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# PROFFERED PAPERS

# A Decade of Population-Based Screening for Colorectal Cancer in Ireland: Lessons from Four Rounds of BowelScreen

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**Background:** Colorectal cancer (CRC) is the second most common cancer in Irish men and third in women, with approximately 2,800 diagnoses annually. BowelScreen, Ireland's national bowel screening programme, launched in 2012 to reduce CRC mortality through early detection and treatment of pre-cancerous lesions. The programme offers biennial faecal immunochemical testing (FIT) to eligible adults, initially aged 60-69, with planned expansion to ages 55-74. We report screening uptake, colonoscopy outcomes, and performance characteristics of FIT during BowelScreen's first decade, providing insights for programme optimization and international comparison.

**Materials and Methods:** We analyzed data from four screening rounds (2012-2022) including FIT participation, colonoscopy attendance, and clinical outcomes. FIT uptake was compared by sex, age group (60-64 versus 65-69 years), and over time. We calculated FIT positivity rates, positive predictive value (PPV) for CRC, cancer detection rates per 1,000 screened, and number needed to scope (NNTScope) to detect one CRC. Statistical significance was assessed using appropriate tests with $p<0.001$ considered significant.

**Results:** Overall FIT uptake reached 42%, significantly higher in women than men (46% versus 38%, $p<0.001$) and in older versus younger participants (45% versus 39%, $p<0.001$). Index colonoscopy attendance was 81%. The programme detected 1.8 CRCs per 1,000 people screened, with PPV for CRC of 5% and NNTScope of 21. Men demonstrated significantly higher FIT positivity (5% versus 3%, $p<0.001$), cancer detection rates (2.5 versus 1.2 per 1,000, $p<0.001$), and PPV (5% versus 4%, $p<0.001$) compared to women, indicating substantial sex-based differences in screening performance.

**Conclusions:** BowelScreen's first decade demonstrates effective CRC detection in the Irish population, though uptake remains below optimal levels. Significant sex-based differences in participation and detection metrics suggest that strategies to increase uptake, particularly among men and younger eligible participants, and sex-tailored screening approaches could substantially improve programme effectiveness and population health impact.

# Identification of Robust MIRNA Biomarkers for Liquid Biopsy-Based Early Oral Cancer Detection Using Integrated Tissue and Literature Evidence

**SY Du**[1,2], M Ward[2], P Ó Broin[3], E O'Regan[1], JEd O'Connell[2,4], JJ O'Leary[1,2], CM Martin[1,2]

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**Introduction:** Early-stage oral cancer has a substantially better prognosis than advanced disease, yet early lesions are often subtle and difficult to distinguish from benign conditions, leading to delayed diagnosis. Over-investigation may also result in unnecessary radiation exposure and biopsies. Therefore, an objective, non-invasive molecular decision aid is urgently needed. Liquid biopsy based on circulating microRNAs (miRNAs) represents a promising solution. This study aimed to identify robust tissue-derived miRNA biomarkers for future liquid biopsy panel development.

**Materials and Methods:** Mature miRNA expression profiles were obtained from isoform sequencing data in The Cancer Genome Atlas (TCGA). Early-stage oral cancer and pooled normal tissues were identified using clinical annotations. Differential expression analysis was performed using limma-voom. Three candidate sets were constructed: tissue-derived differentially expressed miRNAs (SetA), literature-reported liquid biopsy biomarkers (SetB), and their intersection (SetC). Bootstrap stability selection and LASSO-logistic regression were applied for feature selection and model construction. Model performance was evaluated using repeated, nested, patient-level grouped, and batch-grouped cross-validation, as well as permutation testing. External validation was conducted using the Clinical Proteomic Tumor Analysis Consortium (CPTAC) cohort.

**Results:** After stability selection, 7–8 miRNAs were retained in each panel. In patient-level cross-validation, AUCs ranged from 0.977 to 0.998, with repeated and nested cross-validation AUCs above 0.98. Batch-grouped validation yielded AUCs of 0.915–0.960. At optimal thresholds, sensitivities ranged from 95.5% to 97.7% and specificities from 92.2% to 100%. Permutation testing showed a mean AUC of approximately 0.57. External validation achieved AUCs of 0.986–1.000, with perfect sensitivity and specificity.

**Discussion:** By integrating tissue-derived expression profiles and literature-based evidence, this study identified robust miRNA signatures for early oral cancer detection. Consistent performance across multiple validation frameworks supports their reliability and translational potential. These findings provide a solid foundation for developing non-invasive liquid biopsy panels to support clinical decision-making in diagnostically ambiguous early oral lesions.

# Using Structure-Based Modeling to Identify Effective Drug Combinations in RAS-Mutant Acute Myeloid Leukaemia

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**Background:** Mutations activating RAS signalling occur in 15-20% of cancers. These alterations, including mutations in NRAS, KRAS and RAS signalling regulators, are common in acute myeloid leukaemia (AML) and predict poor prognosis. Therapeutic targeting of RAS signalling is challenging, with limited prior success in AML. Here, we used a Systems Biology approach to identify RAF inhibitor (RAFi) combinations as a novel therapeutic strategy in these cases.

**Materials and Methods:** We used a structure-based dynamic model of RAS signalling (RAS/RAF/MEK/ERK), incorporating kinetics of protein-protein and drug-protein interactions, posttranslational modifications, protein structure, and in vivo pharmacokinetics. This approach leverages RAF dimer asymmetry and affinity of different inhibitors (Type 1/1.5/2) for alternative RAF monomer conformations. Combinations of conformation-specific inhibitors were first tested in silico, with computational predictions validated using in vitro and in vivo RAS-mutant AML models.

**Results:** In silico modelling predicted two RAFi combinations as being synergistic against RAS-mutant AML. Lifirafenib (Type 2) + encorafenib (Type 1.5) was highly synergistic in vitro against AML lines harbouring either NRAS or KRAS mutations. Strong synergy was also observed for combined lifirafenib + SB590885 (Type 1) in NRAS-mutant lines, while the combination had an additive effect compared with single-agents in KRAS-mutant lines. Importantly, immunoblotting confirmed that combination efficacy correlated strongly with decreased pathway activation. For further validation in a clinically relevant model, we established a patient-derived xenograft (PDX) of NRAS-mutant AML. Both RAFi combinations were highly effective in vivo, with synergistic increases in overall survival and leukaemia growth delay that correlated with decreased tumour burden and in vivo RAS pathway activation.

**Conclusions:** We have identified novel, non-obvious drug combinations with potent anti-leukaemia effects both in vitro and in vivo. Our systems biology approach was notably validated by high efficacy of well-tolerated treatments in a preclinical mouse model, suggesting rapid clinical translatability for treating RAS-mutant AML.

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# Systemic Inflammatory and Haematological Parameters According to Mismatch Repair Status in Endometrioid Endometrial Carcinoma

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**Background:** Mismatch repair (MMR) deficiency represents a molecularly defined subtype of endometrial carcinoma characterised by distinct tumour biology and enhanced immune activation. Whether routine haematological and biochemical parameters represent systemic signatures of this molecular and immune phenotype remains uncertain. Assessing common preoperative laboratory markers according to MMR status may clarify links between molecular subtype, systemic inflammation and tumour biology.

**Patients and Methods:** A retrospective cohort of 113 patients with histologically confirmed endometrioid endometrial carcinoma were classified as MMR-proficient (pMMR, n=77) or MMR-deficient (dMMR, n=36) as per histopathology reports. Clinicopathological characteristics and pre-treatment laboratory parameters, including haemoglobin, haematocrit, platelet count, white cell count, neutrophil count, albumin, creatinine, c-reactive protein, estimated glomerular filtration rate, platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) were extracted from medical records. Group comparisons were performed using independent t-tests and Chi-square tests.

**Results:** Patients with dMMR tumours had significantly elevated PLR compared to those with pMMR tumours (p=0.0091), while no other routine haematological or biochemical parameters differed significantly. pMMR tumours were more often Grade 1 (p=0.033), whereas lymphovascular space invasion (LVSI) was more common in dMMR compared with pMMR tumours (46.9% vs 23.7%, p=0.023). No differences were observed in FIGO stage, myometrial invasion or lymphadenopathy between groups.

**Conclusion:** These findings suggest that routine preoperative blood parameters are largely similar across MMR subtypes, with the exception of elevated PLR in dMMR patients, consistent with the enhanced systemic inflammatory activity associated with MMR deficiency. The predominance of grade 1 tumours in pMMR patients and higher prevalence of LVSI in dMMR tumours indicates biologically distinct behaviours between these molecular subtypes. Assessing circulating inflammatory biomarkers alongside MMR status may improve understanding of the systemic patterns associated with molecularly defined endometrial cancer subtypes and their interactions with the immune system.

*Thank you to all of the patients who consented to participate in this study, their families, and the staff at St. James's Hospital.*

# Targeting microRNA-31 to Modulate Chemotherapy Response in Pancreatic Cancer

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**Background:** Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest malignancies. Its poor prognosis is in part due to limited patient responses to standard chemotherapy. Platinum agents are the most frequently employed chemotherapeutics, used in the treatment of a wide variety of solid malignancies. However, patients receiving platinum-based chemotherapy eventually succumb to treatment resistance. MicroRNAs (miRNAs) are small, non-coding RNAs that regulate cellular processes involved in chemoresistance. We previously identified miR-31 as a modulator of therapy response in several cancers. Here, we identify a molecular mechanism underpinning miR-31-mediated alterations in chemosensitivity, supporting miR-31 as an important therapeutic target.

**Materials and Methods:** MiR-31 was overexpressed in miR-31–deficient BxPC-3 cells and suppressed in miR-31–abundant Panc-1 cells. Chemosensitivity was assessed via clonogenic assays. Protein expression of candidate miR-31 targets was measured by Western blot. ATOX1 was independently modulated in PDAC cells using overexpression and shRNA vectors. DNA damage response was evaluated by γ-H2AX and 53BP1 detection through Western blot and immunofluorescence. Cytoplasmic and nuclear platinum levels were quantified using ICP-MS.

**Results:** MiR-31 overexpression promoted cisplatin resistance, while miR-31 suppression enhanced sensitivity in PDAC cells. In silico analysis identified ATOX1, a cytoplasmic copper chaperone and transcription factor, as a predicted miR-31 target, with miR-31 expression inversely correlating with ATOX1 levels in PDAC cells. Cisplatin is known to bind the Cu(I)-ATOX1 complex, suggesting a role in intracellular drug transport. Consistently, miR-31 expression inversely correlated with the nuclear accumulation of cisplatin. Moreover, direct overexpression of ATOX1 significantly enhanced cisplatin sensitivity. Conversely, ATOX1 suppression promoted cisplatin resistance but did not alter nuclear platinum levels, suggesting additional mechanisms beyond drug trafficking.

**Conclusions:** Our study demonstrates that miR-31 regulates ATOX1 expression, thereby modulating cisplatin sensitivity in PDAC. Importantly, miR-31–mediated changes in nuclear drug localization are unlikely to represent the primary driver of cisplatin resistance.

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# A Novel Role for Complement in the Pathogenesis and Treatment Resistance of Gastric Cancer

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**Introduction:** Poor pathological response to neo-adjuvant chemotherapy remains a significant clinical problem in gastric cancer. Our lab has previously demonstrated a role for the complement system, a key regulator of immunity, in the progression and treatment resistance in oesophageal and rectal cancer but it's role in gastric cancer is unknown.

**Methods:** RNA sequencing and clinical data for gastric adenocarcinoma (GAC) patients (n=367) were obtained from the Cancer Genome Atlas (TCGA). Complement gene expression was normalised and correlated with clinicopathological factors. The inherent chemosensitivity of GAC cell lines MKN-45 and AGS to oxaliplatin, docetaxel, and 5-fluorouracil (FLOT) was determined by clonogenic assay. mRNA expression was assessed by qPCR. Tumour-conditioned media (TCM) and normal-conditioned media (NCM) was generated from resected gastric tumour tissue and normal adjacent gastric tissue (n=12), with matched metabolic and chemokine/cytokine analysis performed. Metabolic and inflammatory profiles were correlated with C3 expression and clinicopathological factors.

**Results:** C3 and C3aR1 mRNA were significantly upregulated in gastric tumours and correlated with advanced tumour and overall disease stage. High C3 expression was associated with significantly poorer progression-free survival (PFS). Complement upregulation was demonstrated in vitro and ex vivo. In vitro, AGS cells were inherently resistant to FLOT, and demonstrated significantly higher C3 expression, when compared to chemosensitive MKN-45 cells. Ex vivo, TCM contained significantly higher C3 levels than NCM, with C3 secretion correlating with enriched oxidative phosphorylation (OXPHOS) in gastric tumours. Correlation of C3 and OXPHOS revealed significant association with several inflammatory/angiogenic mediators including VEGF, TIE-2, IL-1β, IL-1RA, IL-17.

**Conclusion:** Gastric tumours demonstrate significantly increased expression of C3 and C3aR1, which is associated with advanced disease stage and poorer PFS. C3 is enriched in chemoresistant AGS cells and patient tumour secretomes and is associated with increased OXPHOS and proinflammatory/angiogenic mediators, implicating a novel C3-driven inflammatory-metabolic axis in GAC. This highlights C3 as a potential novel therapeutic target in GAC.

# International multicentre open label randomised controlled trial of intensive surveillance vs. standard postoperative follow-up in patients undergoing surgical resection for esophageal and gastric cancer (SARONG-II)

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**Background:** Follow-up practices after curative resection for oesophageal and gastric cancer vary internationally and there is no high-level evidence that routine imaging surveillance improves survival or health-related quality of life compared with standard clinical review alone. The SARONG-II trial aims to determine whether more intensive postoperative surveillance with scheduled radiological imaging and endoscopy, leads to earlier detection of recurrence and ultimately improves survival and quality of life for patients following curative surgery for oesophageal or gastric cancer.

**Methods:** SARONG-II is an international, multicentre, open-label, two-arm randomised controlled trial(RCT). Patients (>18yrs) who have undergone curative-intent surgical resection for oesophageal or gastric adenocarcinoma or squamous cell carcinoma, with or without neoadjuvant or adjuvant therapy, are eligible. The recruitment target is 952 participants over 3 years to be recruited across participating European centres and randomised 1:1 to either intensive surveillance or standard follow-up. The intensive surveillance arm includes clinical review and CT scans of the chest, abdomen and pelvis every 6 months for 3 years and an upper gastrointestinal endoscopy at 12 months. The standard follow-up arm involves clinical reviews at 6-month intervals for 36 months according to local practice. The primary outcome is overall survival. Secondary outcomes include disease-specific survival, recurrence patterns, recurrence treatment, rates of oligometastatic and multi-metastatic recurrence and health-related quality of life (measured by EQ-5D-5L, EORTC QLQ-C30, QLQ-OG25, and the Cancer Worry Scale).Embedded translational studies include a machine learning study designed to identify early radiological features of recurrence on routine CT imaging, and a liquid biopsy workflow evaluating the utility of circulating tumour DNA(ctDNA) to detect recurrence prior to radiological evidence.

**Results:** As of 05/02/2026,176 patients have been recruited across eight active participating sites. Eleven additional sites are at feasibility stage and a further four sites are at pre-contract stage, with all expected to initiate recruitment within the next six months. Recruitment is ongoing across all active centres.

**Conclusions:** SARONG-II will provide high-quality RCT evidence to inform postoperative surveillance strategies following curative resection for oesophageal and gastric cancer, with integrated translational studies aimed at enabling earlier and more personalised detection of recurrence.

