand 212Pb-AB001 as an alternative treatment for mCRPC in comparison to 223Ra and 225Ac-PSMA-617.

Methods

The cytotoxic effects of 212Pb-AB001 and 225Ac-PSMA-617 were determined in C4-2 and LNCaP 3D multicellular tumour spheroid models. Fluorescein Diacetate/Propidium Iodine staining was performed to assess spheroid viability. Cellular survival, viability and DNA damage after 223Ra, 212Pb-AB001 or 225Ac-PSMA-617 treatment was examined using immunofluorescence staining of γH2AX, CellTitre-Glo and clonogenic assays. Further, cell cycle distribution, apoptosis and necrosis was assessed using flow cytometry.

Results

Treatment with 223Ra significantly reduced cell survival compared to untreated controls ($p < 0.001$) and was induced a slow rate of DSB repair (< 0.01) and increased G2/M arrest ($p < 0.05$). Similarly, 212Pb-AB001 and 225Ac-PSMA-617 significantly reduced cell survival and viability in a dose-dependent manner at activity concentrations of 3-50 kBq/mL of 212Pb-AB001 and 0-2 kBq/mL of 225Ac-PSMA-617. 212Pb-AB001 (5 kBq/mL) and 225Ac-PSMA-617 (1 kBq/mL) significantly inhibited spheroid growth in comparison to untreated spheroids. The doubling time for C4-2 spheroids 21-days post treatment, increased from 4 days for untreated to 7 days for spheroids treated with 212Pb-AB001 (5 kBq/mL) and 8 days for spheroids treated with 225Ac-PSMA-617. Furthermore, 212Pb-AB001 (12.5 kBq/mL) induced significant G2/M phase cell cycle arrest (untreated (15.38%), 212Pb-AB001 (24.5%) ($p < 0.001$).

Conclusion
The next generation of TRT radiopharmaceuticals are gaining significant attraction as therapeutic options for mCRPC however, availability of radionuclides remains a challenge. The present study highlights, for the first time, the potential of utilising the PSMA-targeted radioligand 212Pb-AB001 as an alternative treatment strategy for mCRPC. Taken together, our findings support further pre-clinical evaluation of 212Pb-AB001 in vivo.

O4: Investigating RNA Damage Induced by Ionizing Radiation in Various Radioprotective Environments
Mr Jordan Elliot[2]
[1]The University Of Manchester, Manchester, England, [2]The Dalton Cumbrian Facility, Moor Row, England
Jordan Elliot, Fred Currell, Aliaksandr Baidak, Mel O'Leary, Ruth Edge, Aidan Milston.

When compared to the role of DNA and protein damage contributing to various diseases, such as cancer [1], it is easy to overlook the role of damaged RNA. The structure of RNA (coding and non-coding) is directly linked to its function. For example, mRNA contains structural motifs that play a vital role in the regulation of translation [2]. Therefore, any damage to RNA critically impacts RNAs role within the body, as many essential biochemical interactions between RNA and other biological molecules rely on RNA having the correct optimal folded structure [3]. Causes of damage towards RNA come from both internal and external sources. Nucleic acid strands are particularly vulnerable to damage from reactive oxygen species (ROS) and ionizing radiation due to the reactive oxygen and nitrogen on the nucleobases and the oxygen on the sugar-phosphate backbone [4].

This investigation identifies the damage caused to RNA via two types of ionizing radiations, alpha and X-ray, as well as evaluating the effectiveness of radical scavenger buffers in preventing this damage. In this investigation, two RNA samples were used, MS2 bacteriophage RNA and single stranded (ss)RNA Ladder, which both serve as cost-effective and readily available samples for this experiment. Based on literature, ascorbic acid, and phosphate buffers [5][6] were chosen as the radical scavengers due to their biochemical importance. The samples were irradiated using sources and equipment supplied by the Dalton Cumbrian Facility and the samples were handled using a bespoke sample handling system dubbed "The Spinning Wineglass". This system allowed for the semi-automated dispensing, irradiation, and collection of small sample volumes necessary for this experiment. The irradiated samples were analysed via gel electrophoresis and image processing programmes to verify RNA damage and calculate the relationship between irradiated dose and damage to RNA.

The results of this experiment have yielded new and exciting information such as relating RNA damage to total dose, comparison of the RNA damage mechanisms through high and low LET irradiations, the evaluation of scavenger environments and their effect on RNA damage during those high and low LET irradiations, as well as other promising findings we hope to present at the conference.

O5: OGG1 and PARG play critical roles in the biological response to protons of increasing LET

Dr Maria Fabbrizi[1], Dr Jonathan R Hughes, Mr Karthik Vaidya, Professor Helen Bryant, Professor Thomas Helleday, Professor Jason Parsons
[1]University of Birmingham, Birmingham, UK

Proton beam therapy (PBT) is increasingly being utilised as a precision targeted approach for the treatment of solid tumours, including head and neck squamous cell carcinoma (HNSCC), where it largely acts through damaging the DNA. However, there is significant biological uncertainty due to increases in linear energy transfer (LET) at and around the Bragg peak where most of the radiation dose is delivered. In particular, the densely ionising radiation tracks lead to formation of complex DNA damage (CDD), where several lesions are induced in proximity within one or two helical turns of the DNA, which causes enhanced biological effectiveness. However, the cellular response to CDD and the pathways co-ordinating the repair are currently unclear. Consequently, further preclinical research using the appropriate HNSCC models is needed to fully understand the biological response to PBT and the comparisons to photon radiotherapy to optimise cancer treatment in the clinic.
We have performed a focussed small interfering RNA (siRNA) screen to identify specific DNA damage response proteins crucial for the cell survival of HNSCC cells in response to high-LET versus low-LET protons. From this screening, we have validated two enzymes (8-Oxoguanine DNA Glycosylase (OGG1) and Poly(ADP-ribose) glycohydrolase (PARG)) whose depletion using siRNA leads to significantly decreased survival of HNSCC cells after treatment with high-LET protons, whilst no effect was detected after low-LET radiation (protons and X-rays). We have also used specific inhibitors for OGG1 (TH5487) and PARG (PDD00017273), which further demonstrate that this selectively enhances radiosensitivity of HNSCC cells in response to high-LET protons. We subsequently identified that OGG1 and PARG depletion/inhibition leads to further persistence of CDD post-irradiation after high-LET proton irradiation, providing a mechanism through which reduced survival is achieved. These results suggest a pivotal role for the DNA damage response pathways in resolving CDD following high-LET protons, and that their targeting could represent a future potential therapeutic strategy for the treatment of HNSCC*.

* Fabbrizi MR et al., Targeting OGG1 and PARG radiosensitises head and neck cancer cells to high-LET protons through complex DNA damage persistence. Cell Death Dis. 2024 Feb 17;15(2):150. doi: 10.1038/s41419-024-06541-9.

O6: Radiotherapy combined with TLR stimulation elicits adaptive immune response against prostate cancer

Ms Irene Fischetti, Mrs Laura Botti[1], PhD Valeria Cancila[2], PhD Giorgio Bertolazzi[2], Ms Valeria Pinna[1], Mrs Renata Ferri[1], Prof. Claudio Tripodo[2], PhD Mario Paolo Colombo[1], PhD Claudia Chiodoni[1], PhD Elena Jachetti[1]
[1]Fondazione IRCCS Istituto Nazionale Dei Tumori, Milan, Italia, [2]Università di Palermo, Palermo, Italy

Immunotherapy has notably improved cancer therapy. However, prostate cancer patients still do not respond, necessitating new approaches. Radiotherapy (RT) has both immuno- suppressive and immuno- stimulatory functions. Besides local effects, elicited anti-tumor immunity could mediate the regression of untreated metastases, an effect called “abscopal”. To boost this response, combining RT- and immuno- therapy could be a promising strategy.

We are evaluating the efficacy of local administration of two different toll-like receptor (TLR) agonists and fractionated RT in murine prostate cancer models. Tumor cells are injected in both flanks of mice but only one side is treated in order to assess the abscopal effect on the other. The growth of both treated and contralateral not-treated tumor lesions is evaluated. At the end of the experiments, tumor immune microenvironment is analyzed by flow cytometry and immunohistochemistry.

Our results showed that in the T23 poor differentiated adenocarcinoma model a TLR9 agonist had no therapeutic effect. Conversely, the local administration of a TLR3 agonist significantly impaired tumor growth; combination with RT had no apparent additive effects on volume reduction. Yet, despite similar tumor size, the microenvironment composition in treated lesions was significantly different. In detail, the combination of TLR3 agonist and RT increased the infiltration of CD8+ T cells and germinal center B cells. The TLR3 plus RT combination also induced a tiny but significant abscopal effect on the contralateral lesion. These results were confirmed in two additional models of adenocarcinoma and neuroendocrine prostate cancer.
Mechanistically, digital spatial pathology analyses revealed the formation of putative tertiary lymphoid structures (TLS) in T23 lesions locally treated with the TLR3 and RT combination, which are being validated by immunohistochemistry.
Finally, survival experiments showed a superior benefit of the TLR3 and RT combination treatment than other groups, with regression of 40% of tumors.

These data show that coupling RT with TLR3 agonist can improve the immune response in prostate cancer, which is normally poorly immunogenic and unresponsive to immunotherapy. Further studies are needed to endorse the results and to deeply dissect the immune-mediated mechanisms behind the therapeutic effects. Besides, additional combination with immune checkpoint inhibitors will be considered to boost both local and abscopal effects.

O7: Bilateral DAD contributes to the fatal toxicity of pre-existing ILD mice after partial thoracic irradiation

Dr Jiamei Fu[1,4], Xinglong Liu[2], Yuchuan Zhou[2], Shengnan Zhao[3], Jianghong Zhang[2], Kevin M Prise[4], Chunlin Shao[2], Yaping Xu[1]

[1]Department of Radiation Oncology, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai , China, [2]Institute of Radiation Medicine, Shanghai Medical College, Fudan University, Shanghai, China, [3]Department of Pathology, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai, China, [4]Patrick G. Johnston Centre for Cancer Research, Queen's University Belfast, Belfast, UK

Purpose

Radiotherapy has been a critical strategy for treating lung cancer (LC) patients both in localized and advanced stages. However, accumulating studies suggested that LC patients with comorbid Interstitial lung disease (LC-ILD) patients are at increased risk of developing serious lung toxicities after thoracic radiotherapy, resulting in significantly worse survival and poor quality of life. The pathogenesis and mechanisms underlying the radiotherapy-related severe lung injury are yet to be clarified. We aimed to establish a severe radiation-related lung injury (RRLI) model based on pre-existing pulmonary fibrosis mice. We then clarified the histopathologic characteristics and molecular mechanisms responsible for the severe and fatal toxicities in vivo.

Methods

C57BL/J6 mice were used to develop different lung injury models, including Ctrl, radiation-induced lung injured (RILI), bleomycin induced pulmonary fibrosis (BIPF), and severe radiation-related lung injure (RRLI) group. Lung function changes were measured at week 24 post-irradiation (post-IR). The pathological evaluation of lung lesion phenotypes was performed on hematoxylin and eosin (H&E), Masson's trichrome, immunohistochemistry (IHC) stained sections. RNA extracted from mouse lung tissues was sequenced on the Illumina Novaseq platform. The bioinformatic analysis was performed.

Results

A severe lung injury murine model post-IR was built here based on pre-existing ILD mice induced by BLM administration. Compared to the mono-treatment groups, enhanced damage was observed in the severe RRLI model with higher mortality rate and declined pulmonary function within a six-month period post-IR. The histological phenotypes showed that exudative diffuse alveolar damage (DAD) was manifested in the early phase and proliferating DAD pattern predominated the progression of severe lung injury in the late phase. Morever, autopsy examination presented overlapping of exudative, proliferative and fibrosing DAD patterns in bilateral. RNA sequencing (RNA-seq) analysis showed that common signaling pathways relevant to inflammation, cellular damage and repair responses, involving p53, PI3K-Akt, MAPK, JAK-STAT, HIF-1 and cellular senescence were activated in different lung injury models. Additionally, the participation of epithelial cell and immune cells infiltration during the progression of lung remodeling was validated, and the upregulation of macrophages and CD4+ lymphocytes play important roles in inducing severe lung injury in the irradiated ipsilateral lung and remarkable abscopal responses in the non-IR contralateral lung.

Conclusion

The present study highlighted that the severe or even fatal toxicity was due to diffuse alveolar damage with progressive inflammation and fibrosis in bilateral lungs post-IR. Bioinformatics analysis aided in the discovery of critical signaling pathways, involving immune cell migration and chemotaxis, epithelial cell development, and extracellular structure organization. The hyperactivation of inflammatory responses, including macrophages and CD4+ lymphocyte infiltration, was further validated. However, further studies are needed to clarify the mechanisms underlying disease progression. Rigorous pre-clinical and clinical trials are still required to elucidate potential biomarkers and develop effective therapeutic targets for predicting and preventing severe complications in LC-ILD patients following radiotherapy.

O8: Characterising the role of SSB repair pathways in response to low and high LET radiation

Miss Lydia Gardner[1], Dr Francisco Liberal[1], Dr Karl Butterworth[1], Dr Stephen McMahon[1]
[1]Queen's University Belfast, Belfast, United Kingdom

To build the robust models of individual radiosensitivity which are needed for personalised radiotherapy treatments, a detailed understanding of the impact of alterations in key DNA repair pathways is essential. The disruption of Double Strand Break (DSB) repair pathways is known to significantly impact cellular radiosensitivity, however the involvement of other repair pathways hasnot been as well characterised. While DSBs are known to be the primary driver of radiation-induced cell killing, mutations in the repair pathways are more common than in DSB repair pathways, meaning even small impacts on radiosensitivity may be clinically relevant. The aim of this work was to quantify the impact of genetic alterations in Single Strand Break (SSB) repair pathways on cellular radiosensitivity in response to different radiation qualities.

A panel of cell line models with SSB repair defects was developed using CRISPR-Cas9 to knock out key genes in the base excision repair (BER) pathway: PARP1, XRCC1 and APE1, nucleotide excision repair (NER) pathway: XPC and ERCC1, and mismatch repair (MMR) pathway: MSH2, in the normal human cell line RPE-1. The impact of gene loss on radiosensitivity was assessed by measuring clonogenic survival and levels of DSB and SSB damage, via 53BP1 immunofluorescence and comet assay respectively, following exposure to low LET X-ray irradiation and high LET (129.3±15.2 keV/µm) alpha particle irradiation. The frequency of mutations in the repair pathways was obtained from the CCLE mutations dataset.

Small increases in radiosensitivity were observed in the SSB repair deficient cells following X-ray irradiation (sensitiser enhancement ratio (SER) values ranging from 0.96-1.36), with statistically significant increases in radiosensitivity observed in the BER and NER deficient cells. Disrupting SSB repair pathways also resulted in higher levels of residual DSB damage 24 hours following treatment with 2 Gy, with residual DSBs in knockout cells ranging from 4.1±1.5 to 7.2±1.5 compared to 1.3±0.9 in the parental cells. Measurement of SSB damage following X-ray irradiation indicated that thisincreased sensitivity may be due to the higher levels of unrepaired SSBs and base damages which remain within the cells being converted into additional or more complex DSB damage, which is then left unrepaired. Significant increases in radiosensitivity were observed in all SSB repair deficient cell lines following alpha particle irradiation (SER values ranging from 1.16-1.31), with higher levels of residual damage remaining in all cell lines compared to the parental cells. This increased sensitivity to high LET radiation may be due to an increase in the complexity of damage induced.

