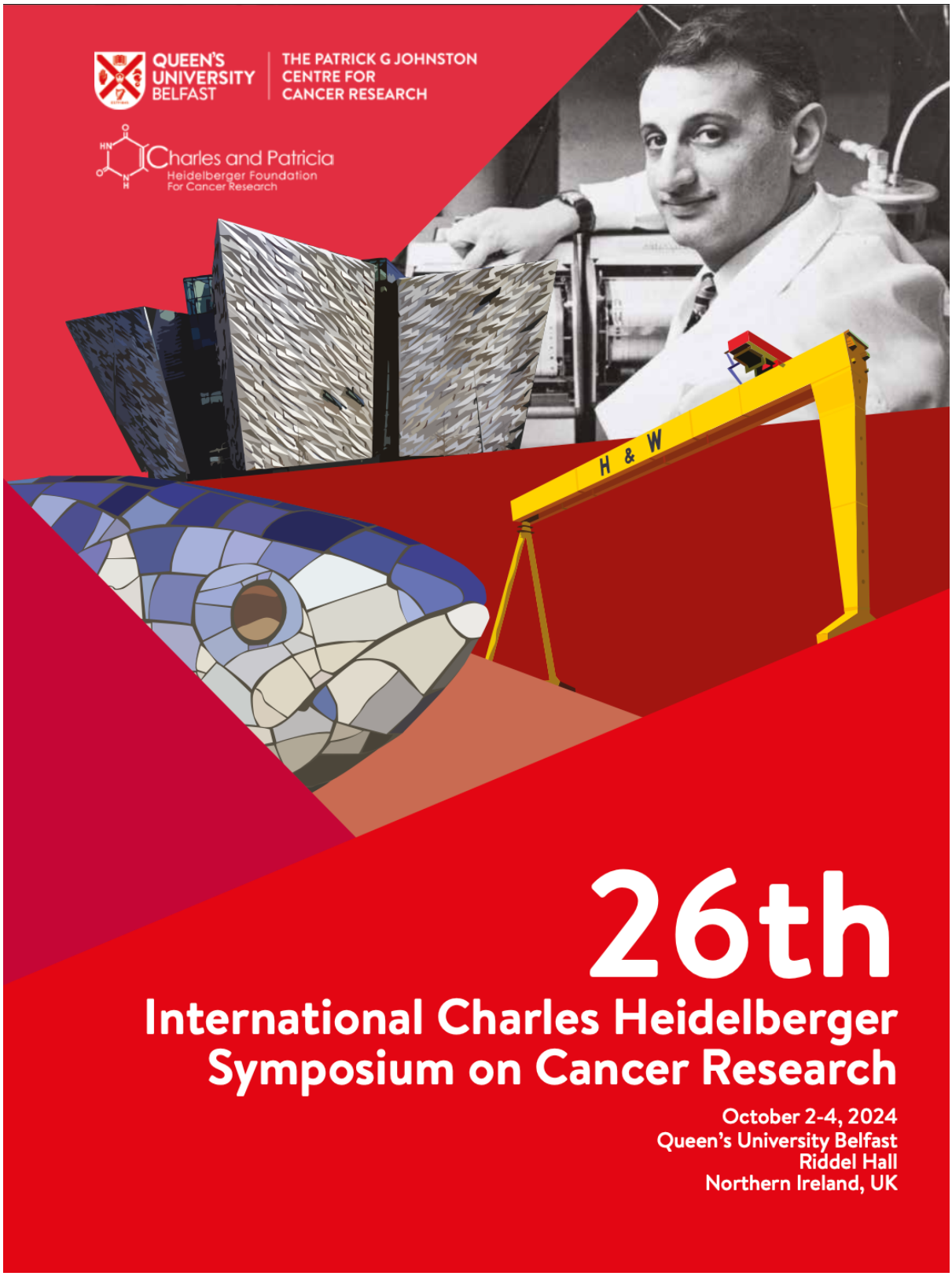

Poster Abstracts

Session 1: Wednesday, October 2, 2024

11:30 - 12:00	POSTER SESSION I

Leanne Bradley
'Identification of novel immune activating agents for rationalised combination therapy in breast cancer'
Patrick G Johnston Centre for Cancer Research; Queen's University Belfast

Alessandro Cazzolla
'Enhancing chemotherapeutic efficacy through liposomal nanoparticles and ultrasound'
Technological University Dublin

Sarah Chambers
'18-colour immunological profiling of a syngeneic tumour model of prostate cancer after delivery of a radio sensitising molecular targeted gold nanoparticle'
School of Pharmacy; Queen's University Belfast

Jie Feng
'Drug-Induced activation of the ISR/eIF2a signaling axis mitigates metabolic radio sensitisation'
School of Pharmacy; Queen's University Belfast

Oscar Pooley
'Countering the proteinase activated receptor 1 (PAR-1) pro-tumour phenotype using a novel nanotherapeutic approach
School of Pharmacy; Queen's University Belfast

Mary-Kate Riley
'Modelling longitudinal impact of standard-of-care treatment on gene expression in colorectal cancer PDX models to identify novel vulnerabilities'
Patrick G Johnston Centre for Cancer Research; Queen's University Belfast

Michael Ryan
'In vitro modelling of ovarian cancer cell lines for the development of novel liquid biopsy tests'
School of Pharmacy; Queen's University Belfast

Tianxin Liu
'Development of a Novel Targeting Antimicrobial Peptide Conjugate for Selective Membrane Disruption in Non-Receptor-Targetable Cancer Cells'
School of Pharmacy; Queen's University Belfast

Tongchuan Wang
'Mannose enhances radiosensitivity in HPV-Negative head and neck cancer via metabolic reprogramming and impaired DNA damage repair'
School of Pharmacy; Queen's University Belfast

Arporn Wangwiwatsin
'Extrachromosomal circular DNA in cholangiocarcinoma: Exploring potential functions in RAS/BRAF signalling'
Khon Kaen University, Thailand

Janith Wanigasekara
'Novel therapeutic approaches for brain cancer treatment using 3D tumor spheroid and co-cultured multicellular tumor models'
Technological University Dublin

Session 1: Abstracts

Leanne Bradley

Identification of novel immune activating agents for rationalised combination therapy in breast cancer