# Integrating Mean Heart Dose Surrogates into an AI-Compatible Workflow for Daily Cardiac Monitoring in Breast Radiotherapy

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**Background:** Estimating mean heart dose (MHD) during breast radiotherapy is challenging when cone-beam CT (CBCT) field-of-view (FOV) only partially visualises the heart. This limitation persists even with AI-based synthetic imaging for monitoring and adaptation. It restricts confidence in daily cardiac dose assessment despite established associations between MHD and late cardiac morbidity in breast cancer survivors. This study identified robust dosimetric surrogates for estimating MHD from truncated cardiac structures, enabling workflow-friendly, RTT-led daily heart-dose monitoring.

**Materials and Methods:** 59 patients (48 deep inspiration breath-hold (DIBH), 11 free-breathing (FB)) receiving 40 Gy in 15 fractions were retrospectively analysed, representing a broad range of cardiac positions and dose levels. Full-heart structures were contoured on planning CTs. Limited-heart structures simulated CBCT truncation by constraining full-heart contours to the CBCT FOV. Heart structures were shifted 3, 5, and 7 mm toward treatment fields to simulate variability, resulting in 205 structures. Practical dose-volume surrogates (D20cc_lim, D40cc_lim, D100cc_lim) and limited mean heart dose (MHD_lim) were compared with true MHD using Pearson and Spearman correlations.

**Results:** All limited-heart surrogate metrics correlated significantly with MHD ($p < 1e-4$). D20cc_lim and D40cc_lim showed strong correlations (r 0.81–0.85, $\rho$ 0.85–0.88; $R^2$ 0.65–0.72, RMSE 0.69–0.77 Gy). D100cc_lim remained moderate to strong (r 0.77, $\rho$ 0.75; $R^2$ 0.59; RMSE 0.83 Gy). MHD_lim demonstrated strongest agreement (r 0.956, $\rho$ 0.948; $R^2$ 0.914, RMSE 0.381 Gy), maintaining linearity within the 1–3 Gy range. Limited heart volumes ranged from 15.5%–94.3% of full-heart volume, yet MHD_lim remained highly predictive across both DIBH and FB cohorts.

**Conclusion:** These findings introduce the first robust dosimetric surrogates for monitoring daily heart dose even when only 15.5%–94.3% of the heart is visible on CBCT imaging. MHD_lim can be integrated into AI-assisted adaptive workflows, supporting RTT-led decisions and long-term cardiac risk reduction in breast radiotherapy.

# Galectin-3 inhibition as a selective treatment to oesophageal adenocarcinoma: a promising new approach

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**Introduction:** Oesophageal adenocarcinoma (OAC) is a dismal prognosis cancer arising from Barrett's oesophagus (BO), a chronic inflammatory disease induced by gastro-oesophageal reflux disease (GORD). Galectin-3 (Gal-3), a β-galactoside-binding lectin, modulates the tumour microenvironment, immune activation and therapeutic resistance. This study investigated Gal-3 in OAC progression and its inhibition as a therapeutic strategy.

**Methods:** Gal-3 and PD-L1 protein levels were assessed via Flow Cytometry and ELISA. Viability and proliferation was assessed using CCK8 and BRDU assays respectively. RNA analysis was done on publicly available datasets and Illumina sequencing of progressors vs non-progressor BO patients.

**Results:** Gal-3 expression significantly increased throughout disease progression in both the cytoplasmic and nuclear compartment of oesophageal epithelia, with PD-L1 surface expression following suit. These expressional increases following metaplastic transition can be induced in normal epithelia following bile acid exposure at concentrations observed in BO patients, significantly increasing up to 72 hours after bile acid exposure.

Gal-3 is significantly upregulated in OAC at the premalignant BO stage. This likely plays an important role in the transition from BO to OAC, as we have shown that BO patients who progress to OAC have significantly higher Gal-3 expression levels compared to non-progressors. Gal-3 inhibition significantly reduced OAC cell viability and proliferation in a dose dependent manner, not seen in the dysplastic, metaplastic or normal cells or primary stromal cells. Gal-3 inhibition significantly reduced PD-L1 OAC expression, making them less immune-evasive and significantly increased cellular calreticulin expression, transitioning the malignant cells to a more immunogenic phenotype without affecting expression in normal or premalignant cell lines.

**Discussion:** The significant increase in Gal-3 levels likely reflects an important homeostatic role in epithelial cells following malignancy transformation. These results suggest that Gal-3 inhibition could offer a selective treatment to OAC by reducing cancer cell survival and enhancing immune response without affecting non-malignant tissue.

*I would like thank all the patients who kindly donated their samples to research which facilitated this study and ensured translational outputs.*

# NK Cells for Glioblastoma: Engineering NK cells to function in the TME

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**Background:** Glioblastoma (GBM) is an aggressive brain cancer with a poor prognosis for patients. Current therapies, such as surgery, radiotherapy and chemotherapy do not substantially improve life expectancy for those diagnosed. Therefore, it is crucial that new therapies are developed. One of the difficulties in the development of new therapies, in particular immunotherapies, is that gliomas are immunologically cold, with very little immune activity. NK cells are one such immune cell that enter the TME in GBM, but they do not succeed in clearing the tumour. This project aims to understand how NK cells are affected by the TME to better develop new immunotherapies.

**Materials and Methods:** NK cells from healthy controls and both the tumour and blood of patients with GBM were analysed via flow cytometry to describe nutrient uptake, cell surface receptors and activation markers. Metabolomic analysis was carried out on interstitial fluid from the tumour and serum of patients and controls to discern the nutrient environment of GBM.

**Results:** Tumour infiltrating NK (TiNK) cells are phenotypically distinct from peripheral blood NK cells. Although most TiNKs have low CD56dim expression, very few co-express CD16. Mitochondrial phenotype is also chaged with increased mitochondrial membrane potential (MMP) and increased mitoROS levels. NK cells from the TME have increased nutrient receptor expression on the cell surface with increased iron uptake, while amino acid uptake was similar to that of peripheral NK cells. Finally, nutrient levels are altered in the TME in comparison to the serum.

**Conclusions:** NK cells found in the TME have increased mitoROS and MMP indicating stress or activation. With reductions in CD16 and GranzymeB these cells are likely less able to kill tumour cells. There are also major changes in the TME in relation to nutrient availability which we are currently dissecting to see the effect on NK cells.

*We would like to thank Mr Stephen MacNally and Eloise Cowie, our clinical collaborators at Beaumont Hospital and all patients who agreed to partake in this study.*

# POSTERS

# All-Ireland Real-World Insights into Young-Onset Gastroesophageal Adenocarcinoma: A Population-Based Analysis

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**Background:** Population-based studies have reported a rising incidence of young-onset (YO) oesophageal and gastric cancer. We examined age-specific differences in clinicopathological features, treatment and outcomes in YO (18-49 yr), average-onset (AO, 50–70 yr), and late-onset (LO, >70 yr) oesophageal and gastric adenocarcinoma.

**Methods:** Population-based data were collected from the National Cancer Registry Ireland and Northern Ireland Cancer Registry. We analysed 21,706 patients diagnosed with oesophageal or gastric adenocarcinoma, (ICD10 C15.0-C16.9) from 1999 to 2022 (censored 31.12.22). Socioeconomic status was categorised into deprivation quintiles using the NICR's Northern Ireland Multiple Deprivation Measure and the NCRI's Pobal HP Deprivation Index. Categorical variables were compared by Chi-squared tests and trend analysis by Cohran-Armitage. Kaplan-Meier (KM) survival curves and log-rank tests were used to present and assess survival differences. Data analysis was undertaken using R v4.4.2. Average annual percentage change (AAPC) was calculated using the JoinPoint software (v5.3.0).

**Results:** The YO cohort accounted for 6.8% of cases. A higher proportion of oesophageal and gastric cancers occurred in the most socioeconomically deprived quintile. YO gastric cancer patients had significantly more diffuse and poorly differentiated tumours ($p<0.0001$). YO patients also presented more frequently with stage IV disease in lower oesophageal and gastric subsites ($p<0.0001$). Incidence increased significantly across YO (AAPC 2.6%), AO (AAPC 3.3%) and LO (AAPC 2.1%) age groups for oesophageal cancer. Gastric cancer incidence was stable in YO but declined in AO (AAPC -2.6%) and LO (AAPC -2.1%) groups. Compared with YO, LO had worse survival (HR 1.23, 95% CI 1.13–1.33, $p<0.001$, multivariable Cox analysis).

**Conclusion:** We identified rising incidence of lower oesophageal cancer across all age groups, while gastric cancer is stable in the young and declining in older adults. Despite more advanced and aggressive disease at presentation, young-onset patients had better adjusted survival, highlighting the need for dedicated studies to clarify mechanisms and optimise care. *This work was supported by Irish Clinical Academic Track Programme and the HRB–Wellcome Trust. We thank the National Cancer Registry Ireland and the Northern Ireland Cancer Registry for data provision and support.*

# Gene enhancers as novel and rational therapeutic targets for impeding colorectal cancer progression

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**Background:** Almost 50% of all colorectal cancer (CRC) patients develop metastatic CRC (mCRC), which has a survival rate of 14% which is further exacerbated by lack of treatment modalities to impede progression of CRC to mCRC. Variable enhancer loci (VEL) represent non-coding DNA sequences regulating target genes, shown to be gained or lost in mCRC and likely regulates CRC progression. The aim of this study was to ascertain the functional impact of these VELs on driving CRC progression and rationalize their role as a potential therapeutic target.

**Material and Methods:** A CRISPRi loss-of-function screen targeting 1,524 VELs (gained or lost in mCRC) was performed in HCT116 cells. The two VELs whose repression caused the greatest cell death were further evaluated for effects on anchorage-independent growth, apoptosis, cell cycle, and migration. Transcriptome-wide impacts were assessed using Hi-C and RNA-seq following VEL silencing. DNA-binding proteins interacting with VELs were identified via mass spectrometry. For in vivo validation, HCT116 cells with VEL knockdown were implanted in a colorectal orthotopic xenograft model, and tumor growth was analyzed alongside Ki-67 and MMP1, expression by immunohistochemistry.

**Results:** CRISPRi screening identified 12 VELs whose repression significantly increased HCT116 cell death; top 2 VELs from screen - VEL_1243 and VEL_1084 (p-adjust < 0.001) were prioritized. Silencing these VELs reduced proliferation and migration across HCT116, SW620, and SW480, with no effect on apoptosis or cell cycle. Hi-C and qPCR confirmed interactions with GINS3 (VEL_1243) and SSB (VEL_1084). RNAseq analysis following VEL repression in HCT-116 cells revealed downregulation of inflammatory and chemokine pathways. VELs were enriched for transcription factors binding sites (RFX7, ELF3), while mass spectrometry identified binding proteins including EEF1D, PARP1, and HSPB1. In vivo experiments showed that VEL knockdown markedly inhibited tumor growth and reduced Ki-67 and MMP1 expression in orthotopic xenografts.

**Conclusions:** VEL_1243 and VEL_1084 function as oncogenic enhancers that promote CRC progression through transcriptional regulation, supporting enhancers as promising therapeutic targets in CRC.

# Challenges of Undergoing Colonoscopy Surveillance for People with Lynch Syndrome

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**Background:** People with Lynch syndrome (LS) have an increased colorectal cancer (CRC) risk and are recommended 2-yearly colonoscopy surveillance throughout adulthood. We assessed challenges of colonoscopy surveillance for people with LS in England.

**Patients and Methods:** We used the 'LS research registry pilot' involving people with LS who previously participated in the Cancer Prevention Programme 3 (CaPP3) trial at four sites (Birmingham, London, Manchester, Newcastle). We developed and administered the 'Views, experiences, and challenges of colonoscopy check-up questionnaire' to 250 eligible people (those aged ≥25 years) from 01/24-04/24. We performed factor analysis of items assessing views/experiences regarding CRC, colonoscopy surveillance, and overall LS care to identify factors and derive factor scores, comparing scores by site, sex, and age group using multiple linear regression.

**Results:** After exclusions, we included 231 participants (92%). The biggest challenges were drinking the laxative due to the taste and volume and feeling anxious about the colonoscopy and possible results. We identified six factors: 1='Perceived limitations in healthcare coordination, information, and support', 2='Satisfaction with most recent surveillance colonoscopy', 3='Difficulty with the bowel preparation and colonoscopy', 4='Perceived benefits of surveillance', 5='Time and financial barriers to surveillance', and 6='Perceived CRC risk and necessity of surveillance'. Factor 1 and 3 scores were higher in women (means=0.17 and 0.38, respectively) than men (means=-0.19 and -0.42, respectively; p-value=0.005 and p-value<0.001, respectively). Factor 1 and 5 scores decreased from the 25-44-year group (means=0.16 and 0.46, respectively) to the 45-64-year group (means=0.08 and 0.00, respectively) and 65-84-year group (means=-0.25 and -0.27, respectively; p-trend=0.04 and p-trend<0.001, respectively).

**Conclusions:** Key challenges with colonoscopy surveillance for people with LS were the bowel preparation and anxiety about the colonoscopy and possible results. There were differences in perceived challenges by sex and age. Participants received care at leading LS centres, and data were largely collected before surveillance moved to the Bowel Cancer Screening Programme. *We thank our patient representatives for their time and invaluable contributions to the study, the CaPP3 trial team, Cancer Research UK, and 40tude for their support, and all study participants for sharing their views and experiences with us.*

# Altered expression of kinetochore proteins nuf2 and hec 1 in cervical pre-cancer and cancer

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**Introduction:** The Nuf2-Hec1 kinetochore heterodimer overexpression results in mitotic checkpoint hyper-activation and the resultant development of aneuploidy. Gene expression work and immunohistochemistry work in our laboratory have shown that Nuf2 and Hec1 were among the most significantly overexpressed genes in cervical cancer cell lines compared to normal cervical tissue, and strong immunostaining positivity was seen in cervical intraepithelial neoplasia. Based on these findings, this study explores Nuf2 and Hec1 over-expression in cervical intraepithelial neoplasia (CIN) as potential biomarkers for cervical pre-cancer and the detection of DNA ploidy status.

**Methods:** Following histopathologist review, 51 cervical tissue blocks from 33 LLETZ were chosen for immunohistochemistry for this initial clinical proof-of-concept study (13 normal tissues from 9 LLETZ, 15 CIN1 tissues from 9 LLETZ and 23 CIN3 tissues from 15 LLETZ). Rabbit polyclonal Nuf2 antibody (Abcam ab230313) and Rabbit polyclonal Hec1 antibody (Thermo-Fischer Scientific PA5-102765) antibodies were optimised, and tissue samples were processed using the Benchmark ULTRA immunostaining system (Benchmark LT, Ventana, Tucson, Arizona, USA) and the OptiView DAB detection Kit (Ventana, USA). The HER2 and Chromosome 17 probes were detected using two-colour chromogenic in situ hybridisation (ISH) in formalin-fixed, FFPE cervical tissue specimens, following staining on BenchMark ULTRA IHC/ISH instruments to detect ploidy status of CIN cases.

**Results:** The study demonstrated that Hec1 and Nuf2 are over-expressed in cervical pre-cancer and cancer. The overexpression of Hec1 and Nuf2, accompanied by the demonstration of aneuploidy with D-DISH staining in CIN3 cervical, indicates that Hec1 and Nuf2 dysregulation is associated with chromosomal instability leading to aneuploidy.

**Conclusion:** The results of this study suggest Hec1 and Nuf2 are potentially useful prognostic and/or diagnostic biomarkers in cervical pre-cancer and cancer, and warrant further exploration.

## P4 Cancer Screening: a multi-modal approach to screening for cervical cancer

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**Background:** Precision medicine plays an important role in cervical cancer screening in an era where HPV-based screening will overtake traditional cytology-based screening. It is important to identify with precision those women requiring appropriate follow-up and management for cervical abnormalities. This study adopts a multi-modal approach for cervical screening which includes a panel of molecular triage options including HPV16/18 genotyping, extended HPV genotyping, cytology, p16/Ki-67 and methylation markers [CADM1, MAL, miR-124, EBP4 and FAM-19] in women who test positive for HPV in primary screening.