The disruption of SSB repair pathways has a small but significant impact cellular radiosensitivity (average change in the mean inactivation dose, ΔMID = 0.28±0.19). While this impact may not be as significant as DSB repair pathway disruption (ΔMID = 0.8±0.5), mutations in these SSB repair pathways are more common, therefore the cumulative effect of mutations may be clinically relevant and important to consider for personalised radiotherapy, particularly for treatments using high LET radiation. Ongoing work is applying high-throughput CRISPR-Cas9 screening techniques to identify possible interactions between genetic defects in multiple pathways.

O9: Multi-omic analysis of the hypoxic extracellular matrix identifies a gene signature that predicts radiotherapy benefit

Dr Conrado Guerrero Quiles[1], Julia Gonzalez Abalos[2], A.S. Foussat[3], Mark Reardon[1], Taha Lodhi[1], Victoria Smith[5], Rekaya Shabbir[1], Sapna Lung[1], A. Baker[4], Michael Eyers[5], T.A.D. Smith[6], Peter Hoskin[1], N.D. James[7], Emma Hall[7], R.A. Huddart[7], Nuria Porta[7], J.D. Humphries[8], M.J. Humphries[1], A. Choudhury[1], Catharine West[1]

[1]University of Manchester, Manchester, UK, [2]Institute Curie, Paris, France, [3]Sorbonne Nouvelle, Paris, France, [4]Cancer Research UK Manchester Institute, Manchester, UK, [5]Medicines Discovery Catapult, Cheshire, UK, [6]Bangor University, Bangor, UK, [7]The Institute of Cancer Research, London, UK, [8]Manchester Metropolitan University, Manchester, UK

Bladder cancer is a prevalent disease (>550,000 new cases worldwide), classified as non-muscle invasive (NMIBC) or muscle-invasive (MIBC)1. MIBC is a poor prognosis disease (21% 5-year overall survival) representing 20-25% of all new bladder cancer diagnoses (>130,000 yearly cases worldwide)1. Currently, radiotherapy and cystectomy are both treatment options with similar outcomes for MIBC2. However, there is no approach available to identify which patients would benefit from radiotherapy rather than cystectomy. Radiotherapy (RT) allows for bladder preservation3, but hypoxia (<2% O2) promotes radioresistance4,5. Among other mechanisms, hypoxia drives RT resistance by promoting extracellular matrix (ECM) remodelling4,5. Here, we aimed to: (1) characterise the hypoxic ECM, and (2) identify a predictive signature of metastasis and RT benefit.

To characterise ECM gene expression changes, we conducted a multi-omics analysis (proteomics, transcriptomics, ChIP-Seq) in four bladder cancer cell lines (T24, UMUC3, J82, RT4) cultured in 21% (normoxia) or 0.1-0.2% (hypoxia) O2. To identify a candidate signature, results were validated in clinical samples (n=34) using spatial transcriptomics. Retrospective validation was done using three RT (BC2001 [n=313], BCON [n=151], Christie [n=180]) and five cystectomy (TCGA-BLCA [n=405], GSE5287 [n=30], GSE13507 [n=62], GSE19915 [n=48] and GSE31684 [n=78]) cohorts. Further validation was conducted through a meta-analysis of all available cohorts while accounting for cohort differences. Mechanistic validation of the findings was then conducted in vitro through attachment, scratch and immunofluorescence assays in non-RT and RT (2-8 Gy) cells seeded onto hypoxic and normoxic ECMs. Respectively, those assays studied cell adhesion, migration, and ECM fibres morphology (collagen [COL] 1, COL5, fibronectin [FN]) and co-localisation (cadherin [CDH], paxillin [PXN], vinculin [VCL]).

Hypoxia affected 350 ECM proteins and RNAs (p. adj.<0.05, fold change>2 or <-2), 203 regulated by HIF1/2. Changes were associated with ECM remodelling and immune pathways, identifying COL and FN as central players. Spatial transcriptomics confirmed the results, identifying a 5-genes hypoxic-ECM signature predictive of metastasis (BCON [p=0.023], BC2001 [p=0.013], Christe [p=0.037]) and prognostic (OS; BCON [p=0.025], B2001 [p=0.013]) only in RT cohorts. “Medium” score patients had increased metastatic risk and worse prognosis when undergoing radiotherapy, suggesting the signature can predict RT benefit. A meta-analysis confirmed the results, showing “high” and “low” hypoxic-ECM score patients benefit from RT (p=0.00023), while “medium” score patients undergoing radiotherapy had an increased risk of mortality (HR=1.61, p<0.001). In vitro analysis provided mechanistic validation of the findings, showing highly hypoxic ECMs (0.2% O2) increased cell adhesion and impaired cell migration, effects enhanced by RT. Hypoxia (0.2% O2) also decreased COL but increased FN fibrogenesis. In addition, RT impaired CDH, PXN and VCL co-localisation with COL and FN, suggesting RT affects cell/ECM interactions and providing further mechanistic context. We concluded hypoxic ECMs affect immune pathways through HIF1/2-dependent and independent mechanisms. Our signature shows patients with “high” and “low” hypoxic-ECM scores benefit from RT. Mechanistically, in vitro findings suggest RT reduces cell migration by impairing focal adhesion signalling, an effect reflected by the signature. Prospective validation of the signature is warranted.

O10: Modelling reveals the impact of LET on the cell cycle and release of DNA fragments

Dr Charlotte Heaven[1,2], Dr John-William Warmenhoven[1,2], DR Nicholas Henthorn[1,2], Dr Amy Chadwick[1,2], Prof Karen Kirkby[1], Dr Michael Merchant[1,2]

[1]University Of Manchester, Manchester, United Kingdom, [2]The Christie Hospital, Manchester, United Kingdom

Radiotherapy is used to treat one third of all cancer types and is often used in conjunction with other treatments to create a more amplified response. Particle therapy, particularly proton beam therapy, is becoming more commonly used and has advantages over conventional radiotherapy in some cancers due to the physical properties of its dose distribution. The cGAS/STING pathway can be activated by the release of DNA fragments following radiation, however the best dose and any effect of LET on the fragment release is poorly understood. Mathematical modelling has been used to evaluate DNA damage and repair following radiation, predicting chromosome aberrations and cell death. This work aims to further one of these models (the DNA Mechanistic Repair Simulator, DaMaRiS), by incorporating the cell cycle in order to analyse DNA fragment release following irradiation. The experiment simulates 2 Gy and 4 Gy of radiation damage from photons and three LETs of protons (0.6 keV/µm, 3.5 keV/µm and 6.5 keV/µm) to study the effect on fragment release at cell division. The study reveals an increase in the number of acentric fragments released with dose and LET as well as a greater delay in cell division at higher dose and LET. There is an unusual effect on cell division following radiation with 2 Gy producing two peaks of cell division, and 4 Gy producing one, later peak. There is also a dose dependent increase in the cumulative amount of DNA released, which is higher with protons than photons, however this is not strictly LET dependent, with the greatest cumulative DNA released following 3.5 keV/µm protons. These results indicate an optimal dose and LET for inducing immune responses to radiation and describe why higher dose/LET does not necessarily result in a greater immune effect.

O11: Utilising ultra-high dose rate proton radiation to observe the FLASH effect in HNSCC

Dr Jonathan R Hughes[1], Mr Alex Bembridge[1], Dr Ben Phoenix[1], Professor Jason Parsons[1]
[1]University of Birmingham, Birmingham, UK

Background: FLASH radiotherapy is an emerging treatment strategy that utilises ultra-high dose rate radiation (>40 Gy/s vs ~5 Gy/min for conventional, CONV, radiotherapy) which elicits normal tissue sparring whilst maintaining tumour control (FLASH effect). The mechanisms behind the FLASH effect have not yet been fully elucidated but emerging hypotheses include oxygen depletion, radical-radical recombination, and DNA damage repair. Proton beam therapy (PBT) is a promising FLASH delivery method as the dose can be deposited deeper within the tissue and targeted to the tumour due to the characteristic Bragg peak region of the beam. Whilst the in vivo effects of FLASH have been heavily studied, investigations into in vitro cell models is lacking, particularly in response to PBT, which are vital for providing important mechanistic analysis and information.

Aims: Investigate the effect of PBT at both FLASH and CONV dose rates using head and neck squamous cell carcinoma (HNSCC) 2D cell lines, 3D spheroids and patient-derived organoids in order to enhance our knowledge of the underlying molecular and cellular biological mechanisms of the FLASH effect, with a particular focus on DNA damage repair.

Methods: Clonogenic survival was assessed using several cancer cell lines following PBT irradiation utilising a 40 MeV cyclotron accelerator at FLASH (60 Gy/s) or CONV (7 Gy/min) dose rates under both normoxic (21% oxygen) and hypoxic (1% oxygen) conditions. Furthermore, the growth of 3D spheroids was monitored over a 15-day period and volumetric increase was measured every 2 days. Organoid viability was measured 7 days post-IR. DDR was investigated via immunoblotting for key DDR pathway protein activation and through immunofluorescent microscopy for radiation-induced DDR foci at various time points post-irradiation.

Results: Increased clonogenic survival was observed following proton FLASH irradiation vs CONV, particularly in HeLa, FaDu and UMSCC12 cell lines, following 15 Gy in normoxic conditions. Furthermore, the presence of hypoxia increased this FLASH sparring effect at a dose of 15 Gy. Patient-derived HN041 HNSCC organoids showed an increased cell viability (~15%) following 15 Gy FLASH protons compared to CONV. No differences were observed in 53BP1 foci or cell cycle distribution.

Conclusion: These results suggest that FLASH is a promising therapeutic strategy for HNSCC. Interestingly, the FLASH effect was induced under normoxia (21% O2) which suggests alternative contributing mechanisms other than the oxygen depletion hypothesis. Therefore, further investigations into the specific biological differences between different cancer types and normal tissue must be undertaken in order to identify the mechanisms behind the FLASH effect. We plan to uncover this through the use of more advanced 3D culture techniques such as utilising pair-matched (normal and HNSCC) patient-derived organoids and the chicken chorioallantoic membrane (CAM) model, an in ovo model that offers the ability to study tumorigenic growth, microenvironment and metastasis.

O12: Chk1 and Wee1 inhibition increases radiosensitivity of HNSCC to X-ray and proton radiation, both low/high-LET

Miss Emma Melia[1], Professor Jason Parsons[1]
[1]University Of Birmingham, Birmingham, United Kingdom

Background: Ionising radiation (IR) relies heavily on the introduction of DNA damage to induce cell death, specifically double strand breaks (DSBs) and complex DNA damage (CDD). These lesions are commonly repaired via the error-prone non-homologous end-joining (NHEJ) pathway, although, these can be repaired more accurately via homologous recombination (HR). However, HR is only active in S and G2 phases of the cell cycle. Activation of Chk1 and Wee1 cell cycle kinases results in cell cycle arrest at the G2/M checkpoint to allow for DSB repair via HR. Consequently, these kinases pose as potential targets to increase the efficacy of radiotherapy. It has been shown that targeting either Chk1 or Wee1 kinases increases radiosensitivity of cells to x-rays, however, evidence for other radiation modalities, such as proton beam therapy (PBT), is lacking. PBT is of specific interest due to variations in linear energy transfer (LET) across the Bragg peak, of which the relatively high LET could contribute to CDD.

Aims: Investigate the radiosensitising effects of cell cycle kinase inhibitors for Chk1 and Wee1, to x-ray and proton IR, and to explore the possible underlying biological mechanisms.

Methods: Clonogenic survival assays were used to assess the cellular survival of head and neck cancer (HNC) cell lines following IR alone (x-ray and low/high-LET protons), or in combination with either the Chk1 (MK-8776) or Wee1 (MK-1775) inhibitors. This combinatorial treatment was also applied to 3D HNC spheroid models, which monitored the volumetric growth over a 10-day period and HNC patient-derived organoids (H-PDO), which were assessed via viability assays. DSB repair kinetics were assessed utilising neutral comet assays and γH2AX/Rad51 foci analysis following combinatorial treatment with radiation.

Results: We show that pre-treatment with either MK-8776 or MK-1775 increases the radiosensitivity of FaDu, UMSCC-12 and A253 HNC cell lines to x-ray and proton IR, of both low- and relatively high-LET. We demonstrate that this phenotype is maintained in more relevant 3D spheroid models of both FaDu and A253 cell lines, which was also translated into H-PDO. γH2AX foci analysis revealed that this increased sensitivity is due to a delayed repair of DNA DSB damage, which was further supported by neutral comet assays. Furthermore, our evidence suggests this lack of DSB repair could in part be due to a reduction in the activity of the HR pathway, due to inhibition of the radiation-induced G2/M arrest, seen via analysis of Rad51 foci.

Discussion: Collectively, our data supports the notion of cell cycle kinases being potential therapeutic targets to enhance the efficacy of both conventional radiotherapy and PBT. Our data also suggests that the increased sensitivity could be a result of delayed or insufficient DSB repair, possibly by reducing HR capacity of the cells. We have also been able to show that this radiosensitivity phenotype is maintained in H-PDO. In the future, we aim to further validate the mechanisms underlying this radiosensitivity in H-PDO and apply this to pair-matched H-PDO derived from normal tissues, to allow for investigative studies into the potential cytotoxicity effects of this combinatorial treatment.

O13: Effect of FLASH radiotherapy on muscle-invasive bladder cancer

Dr Jia-ling Ruan[1], Dr Iain Tullis[1], Dr Christin Cooper[1], Ms Nathalie Lovgren[1], Dr Salome Paillas[1], Dr Doreen Lau[4], Dr Shihong Wu[2], Mr Carl Lee[3], Dr Shiyan Tang[5], Mr Matthew Brown[1], Dr Kristoffer Petersson[1]
[1]Department of Oncology, University Of Oxford, Oxford, UK, [2]Department of Biochemistry, University of Oxford, Oxford, UK, [3]Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK, [4]Centre for Immuno-Oncology, University of Oxford, Oxford, UK, [5]Radcliffe Department of Medicine, University of Oxford, Oxford, UK

Recent research has demonstrated that irradiation delivered at ultra-high dose rates, known as FLASH radiotherapy (FLASH-RT), shows promise in reducing normal tissue toxicity while maintaining the efficacy of tumour eradication compared to conventional dose rate irradiation (CONV-RT). In this study, we investigated the response of murine muscle-invasive bladder cancer (MIBC) models representing different molecular subtypes to CONV-RT and FLASH-RT.

To establish these MIBC models, murine bladder cancer cell lines (MBT2; UPPL1541, representing luminal MIBC, and BBN963, representing basal MIBC) were either injected subcutaneously on the right flank or orthotopically into the bladder of C3H or B6 mice. The conventional dose rate irradiation (CONV IR, 0.01 Gy/s) and FLASH irradiation (FLASH IR, ~ 2,000 Gy/s) were administered using the horizontal beamline of our in-house 6 MeV electron linear accelerator. Mice were positioned inverted on an upright holder to minimise gut exposure, with the radiation beam targeting the lower pelvic region.

In our investigation, we observed significantly enhanced overall survival rates with FLASH IR compared to CONV IR in orthotopic MIBC C3H models. Specifically, the median survival following 15 Gy of CONV IR was 1.5 weeks, whereas it exceeded 30 weeks with FLASH IR (p-value < 0.05). However, our analysis revealed no discernible disparity between CONV IR and FLASH IR in the C3H subcutaneous models.

Interestingly, while the basal subcutaneous MIBC model exhibited superior tumour control with CONV IR over FLASH IR, a contrasting trend emerged in the basal orthotopic MIBC model. Here, FLASH IR demonstrated more favourable outcomes than CONV IR. No significant difference was observed between the two radiation modalities in luminal MIBC models. These findings underscore the complex interplay between treatment modalities and tumour microenvironments, emphasising the need for further investigation to elucidate underlying mechanisms and optimise therapeutic strategies.