Patrick G Johnston Centre for Cancer Research; Queen's University Belfast

The DNA-damage driven immune-response (DDIR) is an immune-driven gene expression signature that has been identified in several cancer types. The DDIR signature is driven by cGAS/STING signalling, which is activated by DNA repair deficiencies and characterised by a classical type I interferon response, with activation of immune checkpoint signalling. Notably, DDIR-positive breast tumours display improved therapeutic responses, increased presence of tumour infiltrating lymphocytes (TILs) and upregulation of various immune checkpoint genes including PD-L1, CTLA-4 and TIM3. Therefore, they may be responsive to immune checkpoint blockade (ICB) therapy. Indeed, metastatic triple negative breast cancers, which are considered relatively 'hot' in the context of other breast cancers, have shown increased responsiveness to the combination of atezolizumab with nab-paclitaxel (IMpassion130 trial), highlighting the potential benefits of developing ICB therapies for breast cancer patients. Moreover, several studies in other solid tumours have demonstrated that the DDIR signature predicts response to ICB, including melanoma and oesophageal cancers.

We hypothesised that 'conversion' of DDIR-negative breast tumours to DDIR-positive tumours may lead to improved responses in those patients. Furthermore, activation of the DDIR signature may lead to increased lymphocytic infiltration into DDIR-negative, 'cold' tumours, thus expanding the potential therapeutic benefits of ICB therapies for a wider range of breast cancer patients. We proposed to 'convert' DDIR-negative tumours using novel immune activating agents, that could be rationally combined with ICB to improve patient responses. To maximise translational potential, we screened 2,321 FDA-approved drugs for their ability to stimulate an immune response across various cell lines. Ten drugs of interest were identified and validated and the top 'hits' were taken forward for further testing. Our data demonstrates the ability to activate innate immune signalling using currently approved drugs, providing a promising foundation to further explore repurposing of these compounds for therapeutic benefit in breast cancer.

Alessandro Cazzolla

Enhancing chemotherapeutic efficacy through liposomal nanoparticles and ultrasound

Alessandro Cazzolla

Technological University Dublin; alessandro.cazzolla@tudublin.ie

Traditional chemotherapy agents like doxorubicin (DOXO) are widely used to treat invasive cancers but often fall short in achieving optimal therapeutic outcomes. As a result, researchers are exploring non-invasive adjuvant therapies to enhance drug delivery and efficacy. One such approach involves the use of ultrasound (US) in conjunction with drug delivery technologies to improve treatment precision and effectiveness. Ultrasound has a dual role: breaking down liposomal membranes to release DOXO and creating transient pores in the plasma membrane, facilitating the entry of lipid nanoparticles into cells. Under favorable conditions, cells can recover normal function within hours while retaining a significant concentration of intracellular drug molecules.

This study aims to utilize liposomal nanoparticles as carriers of chemotherapeutic agents, such as DOXO, at high concentrations. Exposure to ultrasound provides controlled release of the drug at specific sites and tissues. The cytotoxic effects of Lipo-DOXO nanoparticles were compared with those of plain liposomes under varying conditions of ultrasound exposure and incubation times. The study found that low-temperature incubation was crucial in confirming the increased cytotoxicity, attributable to passive drug release mechanisms rather than active transport. These findings suggest that combining liposomal nanoparticles with ultrasound offers a promising strategy for improving chemotherapeutic delivery and efficacy.

Sarah Chambers

18-colour Immunological Profiling of a Syngeneic Tumour Model of Prostate Cancer after Delivery of a Radio Sensitising Molecular Targeted Gold Nanoparticle

Sarah EJ Chambers, Niall Byrne, Jie Feng, Jonathan Coulter

School of Pharmacy, Queen's University Belfast, UK

Gold nanoparticles are solid colloidal particles that are effective radiosensitisers through physical and chemical interactions with radiation, increasing the tumour cell killing effect of radiotherapy. We have formulated a molecular targeted AuNP for specific uptake by tumour cells. To study the necessary pre-clinical safety / toxicity of our targeted AuNP, we developed an 18-colour immune panel to characterise immune cells within tumour tissue, peripheral blood, and splenocytes isolated from a syngeneic tumour model of prostate cancer (DVL3 cells). Treatment groups included SARRP irradiation (IR) (n=10), AuNP treatment (n=10), and a combination of both IR + AuNP (n=11), compared to untreated tumours (n=7). Samples were harvested at 24 h and 7 days post treatment and processed for flow cytometry immunophenotyping on the FACSymphony. Lipopolysaccharide (LPS) was used as a positive control stimulant of the immune system (n=4). A gating strategy was used to exclude debris, select viable cells, exclude doublets, and select for CD45+ Leukocytes. An average viability of 68% in tumours, 88% in blood, and 86% in spleen was achieved. Specifically, we quantified innate immune cells (neutrophils (CD11b+CD11c-Ly6G+), macrophages (CD11b+F4/80+) expressing either MHC II (M1) or CD206 (M2), dendritic cells (CD11c+MHCII+), and natural killer cells (CD3-NKp46+)) and adaptive immune cells (helper T cells (CD3+CD4+), cytotoxic T cells (CD3+CD8+), and B cells (CD3-CD19+)). Overall, we observed no significant activation of immune cells treated with AuNP alone or combined with IR, compared to LPS. This data confirms the biocompatibility of our targeted AuNP as safe and non-toxic, which forms an important foundation for its further pre-clinical development towards the clinic.

Jie Feng

Drug-Induced Activation of the ISR/eIF2α Signaling Axis Mitigates Metabolic Radiosensitisation

Jie Feng[a], Varun Pathak[b], Niall Byrne[a], Sarah Chamber [a] , Tongchuan Wang[a] , Rayhanul Islam[a], Reinhold Medina[b], Jonathan Coulter[a]

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Hypoxia, a hallmark of most solid tumours, including head and neck cancer, significantly undermines the efficacy of radiotherapy, promoting resistance both though an altered microenvironment and at the genomic level, limiting treatment outcomes. As such, understanding the underpinning mechanisms of radiation resistance while implementing strategies to overcome such responses are attractive, particularly as radiotherapy remains a cornerstone treatment of locally advanced disease. Atovaquone, a selective inhibitor of the mitochondrial electron transport chain complex III, has shown promise in alleviating tumour hypoxia in preclinical models, however, this does not consistently result in increased radiation sensitivity. This study investigates the potential of atovaquone to modify the hypoxic response to radiation in models of head and neck squamous cell carcinoma (HNSCC), identifying adaptive resistance mechanisms that limit the radiosensitising potential of atovaquone. Our research reveals that atovaquone significantly disrupts mitochondrial respiration, triggering the phosphorylation of eIF2α, a pivotal player in the integrated stress response (ISR) and a master regulator of protein synthesis. Notably, atovaquone also increased the autophagic load under hypoxia, with autophagy inhibition significantly enhancing apoptosis and improving radiation sensitivity in the presence of atovaquone. These findings uncover, for the first time, that atovaquone-induced ISR promotes an adaptive response that protects against drug induced metabolic radiosensitisation. Importantly, this resistance can be counteracted through the inhibition of ISR signaling.