**Design:** In partnership with CervicalCheck, The National Cervical Screening programme, CERVIVA are undertaking a longitudinal observational HPV primary screening study which is evaluate different precision medicine strategies for management of an HPV-positive cervical screening test. Cervical cytology samples from approximately 13,000 women undergoing routine cervical screening were tested for HPV. All HPV-positive women were further assessed with HPV16/18 genotyping, extended HPV Genotyping (BD Onclarity), cytology, p16/Ki-67 dual staining and methylation markers [CADM1, MAL, miR1-124, EBP4 and FAM-19]. The performance of different strategies has been examined both cross-sectionally and longitudinally over one screening round (to date) for detection of CIN2+.

**Results:** From the overall study population, the prevalence of HPV was 15.7% (1597/10,177). Overall, 31.3 % (500/1597) of HPV DNA positive women were positive for HPV16/18, 33.9% (534/1574) had an abnormality on cytology, 33.9% (371/1092) tested positive for p16/Ki-67 and 39.4% (390/990) tested positive for methylation markers. Clinical performance was assessed in a range of combinations for detection of CIN2+, sensitivity ranged from 44-96% and specificity from 46-96%.

**Conclusion:** It is likely that the future of cervical screening will involve a precision medicine-based approach. This study evaluates different approaches in a longitudinal study which has followed women through at least one round of cervical screening.

# Green-Synthesised Metal Oxide Nanoparticles as Preclinical Anticancer Agents in Hepatic, Colorectal, and Leukemia Models

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**Background:** Green synthesis of nanomaterials using medicinal plant waste offers a sustainable strategy for developing bioactive agents with potential anticancer applications. Fagonia arabica (Dhamasa booti) possesses a rich phytochemical profile that may act as a natural reducing and stabilising agent for nanoparticle synthesis. However, systematic evaluation of F. arabica-mediated metal oxide nanoparticles in both solid tumours and hematological malignancies remains limited.

**Materials and Methods:** Herbal waste of Fagonia arabica was used for the green synthesis of zinc oxide (ZnO) and iron oxide (Fe2O3) nanoparticles. The nanoparticles were characterised for particle size, crystallinity, morphology, surface charge, and dispersion stability using standard physicochemical techniques. Antioxidant activity, haemolytic compatibility, and cytotoxic potential were assessed in vitro. Anticancer efficacy was evaluated against HepG2 (hepatocellular carcinoma) and HT-29 (colorectal carcinoma) cell lines using MTT assay. In vivo therapeutic performance was further examined in a benzene-induced chronic myeloid leukemia (CML) rat model by analysing haematological parameters, body weight changes, and spleen histopathology.

**Results:** Both ZnO and Fe2O3 nanoparticles exhibited nanoscale dimensions (<35 nm), polycrystalline structure, and stable colloidal behaviour. ZnO nanoparticles demonstrated significantly higher antioxidant activity and stronger cytotoxic effects compared with Fe2O3 nanoparticles and the crude extract, while maintaining minimal haemolytic activity. In vitro, ZnO nanoparticles showed lower IC50 values and greater growth inhibition in HepG2 and HT-29 cells. In the CML rat model, ZnO nanoparticles markedly improved haematological indices, reduced disease-associated cachexia, and restored spleen architecture compared with untreated controls.

**Conclusions:** Fagonia arabica-derived ZnO nanoparticles exhibit superior anticancer efficacy and biocompatibility compared with iron oxide nanoparticles. These findings support their potential as sustainable, plant-mediated nanotherapeutics for both solid tumours and hematological malignancies, while promoting the value-added utilisation of medicinal plant waste within green and translational cancer research. The authors acknowledge the financial sport provided by the Princess Nourah bint Abdulrahman University Researchers Supporting Project (PNURSP2025R158), Princess Nourah bint Abdulrahman University, Riyadh. The authors also thank the Deanship of Graduates Studies and Scientific Research at the University of Bisha for funding through the Fast-Track Research Support program.

# Methylation of miRNA-124 is associated with higher risk of cervical pre-cancer in older women

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**Introduction:** Cervical cancer remains a major global health burden. In Ireland, around 300 women are diagnosed annually, with more than 90 deaths and ~6,500 women requiring treatment for cervical intraepithelial neoplasia (CIN). Effective triaging strategies are required for improved risk stratification of HPV-positive women. DNA methylation biomarkers have demonstrated their utility as potential triage markers for the detection of CIN2+ among HPV-positive women. However, age-related biological changes, particularly in women aged ≥50 years, may influence methylation patterns and be potentially useful biomarkers for managing older women.

**Methods:** This is a retrospective observational analysis conducted using data from The CERVIVA HPV Primary Screening Study. A total of 13,446 women were initially enrolled, of whom 12,169 were fully consenting. HPV-testing was performed using the Cobas HPV test. Methylation analysis was performed on the HPV-positive (n=993) using a panel of methylation biomarkers (CADM1-M18, MAL-M1, and hsa-miR-124-2). In this study, 901 samples with histological follow-up were included. Receiver operating characteristic (ROC) analysis was performed to determine cut-off values for each marker and combined marker panels based on the histology. Methylation status was dichotomised, and associations between age (<50 vs ≥50 years) and methylation status were assessed using fisher's exact test.

**Results:** We observed a significant association ($p<0.001$) in the expression patterns of hsa-miR-124-2 and the combined panels (CADM1-M18 + hsa-miR-124-2), (MAL-M1 + hsa-miR-124-2) and (CADM1-M18 + MAL-M1 + hsa-miR-124-2) among older women>50 years compared with younger women<50 years. No statistical significance was observed with CADM1-M18 ($p=0.176$) or MAL-M1 ($p=0.298$) or the marker combination CADM1-M1 + MAL-M1 ($p=0.203$).

**Conclusion:** Following Dr. Reynold's PhD work, this study demonstrates that methylation patterns of specific biomarker panels are higher in older women suggesting hsa-miR-124-2 methylation may be a useful biomarker for triage and stratification of older women who may be at higher risk of developing cervical pre-cancer/cancer.

# Synthetic Health Information for Enhancing the Prevention, Early Diagnosis and Treatment of Gynaecological Cancers

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The application of artificial intelligence and machine learning for healthcare continues to face fundamental constraints: strict GDPR compliance requirements, limited access to diverse patient cohorts, and a scarcity of comprehensive longitudinal datasets. The Synthetic hEalthcare_dAta_goveRnanCe_Hub (SEARCH) programme, led by TCD, supported by an IHI JU grant, aims to integrate synthetic data generation technologies with federated learning models to generate secure, privacy-preserving synthetic health datasets to ensure protection of sensitive patient data while accelerating data-driven innovation.

Within SEARCH, TCD is leading two pioneering clinical studies to address specific research questions in gynaecological cancers:

**1.** To explore how synthetic data can help optimise cervical cancer screening protocols, especially in the context of HPV-vaccinated women, who may be at lower risk. Using SEARCH, we can generate a simulated population based on CERVIVA HPV Primary Screening Study data that reflects these variables (e.g. age, HPV genotype, p16ki67, methylation biomarkers, and cytology). This enables us to model different screening algorithms to assess how they might impact cancer detection and outcomes. **2.** To address the urgent need for improved prognostic stratification in high-grade serous ovarian cancer. Since clinical and genomic factors alone inadequately explain outcome variability. By combining clinical data from 1,750 patients with genome sequencing of 150 cases from the Trinity St James's Cancer Institute Biobank, we aim to generate synthetic multimodal datasets that preserve complex genomic-clinical relationships and allow us to refine triage strategies and treatment decisions.

Both studies employ state-of-the-art generative models with rigorous privacy safeguards and comprehensive validation. A federated learning platform underpins the entire process, enabling decentralised analytics while maintaining data sovereignty. Together, these studies establish a new paradigm for ethical, privacy-preserving, and scalable AI-driven clinical decision support in gynaecological oncology, bridging clinical research, data governance, and patient trust to accelerate the next generation of evidence-based cancer prevention, diagnosis and treatment.

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# A Patient and Public Involvement Approach to Exercise Oncology: Co-Designing a Pre-Radiotherapy Exercise Programme for Lung Cancer Patients

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**Background:** Exercise oncology research has grown significantly, with the latest evidence showing post-treatment exercise can reduce the risk of cancer recurrence and mortality. High-quality efficacy trials remain a priority, however a recent systematic review identified mean recruitment as only 38%. Patient and public involvement (PPI) is increasingly being adopted to overcome barriers to research engagement. This PPI project sought to collaborate with people impacted by lung cancer (LC) to co-design an acceptable pre-radiotherapy exercise program tailored to the needs of this population.

**Materials and Methods:** This project was underpinned by Boyd's (2012;2010) co-design approach and the principles of accelerated experience-based co-design. People impacted by LC were invited to attend two online workshops. Additional meetings with an expert advisory group were hosted before and after each workshop. Workshops were recorded, transcribed and analysed using reflexive thematic analysis. Findings relevant to programme design were deducted into the FITT principles, and barriers and facilitators deducted into the five domains of the PRACTIS guidelines.

**Results:** Seven participants attended the co-design workshops. Regarding programme design, attendees reported that daily exercise is suitable for radiotherapy treatment plans of four weeks or less, providing sessions are integrated with radiotherapy treatment and 20 minutes in duration. Intensity levels must be personalised and autoregulated. Furthermore, attendees identified 15 barriers and 40 facilitators to maintain patient-centred research, including the importance of supervised exercise, appropriate timing of study presentation, and the role of a relational exercise coach. Strategies to sustain participant motivation included integrating exercise education, tracking, and self-reflection throughout the programme

**Conclusions:** Co-design with people impacted by LC resulted in a pre-radiotherapy exercise programme that was perceived as acceptable to LC patients' needs during radiotherapy. Integrating participant-identified design features and implementation strategies may enhance engagement and recruitment in future exercise trials, helping to address persistent challenges in exercise oncology research.

This work was supported by the Department for the Economy and Queen's University Belfast. The authors gratefully acknowledge the support of all advisory group members and workshop attendees who generously volunteered their time to support and facilitate this project. We also thank the Northern Ireland Cancer Research Consumer Forum for providing initial feedback on the project conception. In addition, we acknowledge the support of PPI groups and local charities across the UK and Ireland who assisted by sharing advertisements for the workshop events with their members.

# Extended Genotyping of High-Risk HPV Types Infecting the Anal Canal Among an Irish Population of Men Who Have Sex with Men (MSM)

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**Background:** The incidence of anal carcinoma is rising, particularly among high-risk groups such as HIV-positive individuals and men who have sex with men (MSM). HPV infection is a major risk factor for development of anal carcinoma leading to increasing interest in developing HPV-based methods for anal cancer screening. Ireland's HPV vaccination policy has evolved during this project, with Gardasil 9 now offered as part of national programme to HIV+ MSM up to 45y of age. Our objective was to conduct extended genotyping for high-risk (hrHPV) types among a cohort of MSM's with hrHPV infection in the anal canal to understand HPV type prevalence and impact of HPV vaccination and potential screening strategies.

**Materials and Methods:** This research was part of a larger study exploring anal cancer prevention in a cohort of HIV-Positive and HIV-Negative MSM attending the Department of GU Medicine and Infectious Diseases at St James's Hospital and other community based STI clinics. Anal swabs (n=252) collected in Preservcyt were screened for hrHPV using the cobas® HPV test. hrHPV positive cases were subjected to extended genotyping using the BD OnclarityTM HPV Assay. This assay detects 14hrHPV genotypes, with HPV 16,18,31,45,51 and 52 individually reported and types (33/58), (56/59/66), and (35/39/68) reported in groups.

**Results:** Samples from 153 patients were tested using the BD Onclarity™ assay of which 115 gave valid result. 71% (82/115) tested positive for HPV using the BD Onclarity™ assay. HPV 16 had the highest prevalence at 50% (41/82), followed by HPV genotypes 56/59/66 at 28% (23/82). Of those with high-grade lesions on cytology (n=28), HPV 16 was the most predominant type, 57% (16/28) followed by types 56/59/66 29% (8/28).

**Conclusions:** This data provides important insights into the specific hrHPV types prevalent in the anal canal of a cohort of MSM in Ireland. This will inform future screening and cancer prevention policies.

# Aptamer SELEX as a serum biomarker discovery platform for ovarian cancer: optimising the pipeline using HRP as a target

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**Background:** Ovarian cancer remains the deadliest gynaecological malignancy, largely due to the absence of reliable biomarkers for early detection or population screening. Aptamers are short single-stranded oligonucleotides capable of binding specific molecular targets, offering a powerful approach for identifying subtle biochemical differences, such as those distinguishing malignant from benign serum. We developed an HRP-based proof-of-concept experimental pipeline to model and validate the discovery of novel serum biomarkers using an aptamer-based selection and detection system.

**Materials and Methods:** Systematic Evolution of Ligands by Exponential Enrichment (SELEX) was carried out using Horseradish peroxidase (HRP) as a model target, spiked into human serum at physiologically relevant biomarker concentrations mimicking real-life conditions. In parallel, a pure-HRP SELEX experiment was conducted to generate HRP-specific aptamers. 3 rounds of selections were carried out to enrich for HRP specific aptamers. Following the selection, a magnetic beads-based assay was developed to test whether HRP could be detected using generated aptamers. Resulting aptamer pools were sent for Next-Generation Sequencing.

**Results:** Sequencing data is currently underway; successful proof-of-concept outcomes are expected to show reproducible enrichment of identical or closely related aptamer motifs across both SELEX streams (serum spiked and pure HRP). These overlapping sequence clusters would validate the platform's ability to identify target-specific binders even within complex biological fluids. Pure HRP sequencing data showed enrichment suggesting HRP-specific aptamers, while the serum HRP sequencing has been delayed and is yet to be analysed. The HRP-detection assay is being optimised, following preliminary experimental runs suggesting adjustments to improve binding and signal detection.

**Conclusions:** This work establishes a clinically relevant framework for serum biomarker discovery using aptamer technology. The HRP proof-of-concept demonstrates the feasibility of detecting specific protein signatures in serum without prior biomarker knowledge. This approach lays the foundation for identifying novel circulating biomarkers for ovarian cancer, ultimately supporting earlier diagnosis and improved patient outcomes.

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# Characterisation of the Inherent Radiosensitivity of Lung Cancer Cells

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**Background:** Lung cancer (LC) is the leading cause of cancer-related deaths in Ireland, with a 5-year survival rate of <18%. Approximately 60% of patients with LC will receive radiotherapy as part of their treatment pathway. Unfortunately, resistance to radiotherapy is a significant clinical challenge resulting in treatment failure, tumour recurrence and poor prognosis. Investigating mechanisms underlying radioresistance is crucial to identify biomarkers and novel therapeutics to overcome this radioresistant phenotype. Currently, there is a lack of radioresistant research models to investigate mechanisms of treatment resistance in LC. This study aimed to address this global unmet need by identifying an in vitro model of inherent radioresistance in this cancer type.

**Materials and Methods:** Clonogenic assays were used to determine the inherent radiosensitivity profiles of non-small cell lung cancer (NSCLC) A549 and SKMES-1 cell lines and the small cell lung cancer (SCLC) CRL-2066 cell line, following treatment with X-ray irradiation (Xstrahl RS225 X-ray irradiator) at doses of 2 Gy, 4 Gy and 6 Gy.

**Results:** A549 NSCLC (adenocarcinoma) cells were significantly more radioresistant than SKMES-1 (squamous) cells, following treatment with 4 Gy ($p<0.05$) and 6 Gy ($p<0.01$) X-ray irradiation. While X-ray irradiation significantly ($p<0.0001$) reduced the surviving fraction of CRL-2066 SCLC cells at 2 Gy, 4 Gy and 6 Gy, the A549 cell line was shown to represent an in vitro model of inherent radioresistant LC at 6 Gy, when compared to SKMES-1 ($p<0.01$) and CRL-2066 cells ($p<0.001$).