O14: ER stress-induced intracellular C5aR1 increases cancer cell survival in hypoxia

Dr Tatsuya Suwa[1], Mr Ian Chai[1], Ms Kelly Lee[1], Mr David MacLean[2], Prof Ester M Hammond[1], Dr Monica M Olcina[1]

[1]Department of Oncology, University of Oxford, Oxford, United Kingdom, [2]CRUK Scotland Institute, School of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom

Background and objectives

Complement component C5a receptor 1 (C5aR1) is an innate immune receptor highly expressed in a variety of tumours (relative to normal tissue). High C5aR1 expression is usually associated with poor outcome. In tumours with immunosuppressive microenvironments, we recently reported that targeting C5aR1 improves tumour radiation response[1]. While C5aR1 is well-known for its role in the immune compartment, we found that C5aR1 is also robustly expressed on malignant epithelial cells, highlighting potential tumor cell–specific functions. The molecular mechanisms underlying dysregulated C5aR1 expression and function in the tumour microenvironment are not well understood[2]. Since hypoxia is a common feature of solid tumours and is known to mediate immunosuppression and radiation resistance, we hypothesised that tumour hypoxia may contribute to dysregulated C5aR1 expression and function in the tumour microenvironment.

Methods and Results

Immunohistochemistry and TCGA analyses suggest that C5aR1 is highly expressed in hypoxic tumour regions in xenograft models and in patient samples. In vitro experiments using a variety of tumour cell lines show that C5aR1 expression is induced in an ER-stress dependent but HIF-1 and p53-independent manner in response to severe hypoxia. As a G-protein coupled receptor, C5aR1 is usually expressed on the cell membrane but surprisingly we find that ER-stress induces intracellular C5aR1 expression in tumour cells. Furthermore, we demonstrate that hypoxia-induced C5aR1 attenuates apoptotic cell death, and regulates mTOR signalling and autophagy to increase cancer cell survival.

Conclusion

Taken together, these results show that ER stress-induced intracellular C5aR1 can contribute to cancer cell survival via mTOR signalling. This study improves our understanding of the mechanisms underlying dysregulated C5aR1 expression and function in the tumour microenvironment. The identification of hypoxia and ER-stress-induced intracellular rather than extracellular C5aR1 expression highlights the importance of developing specific inhibitors targeting this receptor in relevant cellular compartments for optimal therapeutic targeting of hypoxic radioresistant tumours.

O15: Entresto as a novel radioprotectant in a partial heart irradiation mouse model

Dr Gerard Walls[1], Dr Mihaela Ghita-Pettigrew[1], Mr Narainrit Karuna[1], Dr Kevin Edgar[1], Dr Kathryn Brown[1], Dr Chris Watson[1], Dr Karl Butterworth[1]
[1]Queen's University Belfast, ,

OBJECTIVE

Radiation cardiotoxicity (RC) is an important treatment sequela for patients with intrathoracic cancer. Heart failure, arrhythmias and acute coronary syndrome can arise in the months–years after treatment. Despite modern radiotherapy advances, the avoidance of incidental cardiac irradiation is not possible for many patients with intrathoracic malignancies. The natriuretic peptide axis represents a homeostatic cardiac hormone system that has not been thoroughly examined in relation to RC. Atrial natriuretic peptide (ANP) has gained interest in recent years for its role in heart failure. In response to stress, atrial cardiomyocytes release additional ANP, which acts on myocytes and fibroblasts of the myocardium and systemic vasculature to improve contractility and reduce harmful remodelling. Entresto inhibits neprilysin, the enzyme which degrades ANP, and reduces heart failure hospitalisation and death. We hypothesised that Entresto may alleviate the RC phenotype in a mouse model.

METHODS

Female 8-week old C57BL/6J mice were randomly assigned to receive sham irradiation (controls), sham irradiation plus Entresto (Entresto-only), irradiation (XRT-only) or irradiation plus Entresto (XRT+Entresto). Irradiated mice received a 20 Gy single-fraction to the superior 2/3 of the heart as a 90º arc field arrangement using a small animal radiotherapy research platform (SARRP, Xstrahl). Entresto was administered in drinking water, from one week prior until the end of the study, at a dose of 100mg/kg/day. Cardiac health was longitudinally monitored by transthoracic echocardiography (TTE) at 10-weekly intervals for 30 weeks using the Vevo module (Visual Sonics), allowing assessment of systolic and diastolic structural and functional parameters, as well as a 3-lead electrocardiogram (ECG). Plasma was analysed for NT-proANP levels by ELISA at 30 weeks.

RESULTS

Perturbations in heart structure and systolic cardiac function were detected at 10 and 20 weeks, and were accentuated at 30 weeks. At 30 weeks, the XRT+Entresto arm exhibited increased global longitudinal strain, compared with the XRT-only arm (means –10% versus –15%, $p<0.0001$), indicating protection of cardiac pump function. Less pronounced improvements in ejection fraction and fractional shortening were also observed. ECG analysis revealed prolongation of P wave ($p=0.001$) and PR interval ($p=0.0001$) in the XRT-only arm compared with controls which were less apparent in the XRT-Entresto arm ($p=0.01$, $p=0.68$). At 30 weeks there was equal narrowing of the QRS duration in both the XRT-only ($p=0.0001$) and XRT-Entresto arms ($p=0.0001$). NT-proANP levels were found to be elevated compared with controls in all groups. There was significant elevation of ANP in the XRT-Entresto compared to XRT-only (435 vs 369pg/mL, $p=0.003$). Ventricular mass was increased in XRT-only animals compared with controls, suggestive of pathological left ventricular hypertrophy. This was not observed in XRT+Entresto animals however. There was evidence of heightened early lung, oesophagus or skin toxicity.

CONCLUSION

Entresto attenuated the RC phenotype as assessed from cardiac function, structure and plasma endpoints, without increasing other radiation-related toxicities. Global longitudinal strain may have utility as a reliable early biomarker of radiation cardiotoxicity. Tissue immunohistochemistry and spatial transcriptomic analyses are underway. Further studies to investigate Entresto as a novel radioprotectant are warranted based on this preliminary data.

O16: A Novel Design of 64/67Cu-based Nano-Radiopharmaceuticals

Mr Volkan Yasakci[1], Dr Aliaksandr Baidak[1], Prof Fred Currell[1]
[1]The University of Manchester, Manchester, United Kingdom

With the increasing number of cancer cases globally, the diagnosis and treatment agents developed against cancer are increasing daily and achieving success. For this purpose, scientists are producing various radionuclides. This production usually takes place using medical cyclotrons or nuclear reactors. Ideally, the radionuclide to be obtained should be well suited for both diagnosis and treatment, while its half-life should sufficient to facilitate the labelling, imaging, and treatment processes. Furthermore, the target material should be accessible and cheap, and it should yield high-quality results [1-3]. For this purpose, as a clinical approach, alongside surgical intervention, chemotherapy, and radiotherapy, scientists are developing both diagnostic and therapeutic strategies by using various radioisotopes labelled with antibodies. Drug delivery is made more effective by using various nanoparticles. In addition, in recent years, theranostic approaches towards nano-radiopharmaceuticals have been rapidly increasing [4]. The aim of this project is to develop Cu-based nano-pharmaceuticals using a fully automatic infrastructure that includes transmutation, dissolution and separation, and nanoparticle synthesis/antibody labelling stages. This work is taking place within the framework of a larger project 'Optimised Production of Theragnostic Isotopes of Copper and Scandium (OPTICS) which has the aim of automated manufacture of radiopharmaceuticals from transmutation to synthesis, in a modular fashion. We expect to be able to deliver 61-Cu, 64-Cu, 67-Cu, 43-Sc, 44-Sc, 47-Sc and 48-Sc through transmutations using this system at the Dalton Cumbrian Facility. Upon its completion the automated manufacture platform will be available to external users.

In the first stage, inactive Cu nanoparticles were synthesised using a variety of methods selected. These methods were chosen based on the reaction time, temperature, the harmlessness of the chemicals used in terms of sustainable environment, and compatibility with biological organisms. In the studies conducted as wet chemistry processes, nanoparticle size control was attempted with FDA-approved stabilizers such as PVP, Tween-20, and Tween-80, alongside copper salts like CuCl2, CuSO4.5H2O, Cu(NO3)2.3H2O, and Cu(CO2CH3)2·H2O. As reducing agents, fructose and glucose were used. NaOH or NH4OH were used for pH adjustment. The reaction temperature was trailed between 60-150°C, and the reaction time varied between 1-4 hours for the optimization of different methods [5-10]. Additionally, a reaction experiment with CuCl2 and ascorbic acid at 80°C for 16 hours was also conducted [11]. The hydrodynamic radius of the particles was determined with DCS and DLS, the particle morphologies with SEM, the chemical bond formations with FT-IR, and the CuO/Cu2O transformations with XRD.

According to the size results obtained using DLS and DCS methods, the synthesis performed using the Cu(CO2CH3)2·H2O precursor has been found to be 192.1 nm. Morphologically, spherical particles have been obtained by using the Cu(NO3)2.3H2O precursor. Again, the most distinct Cu2O structure was observed with these methods. Moreover, despite the reaction time being 16 hours, the size of the nanoparticles synthesized with CuCl2, and ascorbic acid has been measured as 25 nm with DCS. In the synthesis conducted using PVP as a stabilizer, the size falls below 10 nm.

POSTER PRESENTER ABSTRACT

P1: Identification of Novel Radiosensitization Strategies in Head and Neck Squamous Cell Carcinoma

Mrs Aderonke Abah1, Dr. Gabrielle Grundy, Dr. Jason Fleming, Professor Jason Parsons, Dr. Lakis Liloglou, Dr. Caroline McCarthy
1University of Liverpool

Background: Head and Neck Squamous Cell Carcinoma (HNSCC) originates from the epithelial tissues of the oral, nasal, larynx and pharynx regions. Predisposing factors include smoking, excessive alcohol intake and HPV infection. Current therapeutic approaches include surgery and chemotherapy often in combination with radiotherapy, or alternatively immunotherapy. The efficacy of radiotherapy varies depending on factors such as cancer subtype and radiation dosage. Investigation of commonly prescribed medications for prevalent medical conditions could potentially enhance radiosensitivity. Thus, this study aims to identify FDA-approved inhibitors that target metabolism, thereby augmenting the radiosensitivity of HPV-negative HNSCC, and elucidate their underlying mechanisms of action.

Methods: The sensitivity of 3D spheroid cultures of HNSCC cells to X-rays was assessed using fifty FDA-approved metabolic drugs. Clonogenic growth assay was employed to determine post-treatment survival in HPV-negative cell lines. Subsequently, Neutral Comet Assays and γ-H2AX immunofluorescence (IF) were conducted to investigate whether the drugs induce double-strand DNA breaks. The inhibitors were further analysed using immunoblotting. Additional measures will include RNA sequencing to evaluate the drug's impact on cellular expression and an alcohol dehydrogenase assay to determine the role of Fomepizole as an alcohol dehydrogenase.

Results: Among the fifty drugs screened on the FaDu cell line, four drugs (Leflunomide, Syrosingopine, Ezetimibe, and Fomepizole) exhibited potential radiosensitization. Subsequent validation of these four candidates revealed that Fomepizole showed the most promising results in clonogenic survival assays conducted on four additional cell lines. However, Immunofluorescence (IF), Comet assays, and immunoblotting did not detect significant differences between the control group and the Fomepizole-treated samples suggesting radiosensitization was not due to increases in DNA damage but relied on alternative mechanisms.

Conclusion: Effective chemotherapy options with favourable therapeutic profiles will enhance the quality of life for individuals battling cancer.

P2: Predicting Arm Lymphedema Using Machine Learning Techniques

Mrs Abeer Al Janapy1, Prof Chris Talbot2, Dr Tim Rattay3, Dr Tim Lucas4
1University of Leicester, Department of Genetics and Genome Biology, 2University of Leicester, Department of Genetics and Genome Biology, 3University of Leicester, Leicester Cancer Research Centre, 4University of Leicester, Population Health Sciences

Objective: Arm lymphedema is one of the long-term (chronic) side effects that breast cancer patients can experience after treatment. Up to a quarter of all women may go on to develop arm lymphedema after radiotherapy, so identifying women at risk before the start of treatment will help target healthcare resources required for supportive management of the condition or even allow for a change in the treatment plan. Patient factors such as age, BMI, surgery type, age, and many treatment factors are considered important in the prediction of arm lymphedema but radiomic and genomic data are thought to improve the accuracy of predictive models.

Material and Methods: In our analyses, we applied machine-learning algorithms such as Random Forest, Logistic Regression, decision tree, KNN, SVC, etc... to the broad array of patient and treatment features available in the REQUITE dataset with up to 2 years of follow-up on 2059 patients and 409 features such as age, BMI, smoking, surgery type, RT, etc. The genetic features were selected based on previous GWAS and 30 SNPs were extracted for each patient.

Results: The results show that some factors such as node examined, rt_axilla_level, breast volume, and chemo could be good predictors for arm lymphoedema. The best-performing models were NB, Logistic regression, and MLPClassifier with an AUC of 0.86, 0.84, and 0.83. and for sensitivity/ specificity, the best model is NB with a Sensitivity: of 54.55% and a Specificity: of 87.91%. Aggregating the patient genetic data (SNPs) with the clinic data enhances the score of AUC for most ML models to 0.88, which means that the genetic data improves the accuracy of our predictive model.

P3: Utilising collaborative 3D cell models to investigate proton-drug combinations

Dr Jennifer Antrobus1, Dr Elham Santina1, Dr Emma Biglin1, Prof Anthony Chalmers2, Dr Natividad Gomez-Roman3, Dr Kirsteen Campbell2, Dr Amy Chadwick1
1University of Manchester, 2University of Glasgow, 3University of Strathclyde

Introduction:

Many 2D in vitro cell culture models do not accurately mimic the in vivo microenvironment. Data acquired using these models, particularly in radiotherapy (RT)-drug combination studies in radioresistant cancers, has not translated into in vivo or clinical efficacy. Here we have employed 3D in vitro models developed by collaborators in Glasgow to investigate radiotherapy-drug combinations in radioresistant cancer types inducing glioblastoma (GBM) and pancreatic ductal adenocarcinoma (PDAC). Both GBM and PDAC have an extremely poor prognosis, and inherent and acquired radioresistance mechanisms in these cancers can be a significant contributor to treatment failure. Use of 3D in vitro models that better mimic the in vivo situation is crucial to identify and target radio-resistance mechanisms. Proton beam therapy (PBT) is of interest clinically in these diseases, but little is known about how these mechanisms may differ with PBT compared to RT.

Methods:

Using the Proton Research Room at the Christie Proton Therapy Centre, we have utilised scaffold- and matrigel-based 3D models developed by Glasgow collaborators to culture GBM and PDAC cells, respectively, and tested combinations of either DDRis or HDACis with PBT or photons with the aim of improving cellular radiosensitivity and understanding potential resistance mechanisms. Cell survival has been investigated using multiple end points, including clonogenic and CellTiter-Glo assays, DNA damage and repair kinetics are currently under investigation.

Results:

Preliminary clonogenic studies with a 3D in vitro model using a patient-derived PDX GBM cell line (G7) suggest that the DNA repair inhibitors Talazoparib (PARP inhibitor), AZD1390 (ATM inhibitor) and BAY1895344 (ATR inhibitor) sensitise G7 cells grown in 3D to both protons and photons. Studies in the PDAC model shown that the HDACi vorinostat increases cellular radiosensitivity to photons in 2D clonogenic assays. These preliminary results will be further investigated using the proton beam during planned proton beam time in April and expanded to include the study of DNA repair kinetics.

Conclusions:

Our initial results expand of published work from Glasgow and suggest that DNA repair inhibition may sensitise GBM cells to both proton, as well as photon irradiation. We have also demonstrated that vorinostat, a HDACi, can increase the radiosensitivity of PDAC cells to photons. Further investigation in the coming weeks will provide more insight into the impact of these inhibitors in combination with both protons and photons, as well as detailed information about their effects on the DNA damage response.