In summary, our results highlight dual counter opposing impacts of atovaquone, serving as a hypoxic radiosensitiser though oxidative phosphorylation (OXPHOS) inhibition, but also in promoting stress induced ISR signaling, conferring resistance. These data provide new insights into molecular approaches to help overcome hypoxia-induced radioresistance through ISR modulation.

Oscar Pooley

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

Oscar Pooley[1], Jonathan Coulter[1]
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Pancreatic cancer (PDAC) is the 7th leading cause of cancer associated death, with 432,000 associated deaths in 2018[1]. Currently tumour resection is the only curative treatment[1]. Over 80% of patients are diagnosed with unresectable tumours. Furthermore, many others have borderline resectable disease, which is treated with neoadjuvant chemotherapy or chemoradiotherapy (CRT) to debulk the tumour and improve the likelihood of a successful surgery[2]. Due to the proximity of the pancreas to sensitive organs and the high occurrence of radio-resistance, the efficacy of radiotherapy is limited[4]. A targeted radiosensitiser, like a gold nanoparticle, that could enhance the curative impact of radiotherapy, whilst not impacting surrounding tissues could significantly improve treatment outcomes. Proteinase activated receptor 1 (PAR-1) is a cleavage-activated, transmembrane, G-protein coupled receptor often overexpressed in PDAC. Excessive PAR-1 activation is oncogenic, promoting cell migration, proliferation, EMT and cell survival[5]. This project aims to determine the importance of PAR-1 signalling in PDAC, using PAR-1 targeted pepducins and a commercially available PAR-1 inhibitor, Voraparaxar to assess their impact on key oncogenic and pro-survival characteristics. It also aims to determine the role of PAR-1 in mediating the radiation response of pancreatic cancer cell lines. Furthermore, it aims to develop a targeted, radio sensitising gold nanoparticle (AuNP) through the conjugation of a PAR1 specific pepducin (P1PAL7) to a PEGylated gold nanoparticle core. Alamar Blue cytotoxicity studies have shown that Vorapaxar and PAR-1 specific pepducins have little impact of pancreatic cancer cell survival. Clonogenic cell survival experiments are currently ongoing to determine the impact of these treatments on the radiosensitivity of PDAC cell lines. AuNP formulations have been synthesized using the Turkevich reduction method and their properties were characterised using Atomic Absorption Spectroscopy (AAS), Dynamic light scattering (DLS) and Zetasizer data. The uptake of different AuNP formulations by PDAC cell lines were compared using AAS data and Cytoviva Hyperspectral Microscopy.

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Mary-Kate Riley

Modelling longitudinal impact of standard-of-care treatment on gene expression in colorectal cancer PDX models to identify novel vulnerabilities

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Patient derived xenograft (PDX) models are a valuable tool to study drug sensitivity and to understand tumour intrinsic effects of anti-cancer treatment, free from the complexities and confounding effects of the human tumour microenvironment (TME) and immune system.

Increasing evidence suggests that treatment of patient derived xenografts is a powerful predictor of patient response for standard-of-care (SoC) or novel treatments. Genomic analysis of such patient "avatar" trials therefore represents a potentially powerful tool for the discovery of novel predictive biomarkers and treatment combinations. Moreover, given our increasing understanding of the importance of epigenetic plasticity enabling cancer cells to persist treatment, analysis of PDX longitudinal samples has the potential to deepen our understanding of the targetable mechanisms that could improve efficacy of these treatments. Colorectal cancer (CRC) is the third most diagnosed cancer and the second most common cause of cancer-related deaths worldwide. Outcomes for late-stage patients treated with SoC are generally bleak due to recurrence of treatment refractory disease, hence there is an unmet need for novel biomarkers, treatment strategies or combinations to improve patient outcomes.

Using archived formalin fixed paraffin embedded (FFPE) tissue, RNA was extracted from unique set of longitudinal samples from CRC PDX models treated with SoC FOLFIRI (folinic acid + 5-fluorouracil (*5-FU*) + irinotecan).

Preliminary analysis identified significant changes in gene expression in response to treatment as well as between responsive and non-responsive groups. This analysis ongoing and will include integrating basal genetic data, with the goal to determine whether this information can be leveraged to identify novel biomarkers or targetable vulnerabilities.

We hope that this will inform the development of new personalised therapies, either from new or existing drugs, with the overall objective to improve the prediction of patient response to therapy and enhance overall treatment outcome.

Michael Ryan

In vitro modelling of ovarian cancer cell lines for the development of novel liquid biopsy tests

Micheal Ryan[1,2], Alexander McIntyre[1,2], Niamh Buckley[2,3], Paul Mullan[1,2].

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Introduction: Ovarian Cancer is notoriously difficult to diagnose due to its late presentation and lack of accurate diagnostic biomarkers. Liquid biopsies detecting Circulating tumour DNA (ctDNA) has emerged as promising strategy for improving Ovarian Cancer diagnosis from blood samples. Identifying fragments of DNA intact at the time of a blood draw remains a challenge. We aimed to establish an in vitro model for ctDNA by overexpressing *DNase1L3*, a key enzyme involved in the generation of ctDNA, in Ovarian Cancer cell lines. It was hypothesized that by overexpressing *DNase1L3*, the release and degradation of ctDNA could be simulated, thus mimicking the dynamics observed in patients.

Methods: DNase1L3 was overexpressed by liposomal transfection in ovarian cancer cell lines OVCAR3 and OVCAR4. Successful transfection was confirmed by qPCR. Cell free DNA was extracted from the media of these cell lines and the effect of *DNase1L3* overexpression was examined using gel electrophoresis and DNA fragmentation analysis.