**Conclusions:** Establishing a research model of inherent radioresistance in LC is paramount for addressing the global unmet need to investigate mechanisms of radioresistance in LC. These models can be used to enable the investigation of molecular mechanisms underlying radioresistance in LC, help identify biomarkers of radioresistance, in addition to testing novel therapeutics to overcome radioresistance and enhance treatment response to radiotherapy in LC patients.

# Examining Irreversible Electroporation as a potential therapy for Barrett's Oesophagus

**L Smith**[1], C Gargan[1], M McElheron[2], R Lyons[1], S Cahill[1], A Uí Mhaonaigh[1], F O'Connell[1], C O'Donovan[1], M O'Brien[1], C Kennedy[3], A Alasmar[3], N Donlon[3], C Donohue[3], C Dunne[3], D Kevans[3], N Ravi[3], D O'Toole[3], J Reynolds[1], D Soden[4], J O'Sullivan[1]

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Barrett's Oesophagus (BO) is the only known precursor for oesophageal adenocarcinoma. 57% of oesophageal adenocarcinoma patients when diagnosed have Barrett's in their oesophagus. If Barrett's progresses to dysplasia, patients receive Radio Frequency Ablation, a thermal treatment with side effects. We hypothesise that Irreversible electroporation (IRE) which delivers high voltage pulses to trigger non-thermal cell death is a feasible new treatment option. It is already in trials for various malignancies with encouraging results.

This study recruited 57 patients. Initially, we compared the response of 47 Barrett's and normal human ex vivo tissue biopsies to IRE. Following treatment for 24 hours, a 54-plex ELISA was performed to examine the inflammatory profile of the BO microenvironment. IRE reduces many inflammatory mediators notably IL-6, IL-8 and TNF-$\alpha$ ($p<0.0001$) while increasing some cytokines such as IL-1Ra and IL-7 ($p<0.01$) which can have protective roles in malignant transformation. There was no significant difference in response depending on disease severity nor segment length.

Flow cytometric analysis was performed on digested tissue after treatment on 10 matched Barrett's and normal adjacent tissue biopsies to assess cell viability, surviving cell populations and DAMP expression. For 7 of these, matched haematoxylin and eosin analysis was performed. The viability of Barrett's tissue was significantly decreased after IRE ($p<0.0001$). Expression of Calreticulin was significantly increased on live cells in Barrett's tissue after treatment. HMGB1 and Calreticulin expression was significantly increased on normal tissue. DAMP expression was predominantly on epithelial cells for both tissue types. IRE significantly reduced epithelial and stromal cells in Barrett's tissue but did not significantly decrease CD4+, CD8+ or dendritic cell populations in either tissue type.

IRE significantly reduces inflammatory mediators in the BO microenvironment and reduces the viability of BO tissue without affecting immune populations. We hope this could be an alternative, gentler treatment option.

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# Age-Specific Colorectal Cancer Incidence Trends in England: A 25-Year Population-Based Study

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**Background:** Colorectal cancer (CRC) incidence trends can reflect emerging patterns in disease, inequities in cancer burden and effectiveness of screening interventions. Changes to the screening programme and reports of increasing CRC incidence in younger people underscore the importance of examining temporal trends in incidence.

**Patients and Methods:** Using data from CORECT-R (1997-2021), age-specific CRC incidence trends by sex, year of diagnosis, and cancer subsite were examined. Rates were calculated for age groups: 15-34, 35-39, 40-49, 50-59, 60-69 and ≥70 years. Temporal trends in CRC incidence were analysed using Joinpoint Regression, with annual percentage changes (APCs) estimating the rate of change over time.

**Results:** Among 793,407 incident CRC cases (55% men), 63% were distal and 37% were proximal; 95% were late-onset (age ≥50 years). For distal CRC, incidence declined in ≥70 year-old men from 2010 (APC -1.83%; 95% CI-3.68 to -1.10) and from 2011 in women (APC -2.41%; 95% CI -3.93 to -1.66), remained stable in those aged 60-69 years and in 50-59 year-olds, increased in women across the entire period (APC 0.73%; 95% CI 0.42 to 1.07) but not in men. For early-onset distal CRC, incidence increased from 2009 in 40-49 year-old men (APC 3.37%; 95% CI 2.40 to 5.72), and throughout the entire period in women (APC 2.17%; 95% CI 1.65 to 2.77), increased from 2009 in men and 2010 in women aged 35-39 years, and in 15-34 year-olds, increased throughout the time period in women and from 1997-2014 in men. Proximal cancer incidence generally increased over time across most age and sex groups.

**Discussion:** Declining distal CRC in ≥70-year-olds post-2010 may reflect screening impact. While only 5% of CRCs occurred in adults <50 years, incidence increased substantially in this screening-ineligible population. These findings are consistent with rising early-onset CRC rates, highlighting an emerging public health concern that warrants screening policy consideration.

# Dealing with success - Implementing a pathway for re-irradiation in radiation oncology

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**Background:** The success of modern radiotherapy (RT) has resulted in increasing numbers of patients returning for retreatment (reRT). At SLRON SJC, almost 20% of patients attending for treatment have had previous RT. Failure to account for reRT can result in toxicity to unaffected organs at risk (OAR) and/or poor local control of gross disease. Implementing a workflow in the RT process that adequately accounts for reRT is a considerable technical, scientific and clinical challenge and must be tackled collaboratively by the multidisciplinary team.

**Materials and Methods:** At SLRON SJC a standardised workflow has been developed incorporating (i) the data retrieval of 3D dose and anatomical information from previous treatment (ii) the registration of current and previous images series (iii) the use of radiobiological summation to present accumulated dose from multiple RT courses (iv) the development of site specific dose volume constraints (DVC) for reRT (v) the integration of this workflow in the generic RT process without introducing delay.

**Results:** The reRT pathway was introduced in August 2025 and to date 124 patients have been successfully treated. The initiative has resulted in the development of a platform for sharing patient data both nationally and internationally, the configuration of specialised software for registering image series and graphically displaying radiobiologically summed dose and collaboration with international partners to determine safe but effective DVC's. Patients are accrued onto the multi-institutional E2-Radiate EORTC clinical trial where follow up will be shortly available.

**Conclusions:** The multidisciplinary team at SLRON helped design and implement a reRT workflow that would ensure that the main challenges of reRT were met and addressed for each individual patient. Similarly the workflow was designed such that sufficient information was available at each step of the process for clinical decision making.

# Trusted AI Across Four National Cancer Screening Programmes: Ireland's Governance Framework

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**Background:** Artificial intelligence (AI) presents transformative opportunities for population screening through enhanced diagnostic accuracy, improved efficiency, and expanded access to services. Ireland's National Screening Service (NSS) operates four national programmes (BreastCheck, CervicalCheck, BowelScreen, and Diabetic RetinaScreen) serving over 1.3 million participants annually. AI implementation in screening presents unique challenges including clinical risk management, unclear governance pathways, regulatory compliance, and ensuring public trust. Ireland's evolving policy landscape, including Digital for Care, AI for Care, and the HSE AI Implementation Framework, necessitates robust governance structures for responsible AI deployment.

**Materials and Methods:** We developed a comprehensive AI governance programme through a multi-phase approach: defining scope across clinical and administrative use cases; assembling an MDT balancing AI expertise with operational leadership; procuring external expertise; establishing ethical principles aligned with clinical governance standards; and designing a tiered governance structure. The framework comprises a Strategic Advisory Committee providing ethical and strategic guidance, supported by programme-specific project governance using risk-based approaches. Terms of reference encompass patient perspectives, transparency, equity, data governance, privacy considerations, and performance monitoring.

**Results:** The governance structure successfully guides AI evaluation across multiple screening contexts including mammographic imaging analysis in BreastCheck, digital pathology applications in CervicalCheck and BowelScreen, and retinal image interpretation in Diabetic RetinaScreen. The framework integrates emerging national policies while maintaining flexibility for programme-specific requirements. Key learnings include: investing appropriately in governance infrastructure; balancing command-and-control with adaptive approaches; managing stakeholder expectations while identifying quick wins; integrating research methodologies; and fostering AI literacy among staff who demonstrate enthusiasm for innovation.

**Conclusions:** Implementing AI in population cancer screening requires structured governance that balances innovation with safety, transparency, and public trust. Ireland's experience demonstrates that successful AI governance necessitates multidisciplinary collaboration, alignment with evolving policy frameworks, patient-centered perspectives, and iterative learning. This governance programme provides a replicable model for service providers in the HSE navigating AI implementation.

## Uptake of Cervical Screening and Attitudes to HPV Self-Sampling in Irish Traveller Women, an Ethnic Minority Group

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**Background:** The Traveller population is a distinct minority ethnic group, formally recognised by the Irish Government in 2017. Irish Travellers have a lower life expectancy and poorer health expectancy than the general population. Internationally, HPV self-sampling is being considered as an option to improve uptake in cervical screening, particularly in underrepresented groups. It is not currently offered in Ireland, but assessment of acceptability is ongoing. The aim of this study was to determine the uptake of cervical screening and the acceptability of HPV self-sampling in eligible Traveller women.

**Materials and Methods:** A face-to-face survey by and with Travellers, adapted from the National Cancer Control Programme's (NCCP) 2022 National Survey on Cancer Awareness, was conducted by Traveller Community Health workers in partnership with Pavee Point Traveller and Roma Centre.

**Results:** 483 Travellers participated; 306 (63.1%) were women and 191 (39.5%) were in the eligible group for cervical screening (women aged 25-65). Of these, 142 (74%) had ever received an invitation; of those who received an invitation, 125 (89%) attended at least once. Over 73% of those attending cervical screening advised they attended all screening appointments. 37 (19.4%) reported HPV self-sampling as acceptable, 87 (45.5%) as unacceptable to them and 31 (16.2%) were unsure and would like more information. Of the 37 who reported HPV self-sampling as acceptable, 18 (50%) were non (7) or irregular (11) attenders at screening.

**Conclusions:** Traveller women engage well with cervical screening overall but there is room for improvement. HPV self-sampling has the potential to overcome, for some Traveller women, the practical and personal barriers which may prevent them from responding to standard cervical cancer screening.

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## Listening to Patients in Real-Time: Ireland's Digital Patient Experience Programme Across Cancer Screening Programmes

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**Background:** Systematic real-time capture of patient-reported experience measures (PREMs) remains uncommon in population screening. Ireland's National Screening Service developed a digital PREMs programme gathering continuous patient feedback across three national cancer screening programmes (BreastCheck, BowelScreen, Diabetic RetinaScreen) serving over one million participants annually. This digital-first approach aligns with health service digitalisation while addressing the need to listen to patient experiences. The programme aims to identify quality improvement opportunities rapidly, test SMS-based survey feasibility, and position Ireland internationally in patient experience measurement.

**Materials and Methods:** We designed pathway-specific digital surveys capturing experiences from invitation through results across BreastCheck (invitation, accessibility, communication, environment, discomfort, satisfaction), BowelScreen (FIT test, colonoscopy preparation and procedure, post-procedure care), and Diabetic RetinaScreen (invitation, appointment, timeliness). Surveys utilise validated questions where available and operate in "always-on" mode. Invitations are delivered via SMS following screening episodes. All responses are anonymized, capturing quantitative ratings and qualitative feedback. An interactive dashboard enables real-time monitoring and rapid identification of improvement opportunities.

**Results:** Response rates ranged from 38-64% for initiation and 23-49% for completion. BreastCheck achieved exceptional net promoter scores (NPS) of 84, with 86.5% rating experiences as good or very good, representing the largest patient experience survey in breast cancer screening worldwide. BowelScreen achieved 92.2% good/very good ratings with NPS of 76. Diabetic RetinaScreen launched October 2025. The digital approach captured granular feedback identifying "marginal gains" opportunities while demonstrating feasibility in older populations. CervicalCheck implementation planned Q2 2026 completes national coverage.

**Conclusions:** Ireland's digital PREMs programme demonstrates that real-time, continuous patient experience measurement is feasible across national screening programmes using SMS-based surveys. The interactive dashboard transforms patient voices into actionable insights, enabling rapid quality improvements while positioning Ireland internationally in patient experience measurement. This model provides a scalable framework for embedding patient partnership systematically into service delivery and continuous improvement.

# Raman Spectroscopy Approaches for Early Detection of Gynaecological Cancer Using Liquid Biopsy

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**Background:** Ovarian-cancer continue to elude early detection due to lack an effective screening test, with over 70% of cases detected at FIGO stage III–IV, where five-year survival drops below 30%, a diagnostic gap that contributes significantly to mortality. For Endometrial-cancer, current diagnostic pathways rely on invasive tissue sampling. Minimally invasive liquid biopsy capable of detecting molecular alterations early with high accuracy would offer substantial clinical benefit. Raman spectroscopy provides a label-free biochemical fingerprint of biofluids with minimal processing. The aims of this study were to (1) investigate the influence of pre-analytical variables (sample processing/storage conditions) on Raman spectra of plasma & serum, (2) differentiate ovarian and endometrial-cancers from benign gynaecological-conditions using Raman spectroscopy, and (3) compare diagnostic performance between plasma & serum

**Materials and Methods:** Matched plasma and serum samples from healthy donors (n=3) and from patients with gynaecological malignancies (n=10 High-Grade-Serous-Carcinoma-Ovarian-Cancer, n=10 endometrioid-endometrial adenocarcinoma) and from patients with benign gynaecological conditions (n=10) were analysed.

Raman measurements of plasma and serum (60 ml in 96-well plate) were performed using a Horiba-Jobin-Yvon-LabRAM HR800-spectrometer (785nm source laser, 60× water immersion objective lens). Ten spectra per sample were recorded (20s acquisition time, 2 accumulations). Spectra were pre-processed before multivariate analysis.

**Results:** Raman spectra capture characteristic molecular constituents, including protein, lipid, and carbohydrate bands. Raman spectra were stable across repeat freeze–thaw cycles and anticoagulant tube types. In contrast, repeated rapid-thawing produced reproducible spectral shifts in plasma and serum consistent with protein conformational change. Preliminary comparisons showed subtle but consistent differences between Stage IA endometrial-cancer and benign endometrial-conditions in both plasma and serum. Advanced ovarian-cancer exhibited larger deviations from benign-ovarian controls. PCA and PLS-DA models were applied to evaluate discrimination.

**Conclusions:** Optimising pre-analytical handling and biofluid selection enhances reproducibility in Raman-based analysis. Distinct spectral differences between cancer and benign groups highlight Raman spectroscopy's potential as a rapid, non-invasive tool for early detection of gynaecological malignancies.

# Exposing the Mechanisms Underpinning Pancreatic Cyst Progression to Pancreatic Cancer

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**Introduction:** Pancreatic cancer (PC) has the worst 5-year survival rate of any cancer at just 13%. Pancreatic cystic lesions (PCLs) are fluid-filled sacs on the pancreas, which can be benign or pre-malignant. While 8% of the population will develop a PCL, and 15% of all PCLs progress to PC, the mechanism of progression from PCL to PC is completely unknown. We hypothesise that the biologically rich fluid within PCLs, pancreatic cyst fluid (PCF), contains factors which drive this progression.

**Methods:** H6c7-normal and HPNE-intermediary human pancreatic cell lines were cultured with 5% v/v of either low- or high-risk patient PCF for 24 h. Proliferation (CCK-8), cytotoxicity/viability/apoptosis (ApoTox-Glo), metabolism (Seahorse), EMT/immune evasion/growth (Flow Cytometry), invasive potential (Invasion assay), and EMT/morphology (fluorescent microscopy) were assessed. Functional outcomes were correlated with PCF proteomics (LC-MS), and Reactome pathway analysis elucidated potential mechanisms of progression.