P4: In vitro study of radiosensitivity in colorectal cancer cell lines associated with Lynch Syndrome

Dr Stephen Barnard1, David Burling2, Jayne Moquet1, Nicola Anyamene4, Kevin Monahan3, Andrew Latchford3, Rachel Baldwin-Cleland2, Simon Bouffer1, Liz Ainsbury1,5, Hannah Mancey1, Mingzhu Sun1, Dr Christophe Badie1
1United Kingdom Health Security Agency, Department of Radiation Effects, Cytogenetics and Path, 2Intestinal Imaging Centre, St Mark's Hospital, London North West UniversityHealthcare National Health Service Trust, 3Lynch Syndrome Clinic, Centre for Familial Intestinal Cancer, St Mark's Hospital, London North West University Healthcare National Health Service Trust, 4East and North Hertfordshire National Health ServiceTrust, Mount Vernon Cancer Centre, 5Environmental Research GroupWithin the School of Public Health, Faculty of Medicine at Imperial College of Science, Technologyand Medicine

Lynch syndrome patients have an inherited predisposition to cancer due to a deficiency in DNA mismatch repair (MMR) genes which could lead to a higher risk of developing cancer if exposed to ionising radiation. This pilot study aims to reveal the association between MMR deficiency and radiosensitivity at both a CT relevant low dose (20 mGy) and a therapeutic higher dose (2 Gy). Human colorectal cancer cell lines with (dMMR) or without MMR deficiency (pMMR) were analysed before and after exposure to radiation using cellular and cytogenetic analyses i.e. clonogenic assay to determine cell reproductive death; sister chromatid exchange (SCE) assay to detect the exchange of DNA between sister chromatids; γH2AX assay to analyse DNA damage repair; and apoptosis analysis to compare cell death response. The advantages and limitations of these assays were assessed in vitro, and their applicability and feasibility investigated for their potential to be used for clinical samples.

Results from the clonogenic assay indicated that the pMMR cell line (HT29) was significantly more radio-resistant than the dMMR cell lines (HCT116, SW48, and LoVo) after 2 Gy X-irradiation. Both cell type and radiation dose had a significant effect on the yield of SCEs/chromosome. When the yield of SCEs/chromosome for the irradiated samples (2Gy) was normalised against the controls, no significant difference was observed between the cell lines. For the γH2AX assay, 0, 20 mGy and 2 Gy were examined at post-exposure time points of 30 minutes (min), 4 and 24 hours (h). Statistical analysis revealed that HT29 was only significantly more radio-resistant than the MLH1-deficient cells lines, but not the MSH2deficient cell line. Apoptosis analysis (4 Gy) revealed that HT29 was significantly more radio-resistant than HCT116 albeit with very few apoptotic cells observed.

Overall, this study showed radio-resistance of the MMR proficient cell line in some assays, but not in the others. All methods used within this study have been validated; however, due to the limitations associated with cancer cell lines, the next step will be to use these assays in clinical samples for the understanding of the biological and mechanistic effects of radiation in Lynch patients.

P5: Utilising ultra-high-dose-rate proton radiation to observe the FLASH effect in head and neck cancer

Mr Alex Bembridge1, Dr Jonathan R Hughes1, Professor Jason Parsons1, Dr Ben Phoenix1, Professor Stuart Green1
1University Of Birmingham

FLASH radiotherapy is an emerging treatment strategy that utilises ultra-high dose rate radiation (>40 Gy/s vs ~5 Gy/min for conventional, CONV, radiotherapy) which elicits normal tissue sparing whilst maintaining tumour control (FLASH effect) but the mechanisms behind this effect have not yet been fully elucidated. Proton beam therapy (PBT) is a promising FLASH delivery method as the dose is deposited deeper within the tissue and targeted to the tumour (Bragg peak). Whilst the in vivo effects of FLASH have been heavily studied, investigations into in vitro cell models is lacking, particularly in response to PBT, which are vital for providing important mechanistic analysis and information.

We assessed clonogenic survival using several cancer cell lines following PBT irradiation at FLASH (60 Gy/s) or CONV (7 Gy/min) dose rates under both normoxic (21% oxygen) and hypoxic (1% oxygen) conditions. Increased clonogenic survival was observed following proton FLASH irradiation vs CONV, at a dose of 15 Gy in both normoxic and hypoxia conditions. Patient-derived HNSCC organoids showed an increased cell viability (~15%) following 15 Gy FLASH protons compared to CONV.

These results suggest that FLASH is a promising therapeutic strategy for HNSCC. However, further investigations into the specific biological differences between different cancer types and normal tissue must be undertaken in order to identify the mechanisms behind the FLASH effect. We plan to uncover this through the use of more advanced 3D culture techniques such as utilising pair-matched (normal and HNSCC) patient-derived organoids and the chicken chorioallantoic membrane (CAM) in ovo model.

P6: A preclinical proton dosimetry audit using a tissue equivalent murine phantom and Gafchromic EBT4 film.
Dr Adam Aitkenhead, DR Nicholas Henthorn, **Dr Emma Biglin**
1University Of Manchester

Purpose/objective:
One concern with preclinical radiation research is a lack of robust dosimetry protocols that provide traceability to a primary standard. Without this, accuracy and reproducibility between studies can be compromised. Furthermore, with proton beam therapy, various techniques and equipment are required to scale down the size of the beam to target small animals. Often the proton beam is used in transmission mode where the dose is delivered using the entrance region of the beam, rather than using the Bragg peak. Consequently, healthy tissue beyond the target is irradiated, potentially increasing toxicity and not an accurate depiction of a clinical treatment.

Building on a previous preclinical QA dosimetry audit carried out across UK institutions using a phantom designed for X-rays, the aim of this study is to create a 3D printed murine dosimetry phantom to examine the current state of preclinical proton dosimetry.

Methods
To create the murine phantom an Ultimaker S3 3D printer and PLA plastic were used. The body, skeleton and lung components of the phantom, segmented from a CT scan, were divided into 9 slices to accommodate Gafchromic film.

A questionnaire was then sent to several European institutions to record current QA practices. Following the results of the questionnaire, the phantom and Gafchromic EBT4 film will be sent to compare the planned versus delivered doses and the out-of-field dose.

Results
To create a phantom that is soft tissue equivalent (0.95-1.05 g/cm3) the phantom was printed with PLA at several different infill densities and then weighed to account for any warping during the printing process. An infill density of 85% provided an overall density of ~0.96 g/cm3. To create a higher density, the skeleton was printed at 100% infill density. For convenience, due to the difference in density across the breathing cycle, the lungs were printed as a void.

Preliminary responses from the questionnaire indicate that although a standardised dosimetry protocol is not in place across preclinical proton research, participants agreed on a) setting a ~3%/1mm dose tolerance, b) performing dose output and beam alignment checks prior to every experiment and c) using similar detectors for dosimetry, calibrated to national primary standards. Moreover, differences were reported in a) the techniques/equipment used for achieving targeted beam delivery, b) the treatment planning systems implemented, and c) suggested parameters to be reported in publications.

Conclusions
Implementation of the phantom and Gafchromic film could address the need for standardised QA by providing accurate routine measurements.

P7: Identification of an early gene profile biomarker for molecular dosimetry.

Luisa Biolatti1, MSc Lara Negrin2, MSc Jerónimo Leberle2, Dr Soledad Ausas2, Dr Laura Mazzitelli Fuentes2, Dr Irene Ibañez3, Dr Kimberley Reeves1, Professor Ananya Choudhury1, Dr Nicolas Bellora4
1Translational Radiobiology Group, Division of Cancer Sciences, School of Medical Sciences, Faculty of Biology, Medicine and Health, Radiotherapy Related Research, University of Manchester, 2Laboratory of Radiobiology and Biodosimetry, National Atomic Energy Commission (CNEA), Bariloche Nuclear Medicine and Radiotherapy Integral Center - Institute of Nuclear Technologies for Health Foundation (INTECNUS), , 3National Scientific and Technical Research Council (CONICET), Institute of Nanocience and Nanotechnology (INN), 4Laboratory of Computational Genomics - INTECNUS – CONICET

The molecular study of the radioinduced response by means of new technologies that allow massive analysis of the radiomodulated transcriptome has gained great relevance in recent years. The estimation of absorbed dose is currently performed using cytogenetic techniques over a 48 hour period, the gold standard used in biological dosimetry, radiation protection and radiation oncology's approaches. We hypothesised that we could estimate the absorbed dose during irradiation of human leukocytes and identify an associated expression profile. Material and methods: Peripheral blood leukocytes from healthy individuals were irradiated with 6 MEV X-rays (doses: control, 25 cGy, 100 cGy and 200 cGy) and cultured (37°C and 5% CO2) for 4 hours. RNA was extracted and the transcriptome was sequenced using RNA-seq (Illumina platform, 150bp paired-end). Total transcriptomics were analyzed using different bioinformatics tools. Sequencing reads were mapped to the human hg38 genome (STAR software). Comparative transcriptomic analyses of irradiated vs control samples were performed by applying different bioinformatics pipelines and the DESeq2 and limma:voom tools (false discovery rate, FDR, < 0.05). Eight microarray data from malignant eg heptatocellular carcinoma and non-malignant conditions eg myocardial infarction were used to corroborate gene specificity to radiation.

Results: A total of 240 genes and long non-coding RNAs were observed to change in expression due to the effect of radiation. Seven genes were identified as the most strongly associated with radiation dose, but were independent of underlying pathology. These genes represent the highest dose-dependent transcriptional alteration. The gene profile was measurable four hours after exposure. Conclusion: We propose a gene profile biomarker for molecular dosimetry. This biomarker can estimate the absorbed dose during acute exposures of radiation. This is particularly interesting as the biomarker can be measured early, within four hours of exposure.

P8: AURKB Inhibition Radiosensitises NSCLC by Altering Mitotic Fate

Ms Kathryn Egerton1, Dr Tim Mitchel1, Prof. Claire Eyers1, **Professor Helen Bryant**1
1University of Sheffield

Introduction:

Lung cancer accounts for 18% of cancer-related deaths globally, due to its high incidence and mortality rates. Radiotherapy is standard of care but radioresistance remains an area of clinical need. Aurora kinase B (AURKB) is a mitotic kinase with links to DNA damage repair (DDR). Given AURKB's canonical functions in mitotic progression and its interactions with DDR proteins, we hypothesized that inhibition of AURKB via the clinical inhibitor Barasertib (AZD1152) would radiosensitise NSCLC.

Methods:

H460 cells were used as a NSCLC in vitro model. Survival fraction was assessed by clonogenic assay. Mitotic phenotypes and DDR markers were analysed by immunofluorescence. Cell death wass assessed by Annexin V FACS. Phospho-Mass spectrometry (MS) was carried out on a Thermo Orbitrap LC-MS with TMT tagging and phospho-peptide enrichment using TiO2 beads. In vivo experiments were performed using a H460 xenograft model in Balb/c nude mice.

Results and Discussion:

We found that AURKB inhibition by Barasertib radiosensitises NSCLC in vitro. We assessed changes in peptide phosphorylation using global phospho-MS. Comparing IR and IR-Barasertib conditions, significant changes in phospho-peptides representing proteins involved in cell cycle regulation, DDR and survival signalling were observed. Barasertib increased death and apoptotic cells 72 Hrs after IR. There was also a consistent senescent population in Barasertib+IR cells which reduced over time when treated with IR alone. Consistent with this we found significant changes in the mitotic populations after IR with Barasertib. There were greater mitotic defects in Barasertib treated cells. Trials are underway to test the efficacy of the IR-Barasertib combination on tumour growth in vivo.

Discussion:

Our results indicate that, after IR, Barasertib increases mitotic defects and results in increased interphase death. This targets the proliferative population and reduces repopulation after IR.

Conclusions:

Barasertib radiosensitises NSCLC cells. This holds promise for clinical application in highly proliferative tumours.

P9: ZNF280A links DNA double-strand break repair to human 22q11.2 distal deletion syndrome.

Dr Thomas Clarke[1]
1Boston University

DNA double-strand breaks (DSB) are one of the most deleterious forms of DNA damage, and if unresolved result in DNA mutations and chromosomal aberrations that can cause disease, including cancer. Repair of DSBs by homologous recombination (HR) requires extensive nucleolytic digestion of DNA ends in a process known as DNA end-resection. In recent years, progress has been made in understanding how this process is initiated, however the later stages of this process – long range DNA end-resection, is not well understood. Indeed, many questions remain as to how the DNA helicases and endonucleases that catalyze this process are regulated, a key step to avoid spurious activity in the absence of breaks. The importance of DNA end-resection in human disease is highlighted by several human genetic syndromes which are caused by mutations or deficiencies in key proteins involved in this process. In this study, using high throughput microscopy (HTM) coupled with a cDNA "chromORFeome" library, we have identified ZNF280A as a novel chromatin factor that is essential for DNA double-strand break repair. Mechanistically, we demonstrate that ZNF280A promotes long-range DNA end resection by facilitating the recruitment of the BLM-DNA2 helicase-nuclease complex to DNA double-strand break sites, enhancing efficiency of the enzymatic activity of this complex at DNA damage sites. ZNF280A is therefore a key accessory factor for DNA end-resection and DNA repair by homologous recombination. Importantly, ZNF280A is hemizygously deleted in a human genetic condition, 22q11.2 distal deletion syndrome. Features of this condition include congenital heart disease, microcephaly, immune deficiency, developmental delay, and cognitive deficits – features that are associated with other human syndromes caused by defects in genes involved in DNA repair. Remarkably, cells from individuals with a 22q11.2 distal deletion have defects in homologous recombination and increased incidence of genome instability, providing the first evidence of defective DNA repair as a potential mechanistic explanation for several clinical features associated with this human condition.

P10: A Comprehensive Meta-analysis of Effectiveness/Toxicity Profiles of Lutetium-177/Actinium-225-Prostate-Specific Membrane Antigen-labelled Radiopharmaceutical Therapy in Prostate Cancer

Dr Yang-Hong Dai[1]
1University Of Oxford

Context: Managing metastatic castration-resistant prostate cancer (mCRPC) presents significant challenges.
Objective: This systematic review and meta-analysis assessed the efficacy and safety of prostate-specific membrane antigen (PSMA)-targeted radioligand therapy (PRLT) utilising lutetium-177 (177Lu) and actinium-225 (225Ac).

Evidence acquisition: A detailed literature search across PubMed/Medline, EMBASE, Web of Science, Scopus, and Cochrane Library was conducted, culminating in the inclusion of 98 studies involving 8621 patients. Data on PSA responses, toxicity profiles, and survival outcomes were analysed in detail. For PSA response, time-to-event data were estimated from reconstruction of survival curves. Proportional meta-analyses and meta-regression analyses were performed.

Evidence synthesis: Our analysis indicates that PRLT markedly lowers serum PSA levels by at least 50%, with estimated proportions of 0.49 (95% confidence interval [CI]: 0.46–0.52) and 0.60 (95% CI: 0.51–0.68) following 177Lu-PSMA and 225Ac-PSMA therapies, respectively. For any PSA decline, the estimated proportions were 0.70 (95% CI: 0.66–0.73) for 177Lu-PSMA and 0.80 (95% CI: 0.73–0.86) for 225Ac-PSMA. Meta-regression analysis revealed a significant correlation between the cumulative administered amount of radioactivity (AA) and PSA response, indicating activity-response effectiveness. Positive PSA responses were associated with improved overall survival across therapies. Although anaemia was common, with 177Lu-PSMA, severe toxicities were infrequent. In contrast, 225Ac-PSMA therapy showed a higher incidence of xerostomia and anaemia, yet severe anaemia cases were still uncommon (15%).