Results: DNASE1L3 overexpression leads to increased DNA degradation and fragmentation, resulting in a pattern of protected DNA fragments similar to those observed in ctDNA samples from Ovarian Cancer plasmas. *DNASE1L3* overexpression generated characteristic mono- and di-nucleosome patterns from Ovarian Cancer cell media. By modelling this process we aim to reveal which fragments of DNA will be intact in plasma samples at the time of a blood draw and therefore prioritise genomic regions representing the best prospects of successfully developing novel diagnostic tests for Ovarian Cancer.

Conclusion: More accurate and earlier detection of Ovarian Cancer, through blood testing, could dramatically improve survival rates. This innovative approach provides a valuable tool for investigating the release, degradation, and potential diagnostic value of ctDNA in Ovarian Cancer. Further studies may uncover which fragments of DNA are protected from *DNase1L3* degradation and assist in the development of a PCR based blood test for improved Ovarian Cancer diagnosis.

Tianxin Liu

Development of a Novel Targeting Antimicrobial Peptide Conjugate for Selective Membrane Disruption in Non-Receptor-Targetable Cancer Cells

Tianxin Liu, Lingyu Ke, Chunyuan Zhou, Xiaoling Chen*, Lei Wang, Tianbao Chen and Mei Zhou*

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Introduction: Chemotherapy remains a cornerstone in cancer treatment, but its non-selective toxicity to normal cells often leads to severe adverse effects. Targeted ligand-drug conjugates (TDCs) have emerged as promising alternatives to improve therapeutic specificity. Conventional TDCs rely on overexpressed surface proteins in tumour cells, limiting their applicability. In this study, we present a novel TDC strategy employing antimicrobial peptides (AMPs) as cytotoxic payloads to selectively disrupt cancer cell membranes without the need for specific intracellular targets.

Methods and Results: Compound 174-3 is a structure-refined AMP derived from an amphibian temporin peptide (compound 111). Using compound 174-3 as potent cytotoxic payload tagged at the C-terminus with a protease-cleavable linker and PEG(n), we synthesised a peptide conjugate, namely compound 209.
Compound 209 exhibited a much wider therapeutic window compared to it precursors, paclitaxel, etoposide, 5-fluorouracil and cisplatin. LDH release and Sytox Green uptake assays were performed to assess membrane disruption in both NCI-H460 cancer cells and normal cell lines (MRC-5, HEK-293, HMEC-1). At the IC50 concentrations, compound 209 induced 20% LDH release and 35% Sytox Green uptake in NCI-H460 cancer cells, with no significant effects on normal cells, while compound 111 and compound 174-3 caused considerable membrane damage in both cancer and normal cells, indicating that compound 209 exhibits significantly improved membrane selectivity. The antiproliferative effect of compound 209 was also confirmed in chemoresistant NCI-H460/R cells, where it retained significant activity.

Conclusion: This novel strategy of using AMPs in TDCs offers a promising approach for selectively targeting non-receptor-targetable cancer cells. Compound 209 demonstrated enhanced selectivity and retained activity in drug-resistant NCI-H460 variants, indicating its potential as a targeted therapeutic with reduced toxicity to normal cells. This approach could expand treatment options for heterogeneous tumours that evade receptor-dependent and traditional chemotherapy, helping to address the challenge of drug resistance in cancer treatment.

Tongchuan Wang

Mannose enhances radiosensitivity in HPV-Negative head and neck cancer via metabolic reprogramming and impaired DNA damage repair

Tongchuan Wang[a], Niamh Doherty[b], Connor Brown[b], Niall M. Byrne[a], Rayhanul Islam[a], Meabh Doherty[a], Jie Feng[a], Cancan Yin[a], Sarah Chambers[a], Lydia McQuoid[a], Karl Butterworth[b], Emma Kerr[b], Jonathan A. Coulter[a]

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HPV-negative head and neck cancer (HNC) is one of the most aggressive malignancies worldwide and often exhibits radiation resistance, particularly under hypoxic conditions. Current therapeutic strategies, while effective, are associated with significant adverse effects. Thus, there is a growing need to develop novel methods to enhance the efficacy of radiotherapy in HNC. Mannose has recently shown promising potential in enhancing anti-cancer therapies. In this study, we show that mannose, in combination with phosphomannose isomerase (PMI) knockout, a key mannose metabolising enzyme, enhances radiosensitivity in HPV-negative HNC cells by altering cellular metabolism. Specifically, mannose reduces ATP production, thereby impairing the ATP-dependent DNA damage repair response following ionising radiation (IR), leading to a 2.5-fold increase in unresolved DNA double-strand breaks in PMI knockout (KO) cells. Moreover, mannose and PMI knockout (KO) reprograms the metabolic profile of irradiated cells, resulting in a 60% reduction in glycolysis and a 45% decrease in oxidative phosphorylation. Additionally, key components of DNA and RNA metabolism, such as thymidine, uridine, and adenine, were significantly altered following irradiation. Notably, mannose significantly enhances the radiosensitivity of PMI KO cells, with a more pronounced effect under hypoxic conditions (SER: 1.39). Similarly, mannose markedly delays the growth of irradiated PMI-deficient HNC spheroids by suppressing the formation of the hypoxic core. This effect was attributed to mannose-induced downregulation of HIF-1α , the master regulator of oxygen homeostasis, via the accumulation of mannose-6-phosphate in PMI KO cells. These findings demonstrate the radiosensitising potential of mannose, uncover novel mechanisms, and identify a molecular target for regulating mannose's effects. As such, mannose could have significant clinical implications for treating HPV-negative HNSCC tumours, as a promising adjuvant to radiotherapy.