**Results:** PCF elicited differing biological effects between the H6c7-normal and HPNE-intermediary cells. In H6c7-normal cells, PCF exposure: significantly increased cell viability, %invasive, and vimentin expression; significantly decreased apoptosis and PD-L1 expression; caused a significant metabolic shift towards glycolysis; and significantly altered cell morphology ($p<0.05$). In HPNE-intermediary cells, PCF exposure: significantly decreased proliferation; and significantly increased apoptosis and EGFR expression ($p<0.05$). PCF was cytotoxic to both cell lines, with PCF from high-risk patients being most cytotoxic in both cases.

PCF-contained proteins correlated with significantly altered functional outcomes enabling pathway mapping. For example, cytotoxicity significantly correlated with proteins associated with upregulation of MMP activation and ECM degradation (CTRB1/CTRB2/PRSS2), and downregulation of immune system functions and complement cascade activation (C9/CPN2/FCN3/IGHG2).

**Conclusions:** Normal pancreatic cell functions can be significantly altered following 24 h exposure to 5% v/v patient-derived PCF. These data suggest a role for PCF-contained proteins in the progression to PC, thus indicating that routine aspiration of this fluid from all PCL patients may prevent the development of PC.

# Temporal Patterns of Invasive Breast Cancer Incidence in Ireland Between 2004 and 2019: A Comparative Age-Period-Cohort Analysis

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**Background:** International evidence highlights increasing breast cancer (BC) incidence and changing incidence patterns according to tumour subtype, particularly among younger women. At present, it is unclear if these patterns are apparent in Ireland. Therefore, the study aimed to describe changes in invasive BC incidence in Ireland by age and tumour subtype over time.

**Methods:** Demographic and clinical characteristics of female invasive BCs diagnosed from 2004 to 2019 were ascertained from the National Cancer Registry of Ireland (NCRI). Missing data on tumour characteristics were imputed using fully conditional specification. Incidence rates, age-standardised to the 2013 European Standard Population, were stratified by age and tumour subtype defined by hormone receptor (HR) status (combined oestrogen receptor (ER)-progesterone receptor (PR) status) and human epidermal growth factor receptor 2 (HER2) status. Average annual percent changes and 95% confidence intervals in incidence rates were estimated from joinpoint regression models. Pattern heterogeneity in subtype-specific incidence over the study period was evaluated using comparative age-period-cohort modelling.

**Results:** Between 2004 and 2019, 46,005 invasive BC cases were confirmed by NCRI. Among all age groups except women aged 40–49 years at diagnosis, BC incidence increased between 2004 and 2019. Similarly, the incidence of the HR+/HER2- subtype increased among all age groups except women aged 40–49 years at diagnosis. For all other subtypes (HR+/HER2+, HR-/HER2+, HR-/HER2-), incidence increased only among women younger than 40 years at diagnosis. When adjusted for age, period and cohort effects, markedly increasing incidence was apparent among these three subtypes after 2014 among women aged younger than 40 years.

**Conclusions:** These findings highlight temporal patterns of increasing BC incidence in Ireland, with changes most apparent among women younger than 40 years at diagnosis from 2014. This study is currently ongoing and further analyses will evaluate subtype-specific incidence trends according to age and stage at diagnosis.

# Benign Biopsy Patterns Within a Population-Based Breast Screening Setting in Ireland, 2008 to 2019

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**Background:** Approximately 40–75% of breast biopsies prompted by a screen-detected abnormality yield benign findings. Depending on the histology of the lesion, benign biopsies are associated with one- to four-fold increased breast cancer risk. Describing trends in the incidence of benign biopsies in a population-based screening setting may provide opportunities for enhancing aetiological understanding of breast cancer.

**Methods:** This analysis utilised aggregate anonymous data from women aged 50–69 years who attended at least one mammographic screening episode within the breast cancer screening programme in Ireland from 2008 to 2019, were recalled for further assessment, and underwent a biopsy that resulted in benign findings (n=12,430). Incidence rates per 10,000 women screened were calculated by screening round (prevalent, incident), 5-year age at biopsy, biopsy method (percutaneous, surgical) and final benign result. Joinpoint analyses were used to evaluate changes in incidence by estimating the average annual percentage change (AAPC) and corresponding 95% confidence intervals (CI).

**Results:** Benign biopsies within prevalent screening examinations, i.e., first attendances at screening, accounted for 66% of all benign biopsies, and the incidence rate increased between 2008 to 2019 (AAPC 4.6, 95% CI 3.7–5.4, $p<0.01$). When further examined by age at biopsy, this increase occurred only among women aged 50–54 years (AAPC 9.8, 95% CI 8.0–11.8, $p<0.01$). Rates of benign biopsies within incident screening examinations were stable over time and across age groups.

**Conclusion:** Findings from this analysis shows that from 2008 to 2019, the incidence of benign biopsies increased within prevalent screening rounds of the breast screening programme in Ireland among women aged 50–54 years. This study highlights a growing population of women undergoing biopsies who may be at increased risk of future breast cancer.

# Supporting the AYA Journey from Radiotherapy Treatment and Beyond: A Multidisciplinary Perspective on Information, Empowerment and Survivorship in Hodgkin Lymphoma Care

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**Background:** Hodgkin's Lymphoma (HL) is one of the most common cancers among Adolescents and Young Adults (AYAs) in Ireland, accounting for approximately 20% of annual cases. With five-year overall survival rates of around 95%, most AYAs will transition into survivorship. However, AYA cancer care in Ireland remains in a developmental phase, characterised by fragmented pathways, inconsistent access to supportive services, and limited structured long-term follow-up. As a result, self-management is a critical component of survivorship to mitigate late effects of treatment. This study explored multidisciplinary perspectives on information provision and the empowerment of self-management for AYAs with HL across the care continuum.

**Materials and Methods:** A descriptive qualitative design was adopted. Purposive sampling recruited healthcare professionals involved in the multidisciplinary care of AYAs with HL to take part in semi-structured interviews which were analysed using thematic analysis.

**Results:** 15 participants included oncologists, haematologists, clinical nurse specialists, physiotherapists, and occupational therapists took part in the study. Five interrelated themes were identified: context of AYA HL care; information provision and communication; self-management development; professional roles and relationships; and rehabilitation and recovery post-treatment. Care was described as fragmented, with variable implementation of AYA pathways. Information provision focused primarily on acute treatment issues, with limited attention to long-term recovery and late effects. Allied health involvement was often reactive rather than routine, leading to inequitable access. Support for self-management was commonly delayed until after treatment, when AYAs were perceived as better positioned to engage. Follow-up practices varied across sites, ranging from structured end-of-treatment summaries to informal, ad hoc approaches.

**Conclusions:** AYA HL care in Ireland continues to evolve, yet inconsistencies in service provision and timing of information persist. Structured survivorship resources with increased allied health involvement, and timely information delivery may strengthen self-management and long-term outcomes for AYAs with HL.

# "I Don't Know if I'll Be OK, So You Can't Call Me a Cancer Survivor." Content Analysis of Free Text Responses in the Palliative Care and Oncology Survey on Terminology (POST).

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**Background:** Cancer survivorship terminology is commonly applied to people with advanced or metastatic cancer in clinical and academic settings. Interpretation and endorsement of the term "cancer survivor" is variable amongst people with a history of cancer, but the perceptions of people with active cancer have been previously under-represented. The aim of this aspect of the POST study is to understand the perceptions of people with cancer known to specialist palliative care and oncology services about the term "cancer survivor".

**Patients and Methods:** Individuals with cancer were recruited from cancer centres and specialist palliative care units across Ireland and the UK, and completed a study-specific questionnaire. Inductive content analysis was used to identify themes from optional free text responses about the term "cancer survivor".

**Results:** 1328 participants completed the study, and 766 (57.7%) provided comments about the term "cancer survivor". The majority of comments were negative (n=547; 71.4%). Participant perceptions were summarised into five themes: there is uncertainty around "cancer survivor" ("cancer survivor" can be unclear; it can create misperceptions; its need is questionable); there are challenges applying "cancer survivor" universally (the term's use should be an individual's choice; labels are not always appropriate; the term is inappropriate for people who still have cancer); "cancer survivor" may be used to describe certain groups (referring to an event that is over; dependent on certain conditions); "cancer survivor" often evokes negative feelings, but can also be viewed positively; and different wording may be preferable (alternative terminology is available; alternative options are limited).

**Conclusions:** People with cancer known to specialist palliative care and oncology services provided overwhelmingly negative comments about cancer survivor terminology and demonstrated a range of interpretations of the meaning. The potential confusion, alienation and offence this term can cause highlights the need to reconsider its use in clinical and academic settings.

## Incidence, Severity, and Management of Chyle Leak Following Oesophagectomy at a High-Volume Centre

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**Background:** Chyle leak is an uncommon and potentially serious complication following oesophagectomy, associated with impact on nutrition and immune function, and prolonged hospital stay. The optimal management of chyle leak varies and lacks a standardised approach. The aim of this study is to understand the incidence, severity, and outcomes of chyle leak at a high-volume centre.

**Materials and Methods:** Consecutive patients undergoing oesophagectomy between 2015 and 2025 were included. Demographics, tumour characteristics, operative details, diagnosis and volume of chyle leak, management, and patient outcomes were analysed. Chyle leak was defined and graded using the Esophageal Complications Consensus Group (ECCG) standardised format.

**Results:** Some 597 patients were studied. The incidence of chyle leak was 14.7% (n=88). 19.3% (n=17) of chyle leaks could be managed with dietary modification and/or medium chain triglyceride enteral feeding (Grade I), while 65.9% (n=58) required total parenteral nutrition (Grade IIa [n=52, 59.0%] and Grade IIb [n=6, 6.8%]). Further intervention was required for 13 patients (14.8%; Grade IIIa (n=3, 3.4%) and Grade IIIb (n=10, 11.4%). Where nutritional therapies were unsuccessful, image guided lymphangiogram and thoracic duct embolisation represented the initial strategy, with reoperation required in 5 patients (0.8%).

**Conclusions:** Most chyle leaks can be managed with nutritional intervention alone, while image-guided lymphangiogram and thoracic duct embolisation represents an effective second line strategy. A prospective study is currently in progress to assess the impact of a targeted nutritional strategy on chyle-leak incidence.

# Implementation of Group-based Psychological Interventions for Fear of Cancer Recurrence: A Scoping Review

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**Background:** Fear of cancer recurrence (FCR) is one of the most common unmet needs among cancer survivors with approximately 59% reporting at least moderate levels of FCR, with close to one in five patients experiencing high or clinically significant levels. A range of interventions for managing FCR has been developed, with meta analyses identifying contemporary CBT as the most effective. International guidelines recommend a matched care model, tailoring interventions to FCR severity. Self guided psychoeducation or exercise suits low FCR; clinician led group programmes suit moderate FCR; and FCR specific CBT by experienced cancer care clinicians is recommended for high FCR. Evidence is limited by wide variation in group programme design, highlighting the need for more standardised models of care.

**Materials and Methods:** This scoping review followed Preferred Reporting Items for Systematic Reviews and Meta-Analysis extension for Scoping Reviews (PRISMA ScR) guidelines. Six databases were searched for studies on group based FCR interventions published up to April 2025. The Consolidated Framework for Implementation Research (CFIR) guided the mapping and interpretation of findings.

**Results:** Thirty six studies were included. Some similarities were identified in relation to the frequency and number of group sessions. Time since diagnosis or treatment varied greatly—from patients still in treatment to others up to ten years post treatment. The effect of grouping patients with different FCR levels together remains unclear. The main implementation barrier was scheduling and time constraints. Collaboration with stakeholders in designing and adapting the intervention facilitated successful implementation.

**Conclusions:** Group-based interventions offer a structured and efficient approach to delivering targeted FCR treatments while fostering a peer support environment. Our review makes a unique and substantial contribution by being the first evidence synthesis to apply the CFIR framework to systematically examine how group-based FCR interventions are designed, delivered, and implemented. We highlight critical knowledge gaps that must be addressed to ensure effective, scalable, and sustainable solutions for cancer survivors.

# Effects of a Strength Training Program on Upper Limb Volume in Breast Cancer Survivors: A Prospective Study

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**Background:** Breast cancer is the most common malignancy among women, with an estimated 37,682 new cases in Spain in 2025 (1). Breast cancer–related lymphedema is one of the most disabling long-term complications in up to 30.1% of patients after axillary lymph node dissection. Strength training is considered safe and may improve upper limb function, but early volumetric changes of 3–5% are clinically relevant and require accurate and reproducible monitoring to ensure volume stability and detect early changes (2, 3).

**Materials and Methods:** A prospective study including 21 breast cancer survivors enrolled in Phase 3 of the TREVOL Project (4-7). After an educational phase, participants completed a supervised strength training program weekly for two months. Upper limb volume of the affected and contralateral arms was assessed at successive visits using conventional tape measurement (TM) applying the truncated cone formula; and three-dimensional scanning with a virtual reality–based system (VR) using automated volume calculation. Mixed-model ANOVA was used to assess the interaction between measurement method and time, with significance set at $p < 0.05$ (8).

**Results:** A high correlation was observed between both measurement methods ($r \approx 0.89$). At baseline, mean affected-arm volume was 2081 $cm^3$ using TM and 2026 $cm^3$ using VR. Longitudinal analysis showed that strength training did not induce pathological volume increases; effect sizes on arm volume were predominantly neutral or low ($p > 0.05$). During follow-up, VR measurements detected significant asymmetries in 66.6% of participants, identifying early volumetric changes potentially underestimated by TM. Mean differences between measurement systems remained within a clinically acceptable range (<3%).

**Conclusions:** Strength training performed weekly is a safe intervention that contributes to upper limb volume stability in breast cancer survivors. Precise volumetric monitoring supports early detection of subtle changes during preventive exercise programs, facilitating timely clinical decision-making and optimizing rehabilitation strategies.

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# Upper Limb Volumetric Stability After One Month of Combined Strength and HIIT Training in Breast Cancer Survivors: A Prospective Study

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**Background:** Breast cancer–related lymphedema (BCRL) affects up to 30.1% of patients following axillary lymph node dissection, and volume variations of 3–5% are considered clinically relevant for early diagnosis (1). Current evidence supports the safety of strength training and high-intensity interval training (HIIT), which may enhance lymphatic flow through a muscular pump mechanism. Accurate volumetric monitoring is required to determine whether short-term combined exercise induces volume reduction or maintains clinical stability (2-3).

**Materials and Methods:** A prospective study including 21 breast cancer survivors enrolled in the TREVOL Project (4-7). Participants completed a combined strength and low-volume HIIT program weekly for one month. Upper limb volume was assessed at baseline (V1) and after one month (V2) using conventional tape measurement (TM) applying the truncated cone formula, and three-dimensional scanning with a mixed reality–based system (VR). Mixed-model ANOVA was used to evaluate temporal changes, with significance set at $p < 0.05$ (8).

**Results:** TM showed almost complete volumetric stability, with mean affected-arm volume changing from 2081 $cm^3$ at baseline to 2080 $cm^3$ after one month ($p>0,05$). VR recorded a baseline mean volume of 2026 $cm^3$, with follow-up values ranging between 2100 and 2150 $cm^3$, reflecting greater sensitivity to anatomical irregularities. Exercise-related effects on arm volume were predominantly neutral or low ($p > 0.05$). Correlation between methods was high ($r \approx 0.89$). VR detected significant arm asymmetries in 66.6% of participants, identifying subtle changes potentially underestimated by TM.

**Conclusions:** One month of combined strength and HIIT training performed one to two times per week is safe and maintains upper limb volumetric stability in breast cancer survivors ($p > 0.05$). Although short-term volume reduction was not observed, early stability supports the role of combined exercise in preventing BCRL progression. Sensitive volumetric monitoring enables early identification of subtle changes during preventive exercise programs.