Conclusions: Our findings suggest that PRLT effectively reduces PSA levels and enhances survival in mCRPC, with a low incidence of severe toxicity. This finding supports the increasing use of PRLT and underscores its potential as an integral part of mCRPC management.
Patient summary: PSMA-targeted radioligand therapy offers a promising treatment option in metastatic prostate cancer. This therapy has been shown to significantly lower PSA levels, indicating tumour response, and is associated with encouraging survival rates, all while maintaining a manageable safety profile.

P 11: Modelling Intrinsic Radiosensitivity and Relative Biological Effectiveness in Clinical Radiotherapy Plans

Mr Mohammed Dakheel[1]
1Queen's University Belfast

Modelling Intrinsic Radiosensitivity and Relative Biological Effectiveness in Clinical Radiotherapy Plans

Mohammed Dakheel, Kevin M. Prise, Stephen J. McMahon

Background

Proton therapy is believed to achieve superior clinical outcomes to conventional radiotherapy due to its better physical dose distribution and its greater Relative Biological Effectiveness (RBE). However, incorporating RBE variability and individual radiosensitivity into treatment planning remains a challenge. This study aims to address these issues by integrating and benchmarking various RBE models and incorporating predictions of individual radiosensitivity into treatment plans. The impact of these changes on clinically relevant predictions will be evaluated by integrating models of tumour response and normal tissue toxicity.

Methods

Our study examines the behaviour of different RBE models across various cancer types, including prostate, head and neck, and liver cancer, utilising clinical data as the basis of analysis. The Dose-Volume Histograms (DVHs) and Normal Tissue Complication Probabilities (NTCPs) for Organs at Risk (OARs) were obtained using MATLAB and CERR (Computational Environment for Radiotherapy Research). MATLAB was used for 13 different RBE models to calculate variations in RBE predictions and subsequently compared their dosimetric characteristics., while CERR provided a platform for generating DVHs and NTCPs based on clinical cases in these cancer types.

Results

Initial findings reveal a dependence of RBE on parameters such as dose, dose-averaged Linear Energy Transfer (LET), dose fraction, and α/β value. Significantly, our analysis revealed substantial heterogeneity among the 13 RBE models utilized, particularly evident in the diverse RBE predictions across typical prostate, liver, and head and neck tumour scenarios. The RBE values spanned from 1.03 to 1.23 for prostate tumour, 1.03 to 1.44 for liver tumour, and 0.98 to 1.5 for head and neck tumour, reflecting the considerable variability in their predictive capabilities.

Moreover, discernible disparities were observed in dose distributions, showcasing varying responses dependent on the given dose and the α/β value. These variations introduced notable uncertainties into the RBE predictions, emphasising the complexity inherent in modelling radiobiological effects accurately.

Furthermore, the analysis underscored the emerging significance of linear energy transfer (LET) as a key determinant in Normal Tissue Complication Probability (NTCP) for organs at risk (OAR), as suggested by the RBE models.

This comprehensive examination not only highlights the wide-ranging impacts of different RBE models on RBE predictions but also underscores the associated uncertainties, particularly in NTCP predictions for OARs.

Conclusions

This study investigates the use of RBE models and radiosensitivity predictions in proton therapy. Initial results demonstrate that RBE depends on dose and LET across all models, but there is substantial variation in the dependence across models. This research offers vital insights for using RBE models and radiosensitivity predictions to personalise and optimise radiotherapy, which could enhance clinical outcomes.

P12: Investigating the cellular responses to complex DNA damage induced by proton beam therapy in HNSCC

Dr Elizabeth Dufficy1, Professor Jason Parsons1
1University Of Birmingham

Proton beam therapy (PBT) is a precision technique for the treatment of solid tumours, including head and neck squamous cell carcinoma (HNSCC), by specifically targeting the tumour via the Bragg peak and minimising any effects to the normal tissues and organs at risk. Here, high linear energy transfer (LET) protons have the propensity to generate increased levels of complex DNA damage (CDD), which is classified as numerous lesions induced within close proximity of one to two helical turns of the DNA, that promotes cell death. Despite this knowledge, there is a need to further understand the cellular response and the repair pathways that resolve this CDD, and which could be targeted in order to further enhance the biological effectiveness of proton beam therapy.

Through siRNA screening and targeted approaches, we aim to identify key cellular DNA repair pathways and enzymes that repair CDD, leading to a greater understanding of PBT radiobiology and to discover novel combinations of therapeutic strategies for the treatment of HNSCC patients. To measure PBT-induced CDD and its repair, we perform enzyme modified neutral comet assays along with measuring the formation of OGG1 protein foci, and which are correlated with the effects on clonogenic survival. We are currently investigating whether key DNA repair proteins essential for homologous recombination (HR) and non-homologous end joining (NHEJ) are required for the repair of CDD-induced by PBT, including ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), DNA dependent protein kinase catalytic subunit (DNA-PKcs) and BRCA2. We have shown that inhibition of DNA-PKcs and ATM particularly sensitised HNSCC cell lines to high-LET proton irradiation, and that this is mediated through inhibition of the repair of CDD. Additionally, the depletion of BRCA2 also led to increased radiosensitivity to high-LET proton IR. Overall, we have shown that key DNA repair proteins are required for the repair of CDD-induced by PBT, and could be targetable for enhancing the effectiveness of the treatment in HNSCC.

P13: Investigating the differential impact of low and high-LET radiation on DNA replication dynamics in HNSCC

Mr George Duffield1, Professor Jason Parsons1
1University of Birmingham

Ionizing radiation (IR) is used as a treatment for up to 50% of all cancer patients and is usually low-linear energy transfer (LET) x-ray radiation. Higher-LET radiation sources, which have higher ionisation densities, such as proton and carbon ion therapy, are becoming increasingly common, especially for patients with tumours close to critical organs, such as head and neck squamous cell carcinomas (HNSCC). Higher-LET radiation is characterised by its ability to form 'complex' or 'clustered' DNA damage (CDD), made up of multiple DNA lesions in close proximity. CDD provides a much greater challenge for DNA repair machinery to resolve in comparison to isolated single or double strand breaks. Simple low-LET radiation associated DNA damage has been shown to impact DNA replication, resulting in replication stress, but little research has been carried out to deduce the impact of high-LET radiation on DNA replication dynamics. Given the ability of high-LET radiation to form persistent CDD, deciphering the impact on DNA replication in cancer cells would increase our understanding of the cellular response to high-LET radiation and could highlight novel therapeutic opportunities.

Here, we primarily use the DNA fibre assay to evaluate the replication stress response in HNSCC cells treated with x-ray radiation (low-LET) and low/high-LET protons. We show that x-ray radiation induces only transient replication fork slowing in HNSCC cells, without any meaningful differences in stalled forks and new origin firing, followed by a rapid increase in replication speed one hour following treatment. Interestingly, this was despite higher levels of DNA damage in replicating cells, as indicated by yH2AX foci. Using siRNA against RAD51 completely abrogates this affect, indicating replication forks might be undergoing temporary fork reversal immediately after IR, potentially followed by a compensatory speed increase to ensure S-phase is completed in a timely manner. Further interrogation of the proteins involved in this response revealed potential roles for ATM and DNA-PK, but not ATR, in modulating the observed fork slowing and subsequent speed increase following IR. Initial data shows that high-LET protons induce greater fork slowing than low-LET protons and x-rays, potentially highlighting that higher-LET radiation may induce more replication stress and fork reversal. Further experiments will look to deduce this response over time and look to target this phenotype clinically to attempt to find novel radiosensitizers to radiation for HNSCC patients.

P14: The Dalton Cumbrian Facility (DCF): An Internationally recognised Radiation Science User Facility

Dr Ruth Edge1, Dr Carl Andrews1, Dr Aliaksandr Baidak1,2, Dr Samir de Moraes Shubeita1, Dr Adam Fisher1, Dr Robert Jones1, Dr Mel O'Leary1, Dr Aidan Milston1, Dr Andrew Smith1, Miss Claire Trevaskis1, Dr Chetna Tyagi1, Mrs Sally Wilson1, Prof Fred Currell1,2
1Dalton Cumbrian Facility, The University of Manchester, 2 Department of Chemistry, The University of Manchester

The Dalton Cumbrian Facility (DCF) is an international radiation-science user facility and leading hub for the UK radiation science community, which fosters and supports the understanding of radiation-driven processes and the responsible use of radiation [1, 2]. Our vision is to support innovation in applications of radiation science and engineering across all disciplines, including through various national access schemes [1].
Our world-leading radiation facilities along with state-of-the-art scientific analytical and characterisation equipment housed in a modern laboratory are situated in one of the most scenic parts of the country (West Cumbria) and at the heart of the UK's nuclear industry. We deliver X-rays and gamma rays at dose rates up to 30 kGy/hour and proton, helium-ion, carbon-ion and heavier ion beams from two accelerators.
The combination of the 60-Co gamma rays and fast ions we offer on one site should be especially attractive to radiobiologists since the 60-Co gamma rays define the standard RBE =1, allowing for interesting comparative studies. We have also developed a neutron-conversion target, able to deliver beams of neutrons, along with bespoke sample handling processes well suited to high throughput irradiation of samples [2]. For the ion beams we offer users a wide range of automated sample handling systems able to automatically irradiate liquid samples from 10s of microlitres up to several millilitres at one time, with multiple samples being irradiated sequentially without the users needing to enter the search area. For the gamma and X-ray irradiators many samples can be irradiated in parallel using various racks/turntable systems we have available. The 60-Co gamma rays are also far more penetrating than users of X-ray cabinet irradiators are used to – this feature offers the ability to irradiate samples one behind the other, doing dose-rate/variable dose studies in parallel.

Although typically thought of as supporting nuclear energy sector research, DCF supports a far wider range of research, currently including research into radiation chemistry and biology, radioprotection, automated development of radiopharmaceuticals and RNA damage and protection studies. We have a dedicated bio-lab equipped with basic DNA/RNA and cell handing capabilities, including a CAT II biosafety cabinet, a CO2 incubator, and an ultra-low temperature freezer. On-site analytical and characterisation capabilities include HPLC, ion and gas chromatography; fluorescence, UV-Vis, IR and Raman spectrometers; a Raman microscope, XRD, EPR and SEM.

We have a highly qualified and responsive technical support team and are adaptable to specific user-requirements. This poster will review the facilities available and act as a gateway for those interested in using the facility.

P15: GSK-3 inhibitor elraglusib enhances apoptotic cancer cell death in glioblastoma through centrosome disruption

Dr Teklu Egnuni1, Ms Jessica Murby1, Dr Alison Taylor, Professor Susan Short1
1University of Leeds

Background: Glycogen synthase kinase-3 (GSK-3) plays vital roles in metabolism, cell proliferation, invasion, immune modulation and therapeutic responses [1, 2]. GSK-3B is highly expressed in glioblastoma (GBM) and is a marker of poor prognosis [3]. In our previous study, using the GSK-3 inhibitor AZD2858 we demonstrated cytotoxicity through centrosome disruption in glioma models [4]. An in vivo study using B16 melanoma tumour model showed increased immune modulatory effect of elraglusib, which led to reduced expression of immune checkpoint molecules [5]. Here, using glioblastoma cell lines, we investigate the effects of the small molecule GSK-3B selective inhibitor elraglusib, an orphan drug which has recently received FDA approval for clinical use in pancreatic cancer patients.

Materials and Methods: The cytotoxic effect of elraglusib, with or without radiation, was determined using 3-(4,5-dimethylthazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and clonogenic assays. Four glioblastoma cancer cell lines; patient derived cell lines (GBM1 & GBM4), established human glioblastoma cell line (U87) and murine glioblastoma cell line (CT2A) were used. Two different combination schedules were tested, either drug first followed by radiation or vice-versa. Western blot and immunofluorescence staining were used to investigate the effect of elraglusib on GSK-3 downstream targets, pro-apoptotic signalling and DNA repair protein expression following treatment.

Results: MTT assay analysis showed dose dependent cytotoxicity of elraglusib in all glioma cell lines. The patient derived cell lines (GBM1 and GBM4) were more sensitive [IC50 = 0.19 and 0.34 uM] compared to U87 [IC50 = 1.08 uM]. Clonogenic assay confirmed these results, showing increased sensitivity of patient derived cell lines to elraglusib monotherapy. Overall, combination of elraglusib and radiotherapy significantly decreased surviving fraction in all four glioblastoma cancer cell lines. Synergy test using Chou Talalay equation of Loewe Additivity model [6-8], demonstrated a synergistic effect of this combination therapy. This synergistic effect was particularly marked when such cells were treated using elraglusib followed by irradiation 3 hrs later. Mechanistically, immunofluorescence staining for pericentrin following elraglusib exposure clearly demonstrated centrosome disruption and consequent mitotic failure leading to cell death. Western blot analysis of elraglusib treated CT2A cancer cell lysates showed dose dependent reduction in total GSK-3B and phosphorylated GSK-3B & Akt expression level. Furthermore, expression of DNA damage associated proteins including Rad51, Cyclin D1, PARP and phospho-P53, which were induced by radiation, were reduced with combination therapy. Cell death by apoptosis was confirmed by increased expression of caspase 3 in a time and dose dependent manner following combination treatment.

Conclusion: This study demonstrated that GSK-3B inhibitor drug elraglusib caused cytotoxicity in glioma cell lines through centrosome disruption, confirming our previous data using AZD2858. Drug-radiotherapy combination demonstrated a synergistic effect in vitro. These investigations demonstrate the potential use of a clinically available GSK-3 inhibitor in combination with radiotherapy in the treatment of glioma patients. In vivo experiments are ongoing to confirm these findings and assess whether this agent also has immune stimulatory effects in relevant glioma models.

P16: Enhancing the radiosensitivty of uveal melanoma to photon and proton radiation through targeting DNA repair

Miss Laura Hawkins1, Professor Jason Parsons1
1University of Birmingham

Uveal Melanoma (UM) is the most common intraocular tumour in adults which not only threatens loss of vision but has a poor median survival of 4-6 months following metastasis. Radiotherapy is the mainstay of treatment for this cancer, with majority of patients receiving proton beam therapy (PBT) over invasive enucleation surgery. Despite either treatment option, approximately 50% of patients will progress to metastasis. There is a pressing need to further understand and optimise the radiation response of this cancer, to improve this to conserve eyesight and prevent progression of the disease.

Spheroids are a 3D model which naturally form oxygen and nutrient gradients to better mimic the tumour microenvironment, as compared to monolayers. Through the use of this model, we aim to evaluate radiation response to both photon and proton radiations in cell lines derived from primary tumour and metastatic tumours, through growth and viability assays and the comet assay, to measure DNA damage. We are currently investigating whether key DNA repair proteins essential for homologous recombination (HR) and non-homologous end joining (NHEJ) are required for the repair of DNA damage induced by either form of ionising radiation; including ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), and DNA dependent protein kinase catalytic subunit (DNA-PKcs).

We have shown that inhibitors for all three kinases sensitised UM spheroids to photon and proton radiation, with the ATM inhibitor in particular creating persistent DNA damage which correlated to the reduced growth phenotype. Overall, we have shown that key DNA repair proteins are required for efficient repair of IR-induced lesions and could be targetable for the enhancing the effectiveness of UM treatment.