Arporn Wangwiwatsin

Extrachromosomal circular DNA in cholangiocarcinoma: Exploring potential functions in RAS/BRAF signalling

Zar Zar Win[1], Hasaya Dokduang[3,4], Siriyakorn Kulwong[2], Watcharin Loilome[2,3], Nisana Namwat[2,3], Jutarop Phetcharaburanin[2,3], Thidathip Wongsurawat[5], Piroon Jenjaroenpun[5], Poramate Klanrit[2,3], Arporn Wangwiwatsin[2,3]

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Extrachromosomal circular DNAs (eccDNAs) have received attention due to their pivotal role in promoting cancer heterogeneity, with potential association to elevated oncogene copy numbers in various malignancies (1). While presence of eccDNAs in both normal and cancer cells is confirmed, its influence on gene-level alterations in cancer cells remains largely unexplored (2). This study delves into the genomic profiles of eccDNA in cholangiocarcinoma (CCA), an aggressive biliary tract malignancy with extensive heterogeneity and diverse molecular alterations, using a modified long-read Circle-Seq method. We unveiled distinct eccDNA characteristics in CCA compared to non-tumor cholangiocyte cells, focusing on genic components and chromosomal origins. Analysing read-depth differences in oncogene-containing eccDNA, we identified potential eccDNA candidates that may be relevant for the development of CCA. Subsequent bioinformatics analysis was performed using a published CReSIL tool, revealing distinct patterns of these oncogenes, particularly genes in the RAS/BRAF pathway, suggesting a potential functional role of eccDNAs in a cancer signalling pathway. The findings highlight the remarkable heterogeneity and diverse origins of eccDNA in CCA. This study established the first profiling of eccDNAs in cholangiocarcinoma and paved the way for further investigation of their contribution to oncogene amplification and disease progression.

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Janith Wanigasekara

Novel therapeutic approaches for brain cancer treatment using 3D tumor spheroid and co-cultured multicellular tumor models

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Glioblastoma (GBM), a grade 4 adult-type diffuse glioma (IDH wild type), is the most common and aggressive form of malignant primary brain tumor in adults, characterized by high vascularity and poor prognosis. The high failure rate of cancer clinical trials is due to inefficient treatment methods and imperfect pre-clinical models, which limit our ability to predict efficacy and toxicity in humans. To mitigate the issue of inaccurate pre-clinical therapeutic outcomes resulting from imperfect pre-clinical models, we have optimized and integrated the use of 3D tumor spheroid models and co-cultured multicellular tumor models into our research. In addressing the issue of inefficient treatment methods, we discovered the potential of novel therapeutic approaches, such as Cold Atmospheric Plasma (CAP), Ultrasound (US), and Plasma Microbubbles (PMB) treatments.

These treatments effectively induced 3D GBM and epidermoid tumor spheroid cell death in a time-, dose-, treatment frequency-, and reactive oxygen and nitrogen species (RONS)-dependent manner. Additionally, these treatments significantly reduced 3D GBM spheroid regrowth, cell proliferation, and metabolic activity, while inducing cytotoxic effects, damage to the tumor sphere's cell membrane, spheroid shrinkage, and disruption of the tumor microenvironment (TME). Our study on drug delivery showed that combining US with chemotherapeutics improves drug diffusion and enhances cytotoxicity in 3D tumor spheres of GBM and epidermoid carcinoma, compared to two-dimensional (2D) cell cultures.

These findings highlight a key limitation in translating CAP, US, and PMB therapies into clinical settings, underscoring the need for careful consideration of the approach. They also emphasize the importance of utilizing 3D cell culture and co-culture models in preclinical research. Relying solely on 2D cell cultures, followed by animal testing and clinical trials, has led to a 95% failure rate due to poor prediction of human efficacy and toxicity.

Session 2: Friday, October 4, 2024

13:30 - 14:00	POSTER SESSION II

Bayan Alkhaldi
'The influence of hedgehog (Hh) signaling in modulating the radiosensitivity of Glioblastoma tumour models'
School of Pharmacy; Queen's University Belfast

Connor Brown
'Investigating cholesterol metabolism as a chemotherapy-induced metabolic vulnerability in colorectal cancer models'
Patrick G Johnston Centre for Cancer Research; Queen's University Belfast

Jiamei Fu
'Nintedanib alleviates radiation-related severe lung injury by remodeling immune microenvironment and inhibiting fibrogenic EMT progression'
School of Medicine, Tongji University, Shanghai, China

Poramate Klanrit
'Development of in vitro 3D cholangiocarcinoma model for personalized drug evaluation'
Khon Kaen University, Thailand

Shauna McClelland
'Targeting CXCR2 and cytokine interactions in the prostate tumour microenvironment: Implications for novel cancer therapies'
Patrick G Johnston Centre for Cancer Research; Queen's University Belfast

Ethna McFerran
'Skin in The Game: The cost consequences of skin cancer diagnosis, treatment and care in Northern Ireland'
Patrick G Johnston Centre for Cancer Research; Queen's University Belfast

Lydia McQuoid
'Early insights into novel AuNPs and the radiation-induced bystander effect'
School of Pharmacy; Queen's University Belfast

Xiyuan Qi
'Discovery and investigation of the mechanisms of synergy between Pol I inhibitors and 5-florouracil in colorectal cancer cell models'
School of Biological Sciences; Queen's University Belfast

Zelin Tan
'Phosphomannose-isomerase gene expression as a novel radiosensitising target in pancreatic cancer'
School of Pharmacy; Queen's University Belfast

Yaqin Zhou
'Investigation and therapeutic exploration of the epigenetic mechanisms underpinning the emergence of Drug-Tolerant Persister (DTP) cells in colorectal cancer'
Patrick G Johnston Centre for Cancer Research; Queen's University Belfast

Session 2: Abstracts

Bayan Alkhaldi

The influence of hedgehog (Hh) signaling in modulating the radiosensitivity of Glioblastoma tumour models

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Introduction: Ionising radiation (IR) is a primary treatment option for glioblastoma multiforme (GBM), an aggressive primary malignant human brain tumour. However, GBM radioresistance represents a major treatment barrier resulting in poor treatment outcomes. The hedgehog (Hh) signaling pathway is mainly active during embryogenesis, with aberrant activation associated with tumour development including GBM. Targeting and antagonising Hh signaling by blocking the smoothened (SMO) receptor, is one approach for interfering with this pro-survival pathway. The overall aim of this work is to establish the impact of inhibiting the SMO receptor, assessing the subsequent activity of down-stream glioma-associated zinc finger transcription factors GLI-1, establishing the impact of pathway antagonism on GBM radiosensitivity.