*To cluster Sivi To The Department of health, of the European University To Impulsame And to the European regional funds that made this project a reality*

# Understanding how clinical decision-making informs hormone therapy changes in a nurse-led breast cancer survivorship clinic

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**Background:** According to the NCCP, over 200,000 people in Ireland are living with or beyond a cancer diagnosis, and this number is expected to double. This growing population increases the need to address the long-term impacts of cancer treatments and the psychosocial needs of cancer survivors. Breast cancer survivors with hormone positive disease are frequently prescribed aromatase inhibitors as part of adjuvant therapy. However, these agents accelerate bone resorption, increasing the risk of osteoporosis and fractures. Nurses working in survivorship roles are well placed to support patients as they navigate complex treatment choices that extend beyond cancer control and influence long term health.

This review aimed to understand clinical decision making within a nurse led survivorship clinic regarding the modification of hormone therapy for breast cancer survivors with increased osteoporosis risk.

**Patients and Methods:** A practice-based service evaluation was undertaken in a nurse led breast cancer survivorship clinic. Retrospective case review and reflective analysis were used to examine the clinical decision-making processes related to hormone therapy modification. The Multi Modal Framework by Tiffin et al. (2012) guided analysis, providing a structured approach that incorporated assessment of survivorship risks, osteoporosis factors, patient values, shared decision making, bias and multidisciplinary collaboration.

**Results:** The nurse led survivorship clinic demonstrated a holistic, non-linear approach to decision making that integrated oncological safety with long term survivorship considerations. Shared decision-making enabled patients to participate in decisions affecting their health, promoting confidence, treatment adherence and self-management. Early identification and proactive management of osteoporosis risk supported safer transitions into long term survivorship.

**Conclusions:** Nurse led survivorship clinics play a pivotal role in supporting individuals living with and beyond cancer through person centred, evidence-based decision making. The use of a structured decision-making model supports consistent, high quality clinical decisions. These clinics prioritise long term health and quality of life to enhance patient outcomes.

# Supportive Care Needs of Men Living with Prostate Cancer: A Qualitative Study to Inform Survivorship Programmes in Ireland

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**Background:** Findings from the Irish Prostate Cancer Outcomes Research (IPCOR) study highlight ongoing unmet supportive care needs, especially in sexuality, psychological well-being, and information. A qualitative study, supported by the Irish Health Research Board (HRB), was conducted to gather patient insights to guide supportive care and survivorship programmes for men with prostate cancer in Ireland.

**Participants and Methods:** Four focus groups were conducted, each with 5–9 participants with lived experience of prostate cancer at various treatment stages. These were held both in person (Dublin, Cork, Galway–Midlands) and online. Discussions were facilitated by a trained moderator with personal lived experience, guided by an interview manual. Sessions were audio-recorded, transcribed, and anonymised. Thematic analysis was conducted by three independent researchers following Braun and Clarke's methodology. The findings were co-developed and validated with a patient advisory panel.

**Results:** Seven major themes were identified: (1) persistent physical symptoms and impact on daily activities; (2) psychological distress and altered sense of identity; (3) social isolation and loneliness; (4) inadequately addressed sexual dysfunction issues, lack of supports and information; (5) strain on relationships and family members; (6) unmet informational needs and lack of coordinated care; and (7) financial burden. Participants described feeling "dropped into outer space" post-treatment, highlighting poor communication, limited guidance, and the need to self-manage complex health decisions. Many emphasised the absence of sexual counselling and psychological support, describing prostate cancer as a "hidden disability".

**Conclusions:** Men's lived experiences highlight significant gaps in survivorship care. Participants suggested a structured "Prostate Cancer Toolkit", a peer "Buddy System", access to a dedicated care coordinator, routine mental health check-ins, and increased awareness of community-based resources. Incorporating these patient insights into national survivorship guidelines through an ongoing collaboration between IPCOR and the Irish National Cancer Control Programme (NCCP) will foster more coordinated, holistic, and patient-centred care.

# Phase One in the Development of a Core Outcome Set for Exercise Interventions Targeting Chemotherapy-induced Peripheral Neuropathy

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**Background:** Chemotherapy-induced peripheral neuropathy (CIPN) is a common and challenging adverse effect of neurotoxic chemotherapy. Currently, no evidence-based treatment exists for CIPN. Exercise is emerging as a possible preventative or therapeutic tool; however, the strength of the evidence generated is low. Contributing factor is the heterogeneity of outcomes used, limiting comparison. Standardisations of key outcomes, through the development of a core outcome set (COS), would facilitate meaningful comparisons across studies, enhancing the quality of evidence. Step one in COS development generates a long list of candidate outcomes for later prioritisation. This study aimed to identify outcomes used in exercise trials and those valued by stakeholders in CIPN exercise interventions.

**Materials and Methods:** A systematic review (SR) and rapid qualitative review (rQES) were completed following the PRISMA guidelines. EMBASE, MEDLINE, CINAHL, and Web of Science–Core Collection were searched (31/03/2025 & 15/10/2025). Title and abstract screening, full-text review and data extraction were completed. Qualitative data were analysed using thematic synthesis. Outcomes from both reviews reflecting the same concept were consolidated to generate a long list of unique outcomes and mapped to the COMET taxonomy.

**Results:** 39 studies were included in the SR and 4 in the qRES. The 285 extracted outcomes were consolidated into 35. The most frequent were CIPN severity (53, 18.7%), balance (28, 9.9%), and quality of life (24, 8.5%). Outcomes were mapped to COMET Taxonomy within Life Impact (24/35, 60.6%) and Physiological/Clinical (11/35, 31.4%). Three themes were identified 'Evolving sensory symptom profile in CIPN', 'Enhanced balance and stability to support functional tasks' and 'Enhanced ability to participate in activities'.

**Conclusion:** Results generated a comprehensive list of outcomes, with identified themes and COMET-mapped outcomes reflecting patients' priorities for function and symptom relief. Next, focus groups with stakeholders will identify any missing outcomes, followed by prioritisation via Delphi survey and consensus meeting.

# A Re-audit of Bone Health Practices in Adult Recipients of Allograft Hematopoietic Stem Cell Transplantation (HSCT) in a single centre over a 3-year period

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**Introduction:** Patients receiving HSCT are at high risk of poor bone health as a result of high dose chemotherapy, radiation, corticosteroid intake, prolonged hospital admissions and the need for protective isolation which limits sunlight exposure. A comprehensive audit against international standards was completed in 2023 in our centre which highlighted areas for improvement including the need for clearer guidance for clinicians with regards to prescribing vitamin D and calcium supplementation, increased uptake of vitamin D screening and DEXA scans. A reaudit of HSCT recipients in 2024 and 2025 was completed to evaluate if improvement in practice has been achieved.

**Method:** This was a single centre, retrospective review of all adults who underwent HSCT in the National Transplant Unit from 2023-2025 (n=297). Patients were identified using the electronic health record. Similar demographics were noted in terms of age and gender across all years.

**Results:** Results showed an increase in screening, rescreening and supplementation which improved in 2024 and again in 2025. Notable improvements included screening of vitamin D levels during inpatient admission for HSCT (3% of recipients in 2023 vs 89% in 2024 and 97% in 2025). This has allowed clinicians to observe frequency of, and treat vitamin D deficiency in this population. Post-transplant, an increase in re-screening in outpatients was noted along with an improvement in number of patients receiving supplementation long term. Frequency of performed DEXA scans in this population did not improve over these timepoints.

**Conclusion:** Increased awareness of the importance of bone health in HSCT recipients has resulted in improvements in vitamin D screening, calcium and vitamin D supplementation and identification of vitamin D deficiency in our centre. There are further improvements to be made via the development and embedding of a standard operating procedure to ensure optimal management of bone health in patients undergoing HSCT going forward.

# Empowering RTTs: A Lean Synthetic CT-Based Plan-of-the-Day Feasibility Study for Prostate Radiotherapy Patients with Bladder Filling Challenges

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**Background:** Bladder filling variability in prostate radiotherapy risks exceeding OAR doses, repeated imaging and increased patient distress. This study evaluates the feasibility and dosimetric impact of a vendor neutral, RTT-led, plan-of-the-day (PoD) workflow using CBCT-derived synthetic CTs (sCT) and AI-assisted contouring on non-adaptive Varian linacs, aiming to create a lean protocol that enables RTTs to manage anatomical variation consistently.

**Materials and Methods:** A retrospective review included 10 prostate cancer patients treated to 60 Gy in 20# who had persistent low bladder volumes (bladder < planned for ≥3 fractions). Daily CBCTs were converted to sCTs. MVision Contour+ auto-contoured OARs. Rigid registration was used to map PTVs to the sCT. The clinical plan was recalculated in Eclipse v18 and evaluated against CHiPP trial DVCs. If bladder DVCs were exceeded, RTTs re-optimised the plan on the sCT generating a PoD option. PoD bladder volumes were compared with subsequent CBCTs to assess (1) PoD adoption potential and (2) achievable bladder dose reduction.

**Results:** All on-treatment bladders exceeded CHiPP bladder constraints; every patient required a PoD to ensure bladder constraints were achieved. RTT re-optimisation preserved PTV coverage in 9/10 patients; one case required reduced PTV coverage and would need full replanning to restore coverage. Sigmoid position was qualitatively checked but not dosimetrically quantified due to limited CBCT field of view. Plan complexity analysis indicated all sCT plans were deliverable without additional PSQA. 91 CBCTs were analysed for potential PoD adoption. Retrospective application of the PoD workflow would allow PoD selection in 50/91 fractions (55%), reducing replans, additional imaging and shortening time in department.

**Conclusion:** An RTT-led sCT and AI-supported PoD workflow on non-adaptive linacs is feasible, deliverable, and dosimetrically valuable. By leveraging existing equipment with modern AI tools and empowering RTTs to lead adaptive decision-making, this PoD approach delivers high value care in resource limited settings. Prospective validation and integration into clinical pathways are warranted.

## Unmet cancer rehabilitation needs and access to survivorship services across the cancer continuum

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**Background:** To investigate perceived rehabilitation needs and service utilisation in people living with and beyond cancer in a large Comprehensive Cancer Centre in Ireland and examine associations between impairment and demographic and clinical parameters.

**Methods:** Utilising a prospective, observational design, a consecutive sample of participants attending outpatient clinics were enrolled in June and July 2025. Demographic data, clinical data and use of allied health professional (AHP) cancer rehabilitation services were self-reported. Unmet needs were captured using the Macmillan Holistic Needs Assessment. Data were analysed using descriptive statistics and multivariate regression models.

**Results:** In total, 660 surveys were analysed. Most participants were female (58.2%) and aged between 56-75 years (54%). Haematological cancers (25.6%) and breast cancers (22.9%) were the two largest cohorts, and most patients (57%) were currently on treatment. Prevalence of AHP cancer rehabilitation needs was high with an average of 71% of patients reporting at least one specialist need and 49% reporting at least two specialist needs across professions. On-treatment status was consistently associated with a greater odds of patients reporting specialised needs. 36% of patients with perceived needs, reported seeing a relevant AHP since their diagnosis

**Conclusions:** Results confirm that patient-reported rehabilitation needs are significant across the cancer trajectory, but that utilisation of cancer rehabilitation AHPs is suboptimal.

**Implications for Cancer Survivors:** Strategic development of cancer rehabilitation AHP workforce and service models is required to mitigate the impact of cancer and its treatment on functional, nutritional, and psychosocial patient outcomes, and optimise health-related quality of life.

*Ms Ganna Eissa was funded by a HRB Summer Student Scholarship SS-2025-026 and supervised by Emer Guinan.*

# The impact of pre-operative frailty and physical function on recovery in patients with head and neck cancer

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**Background:** This study explores pre-operative frailty and physical function and recovery in patients with head and neck cancer (HNC).

**Methods:** Perioperative data of HNC patients referred to the cancer institute's exercise prehabilitation service from 2022 and 2024 were retrospectively analysed. Preoperative variables (e.g., demographic details, physical function measures (Duke Activity Status Index, 6 Minute Walk test, 30 second sit to stand, mobility status), clinical frailty scale and 5MFI were collected, as well as postoperative outcomes eg. hospital length of stay. The Mann-Whitney U and chi square tests examined associations between variables.

**Results:** In total, 158 patients completed a prehabilitation assessment (61% male, mean age 66.19 years (SD 11.24, range 26-89)). The mean pre-operative DASI score was 39.47 (SD 13.13, range 4.5-58.2), average 6MWT was 456.41 m (SD 158.95, range 120-700) and mean 30 sec STS score was 12.73 reps (SD 4.87, range 0-30). The CFS identified 4.16% of participants as frail (CFS ≥5), and the 5MFI 14% of participants as frail (mFI-5 ≥ 2). In total, 30% of patients were achieving aerobic and 7% were achieving strength exercise guidelines pre-operatively. Patients achieving strength guidelines had a shorter length of hospital stay (p=.043). Twelve patients (7.74%) were discharged at a lower functional status than baseline. Patients with higher frailty scores pre-operatively had lower functional status (less independence) on discharge (p<.001).

**Conclusion:** Patients with HNC awaiting surgery presented with a range of frailty and physical function scores. Frailty assessments identified high-risk patients and should guide perioperative interventions to optimise patient outcomes.

# Physical Activity and Head and Neck Cancer: Results of an Exercise Feasibility Study

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**Background:** The aim of this study was to evaluate the feasibility and acceptability of a multi-modal exercise rehabilitation programme for patients post head and neck cancer (NHC) treatment.

**Methods:** This single-arm prospective feasibility study included patients in the first two years following treatment for HNC. Participants completed a 10-week multi-modal exercise programme including twice weekly online or in-person group sessions of aerobic, resistance, flexibility and balance exercise. Feasibility was evaluated via recruitment, adherence and compliance to the programme. Secondary outcomes examined physical function and quality of life. The acceptability of the programme was assessed through patient feedback.

**Results:** In total, 22 participants were recruited (36% (n=8) female, mean age 65.23 (SD 14.53, 27-87). The recruitment rate was 24%. Most participants had a history of surgery including neck dissection (95.5%, n=21) and flap reconstruction (31.8%, n=7). Four people engaged with the online class (average 16.25 classes, range 6-24). Most patients attended the in-person class (n=14, average 9.86 classes range 1-20). One person chose to do both online and in person classes. Two participants enrolled in the online programme did not engage with the intervention. Approximately 54.5% (n=12) of the participants screened positively for lymphoedema. Measures of physical activity levels, strength, frailty and physical well-being all increased post intervention. Engagement was high, and participants provided overwhelmingly positive feedback, particularly valuing the social experience of exercising alongside peers with similar cancer experiences.

**Conclusions:** The intervention appears feasible in a group of complex cancer survivors of HNC, with a preference for in-person exercise classes.

ClinicalTrials.gov Registration Number: NCT06646861

# "Access to a Dietitian Would Have Been Welcome. It Would Still Be Welcome." A Service Evaluation of Nutrition and Dietetic Supports in Cancer Care Services – The Patient Experience

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**Background:** Despite its integral role in supporting patients with malnutrition and other nutritional concerns, dietetics is often overlooked within cancer service planning in Ireland. The aim of this service evaluation is to assess how effectively current cancer services at selected clinical sites provide nutrition and dietetic care, using the lived patient experience to evaluate the adequacy of nutritional supports available to people receiving cancer care.

**Patients and Methods:** Participating sites registered the service evaluation with their local quality teams. Data was collected April – September 2025 via an anonymous patient experience survey, distributed to adult patients/cancer service users with a diagnosis of cancer by the local collaborating dietitian(s). The survey collected data on demographics, the areas of the cancer service being accessed (e.g. inpatients, OPD, therapy day wards), malnutrition risk and experience of nutrition care throughout respondents' cancer journey. Data was analysed using descriptive statistics.