P17: Investigating DNA damaging effects of FLASH vs CONV dose rates via proton irradiation of plasmids

Miss Abigail Hemming1, DR Nicholas Henthorn1, Dr John-William Warmenhoven1
1The University Of Manchester, 2The Christie NHS Foundation Trust

Ultra-high dose rate (UHDR) radiotherapy, typically ≥40 Gy/s, has been shown to induce a normal tissue sparing effect without impacting tumour volume control (the FLASH effect). The mechanism of this effect is unknown. In this work we use the plasmid DNA nicking assay approach as a reductionist technique to investigate the differential DNA damaging effects of proton irradiation at UHDR and conventional dose rates.
PBR322 plasmid samples, dialyzed and then diluted to a concentration of 100 ng/µl, were irradiated with doses of 0, 5, 15, 30, 45, 60, 80, 90, and 100 Gy at both conventional (0.2 Gy/s) and FLASH (122 Gy/s) dose rates using 245 MeV protons under aerobic conditions (21% oxygen). Three irradiation repeats were conducted at each dose. Irradiated and control samples were then analysed through agarose gel electrophoresis and single and double strand break yields were quantified using the fitting method described by McMahon et al. Three electrophoresis repeats were conducted per sample. In addition, samples were incubated at 37°C for 1 hour, with base excision repair enzymes Endonuclease III (Nth) and Formamidopyrimidine DNA Glycosylase (FPG) prior to electrophoresis to quantify base damage. Utilizing this method provided insights into the induction of single strand breaks (SSB), double strand breaks (DSB), isolated and clustered heat labile sites, and isolated and clustered oxidised base damages.

The results of this experiment showed that, for samples not incubated, UHDR irradiation lowered the rate of double strand break induction by 8.15% and the rate of single strand break induction by 26.41%. Samples irradiated at UHDRs also displayed a lower increase in SSB and DSB induction rate following incubation with FPG and Nth. This indicates that UHDR irradiation causes a lower amount of isolated and clustered oxidised base damage. These results show a FLASH sparing effect at the DNA level, independent of other biological processes.

To conclude, a plasmid system is a viable and valuable option to assess DNA damage, with adequate sensitivity to detect differential DNA damage. Our work probes the FLASH effect in terms of DNA damage, removing cofounding factors found within in vitro and in vivo work. Following on from this we plan to investigate the effect of including hydroxyl radical scavengers such as TRIS and DMSO within our samples as well as more biologically relevant scavengers including thiols such as glutathione. We will also explore the effect of differing oxygen concentrations on the DNA damage yields at both dose rates.

P18: c-Met Targeted Immuno-PET for the Detection of Oesophageal Carcinoma

Dr Yi-jhih Huang, Dr Kel Tan, Ms Shelly Lum, Dr Gemma Dias, Professor Katherine Vallis

Purpose/Background: Oesophageal cancer (OC) is a frequently lethal disease that accounts for approximately 0.54 million deaths worldwide annually. High c-Met expression is associated with an unfavourable prognosis in OC patients. The aim of this study was to develop a novel zirconium-89 (^{89}Zr: t1/2 = 78.4 h)-labelled, c-Met targeting monoclonal antibody (EP1454Y) for OC detection.

Methods: p-SCN-Bn-deferoxamine (DFO) was conjugated to EP1454Y, followed by radiolabelling with ^{89}Zr. The purity and radiochemical yield of the immunoconjugate was determined by thin-layer chromatography (TLC). OE33 (high c-Met, 5 x 10^6 cells) and FLO-1 (low c-Met, 2.5 x 10^6 cells) human OC cells were implanted into the right and left flanks, respectively, of 6-week-old female NOD-SCID gamma mice. The inoculations were 10-14 days apart to establish subcutaneous xenografts of approximately equal volume by the start of the experiment. [^{89}Zr]Zr-DFO-EP1454Y (5 MBq) was administered via a lateral tail vein. A control group of animals received a non-specific IgG radioimmunoconjugate, [^{89}Zr]Zr-DFO-IgG. PET images were acquired using a VECTor4CT scanner (MILabs) at 24, 48, and 72 h post-injection (p.i.). Images were analysed using MILabs and PMOD software. Mice were euthanised 72 h p.i., the tumours and organs were removed, and the radioactivity of each tissue was counted in a gamma counter.

Results: [^{89}Zr]Zr-DFO-EP1454Y was synthesised with high purity (>98%) and radiochemical yield (>75%). ImmunoPET images showed high specific uptake of [^{89}Zr]Zr-DFO-EP1454Y in OE33 tumours at 24, 48, and 72 h p.i. and significantly lower uptake in FLO-1 tumours. The per cent of injected dose per gram of tumour tissue (%ID/g) was significantly higher in OE33 than in FLO-1 (19.50±2.46 versus 10.65±1.25, $p<0.0001$). Tumour uptake values for OE33 and FLO-1 xenografts in the [^{89}Zr]Zr-DFO-IgG group were 13.69±1.54 and 8.89±1.17 %ID/g, respectively ($p=0.07$). There was a statistically significant difference in the %ID/g in OE33 following administration of [^{89}Zr]Zr-DFO-EP1454Y versus [^{89}Zr]Zr-DFO-IgG ($p<0.0001$): this was not the case for FLO-1 ($p=0.6683$). This confirmed the specificity of [^{89}Zr]Zr-DFO-EP1454Y and that the signal observed in FLO-1 tumours was non-specific and likely secondary to the enhanced permeability and retention (EPR) effect.

Conclusion: The radioimmunoconjugate, [^{89}Zr]Zr-DFO-EP1454Y, is a novel PET tracer that specifically detects c-Met expression in OC.

P19: Inhibiting the CXCR4/CXCL12 signaling axis with targeted gold nanoparticles sensitizes prostate cancer to radiotherapy.

Professor Jonathan Coulter1, **Miss Xinyi Liu**, Miss Melissa Wilson1
1Queen's University Of Belfast

Purpose: Gold nanoparticles (AuNPs) have long been recognised as effective sensitisers to radiation therapy, an effect due to their high X-ray absorption and unique physicochemical properties. However, the full potential of AuNPs as radiosensitisers is, in part, limited due to large treatment concentrations used for formulations dependent on passive accumulation. As such, modified AuNPs were developed by conjugating an antagonistic CXCR4 targeting ligand to the nanoparticle surface. Importantly, activation of the CXCR4/CXCL12 signalling axis is also associated with resistance to radiotherapy (RT), mediated by increased cell proliferation and migration (Cojoc et al, 2013). The novel nanoparticle formulation termed AuXR4 aims to exploit both molecular effects by antagonising CXCR4 signaling, while utilising G-protein coupled receptor ligand binding to facilitate targeted internalisation of physical radiosensitising gold nanoparticles.

Methods: AuXR4 was prepared using the Turkevich method, reducing gold chloride using sodium citrate. Dynamic light scattering (DLS) and UV-vis spectroscopy were used to measure physicochemical properties, with atomic absorption spectroscopy (AAS) and enhanced dark field/hyperspectral microscopy used to establish AuXR4 internalisation. Clonogenic assays and immunostaining were used to assess the radiosensitising potential and radiation induced DNA double strand breaks (DSB) in the presence of AuXR4. Additionally, molecular analysis of pAKT/AKT alterations and cell migration were established using western blot and transwell assays, to better understand the impact of molecular antagonism of the CXCR4/CXCL12 pathway.

Results: Optimised AuXR4 possessed a hydrodynamic size of 38 nm, exhibiting excellent colloidal stability under physiologically relevant ionic stress. Pre-treatment of CXCR4 expressing prostate cancer cells with AuXR4 resulted in efficient nanoparticle internalisation in several (DU145 and PC3) cell line models of prostate cancer. Importantly, AuXR4 increased radiation sensitivity on average by 20% across cell lines. This effect was underpinned by a significant increase in DNA DSB yields post-irradiation. At the molecular level, AuXR4 potently supressed CXCR4/CXCL12 activation, reducing pAKT/AKT compared to untargeted AuNPs, an effect observed at both the mRNA and protein levels. Interestingly AuXR4 also significantly inhibited tumour cell migration towards CXCL12.

Conclusion: The optimised nanoparticle AuXR4 demonstrates specificity for the CXCR4/CXCL12 pathway, with targeted tumour cell uptake resulting in increased DNA DSB damage and reduced clonogenic survival. Interestingly, our optimised formulation also significantly inhibits tumour cell migrating towards CXCL12, which could potentially reduce the metastatic potential of localised disease, a phenomenon that will be explored more in follow-up studies.

P20: Treatment Planning Software Can Mitigate Impact of Scattering Effects for Bragg Peak FLASH Proton Therapy

Mr Rasmus Nilsson2, Dr Erik Traneus2, **Ms Nathalie Lövgren**1, Dr Kristoffer Petersson1
1Oxford Institute for Radiation Oncology, University of Oxford, 2RaySearch Laboratories AB

FLASH proton therapy (FLASH-PT) aims to use ultra-high dose rates (≥ 40 Gy/s) to elicit a normal tissue sparing effect whilst maintaining the anti-tumour effectiveness of conventional proton therapy. Conventional proton therapy beam delivery, however, is not fast enough to achieve ultra-high dose rates. Bragg peak FLASH-PT beam delivery therefore involves the use of patient- and beam-specific energy degraders which include a "hedgehog", range shifter, and an aperture. The inclusion of the hedgehog and range shifter scatters the beam, increasing spot sizes and range straggling, thus negatively impacting the dose distributions. To investigate how effectively the beam delivery modifications are managed by treatment planning and optimisation software, Bragg peak FLASH-PT plans are produced using two different treatment planning systems (TPS). Additionally, fractionated beam delivery is investigated, and all plans compared to those for intensity modulated proton therapy (IMPT).

Bragg peak FLASH-PT and IMPT plans were generated for bone (n = 3), brain (n = 4), and lung (n = 3) targets. A research version of the RayStation TPS and the open-source MIROpt TPS, working in conjunction with the ConformalFLASH library (www.openFLASH.software), were used to create all treatment plans. Single-fraction (bone) and hypofractionated (brain and lung) plans were produced using one beam per fraction.

A target size constraint was imposed for the MIROpt generated treatment plans as the simulated hedgehogs for some patient cases did not allow enough space to include the range shifter in the nozzle of the beam. This led to the exclusion of all bone cases and one brain case when evaluating the MIROpt TPS. Treatment plans could be simulated for all patient cases using the RayStation TPS. Clinically acceptable Bragg peak FLASH-PT treatment plans were generated using RayStation, fulfilling both target (D95% ≥ 95% and D2% ≤ 105%) and organ at risk (OAR) dose constraints. Plans generated in MIROpt only satisfied the OAR dose constraints. For both systems, FLASH plans were associated with increased OAR doses and reduced dose conformity, compared to IMPT. Intra-fraction FLASH dose rates (defined as ≥40 Gy/s for doses ≥2 Gy) were achieved to irradiated OARs for each patient case. All results extend to both single-fraction and hypo-fractionated treatment plans.

Clinically acceptable single-fraction and hypo-fractionated treatment plans were produced for Bragg peak FLASH-PT, albeit with increased doses to OARs and reduced dose conformity as compared to IMPT. The detrimental impact of scattering effects on dose distributions, due to the Bragg peak FLASH-PT beam modifications, can in part be mitigated by the software used, as shown by RayStation outperforming the MIROpt TPS. This could potentially be mitigated further by lowering the beam starting energies, therefore removing the need for a range shifter (e.g., with the use of a synchrotron-based beam production). Further research is needed, likely involving both hardware and software optimisation, before hypofractionated Bragg peak FLASH-PT can achieve the same treatment plan quality as IMPT.

P21: A novel approach to biologically effective dose calculation for various dose rate scenarios

Mr Mark Macsuka1, Professor Katherine Vallis1, Dr Daniel McGowan1,2
1Department of Oncology, University of Oxford, 2Department of Medical Physics and Clinical Engineering, Oxford University Hospitals NHS Foundation Trust

Purpose

Organ dose limits and tumour dose-response correlations in radiopharmaceutical therapy (RPT) are often sought by considering the biologically effective dose (BED) and the equivalent dose in 2 Gy fractions (EQD2). These metrics depend on models that include not only the physical dose, but also various radiobiological parameters characterising the radiosensitivity and damage repair capacity of tissues of interest. Such mechanistic radiobiological models necessitate the BED and EQD2 to depend explicitly on the dose rate and the rate of sublethal DNA damage repair. These dependencies are usually assumed to take the form of a monoexponential, whereas two or more phases are often more appropriate to describe especially the dose rate function.

Methods

Assuming simple exponential decay for DNA damage repair and a biexponential function for dose rate decay, we rederived the solution for the BED in a closed analytical form. We also arrived at a novel solution for the case of piecewise-defined dose-rate functions, which relies on both numerical and analytical ideas. These two approaches are widely used to model [177Lu]Lu-DOTATATE clearance if comprehensive dosimetry is available. Both expressions were validated using simulated measurements by comparison with a fully numerical method. We further investigated the reliability of the fully numerical, fully analytical, and mixed methods when attempting to simplify a comprehensive dosimetry protocol. Using publicly available clinical data of two patients undergoing [177Lu]Lu-DOTATATE therapy, we defined the ground truth dose rate as the best biexponential fit to four post-injection SPECT measurements on the organ level and explored the differences in BED and EQD2 values when omitting the last measurement.

Results

It was found that our approaches are accurate and the numerical method converges to our analytical solutions with increasing extrapolation time after injection. A 0.1 Gy difference between methods may necessitate running the numerical method for up to 1400 hours, at which point it may fail due to overflow errors. On the clinical dataset we found that the numerical, mixed, and analytical approaches underestimated the ground truth to tumours by 2.9 +/- 3.9 Gy, 0.3 +/- 0.6 Gy, and 0.2 +/- 0.5 Gy, respectively, in EQD2.

Conclusion

As it is often desirable to perform comprehensive dosimetric studies in RPT using more accurate measurements, it is equally important for radiobiological models to match their accuracy, at least theoretically. Our results show that the proposed methods are accurate, scalable, and suitable for radiobiologically motivated RPT dosimetry.

P22: Early Insights into Novel AuNPs and the Radiation-Induced Bystander Effect.

Miss Lydia Mcquoid1, Dr Niall Byrne1, Dr Jie Feng1, Dr Sarah Chambers1, Dr Rayhanul Islam1, Dr Mukesh Kumawat1, Dr Chris Grigsby1, Ms Meabh Doherty1, Mrs Bayan Ahmed A Alkhaldi1, Miss Xinyi Liu1, Mr Oscar Pooley1, Mr Zelin Tan1, Professor Helen McCarthy1, Professor Jonathan Coulter1
1School of Pharmacy, Queen's University Belfast

Purpose:

Ionising Radiation (IR) is a widely used treatment modality for various forms of cancer, however off-target radiation damage is a common occurrence complicating therapeutic responses. Irradiated cells can release factors that propagate radiation damage to neighbouring unirradiated cells, an effect termed the radiation-induced bystander effect (RIBE)1,2. Understanding the impact of radiotherapy-enhancing agents on the RIBE is important to maximise their full clinical impact – whether they enhance tumour cell kill beyond the irradiated volume or stimulate proliferation in neighbouring cells. Novel chemokine-targeting gold nanoparticles (AuNPs) have been developed as potent radiosensitisers, however, they have yet to be investigated for their influence on the RIBE. Two chemokine targeted AuNP formulations have been selected to study this indirect effect. Both CXCR2 and CXCR4 lie upstream of a number of pro-survival pathways and as such we first examined for any potential influence of chemokine antagonism on RIBE prior to investigating the AuNP formulations3,4.

Methods:

DU145 (prostate) and FaDu (head and neck) cancer cell lines were used following confirmation of basal and radiation induced CXCR2 and CXCR4 expression. Clonogenic assays were used to quantify radiation sensitivity following CXCR2 or CXCR4 antagonism using both respective commercial pharmacological small molecule inhibitors (AZD5069 and AMD3100) or novel peptide base inhibitors (x1/2pal-i3 or x4pal-i1). Conditioned media was transferred at either 1 h or 24 h post-irradiation from antagonised donor cells to unirradiated bystander cells, assessed for clonogenic survival. Furthermore, DCFDA assays were used to measure ROS production in AuNP-treated irradiated cells, and again radiosensitivity and bystander effects assessed following AuNP treatment.