Methods: For all *in vitro* studies U251MG, U87MG, and T98G human GBM tumour models were used. Direct toxicity of GDC-0449 and SMOi2-8 were determined using the alamar blue assay. Next using a non-cytotoxic antagonist, concentrations total RNA were isolated at both basal level and 24 h post treatment with GDC-0449 (5, 50 µM) a small molecule antagonist and using an inhibitory peptide SMOi2-8 (300 nM) +/- 2 Gy radiation. Finally the impact of combining SMO antagonist (drug/peptide) with 4 Gy radiation dose was determined by clonogenic assay.

Results: SMO and GLI-1 were expressed in all GBM cells, but at a significantly ($p < 0.001$) higher level in T98G compared with U251 MG and U87 MG cells. T98G cells had 16-fold higher SMO expression and 3-fold higher GLI expression compared to U87 MG. No direct toxicity was reported from GDC-0449 or SMOi2-8 in all three tumour cell models. Pre-treatment with GDC-0449 antagonised GLI-1 expression by ~40% in T98G cells, minimally impacting GLI-1 in U251 or U87 cells. Interestingly, SMOi2-8 proved more effective than the small molecule inhibitor, suppressing T98G GLI-1 expression by ~75%. With respect to radiation modulation, antagonising SMO signaling additionally reduced survival fraction over radiation alone (4 Gy) in all cell lines tested, proving significant ($p<0.05$) in both U87 and T98G cells. Radiation failed to directly impact the expression of the SMO receptor, however, it did produce a potent but transient reduction in GLI-1 expression, returning to basal levels 24 h post radiation treatment.

Conclusion: Antagonising the SMO receptor using SMOi2-8 correlated with decreased GLI-1 expression, which acts as a major nuclear effector of Hh signaling by inhibiting the intracellular signal transduction cascade. Furthermore, the combined action of Hh

antagonism and radiotherapy resulted increased GMB cell death over radiation alone. As such the Hh/SMO signalling axis may represent novel therapeutic target to help overcome GBM radio-resistance. Further studies will be undertaken to expand the range of radiation doses in addition to exploring the mechanisms contributing to drug mediated radiosensitisation.

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Connor Brown

Investigating cholesterol metabolism as a chemotherapy-induced metabolic vulnerability in colorectal cancer models

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Chemotherapy (typically fluoropyrimidine-based) is a common treatment choice for colon cancer patients, alone or in combination with surgery/radiotherapy, and is most heavily relied upon by patients with advanced disease. The antimetabolite 5-fluorouracil (5FU) remains the backbone for fluoropyrimidine (FP)-based chemotherapy, given alone or in combination with Oxaliplatin (FOLFOX) or Irinotecan (FOLFIRI). Despite its widespread use, 30-50% of CRC patients have intrinsic resistance, and up to 90% develop acquired resistance, driving treatment failure. The mechanisms driving 5FU resistance remain poorly understood, confounded by 5FU's multimodal mechanism of action. Reprogramming of cancer cell metabolism has been linked to drug resistance in various cancer types and we hypothesize that it is an attractive target to enhance chemotherapy response in CRC. Here, we report that cholesterol metabolism is acutely upregulated intracellularly in response to 5FU treatment at multiple levels to promote cell survival *in vitro*. Our data demonstrates that reprogramming of cholesterol metabolism imposes a targetable vulnerability to enhance response to 5FU and we will now explore the potential of this combination therapy in more advanced preclinical models.

Jiamei Fu

Nintedanib alleviates radiation-related severe lung injury by remodeling immune microenvironment and inhibiting fibrogenic EMT progression

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Background: Radiation-induced pulmonary toxicities, especially the life-threatening interstitial lung disease (ILD) exacerbation, represent a unique challenge in oncologic management. This study aims to exploit the antifibrotic efficacy and safety of nintedanib combination therapy in pre-existing ILD mice receiving radiotherapy and the underlying mechanisms.

Methods: A C57BL/6 murine model of radiation-related severe lung injury (RRLI) was established with 16 Gy X-ray right thoracic irradiation based on the bleomycin-induced pulmonary fibrosis model. Nintedanib was administered 1 week before irradiation and consistently applied for another 8 weeks by gavage daily. The mice were performed with lung function test at 26 weeks and the lungs were collected for histological examination, immunohistochemistry, and flow cytometry. Transcriptome, proteome, and metabolome sequencing were conducted to identify the prospective biomarkers.

Results: Mice from RRLI group exhibited extensive and progressive fibrotic lung injury. The administration of nintedanib reduced the mean fibrotic score of RRLI mice by 40.0% and improved the declining lung vital capacity by 41.8%. Nintedanib also reduced the infiltration of macrophages and regulatory T cells by 16.1% and 45.6% respectively in injured lung tissues. Mechanistically, it was observed that Nintedanib facilitated the recovery of the immune balance between regulatory T cells and T helper 17 cells in the spleen and the differentiation of myeloid cells in the bone marrow. It also reversed the EMT process in RRLI mice with decreased expression of proteins vimentin, snail, and α-SMA, and increased expression of protein E-cadherin. A multi-omics co-analysis revealed potential molecular targets, such as *Ctsb*, *Ctsk*, *Mmp2*, and *Tgfbr1*, and pathways, such as Rap1 signaling pathway of nintedanib.

Conclusions: Nintedanib significantly alleviated severe lung injury in the survival mice with pre-existing ILDs after partial thoracic irradiation. Our findings provide compelling evidence of the molecular mechanisms for combination and consolidation treatments in pre-existing or subclinical ILD patients undergoing radiotherapy.

Poramate Klanrit

Development of *in vitro* 3D cholangiocarcinoma model for personalized drug evaluation

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The three-dimensional (3D) organoid technology has become more popular among researchers. Organoids are the small structure of tissues and organs that mimic a specific organ's 3D architectures and functions. These 3D constructs represent near-physiological *in vitro* models and support many biomedical applications, including cancer research. Cholangiocarcinoma (CCA) is a malignant tumor of bile duct epithelium that is highly endemic to the northeastern part of Thailand. Several CCA cell lines were established successfully as 2D and 3D cultures; however, multi-phenotypic cells studies are require to improve a precise drug evaluation. Therefore, the tissue-engineering approach might help produce CCA organoids with multi-phenotypic cells in a controllable manner. From the results, explant culture was established under *in vitro* conditions with cells growing out of CCA explants in a 2D plane. The Advanced DMEM with adapted supplements showed superior outcomes, with primary cells obtained within 2-3 weeks. Single cells were further isolated and initially characterized with anti-cytokeratin 19 and anti-α-SMA antibodies to define the CCA cell and the cancer-associated fibroblast (CAF) populations. The CCA cells and CAFs can resemble back into the 3D structure via a tissue-engineering scheme. This technique can precisely control cell number and population related to organoid quality control. The results also showed the generation of extracellular matrix components, as demonstrated in Masson's trichrome staining. Further characterization of cells will be required, and drug responses of self-assembled organoids (CCA-CAF) will be elucidated in the near future.