**Results:** A total of 1,742 respondents participated across 14 clinical sites. Over 70% self-reported muscle strength loss and 28% reported ≥5% weight loss within 6 months. Malnutrition screening (PG-SGA-SF) identified 25% as being at 'medium risk' and 19% at 'high risk' of malnutrition. Nutrition was rated as either 'very' or 'extremely' important by 90% of respondents. Over one-third (36%) reported experiencing diet and nutrition-related problems. However, almost half (43%) either 'rarely' or 'never' had access to a dietitian when they needed one. Of those who had to access dietetic care, 93% found it helpful.

**Conclusions:** The unmet needs highlighted by this service evaluation will be used to make recommendations for submission to the upcoming National Cancer Strategy, as an opportunity to improve care and patient experience. With this, the National Cancer Control Programme can make better informed and evidenced-based commissioning decisions in relation to the much-needed development of improved nutrition and dietetic supports for those accessing cancer care services at these sites.

Representatives from the Dietitians National Cancer Strategy Working Group. The Irish Nutrition & Dietetic Institute. The Irish Society for Clinical Nutrition & Metabolism.

# Exploring the feasiblity of delivering Music Therapy in collaboration with Speech and Language Therapy in an acute hospital setting to patients post Head and Neck Cancer surgery

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**Background:** Music therapy (MT) is an evidence-based profession where the planned and creative use of music-based interventions supports people to improve health, functioning and well-being (IACAT, 2026). Benefits of MT in healthcare are well established in the literature (Morrow-Odom & Swann, 2025; Hart 2009). Collaborative working between the professions of MT and SLT has also been documented with research suggesting that music- based interventions may improve communication (Tamplin & Grocke 2008). Recently funding for MT was secured on a temporary basis, to explore the practicalities and benefits of co-delivering MT & SLT to patients post head and neck cancer (HNC) surgery. This initiative aimed to establish whether delivery of MT was feasible in a post-surgical acute setting and to ascertain if patients were able to engage in the process and gain therapeutic benefit.

**Patients and Methods:** Weekly 1:1 MT & SLT sessions were held with SLTs identifying suitable patients and assessing oromotor function & speech intelligibility. Therapeutic goals to be targeted during sessions were agreed with MT in advance of sessions.

**Results:** 30 sessions took place from Sept 2025-Jan 2026. Mean age of participants was 62yrs. 6 patients had >1 session with 4 patients having >2 or more. Surgery types included glossectomy, laryngectomy & tracheostomy. Patient feedback was overwhelmingly positive with many patients keen to attend again. Delivering a novel service in a fast-paced environment with high patient turnover can be challenging. Support from nursing colleagues from the outset along with flexibility from MT/ SLT allowed for sessions to be delivered consistently. Challenges faced by the project were consistently capturing measurable data on outcomes given high turnover of patients.

**Conclusions:** Music therapy can ease psychologic, physical, and spiritual burdens experienced by patients with cancer (Hart 2009). By collaboration with MT and nursing staff, we demonstrated that codelivery of MT and SLT is feasible and effective in enhancing communication and patients well- being. The project has applicability and scalability to other inpatient cohorts.

*We would like to acknowledge all the patients who attended the Music Therapy sessions, nursing staff on St. John's & Anne Young wards, SCOPE management & Creative Life staff.*

# CAN-REACT: Design and Implementation of a Personalised Exercise Programme for Improving Physical Function in Cancer Survivors

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**Background:** Cancer survivors often experience long-term treatment side effects that substantially diminish their functional capacity and quality of life (QoL). Among the wide range of symptoms reported, chronic fatigue and loss of physical function - particularly reduced cardiorespiratory fitness, flexibility, and muscle strength - emerged as the most debilitating. These symptoms vary widely across individuals, highlighting the need for tailored, multi-modal rehabilitation approaches. Building on previous research and preliminary patient feedback, this study aimed to design, implement, and evaluate a personalised, exercise-based program targeting the physical and fatigue-related challenges faced by cancer survivors.

**Methods:** Eligible participants included individuals diagnosed with cancer at any treatment stage who had no contraindications to exercise. After completing informed consent and a PAR-Q+ form, participants underwent baseline assessments of fatigue, health-related QoL, and physical function, including cardiorespiratory fitness, muscular endurance, flexibility, balance, and body composition. Using these results and patient-reported symptoms, researchers developed individualised 12-week exercise programs incorporating aerobic, resistance, and flexibility training. Each program was supervised by an exercise physiologist or physiotherapist, who provided weekly guidance through online sessions, phone calls, and email check-ins. Programs were adjusted every four weeks based on participant progress.

**Results:** Upon completing the 12-week intervention, participants repeated the physical assessments and questionnaires. Results demonstrated significant improvements in aerobic capacity and muscular endurance, alongside meaningful reductions in cancer-related fatigue. Additionally, a strong positive correlation was observed between post-intervention muscular endurance and reduced fatigue, indicating that gains in strength and endurance may play a key role in managing persistent fatigue.

**Conclusion:** Overall, this study concludes that personalised, multi-component exercise programs can effectively improve physical function and lessen cancer-related fatigue among survivors. Future research should refine assessment and exercise-prescription processes to further optimize recovery and long-term health outcomes for this population.

*PPI members, all our participants, East Galway and Midlands Cancer Centre, Mayo Cancer Centre and Gort Cancer support centre.*

# Effectiveness of Digital-Based Cognitive Training in Pediatric Cancer Survivors: A Systematic Review and Meta-analysis

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**Background:** Cognitive impairments are prevalent yet often underrecognized long-term consequences in pediatric cancer survivors, negatively affecting memory, attention, executive function, and academic performance. This meta-analysis aimed to evaluate the effectiveness of digital-based cognitive training on cognitive outcomes, academic performance, and quality of life among pediatric cancer survivors.

**Materials and Methods:** Search strategy covered seven databases, including PubMed, Web of Science, the Cochrane Library, MEDLINE (OVID), CINAHL, Scopus and Google Scholar. Risk of bias was assessed using the Cochrane ROB 2 tool for RCTs and ROBINS-I for quasi-experimental studies. A random-effects model was applied to calculate pooled effect sizes (Hedges's g) with 95% confidence intervals. Heterogeneity was examined using the $I^2$ statistic and Cochran's Q test, and publication bias was assessed through multiple statistical methods.

**Results:** Twelve studies with 448 pediatric cancer survivors aged 6-19 were analyzed. The meta-analysis results showed a significant effect of cognitive training interventions on immediate verbal working memory and attention (Hedges's $g = 0.375$, 95% CI: 0.091–0.659, $p = 0.010$), verbal working memory (Hedges's $g = 0.420$, 95% CI: 0.029–0.812, $p = 0.035$), visual-spatial working memory (Hedges's $g = 0.506$, 95% CI: 0.224–0.788, $p < 0.001$), and overall working memory capacity (Hedges's $g = 0.353$, 95% CI: 0.001–0.706, $p = 0.050$).

**Conclusions:** This meta-analysis demonstrates that digital cognitive training interventions appear to be effective, producing small-to-moderate improvements in working memory and attention, while also demonstrating feasibility and acceptability among pediatric cancer survivors. Future research should examine the long-term effects and sustainability of these approaches.

# Who Seeks Support to Return to Work Following Cancer Treatment? Demographic and Health Characteristics of Women in a Definitive Trial of CANWORK, a Return-to-Work Intervention for Women with Breast Cancer

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**Background:** Many individuals with cancer experience treatment-related difficulties that interfere with returning to work (RTW), leading to early cessation of work and/or reduced productivity. Little is known, however, about the characteristics of individuals who may require focused support for RTW following cancer treatment. CanWork is an occupational therapy-led intervention to prepare women post-breast cancer treatment to RTW. This study examined demographical, clinical and employment-related data of participants of a definitive intervention trial of CanWork.

**Patients and Methods:** A mixed-methods cluster RCT is underway to test the effectiveness of CanWork. Data collected at baseline included demographic, health and work characteristics, and readiness to RTW. Focus groups explored acceptability of CanWork. Quantitative data were analysed using descriptive statistics. Content analysis was applied to focus group data.

**Results:** To date, 181 women have been randomised to the CanWork intervention or care as usual. The median age of participants is 49 years (range 32-63). There is an even distribution of cancer stages I-III at diagnosis with 66.7% of participants having three treatment types (surgery, chemotherapy and radiation therapy). Prior to treatment, the majority of participants were working in administrative or professional roles (67.2%). Mean readiness to RTW at baseline was 4.61 (SD= 2.22) on a scale of 1-10.

Focus group data suggests acceptability of CanWork: "hugely informative and beneficial" with one participant noting "I am looking forward to going back to work now which I wasn't before". CanWork provides a tailored RTW plan which was highlighted as an important resource when meeting with employers to discuss RTW.

**Conclusions:** This study indicates that women with stage I-III breast cancer who received multiple treatments should be identified early during the course of treatment, assessed for readiness to RTW and offered tailored support to navigate RTW to ensure more women return to work successfully after completion of treatment and remain in the workforce.

*CanWork is funded by HRB Definitive Intervention and Feasibility Award. CanWork research team thanks all the women with breast cancer, staff of community cancer support centres and hospital staff who have supported this study.*

## Thoracic Re-Irradiation: An 8-Year Safety Review to Inform Modern Practice

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The growing number of long-term cancer survivors has led to an increase in patients at risk of relapse or secondary malignancies. Consequently, repeat courses of radiotherapy are becoming more frequent. Despite the expanding clinical adoption of re-irradiation, there remains a lack of robust data on patient demographics, indications, and radiotherapy technical modality including Dose Volume Constraints.

This project focuses on reviewing patients classified as re-irradiation type 1. Institutional data for 110 patients who have undergone irradiation in last 8 years was evaluated. Retrospective reporting of toxicity was completed for the incidence of grade ≥1 toxicities to validate long-term clinical outcomes of the predetermined DVCs. Predetermined DVCs were based on 2019 literature assuming no reparations except for spinal cord. At time of planning max dose to serial organs was evaluated on organ + 5mm expansion to take into account registration uncertainty.

With the median follow up time is 6.7 months (Range 0 -74 months), toxicities seen in 110 patients analysed was Toxicity Grade 1-3 =10%, Grade 4 =0% and Grade 5 = 3%. For Grade 5 toxicities pulmonary acute toxicities were observed. For all patients no serial organs toxicities were seen. Lung volume receiving ≥20Gy (v20) or Lungs –GTV v20 (Gross target volume) is a predictor in radiation pneumonitis. With regards to pulmonary toxicities the combined and most current treatment Lungs – GTV v20Gy mean was 19% and 8.8% respectively and therefore not able to discriminate in patients with high toxicity.

This study formalises a standardised SABR thoracic re-irradiation protocol demonstrating its safety, reproducibility, and adaptability across technologies. Limitations of the study include this is retrospective data and increase the follow-up to 24 months post last treatment to further validate toxicity incidence. For these patients the results provide a validated foundation for further refining dose–volume constraints, potentially expanding treatment eligibility for patients requiring thoracic re-irradiation.

# LOY as a Predictor of Radioresistance: Emerging Clues Across Cancer Types

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**Background:** Loss of the Y chromosome (LOY) is a frequent event in male cancers, but its influence on radiotherapy response has not been systematically investigated. Understanding this relationship could inform precision radiotherapy strategies.

**Methods:** LOY status was curated in 672 adult male cancer cell lines using karyotype mining and computational inference. Radiosensitivity was assessed via surviving fraction at 2 Gy (SF2). Transcriptomic analyses explored pathway alterations associated with LOY. Clinical relevance was evaluated in the TCGA PanCancer cohort of non-metastatic male patients treated with radiotherapy.

**Results:** LOY was detected in 58.3% of cell lines and associated with higher SF2 values, indicating increased radioresistance (median 0.67 vs 0.58, $p = 0.008$). Transcriptomic profiling revealed downregulation of Y-linked genes and remodeling of DNA damage response, senescence, and longevity pathways. In TCGA, LOY occurred in 19% of patients and correlated with worse overall survival ($p = 0.01$) and progression-free interval ($p = 0.02$), with pronounced effects in bladder cancer, mesothelioma, melanoma, and lung cancer.

**Discussion:** LOY emerges as a potential biomarker of radioresistance and adverse outcomes in male cancers. These findings warrant validation in larger, clinically annotated cohorts and suggest that LOY status could guide treatment personalization and dose adaptation in radiation oncology.

# Global proteomic assessment of a novel HDAC6 inhibitor and PROTAC in Multiple Myeloma Identify Metabolic Adaptations

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**Background:** Multiple myeloma (MM), the second most common hematological malignancy, arises from uncontrolled plasma cell proliferation in the bone marrow. Although proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) have improved survival, resistance remains a major obstacle, underscoring the need for agents with novel mechanisms of action. Through high-throughput screening, we identified BAS-2, a selective histone deacetylase 6 (HDAC6) inhibitor that induces apoptosis in chemotherapy-resistant cancer cells and alters glycolytic activity. Building on this, we developed TTCP-10, a BAS-2–based proteolysis targeting chimera (PROTAC) that mediates selective HDAC6 degradation via the ubiquitin–proteasome pathway, representing a distinct therapeutic strategy in MM.

**Materials and Methods:** JJN3 myeloma cells were treated with BAS-2, TTCP-10, or DMSO control. Global proteomic profiling was performed using data-independent acquisition (DIA) mass spectrometry followed by differential expression and enrichment analysis. Functional categories related to metabolism were further examined. Key transporters were functionally evaluated under glutamine deprivation and in the presence of benzylserine, an inhibitor of SLC1A5, SLC3A2, and SLC7A1. Ongoing glutamine tracing experiments aim to delineate the metabolic adaptations that drive these responses.

**Results:** Proteomic analysis revealed significant upregulation of processes associated with *ribosomal biogenesis* and *neutral amino acid transport* following BAS-2 treatment. Transporters SLC1A5 and SLC3A2 were notably increased, indicating a potential reliance on glutamine metabolism. TTCP-10 elicited similar but more pronounced effects, confirming that both HDAC6 inhibition and degradation trigger comparable metabolic reprogramming. Interestingly, glutamine deprivation or benzylserine co-treatment conferred protection against BAS-2-induced cytotoxicity, suggesting a compensatory metabolic adaptation to support survival.

**Conclusions:** Our findings indicate that HDAC6 inhibition and degradation modulate amino acid metabolism in MM cells, enhancing amino acid uptake and utilisation under stress. Targeting components of this adaptive metabolic response, particularly glutamine transporters, may potentiate the therapeutic effects of HDAC6-directed therapies and uncover new metabolic vulnerabilities in multiple myeloma.

# FKBPL and DNA Damage Responses Following Bile Acid–Induced Stimulation in Oesophageal Cancer Progression Models

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**Background:** Oesophageal adenocarcinoma (OAC) develops through a multistep disease progression process associated with chronic gastro-oesophageal reflux, leading to sustained cellular stress and DNA damage. Deoxycholic acid (DCA) is a key bile acid implicated in reflux-associated oesophageal carcinogenesis. FKBPL is a tumour suppressor in other cancer types; however, its regulation in oesophageal adenocarcinoma remains poorly understood.

**Materials and Methods:** A panel of oesophageal cell lines representing disease progression from normal squamous epithelium to oesophageal adenocarcinoma was treated with physiologically relevant concentrations of DCA (150 μM, 200 μM and 300 μM). Cells were harvested at multiple time points following treatment (30 minutes, 1 hour, 6 hours and 24 hours). Protein expression of FKBPL and γH2AX was assessed.