Results:

Chemokine receptor antagonism caused modest radiosensitisation in both cell lines, whilst AuNPs were capable of significantly ($p<0.01$) augmenting both radiation sensitivity and intracellular ROS levels. Basal bystander effects in both tumour cell models were inhibitory, dominating at 1 h post-radiation and at low radiation doses (<1 Gy). Interestingly, chemokine receptor antagonism appeared to reduce this inhibitory effect whilst AuNP treatment of donor cells enhanced the inhibitory bystander response in unirradiated cells.

Discussion:

Increased clonogenic survival post receptor antagonism in unirradiated recipient bystander cells suggest that secreted pro-inflammatory factors confer a pro-survival response that will be further investigated using cytokine arrays. The increase in ROS caused by AuX2R and AuXR4 and strong radiosensitisation of irradiated cells, and the enhancement of bystander effects suggests that the physical gold component of these nanoparticles has a greater impact on direct and indirect radiation effects than chemokine receptor antagonism alone.

P23: A systematic review of neurovascular unit damage after brain irradiation

Ms Annet Nakkazi1, Dr. Duncan Forster2, Dr. Gillian Whitfield4, Dr. Douglas Dyer3, Dr Ben Dickie1

1a. Division of Informatics, Imaging and Data Sciences, School of Health Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK. b. Geoffrey Jefferson Brain Research Centre, Manchester Academic Health Science Centre, Northern Care Alliance NHS Group, University of Manchester, Manchester, UK, 2a. Division of Informatics, Imaging and Data Sciences, School of Health Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK., 3b. Geoffrey Jefferson Brain Research Centre, Manchester Academic Health Science Centre, Northern Care Alliance NHS Group, University of Manchester, Manchester, UK c. Wellcome Centre for Cell-Matrix Research, Lydia Becker Institute of Immunology and Inflammation, Faculty of Biology, Medicine and Health, Manchester Academic Health Science, 4d. The Christie NHS Foundation Trust, Wilmslow Road, Manchester, M20 4BX, UK e. Division of Cancer Sciences, University of Manchester, Manchester Cancer Research Centre, Manchester Academic Health Science Centre, M20 4BX, UK

Background: Radiotherapy is key in the treatment of primary and secondary brain tumours. Some normal tissue is inevitably irradiated, which can lead to cognitive decline. This is a particular issue in children, as well as in adults with low-grade gliomas or curable tumours, who could live with the effects for many years. Radiation-induced damage to the neurovascular unit (NVU) could drive neurocognitive decline by inducing chronic neuroinflammation and neurovascular dysfunction. Here, we review studies investigating radiation-induced damage to the NVU and discuss relevance of these findings in the context of cognitive decline.

Method: Using PubMed and Web of Science, we summarised preclinical and clinical data published between 1st January 1970 and 1st December 2022 on radiation-induced NVU alterations, including timing and severity effects.

Results: Seventy-four rodent, four canine, one rabbit, one macaque, one fish, and five human studies met inclusion criteria. Results were heterogeneous, but most studies found radiation increased blood-brain-barrier (BBB) permeability, led to loss of endothelial cells and extracellular matrix proteoglycans, reduced tight junction proteins, upregulated cellular adhesion molecules expression, induced oedema, and reduced activity of glucose and BBB efflux transporters. In the brain parenchyma, studies observed significant increases in activated glial cells, upregulated metalloproteinases 2 and 9 levels, demyelination and myelin synthesis inhibition, cell death, reduced neural proliferation, inhibited differentiation, disrupted volume (extrasynaptic) transmission, and necrosis. Damage extent varied by radiation type and form, dose, delivery technique, fractionation scheme, animal strain, and presence or absence of a tumour in the irradiated region. Among the 81 preclinical studies, 70 were in vivo, and acute changes were examined in 65, delayed effects in 38 and late effects in 31 studies. Of the five clinical studies, only one was in vivo, and acute changes were examined in four studies while late effects assessed in one study. Only fourteen studies examined acute, delayed and late effects in a longitudinal setting, and all were preclinical. Additionally, out of the 71 in vivo studies, only six performed cognitive testing, and they found NVU changes associated with cognitive impairments, including spatial learning and memory loss and reduced motor coordination reduction. However, the occurrence of these cognitive deficits was partly influenced by the radiation dose and the testing technique employed.

Conclusion: Irradiation of normal brain tissue leads to widespread and varied impacts on the NVU. More studies with comparable end points, subject traits, and radiation characteristics are needed to track longer term changes in the NVU, alongside neurocognitive changes, which could help identify mechanisms of radiation-induced cognitive decline.

P24: MangaCisTEX: A novel texaphyrin-pt(IV) conjugate as potential antitumour agent

Gonzalo Rodriguez Berriguete, Purusotha Thambiayah, **Mr David Nderitu**1, Dr Adam Sedgewick, Geoff Higgins
1University Of Oxford

Cisplatin (II) is the gold standard for chemotherapeutic treatment for various solid tumours and haematological malignancies, especially lung cancer1. However, cisplatin treatment often results in adverse effects, such as renal toxicity and peripheral neuropathy2,3. MMn(NO3) (herein referred to as MMn) is a novel texaphyrin compound that exerts its antitumour effects in a three-step mechanism. Firstly, the molecular weight of the compound allows it to accumulate in regions of increased vascular permeability such as tumour vasculature. Secondly, MMn facilitates the transfer of an electron from ascorbate to oxygen, forming superoxide ions inducing oxidative stress in the cell4. Finally, MMn's ability to "redox-cycle" facilitates the reduction of the platinum prodrug cisplatin (IV) to its active form cisplatin (II). MangaCisTEX is the chemical conjugate of MMn and Cisplatin (IV). This study aims to evaluate whether MangaCisTEX radiosensitises cancerous cells better than the already established cisplatin treatment.

MangaCisTEX was tested in vitro by Resazurin-based cell viability assays in A549 and H1299 cell lines (n=3). Cells were seeded in 96-well plates, with 175 cells/well in the unirradiated plates and 400 cells/well for the single radiation dose (4Gy). Seven drugs were tested: MangaCisTEX, MMn, Motexafin Gadolinium (MGd), Cisplatin (IV), Cisplatin (II), MMn+Cisplatin (IV) and MGd+Cisplatin(IV). Drugs were prepared at concentrations ranging from 0.66µM-20µM with a DMSO control. The primary endpoint was when the DMSO control well reached 70-80% confluency, falling on day 7. The plates were stained and analysed using GraphPad Prism.

The fluorescence readout comes from the reduction of resazurin to resorufin which is directly proportional to the number of viable cells in the well. MMn was compared to an established texaphyrin MGd. A small amount of cytotoxicity was expected as the "redox-cycling" process generates reactive oxygen species that can place the cell under stress. Surprisingly, MMn seemed to confer radioprotection but this was not significant ($p>0.05$). In the A549 cell line, the IC50 for MMn was 5.93e+05 and 2.71e+07 in the 0Gy and 4Gy arms respectively. The change in IC50 in H1299 was less marked at 2.45 and 7.74 for 0Gy and 4Gy respectively. Cisplatin (IV) as a prodrug should have remained non-toxic unless conjugated to the reducing agent, MMn. However, there was a slight reduction in cell viability at increasing drug concentrations for both cell lines. For MangaCisTEX, a clear reduction in cell viability was observed with the drug becoming strongly cytotoxic at concentrations above 2.5µM for both irradiated and unirradiated arms of the H1299 cell line. The conjugate became strongly cytotoxic at concentrations above 10µM for both arms of the A549 cell line. In both cell lines, MangaCisTEX was unable to radiosensitise tumour cells often exerting cytotoxic effects regardless of the presence of radiation.

This work aimed to evaluate whether MangaCisTEX could radiosensitise cancerous cells better than the current cisplatin treatment used in the clinic. MangaCisTEX failed to radiosensitise both cancerous cell lines. The cytotoxicity of the cisplatin prodrug was possibly due to photo-activation, highlighting the importance of having MMn conjugated to act as a photocage5. To further this work, studies using fluorescently-tagged cisplatin prodrug are required to confirm the effects of MangaCisTEX are due to prodrug reduction.

P25: Boosting Oxygen Diffusion in the Radioresistant Oesophageal Tumour Microenvironment to Improve Radiotherapy.

Dr Melissa Anne-Tutty3, Ms Christina Cahill1, Prof Lasse Jensen5,6, Prof Helena Kelly2, Ms Xuehua Lin1,2, Dr Stephen Maher1, Dr Ian Miller4, **Mr Maitiú Ó Murchú**1,2, Prof Jacintha O'Sullivan1, Dr Adriele Prina-Mello3
1Trinity St James Cancer Institute, Department of Surgery, Trinity Translational Medicine Institute, Trinity College Dublin., 2School of Pharmacy and Biomolecular Sciences, Royal College of Surgeons Ireland, 3Nanomedicine Group, Trinity Translational Medicine Institute, Trinity College Dublin, 4National Preclinical Imaging Centre, School of Pharmacy and Biomolecular Sciences, Royal College of Surgeons Ireland, 5Linkoping University, Linkoping, Sweden., 6Bioreperia AB, Linkoping, Sweden.

Purpose/ Objective

Oesophageal adenocarcinoma (OAC) is a poor prognosis cancer with a 20% survival rate [1]. OAC patients receive neo-chemoradiotherapy prior to surgery [2]. Only 30% of patients achieve a pathological complete response to this treatment approach [3]. Hypoxia is present in most solid tumours and significantly contributes to treatment failure. Hypoxia facilitates the selection of cells with low apoptotic potential in the tumour microenvironment [4, 5]. In addition, hypoxia specifically limits radiotherapy efficacy as oxygen is a potent radiosensitiser [6]. Increased oxidative phosphorylation in the OAC tumour microenvironment facilitates hypoxia and contributes to radiation resistance in tandem with increased DNA repair protein expression [7, 8]. Increasing tumour oxygen level is a potential approach to improve radiosensitivity in OAC. Perfluorocarbons are chemically inert compounds which can dissolve large amounts of oxygen due to their chemical structure. However, due to their hydrophobicity they must be formulated into stable nanoemulsions for effective delivery. This project aims to develop perfluorocarbon nanoemulsions (PFC-NEs) for intratumoural delivery of oxygen to reduce hypoxia and increase radiosensitivity in OAC.

Methodology

PFC-NEs were produced using lipoid, phosphate-buffered saline, and perfluorocarbons. PFC-NE stability was measured using Malvern's Zetasizer. Nanoemulsion imaging capacity was measured using ex vivo mice livers via microCT. Oxygen-carrying capacity and oxygen release profiles were measured using a cell-based in vitro Seahorse assay. The toxicity of lead PFC-NEs were assessed using different in vitro and in vivo model systems (isogenic model of OAC radioresistance, 2D and 3D HepG2 models, and zebrafish). Following treatment of an isogenic model of OAC with PFC-NEs, clonogenicity, DNA repair, metabolism, cytokines, and cysteinyl leukotriene receptor expression were assessed.

Results

PFC-NEs are stable, can be visualised ex vivo using microCT, and can load a significant amount of oxygen. Lead PFC-NEs do not induce toxicity in vitro using an isogenic model of OAC radioresistance and in 2D and 3D HepG2 models. Formulation 001UG reduced zebrafish viability at higher concentrations. Formulation 001UG significantly increases supernatant oxygen concentration and assimilates oxygen in vitro under normoxic conditions. Formulation 001UG also reduces the expression of HIF-1α under hypoxic conditions (0.5% O2). In normoxic conditions, formulation 001UG significantly reduces clonogenicity basally, irrespective of oxygen-loading status. Formulation 001UG significantly reduces surviving fraction in combination with radiation (compared to radiation control). Under normoxia, formulation 001UG significantly increased SMUG1 expression basally, however this effect was not seen in the context of radiation (2 Gy). Formulation 001UG significantly reduces oxygen consumption rate and alters cytokine secretion in radioresistant OAC cells. Formulation 001UG may reduce Cysteinyl Leukotriene Receptor 1 expression, a predictor of poor patient response [9], basally and in response to radiation in radioresistant OAC cells.

Conclusion
Hypoxia is a significant tumour microenvironmental factor which facilitates the selection of radiation resistant cancer cells. In addition, the absence of oxygen significantly limits radiotherapy as oxygen is a potent radiosensitiser. These data suggest that our novel PFC-NE functions as an oxygen delivery system with the potential to improve radiosensitivity in oesophageal adenocarcinoma.

P26: Realizing the radiobiological impact of protons and high-LET particles in glioblastoma models
Dr Alice Ormrod1, Dr Chun Li1, Dr Radhika Aiyappa-Maudsley2, Dr Jonathan R Hughes1, Prof Anthony Chalmers3, Dr Ben Phoenix1, Professor Jason Parsons1
1University of Birmingham, 2University of Liverpool, 3University of Glasgow

Glioblastoma multiforme (GBM) is the most common primary brain tumour in adults and has dismal survival prospects (median of ~14.6 months). Conventional radiotherapy consisting of X-ray (photon) treatment has limited efficacy and therefore there is an urgent need for more effective therapeutics. Proton beam radiotherapy has the significant advantage over photons of delivering the maximum energy targeted to the solid tumour with a low entrance dose sparing the healthy tissues and organs. Additionally, by taking advantage of the Bragg-peak to deliver a higher LET (linear energy transfer) will also generate a higher biological effectiveness due to increased ionisation density. However, the optimal use of protons in GBM treatment has yet to be realised.

GBM is thought to be driven by a small niche of cancer stem cells which have unlimited proliferative capability. GBM stem-like cells (GSCs) which express stem cell markers CD133, SOX2 and nestin have been demonstrated to be more radioresistant than their paired bulk non-stem cells. Given this, it is hypothesised that radiosensitising strategies are required to effectively target this population and increase survival for GBM patients. Another important factor in solid tumours such as GBM is hypoxia, which generates a more radioresistant phenotype in response to conventional radiotherapy treatment.

Our data shows that inhibition of key DNA damage response (DDR) pathway proteins combined with low LET photons and protons can significantly reduce GBM cell survival compared to radiation alone. We also demonstrate in hypoxic conditions that some GBM cell lines gain further radioresistance to radiation treatment, but which can be partially overcome with DDR inhibition. Further work is underway to explore the molecular mechanisms underpinning this altered GBM cellular hypoxic response.
Lastly, we have begun to explore the impact of high LET protons achieved at the distal end of the Bragg peak. Here, we have shown that these have a significantly greater biological effect on reducing GBM colony survival, caused by the production of more complex DNA damage (two or more lesions within one DNA helical turn) which has slower DNA repair kinetics. We are now investigating the potential for DDR pathway inhibition to exacerbate the effects of high-LET protons in GBM cell killing.

Our work has indicated the exciting prospect of improving the GBM cellular response to the current standard of care radiotherapy. In particular, this can be achieved through the combination of protons and DDR inhibition, taking into account important factors such as the GBM cancer stem cell population and inherent radioresistance caused by hypoxic conditions.