Shauna McClelland

Targeting CXCR2 and cytokine interactions in the prostate tumour microenvironment: Implications for novel cancer therapies

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Introduction. Prostate cancer (PC) is a prevalent malignancy with a complex aetiology that involves the interplay of various genetic, cellular and molecular components in the tumour and tumour microenvironment (TME). Exciting research shows that the activation of CXCR2 triggers a transformation of the TME, resulting in the recruitment of neutrophils and myeloid-derived suppressor cells (MDSCs), which, in turn, fosters immune suppression and tumour progression and reinforces treatment resistance.
A notable genetic alteration observed in PC is the loss of Phosphatase and Tensin Homolog (PTEN), affecting up to 50% of castration-resistant tumours and 20% of primary PC tumours. PTEN loss leads to heightened tumour growth, survival, and resistance to therapeutic interventions, through the activation of the PI3K-AKT signalling pathway and the augmentation of inflammatory and chemokine signalling, including interleukin-8 and its receptors CXCR1 and CXCR2. This research endeavours to shed light on the pivotal role of targeting CXCR2 and cytokine interactions within the prostate tumour and the TME, presenting a promising avenue for advancing cancer treatment.

Material and methods. Human (C4-2B; CWR-R1) and murine (DVL3) PC models, sensitive and resistant to androgen receptor inhibition, were used to evaluate the efficacy of CXCR2 inhibition and its impact on cytokine-induced vulnerabilities. Phenotypic assessment by cell viability, survival assays and cell cycle analysis were complemented with mechanistic evaluation by siRNA, qRT-PCR, Western blot and ELISA under both normoxic and hypoxic conditions.

Results and discussion: In human and murine PC models, we assessed phenotypic and molecular responses to Enzalutamide both as a standalone treatment and in combination with CXCR2 inhibition, within normoxic and hypoxic contexts. Additionally, we sought to identify potential vulnerabilities intrinsic to the tumour or originating from the TME that may play a role in the development of treatment resistance.

Conclusion: Thus far, our findings underscore the limited efficacy of CXCR1/2 inhibition as a monotherapy in PTEN-deficient prostate cancer cells. However, when combined with Enzalutamide, these inhibitors demonstrate significant promise, highlighting their pivotal roles in the strategic design of combination treatments.

Ethna McFerran

Skin in The Game: The cost consequences of skin cancer diagnosis, treatment and care in Northern Ireland

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Background: Skin cancer is a prevalent cancer in the UK. Its rising incidence and mortality rates are expected to result in substantial financial implications, particularly on diagnostic and treatment services for skin cancer management in Northern Ireland (NI). Such anticipated disease increases underscore the need for prevention and control measures that should help guide policymaking and planning efforts.

Methods: We conducted a cost of illness study to assess the economic impact of skin cancer in NI from the healthcare system's perspective, using a bottom-up method, employing NHS reference costs (UK£) for skin cancer diagnosis and treatment patient pathways in 2021/22. Sensitivity analyses varied diagnostic volumes by applying multipliers for benign cases, assuming a diagnostic conversion rate of 6.8%, and examined an alternative chemotherapy regimen compliance rate of 75%. Additionally, proportional cost increases were projected based on future estimated increases of 9% and 28% to malignant melanoma (MM) cases for diagnostic, treatment, and follow-up volumes.

Results: Significant numbers of non-melanoma skin cancers (NMSC) and MM cases were recorded, 4289 NMSCs and 439 MM cases. The total cost for managing NMSC was £3,365,350. Total costs for MM skin cancer were £13,740,681, including £8,753,494 for procurement, administration, and chemotherapy drug use. Overall healthcare spending on skin cancer care totalled £21,167,651. Sensitivity analysis suggested diagnostic cost may increase significantly to £12,374,478 based on referral volume assumptions. If base case rates rise by 9 or 28% estimated total costs of treating skin cancer will increase to £22.3 million and £24.9 million, respectively.

Conclusions: Skin cancer management costs in NI totalled ~£21.1 million to £32.1 million, depending on diagnostic referral assumptions. Costs have risen ~10-fold over the past decade for MM due largely to chemotherapy costs. A predicted 28% increase in MM cases by 2040 would lead to ~£3.8 million of additional expenditures, providing a significant challenge for cancer health systems.

Lydia McQuoid

Early insights into novel AuNPs and the radiation-induced bystander effect

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Introduction: 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 proven to act as effective radiosensitisers. Both the chemokine-targeting portion alone and the final AuNP formulation were examined for any potential impact on the RIBE in models of prostate and head and neck cancer3,4.

Methods: Clonogenic assays were used to quantify radiation sensitivity following receptor antagonism using commercial pharmacological small molecule inhibitors (AZD5069 and AMD3100) or novel peptide based antagonists. Irradiated Cell Conditioned Media (ICCM) was transferred 1 h or 24 h post-irradiation from antagonised donor cells to unirradiated bystander cells, for assessment of clonogenic survival. The micronucleus assay was also used to examine bystander genotoxicity caused by AuNP ICCM.

Results: Chemokine receptor antagonism caused modest radiosensitisation in both cell lines, whilst AuNPs significantly ($p<0.01$) augmented radiation sensitivity. Basal bystander effects were inhibitory, dominating at low radiation doses (<1 Gy) 1 h post-radiation. Interestingly, chemokine receptor antagonism appeared to reduce this effect, while exposure to AuNP treated donor media enhanced the bystander response.

Discussion: Increased clonogenic survival post receptor antagonism in unirradiated recipient bystander cells suggest that secreted pro-inflammatory factors confer a pro-survival response. The strong radiosensitisation of irradiated cells conferred by AuNPs, 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.