P27: Understanding the biological response to boron neutron capture therapy (BNCT) in head and neck cancer

Miss Leah Punshon[1], Professor Jason Parsons, Dr Maria Fabbrizi, Dr Ben Phoenix
[1]University Of Birmingham

Boron neutron capture therapy (BNCT) is an exciting alternative radiation modality to conventional X-ray radiotherapy that selectively targets tumour cells leading to a reduction in radiotherapy-induced side effects whilst also delivering a more biologically effective dose. Using tumour-targeted boron-containing drugs followed by irradiation of the tumour area with thermal energy neutrons, boron neutron capture fission reactions within cells lead to the production of high linear energy transfer (LET) helium and lithium particles which inflict high levels of damage on cellular DNA. High LET radiation is known to induce substantial complex DNA damage, but the exact profile of damage produced following BNCT is not understood. Access to the UK's sole high flux accelerator-driven neutron facility has enabled us to begin assessing survival of several head and neck cancer cell lines following BNCT using boric acid, comparing this to treatment with conventional X-ray irradiation. Clonogenic survival assays were conducted with increasing concentrations of boric acid (0-500ppm) assessed with increasing neutron fluences (1.9625x1010- 3.925x1011 2200ms-1 /cm-2). Our data shows that, as expected, boric acid has no effect on the sensitivity of cells to X-ray radiation, however increasing boric acid concentrations caused decreased cell survival in a dose-dependent manner with neutron radiation ($p<0.001$). Our accumulating work demonstrates the efficacy of BNCT for decreasing survival of head and neck cancer cells in vitro. This lays the foundations for future research aimed at elucidating the molecular effects post-BNCT treatment, including impact on DNA damage repair, cell cycle progression and cell death analysis.

P28: Use of CDK12/13 inhibitors as radiosensitizing agents.

Miss Morgan Rycroft, Professor Helen Bryant
1University of Sheffield

Most cancer patients benefit initially from radiation treatment; however, all too often intrinsic and acquired radioresistance occurs. Thus strategies to improve radiotherapy response are urgently required. A promising approach is to combine molecularly targeted drugs with radiotherapy.

CDK12 and CDK13 are Ser/Thr protein kinases that regulate cell cycle and transcription. Consequently, selective CDK12/13 inhibitors constitute powerful research tools as well as promising anti-cancer therapeutics, either alone or in combination therapy.

Clonogenic assays demonstrate that CDK12/13 inhibitors can radiosensitize several different cancer cell lines. We also show that CDK12/13 inhibition causes a BRCAness phenotype as demonstrated by sensitivity to PARP inhibitors. DNA damage and repair is investigated using gH2AX foci formation and expression of key repair genes examined by RT-PCR and western blotting.

P29: Characterising VHEE Facilities in Europe with Potential for FLASH Dose Delivery

Dr Kristina Small1,2, Professor Roger Jones1,2
1University Of Manchester, 2Cockcroft Institute

Very High Energy Electron (VHEE) radiotherapy is gaining interest as a novel cancer treatment, with potential benefits including increased penetration into tissue, reduced lateral scattering, rapid delivery, relative insensitivity to inhomogeneities and similar RBE to established radiotherapy modalities[1-4].

Rapid delivery and high production efficiency could make VHEE compatible for FLASH radiotherapy, a technique involving delivery of treatment fractions at ultra-high dose rates – specified as >40 Gy/s in a number of reviews[5-8]. This has consistently shown to significantly reduce radiation-induced toxicity compared with conventional radiotherapy while maintaining similar rates of disease control. Combining VHEE and FLASH offers the potential for a highly effective, patient-friendly treatment.

This study reviews VHEE-FLASH capable facilities in Europe in the context of radiobiological research. This will focus on four facilities: CERN Linear Electron Accelerator for Research (CLEAR) [9], Accelerator Research Experiment at SINBAD (ARES) at DESY [10], SApienza FLASH Electron Source for radioTherapy (SAFEST) at INFN[11] and the Compact Linear Accelerator for Research Applications (CLARA) at Daresbury Laboratory[12,13]. The study objectives are two-fold:

1. Assess the characteristics of VHEE facilities in Europe and determine whether they are FLASH-capable.
2. Predict the dose rate impinging on potential radiobiological samples from fits to various analytical functions.

Each facility electron beam was modelled from the beam exit window using TOPAS, a wrapper for the Monte Carlo particle tracking code Geant4[14,15]. A parameter variation study was performed to determine dependency on transverse beam dimension and source-to-surface distance (SSD). The resulting dose rate to a water volume was calculated from surface dose according to the bunch charge and RF repetition rate.

Dose rate data was used to develop a set of empirical functions in terms of beam sigma (for circular Gaussian beams) and SSD at specified energies for each beamline. Analysis in Mathematica determined an inverse square relationship between dose rate and both beam sigma and SSD. An average discrepancy of <3% between simulation data and fit indicated good agreement, resulting in a set of robust functions to predict VHEE beam dose rate based on experimental setup.

Across the study, electron beam dose rates were compared to the FLASH threshold of 40 Gy/s. All four facilities are capable of producing beams exceeding this FLASH threshold, providing the sigma associated with a Gaussian beam is less than 3mm for CLEAR and CLARA, and less than 2mm for ARES and SAFEST. Gaussian beams with a sigma of 1mm or less allows a dose rate of several hundreds of Gy/s (the details depending on energy and other parameters of the beam facility).

In conclusion, we have validated from initial simulation studies that there are at least four FLASH-capable facilities in Europe. In performing this suite of simulations, we developed a series of analytical functions which may prove useful for users to access on-axis dose rate for various experimental scenarios. In the near future, there is the potential to experimentally validate the dose rate functional behaviour at the CLARA facility in 2025, in preparation for the anticipated first VHEE-FLASH radiobiology studies in the UK.

P30: Development of an explainable AI prediction model for arm lymphoedema following breast cancer radiotherapy

Prof Chris Talbot1, Professor Guido Bologna2, Dr Alexis Bombezin-Domino3, Dr Gabriella Cortellessa4, Prof Andre Dekker5, Dr Francesca Fracasso4, Prof Manuela Joore6, Dr Andre Panisson7, Dr Alan Perotti7, Dr Bram Ramaekers6, Dr Tim Rattay1, Prof Sofia Rivera8, Dr Alessio Romita9, Dr Cheryl Roumen10, Dr Johann van Soest9, Dr Fariba Tohidinezhad5, Dr Karolien Verhoeven5, Dr Adam Webb1, Prof Iordanis Koutsopoulos11
1University of Leicester, 2University of Applied Sciences and Arts of Western Switzerland, 3Therapanacea, 4Consiglio Nazionale delle Ricerche (CNR), 5Department of Radiation Oncology, MAASTRO, GROW School for Oncology and Reproduction, Maastricht University Medical Centre, 6Department of Health Services Research, Care and Public Health Research Institute (CAPHRI), Faculty of Health Medicine and Life Sciences, Maastricht University, 7CENTAI Institute, 8UNICANCER, 9Medical Data Works B.V., 10Patient Advocate, 11Athens University of Economics and Business (AUEB), Department of Informatics

Background

PRE-ACT (Prediction of Radiotherapy side Effects using explainable AI for patient Communication and Treatment modification) is an ongoing multi-disciplinary European collaborative study with the goal of using Artificial Intelligence (AI) to predict long-term side effects (toxicity) from radiotherapy in breast cancer patients. Some of the factors that increase the risk for clinically significant toxicity are already known, but current approaches to risk prediction mainly use low-dimensional statistical approaches and do not use complex genomics and imaging data. AI and machine learning are already used in some aspects of radiotherapy delivery. The aim of PRE-ACT is to leverage its huge potential towards accurate toxicity prediction, and at the same time provide an easily understood explanation to support shared treatment decision-making between patient and physician.

Methods

We developed discretized interpretable multi-layer perceptron (DIMLP) neural network, random forest and gradient boosted tree models for arm lymphoedema following surgery and radiotherapy +/- chemotherapy in three breast cancer patient datasets from European and French multi-centre breast cancer cohorts (REQUITE, Hypo-G, CANTO, total n=6,361). Using patient demographic and treatment-related features (m=32), we trained the models with 10-fold cross-validation in a 90:10 random-split data set to predict arm lymphoedema (defined as ≥10% increase in arm circumference 15 cm proximal and/or 10 cm distal to the ipsilateral olecranon relative to baseline) up to three years from the start of radiotherapy.

Results

The incidence of arm lymphoedema was 6% across the three datasets. Our best-performing model was based on gradient-boosted decision trees retaining all 32 patient- and treatment-related features with an average AUC of 0.84 (±0.003). Average sensitivity and specificity were 81.6% and 72.9%, respectively. We extracted propositional rules from gradient-boosted decision trees to explain their output, for example:

- If cN STAGE is 0, HER2 STATUS is Unknown, and SENTINEL NODE BIOPSY is True, then NO arm lymphoedema.
- If no INTENSITY-MODULATED RADIOTHERAPY and no ADJUVANT_CHEMOTHERAPY and type of surgery is LUMPECTOMY and PR_STATUS is POSITIVE and no DIABETIES, then NO arm lymphoedema

Conclusion

We generated explainable predictions for arm lymphoedema by applying DIMLP algorithms to patient demographic and treatment features. Further inclusion of genomic and radiomic markers are likely to improve accuracy. These AI models will be incorporated into a web interface for providing the explanations to physicians and patients, to identify patients at increased risk of toxicity who may benefit from supportive intervention or even a change in treatment plan. As part of PRE-ACT, the interface which will be tested in a clinical trial and evaluated in a health economic analysis.

P31: The mitotic DNA damage response to IR induced DNA breaks

Alexander Breitweiser, Rachel Gatenby, Priya Lata, Nan Li, **Dr Ruth Thompson**, Afzaal Tufail, Thomas Walne

In the event of DNA damage induced by factors such as irradiation, the cell cycle is slowed or halted to allow for DNA repair. However, the mechanisms underpinning mitotic delay in response to damage are unclear. We have identified Superoxide Dismutase 1 (SOD1) as an essential factor mediating delayed mitotic progression in response to DNA damage and replication stress. Cells depleted of SOD1 display increased levels of damaged centromeres and mitotic defects but no longer exhibit DNA damage dependent mitotic delay. In addition, SOD1-depleted cells show reduced levels of mitotic EdU incorporation in response to either replication stress or DNA breaks, seemingly in tandem with RAD51, and SOD1-depletion induced mitotic progression in the presence of DNA breaks is RAD52-dependent. We suggest that there are two responses to DNA breaks in mitosis; either arrest and mitotic DNA repair or DNA break tethering, progression and post-mitotic repair; and these two pathways exist in a fine balance, controlled by a signaling cascade involving SOD1.

P32:Optimising DNA damage and repair models to predict radiation response

Dr Ian Overton1, **Miss Shannon Thompson**1, Prof Kevin Prise1, Dr Stephen McMahon1
1Queen's University Belfast

Purpose: Ion therapies such as proton have an increased relative biological effectiveness (RBE) compared to conventional X-ray therapy, however the exact magnitude of this increase remains unknown. Mechanistic models that simulate DNA damage and cellular response to radiation exposures could be used to better quantify RBE. However, across models there is large variation in design which may influence the predicted damage yield, and the model detail required to reproduce experimental data remains understudied. This work investigated damage models with varying levels of simulation detail to determine how different model assumptions impact the predicted damage, and moreover the model detail required to reproduce experimental results.

Methods: Five damage models of decreasing detail were set up using TOPAS-nBio and Medras to investigate the inclusion of chemistry, nuclear structure, single-strand breaks (SSBs) and track structure. Each nuclear model was irradiated with 1 Gy of protons across a range of linear energy transfers (LETs). The simplified model parameters were optimised by fitting to the proton DSB yield of the most detailed model. Electron, alpha and carbon ion exposures were then simulated to determine if the damage parameters optimised for proton exposures maintained agreement in DSB predictions for different radiation qualities. The damage predicted from each optimised model was then imported into the Medras biological response model to simulate DSB repair and the formation of lethal chromosome aberrations. Model predictions were then compared against each another and experimental results.

Results and Conclusion: Regardless of model detail, after appropriate damage parameter optimisation, all damage models predicted similar aberration yields, with average model differences remaining within 0.7 aberrations per cell per Gy over the 0.2 to 79 keV/µm LET range. Additionally, by setting appropriate repair parameter values within the Medras biological response model, aberration yields could be predicted in line with the experimental data. The mean absolute error between simulated and experimental data was found to be 0.27 and 0.41 aberrations per cell per Gy for the most detailed and most simplified model respectively.
This work has shown that several simplifications can be made to damage models without compromising their predictive ability at key damage endpoints, suggesting the investigated model assumptions do not fundamentally impact the predicted damage yields. Furthermore, it was found that through appropriate parameter optimisation, simpler, more efficient damage models can predict DNA damage in line with the experimental data.

It can therefore be suggested that further model developments in the simulation of DNA damage would have limited impact on the predictive ability of the models within current experimental limitations. For this reason, efforts may be better focused on improving the experimental data available for model validation. Additionally, model developments could be better directed on the inclusion of mutation specific information which can affect biological responses after irradiation. Through these improvements, a more translatable model could be built to predict patient specific RBE.

P33: Docetaxel reduces CAV-1 expression and sensitizes castration-resistant prostate cancer to radiation

Kevin Tu1, Dr. Sanjit Roy2, Zachery Keepers2, Dr. Manas Gartia3, Dr. Hem Shukla2, Dr. Nrusingh Biswal2
1Cancer Research UK Cambridge Institute, University Of Cambridge, 2Department of Radiation Oncology, University of Maryland School of Medicine, 3Department of Mechanical & Industrial Engineering, Louisiana State University

Purpose/Objective: Docetaxel (DXL), a known radiosensitizer, is used as a first-line treatment for castration-resistant prostate cancer (CRPC). Yet only a fraction of patients responds to DXL, and available therapies for CRPC confer minimal survival advantage. Mounting evidence suggests radiation, commonly used for palliative care in metastatic CRPC, may offer treatment benefits on top of systemic treatments (1). The effectiveness and molecular characteristics of DXL and radiation combination therapy has not been thoroughly investigated. The objective of this study was to test if DXL and radiation combination therapy could offer synergistic tumour killing effects and, if so, to identify biomarkers that could potentially select patients who may benefit from additional radiotherapy.

Material/Methods: We determined the IC50 of DXL for PC3, DU-145, and TRAMP-C1 cells using live/dead assays. Clonogenic assays were used to determine the response to radiotherapy (4, 8, and 12 Gy) in vitro with/without the IC50 dose of DXL. Flow cytometry was used to quantify the proportion of cells arrested within stages of the cell cycle following treatment with the IC50 dose of docetaxel. Protein expression was assessed using western blot. Computationally, we performed gene expression analysis on expression data in public repositories collected from C4-2, PC3, DU-145, and LNCaP cell lines that were treated with DXL for 8, 16, and 72 hours (n=4 technical replicates). Genomic features were associated with clinical data from the TCGA prostate cancer cohort.

Results: Combination therapy with DXL significantly increased CRPC death in PC3 (1.48-fold, $p < .0001$), DU-145 (1.64-fold, $p < .05$), and TRAMP-C1 (1.13-fold, $p < .05$) at 4 Gy of radiation. Gene expression of CRPC treated with DXL revealed downregulated genes related to cell cycle regulation and upregulated genes related to immune activation and oxidative stress. Highly interconnected genes central to gene expression changes included STAT1, IL6, and CCNB1. G2/M cell cycle arrest was significantly increased after treatment with DXL and radiation. DXL decreased CAV-1 protein expression in a dose-dependent manner; furthermore, CAV-1 copy number was significantly associated with poor disease-free survival, Gleason score, and poor response to therapy in CRPC patients. High CAV-1 copy number was observed in the SPOP subtype.

Conclusion: DXL sensitizes CRPC to radiation in vitro and downregulates CAV-1 expression while high CAV-1 copy number seems to be prognostically unfavourable for CRPC treatment and survival outcomes. Thus, combining DXL and radiotherapy may be effective at treating CRPC, and CAV-1 may be a biomarker for patients who could benefit from additional radiotherapy. We speculate that combining DXL and radiotherapy may be especially useful in subtypes associated with high CAV-1 copy number and should be studied further. DXL also alters CRPC gene expression to induce G2/M arrest, immune activation, and oxidative stress, which may offer opportunities for other combinatory treatments.

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