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Xiyuan Qi

Discovery and investigation of the mechanisms of synergy between Pol I inhibitors and 5-florouracil in colorectal cancer cell models

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Until recently ribosome biogenesis (RiBi) was considered merely a 'housekeeping' process, equally important to normal and tumour cells alike, thus drugs targeting this process would fail to achieve a therapeutic benefits. However, our and others work successfully disprove this dogma and demonstrated that a key stage of RiBi, ribosomal RNA (rRNA) transcription, can be targeted to treat cancer while preserving normal cells (for latest reviews see: [1,2,3]). Furthermore, mounting evidences suggest that upregulated rRNA transcription plays important role in the development of metastasis[4,5], making rRNA transcription inhibitors promising agent to target metastatic cells.

5-florouracil (5-FU) is one of widely used chemotherapeutic drugs, however it characterised by relatively high toxicity and development of resistance[6,7]. Combination therapy is well known way to increase efficacy of treatment, lower toxicity and decrease chances to develop a resistance.

In our experiments, we analysed the efficacy of 5-FU in combination with three Pol I inhibitors characterized by different Pol I inhibition mechanism and different off-target effects (CX-5461, BMH-21 and PMR-116) using two isogenic colorectal cancer cell lines (HCT-116 $p53^{+/+}$ and HCT-116 $p53^{-/-}$).

We have found that low doses of Pol I inhibitors (below GR_{10}) can significantly increase the efficacy of 5-FU (5-6-fold) by enhancing an apoptotic response. Interestingly, analysis of dose-response data shows that Pol I inhibitors and 5-FU act synergistically, and the synergistic effect is dependent on p53. Here, we will discuss potential mechanisms of the synergy between 5-FU and Pol I inhibitors.

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Zelin Tan

Phosphomannose-isomerase gene expression as a novel radiosensitising target in pancreatic cancer

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Introduction: Limited therapeutic options makes pancreatic cancer predicted to be the second leading cause of cancer related death in 2030.[1] Due to the immunosuppressive tumor microenvironment, PDAC is extremely resistant to conventional cytotoxic and target therapies including chemo, immune and radiation therapy. [2,3] Clinically, some patients with pancreatic cancer receive stereotactic radiation therapy, and SBRT, which usually refers to five or fewer RT therapy sessions, remains a significant challenge. When high doses radiation are given to pancreatic cancer, organs of the digestive system nearby are susceptible and can have devastating consequences.[4] Studies have shown that mannose increases the chemotherapy sensitivity of cisplatin, as well as radiosensitizing esophageal squamous cell carcinoma.[5,6] Phophomannnose isomerase is a major player in mannose metabolism, and its elimination will increase the sensitization effect induced by mannose. This phenomenon offers the possibility to reach better therapeutic effect with lower radiation dose. Our research group hopes to use less toxic bioreducible poly amino amide to deliver PMI knockdown siRNA as a novel therapy to explore the feasibility of radiotherapy for pancreatic cancer.

Method: Twp human pancreatic cancer cell line Panc-1 and BxPC-3 were used for in vivo studies. The PMI level of both cell lines were obtained by Western blot assay. DharmaFECT 1 transfection reagent was used as an commercial comparation and S-S modified siRNA targeting PMI were used for gene knockdown, bioreducible polymer pABOL (poly(cystamine bisacrylamide-co-4-amino-1-butanol) were obtained by aza-Michael polyaddition of 4-amino-1-butanol (ABOL) to N,N'-cystaminebis(acrylamide) (CBA) synthesised by Dr Christopher. The response of cells to multiple radiation dose (0,2,4,6,8) with/ 20 mM mannose treated after around 50% PMI knockdown were determined by clonogenic assay.

Results: PMI is highly expressed in pancreatic cancer cells particularly in Panc-1 cells. The LD50 (Lethal dose, 50%) for mannose is133.7 mM for Panc-1 and 97.15 mM for BxPC-3 after 48 hours exposure. Long exposure to mannose after radiation significantly radiosensitise ($p<0.05$) Panc-1 cells with SER of 1.16 and radiosensitise BxPC-3 cells with SER of 1.14. Mannose failed to influence the radiosensitivity of both cell lines when treated 24 hours before radiation. 57% PMI knockdown was observed after transfected with S-S modified siRNA in DharmaFECT 1 transfection reagent. pABOL-siRNA nanoparticle failed to knockdown PMI in both tumour cell model.

Conclusion: The combination of mannose long exposure and radiotherapy result in increased cell death compared with radiation alone. pABOL alone is not suitable for siRNA delivery. Future work will include investigating the relationship between PMI level and mannose

radiosensitising effect using PMI knock out pancreatic cancer cell lines as well as Evaluating the siRNA knockdown effect using pABOL-EDA co-polymer.

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Yaqin Zhou

Investigation and therapeutic exploration of the epigenetic mechanisms underpinning the emergence of Drug-Tolerant Persister (DTP) cells in colorectal cancer

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The mainstay Standard-of-Care chemotherapy of colorectal cancer (CRC) is 5-Fluororuacil. However, it is becoming increasingly clear that cancer cells are capable of entering a reversible drug-tolerant persister (DTP) state. This survival mechanism allows them to evade the lethal impacts of various treatments, including 5FU-based chemotherapies. Importantly, the emergence of 5FU-induced DTPs is accompanied with profound changes in epigenome, leading to significant transcriptional reprogramming that we believe supports the emergence of DTP. This hypothesis is further supported by genome wide CRISPR screening, revealing the important role of epigenetic regulatory complexes in 5FU DTPs, that together hint at a key role for histone acetyltransferase and deacetylase (HDAC) complexes in influencing emergence. To understand the 5FU-induced DTP state, our initial investigations focused on the effects of HDAC inhibitors (HDACi) on DTPs after long-term 5FU treatment. Our findings indicate that HDACi are effective in reducing 5FU-induced DTPs by deregulating FLIP expression. Moreover, Gene Set Enrichment Analysis (GSEA) underscored the relevance of HDAC1,2 and 5FU persistent cells and the CRISPR screening hints at key complexes related to HDAC1,2. Additionally, chromatin immunoprecipitation sequencing (ChIP-seq) revealed that the Class-I HDACi Entinostat (ENT) repressee modulation of acetylation that occurs in the response to 5FU. In summary, HDACi helps to reduce 5FU-induced DTPs, supporting the identification of novel therapeutic targets to enhance the effectiveness of 5FU-based treatments.

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