**Results:** DCA stimulation induced cell line-specific and time-dependent alterations in FKBPL expression and DNA damage signalling across oesophageal disease progression models. In HET1-A cells, FKBPL expression was significantly decreased at 24 hours following treatment with DCA 150 μM and 300 μM, alongside a significant increase in γH2AX expression at 24 hours with DCA 300 μM. In Barrett's-associated QH cells, treatment with DCA 200 μM resulted in a significant reduction in FKBPL expression at 24 hours, accompanied by significant increases in γH2AX expression at 1 hour, 6 hours and 24 hours. In dysplastic GO cells, no significant changes in FKBPL expression were detected, while γH2AX expression was significantly decreased at 30 minutes and 6 hours, predominantly following DCA 200 μM treatment. In OE33 oesophageal adenocarcinoma cells, FKBPL expression was not significantly altered, whereas γH2AX expression was significantly increased at 6 hours following treatment with DCA 200 μM.

**Conclusions:** These findings demonstrate that DCA induces cell line-specific and time-dependent responses across oesophageal disease progression models. DNA damage signalling was predominantly increased following DCA exposure, while FKBPL expression was reduced or remained unchanged depending on disease stage and duration of exposure.

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# Extracellular Vesicle-Associated LRG1 in Melanoma-Microenvironment Crosstalk: A Cell Line Study

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**Background:** Cutaneous melanoma is the most lethal form of skin cancer, characterised by rapid progression, early dissemination, and resistance to therapy. Its aggressive behaviour is driven not only by intrinsic tumour biology but also by dynamic signalling between malignant cells and the tumour microenvironment (TME), including immune cells, fibroblasts, and vascular endothelium. Extracellular vesicles (EVs) - nano-sized, membrane-bound particles released by all cells - have emerged as key mediators of this crosstalk. By transferring proteins, lipids, and nucleic acids, EVs actively remodel the TME and promote angiogenesis, immune evasion, and metastatic spread.

Because EV cargo reflects tumour state and function, EV-associated proteins represent an attractive source of biomarkers and therapeutic targets. Leucine-rich alpha-2 glycoprotein 1 (LRG1) has recently gained attention due to its pro-angiogenic and immunomodulatory roles and its association with poor outcomes across multiple malignancies. However, the contribution of LRG1 to melanoma biology, particularly as an EV-associated signalling molecule, remains undefined.

This study aims to characterise melanoma-derived EVs and determine the role of LRG1-mediated EV signalling in shaping TME interactions.

**Materials and Methods:** Melanoma and fibroblast cell lines with confirmed LRG1 expression were cultured under EV-depleted conditions prior to vesicle isolation. EVs were isolated and rigorously characterised using standardised approaches. EV-associated LRG1 was quantified and validated using ELISA, mass spectrometry, and proteomic profiling.

**Results:** Preliminary analyses reveal stage-dependent differences in LRG1 expression, with higher levels observed in primary tumour–derived cell lines compared with metastatic lines. These findings suggest that LRG1 may play a role in early TME signalling. Ongoing work is quantifying EV-associated LRG1 and evaluating its functional effects.

**Conclusions:** LRG1 represents a candidate mediator of EV-driven communication in melanoma. Defining its role may uncover novel mechanisms of progression and support the development of clinically actionable biomarkers and therapeutic strategies.

# Generation and Profiling of Upper Gastrointestinal Adenocarcinoma Patient-Derived Organoids for Personalised Medicine

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**Background:** Over 700 deaths in Ireland are attributed to gastric and oesophageal adenocarcinomas annually. A significant portion of patients don't respond to the standard of care FLOT chemotherapy regimen in the neoadjuvant setting. There is therefore an urgent need to identify chemotherapy refractory patients, expand treatment options and overcome treatment resistance in these upper gastrointestinal (GI) cancers. This project is using patient-derived tumour organoids as mini ex vivo models, which retain genetic and molecular characteristics of parent tumours, for personalised drug testing.

**Materials and Methods:** Oesophageal and gastric tumour tissue were processed, and 80,000 cells/well seeded in a 24-well plate in 50 µl Cultrex™ BME (1:10 ratio) to form patient-derived organoids (PDOs). PDO lines were passaged at ratios of 1:2 to 1:5 every 2-4 weeks. PDO lines were cryopreserved in Gibco Recovery™ Cell Culture Freezing Medium and retained in liquid nitrogen for long term storage. Following formalin fixation and paraffin-embedding, PDOs were sectioned at 3 µm and haematoxylin and eosin (H&E) staining performed. In addition, seeding density optimization for patient-tailored drug screening has been carried out.

**Results:** Upper GI cancer PDO lines were successfully established from pre-treatment and restage endoscopic biopsy tissue. Culturing demonstrated varying growth rates between PDOs and differences in size, density, and morphology. H&E staining facilitated visualisation of PDO architecture, with glandular, solid, and cystic morphologies observed. PDOs were stored for 4, 7, and 12 weeks in liquid nitrogen with successful recovery. Finally, seeding density optimization demonstrated PDO establishment from seeding of 7,000 cells/well in Cultrex™ BME ratios of both 1:2 and 1:4 in a 96-well plate.

**Conclusions:** We have successfully established PDOs for upper GI tumours, and further evaluation will involve characterisation and drug treatments of these PDOs to test how reflective these models are in comparison to the matched in vivo patient tumours.

*Patients and their families*

# Guanidinium-Based Compounds as Potential Anti-Myeloma Agents

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**Background:** Multiple myeloma (MM) is a plasma cell malignancy that accounts for around 10% of all haematological cancers. Despite advances in promising new therapies only 25-35% of patients respond to therapies in the relapsed and refractory settings demonstrating the need for novel or combined targeted therapies. We previously identified VP79s, a novel guanidinium-based compound, with potent anti-myeloma activity which targets the dysregulated JAK/STAT signalling pathway. Here, we further evaluate the effect of VP79s and examine whether VP79s can synergise with current and emerging MM treatments, including the BH3 mimetic venetoclax.

**Methods:** We evaluated the anti-cancer activity of VP79s in a panel of MM cell lines by Alamar Blue assay. Apoptosis was assessed by annexin V/propidium iodide staining followed by flow cytometry analysis. Western blotting was performed to evaluate the expression level of key target proteins.

**Results:** VP79s reduced the cell viability and induced apoptosis in a dose and time-dependent manner in myeloma cell lines. VP79s inhibited both constitutively active and IL-6-induced STAT3 activation with decreased expression of anti-apoptotic Mcl-1, a STAT3 target gene. Co-treatment of NCI-H929 and MM1.S cells with VP79s and the Bcl-2 specific inhibitor venetoclax resulted in a synergistic enhancement of cell death which was associated with an enhanced decrease in Mcl-1 expression.

**Conclusions:** This study has identified further insights into the mechanism of action of VP79s in myeloma which will help to develop its translational potential. To further investigate its effects and mechanisms of action, global gene expression profiling of myeloma cells treated with VP79s is proposed.

# Development of a liquid biopsy test for Ovarian Cancer using Fragmentomic Characterization of blood samples

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**Background:** Liquid biopsies are non-invasive tests that detect genetic material shed from tumors into bodily fluids, such as blood, urine, or tears. This approach allows for real-time or longitudinal monitoring of cancer dynamics, enabling early detection, tracking of disease progression, and assessment of treatment response. Fragment analysis of cell-free DNA (cfDNA) in the biological fluid of interest allows the detection and classification of the circulating tumor DNA (ctDNA), fragments released from cancer cells following apoptosis or necrosis. The characteristics of these fragments, for example, their size and distribution, as well as their molecular profiling (mutations, methylation status etc.) may indicate the type of cancer or its stage, as well as providing a measure of tumor heterogeneity, a crucial step for providing personalized treatment strategies.

**Methods:** This study include plasma samples from 7 non-cancer controls and ascites samples from 7 ovarian cancer patients. Library preparation and sequencing was carried out using PacBio's Onzo sequencing protocol and computational analysis was performed using the FinaleDB, cfDNApipe, and OpenGene ctDNA workflows.

**Results:** Results are grouped into four main categories: fragment profile (fragment length), fragmentation patterns (window protection score, WPS), end-motif analysis, and variant analysis. A significant distinction in fragment length distribution was observed between the cancer and the normal plasma samples as well as significant differences in their end motif k-mer usage. A number of SNPs were also identified for further follow-up.

**Discussion:** Fragmentomic analysis can determine the presence of cancerous cells without the need for invasive biopsies, and may prove especially useful for early detection and continuous monitoring of cancer. Future work will focus on additional multi-omic analysis including the integration of window protection score and methylation profiles.

# Circulating tumour cells in patients with high-grade serous carcinoma of the ovary

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Circulating tumour cells (CTCs) are rare cancer cells shed from primary or metastatic tumors into the circulation, offering a minimally invasive biomarker for prognostic monitoring. While serial CTC sampling is prognostically validated in breast, prostate, and colorectal cancers, its role in ovarian cancers is poorly understood. This study explores the association between CTCs and disease-free interval (DFI) in patients with advanced high-grade serous carcinoma (HGSC), the most common form of ovarian cancer.

Blood samples were collected from 14 patients with FIGO stage III or IV HGSC undergoing neoadjuvant chemotherapy (NACT) followed by interval cytoreductive surgery (ICRS). Peripheral samples were taken at two timepoints: pre-NACT(n = 14) and post-NACT(n = 12), prior to ICRS. An additional blood sample was collected intraoperatively from the ovarian vein during ICRS(n = 7), when deemed suitable by the surgeon. Whole blood was enriched on the Parsortix® PR1 microfluidic system. CTCs were identified using an optimised marker panel (EpCAM+,Cytokeratin(including CK7)+,Hoechst+,CD45-). Patients were stratified as CTC+ (if ≥1 CTC was detected at any timepoint) and CTC- (if no CTCs were detected). Kaplan-Meier curves were used to analyse. DFI was defined as the time from completion of first-line treatment to disease recurrence.

CTCs were detected in 6/14 patients (43%) pre-NACT. Post-NACT, 3/12 (25%) were CTC+ (all pre-NACT positives). CTCs were detected in the ovarian vein at ICRS in 4/7 (57%) patients, including 3 with peripheral negativity. One patient was CTC+ pre-NACT and in the ovarian vein. After a median follow-up of 14 months, 11/14 (79%) patients had disease progression, 9 (82%) of whom were CTC+ at least one timepoint. DFI was significantly shorter in CTC+ patients compared to CTC- patients (p = 0.0282, median DFI: 6 vs. 17 months).

CTC presence was associated with reduced DFI in advanced HGSC patients undergoing NACT-ICRS. Despite the small sample size, this data suggests that serial CTC sampling may have prognostic value for longitudinal monitoring in HGSC and highlights the need for larger prospective trials to validate CTCs as prognostic biomarkers.

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# CLIP-TAC: A Novel Targeted Proteasomal Degradation of BCL-XL in Multiple Myeloma

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**Background:** Multiple myeloma (MM) is an incurable plasma cell malignancy. Dysregulation of BCL2 family proteins (BCLXL, MCL1, and BCL2) contributes to MM drug resistance and relapse. However, clinical translation of conventional BCLXL inhibitors is limited by on-target thrombocytopenia.

To overcome this, a proteolysis targeting-chimera (PROTAC), DT2216, was developed that couples navitoclax (BCL-XL binder) to a von Hippel–Lindau (VHL) E3 ligase-recruiting ligand to selectively degrade BCL-XL in tumour cells. Our goal is to use ex-vivo BH3-profiling to identify MM patient samples that rely on BCL-XL for survival and test vulnerability to DT2216, as a precision medicine approach. Building on this, we are developing a next generation, in-cell self-assembling PROTAC, termed a Clip-TAC, that forms a heterobifunctional degrader inside MM cells via click chemistry.

**Materials and Methods:** Protein degradation, cell viability, apoptotic dependencies were evaluated by western-blotting, flow cytometry, co-immunoprecipitation, and BH3-profiling in a panel of MM cell lines. DT2216 was tested ex-vivo in MM patient samples.

**Results:** DT2216 induced dose- and time- dependent degradation of BCL-XL across the MM cell line panel. Degradation was rescued by pre-treatment with A-1331852 (BCL-XL inhibitor) or MLN-4924 (NEDD8-activating enzyme inhibitor), confirming on-target, proteasome-dependent activity. BH3-profiling and co-immunoprecipitation after treatment revealed a compensatory increase in MCL-1 dependence. Co-targeting this compensation, either directly with MCL-1 inhibitor AMG-176 or indirectly with cyclin-dependent kinase inhibitors (CYC065, THZ1, Samuraciclib), synergistically enhanced DT2216-induced cell death.

MM patient samples, identified as BCL-XL reliant, were sensitive to DT2216 ex-vivo, and BCL-XL degradation was confirmed by flow cytometry. In collaboration with Prof. Griffith, we've developed a novel BCL-XL Clip-TAC and confirmed in-cell click chemistry inducing degradation at nanomolar concentrations; efficacy studies are ongoing in the chicken chorioallantoic membrane (CAM) model.

**Conclusions:** MM exhibits heterogeneous dependence on BCL2 family proteins. BH3-profiling can identify BCL-XL-reliant tumours. Targeted degradation of BCL-XL with DT2216, or a novel Clip-TAC, shows therapeutic potential in MM.

# Inflammatory and Metabolic Landscapes in Gastrointestinal Cancers Using Fresh Human *Ex Vivo* Explants

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**Background:** The tumour microenvironment (TME) profoundly influences cancer metabolism and immunology, critically shaping tumour growth and progression, as well as therapy response. Fresh human ex vivo biopsies are a physiologically relevant model to investigate tumour biology while preserving the TME, thereby enabling comprehensive profiling of the immune-metabolic axis, treatment resistance mechanisms and identification of potential therapeutic targets.

**Materials and Methods:** This study evaluates a cohort of 44 patients (23 Upper Gi and 21 CRC) using fresh resection tumour tissue and matched non-cancer adjacent tissue samples. Metabolic profiles of tumour and normal tissue were analysed using the Seahorse XFe24 platform. Secreted analytes in the normal (NCM) and tumour conditioned media (TCM) were measured using the MesoScale Discovery multiplex platform

**Results:** This ex vivo explant analysis reveals distinct inflammatory and metabolic landscapes across Upper GI and Colorectal Cancers. Both investigated tumour types exhibit significant changes in ECAR indicating an increase in glycolysis. UGI cancers show a decreased level of CytC both intracellularly as well as secreted, whilst CRC cancers show an increase in the secreted CytC level. Both cancer types show alterations in pro-inflammatory cytokines, chemokines, and angiogenic factors (e.g., IL-1α, IL-6, IL-8, MCP-1, VEGF/VEGF-A), indicative of persistent inflammation and vascular remodelling.

**Conclusions:** Whilst Upper GI cancers show a focused pro-inflammatory and Th1-driven profile, CRC cancers present more extensive cytokine dysregulation, encompassing broad panels of cell mediated, humoral and Th17-associated immunity, alongside prominent immunosuppressive and pro-metastatic markers, suggesting a highly complex immune milieu.

*This work would not be possible without the HEALED Consortium and the patients that were brave enough to consent to research during their cancer journey.*

# Erase the Limits: Characterisation of Electroporation Pulse Parameters in Comparison with IRE and H-FIRE

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**Introduction:** Electroporation (EP) therapy applies electric pulses to malignant tissue to create transient pores in cell membranes, enhances drug delivery and tumor ablation while minimizing thermal damage1,2. Reversible electroporation (RE) and ESOPE are gold standards for localised cancer treatment. However, ESOPE can cause painful muscle contractions, tissue damage, and electrode placement challenges1. Irreversible electroporation (IRE) offers a non-thermal alternative but often requires anesthesia and muscle relaxants due to severe muscle contractions3,4. Optimising EP pulse parameters to increase the ablation zone while minimising adverse effects is crucial for advancing cancer therapies.

**Methods:** We investigated Mirai Medical's novel EP technology, focusing on new pulse parameters and electrode designs aimed at minimising muscle contractions and thermal ablation. The CT26 CRC isogenic subcutaneous tumor model was used to assess tumor growth, thermal ablation, and cell death via macroscopic observation and histological analysis. Tumor response was characterized by examining necrosis and viable tissue. Temperature fluctuations during pulsing were monitored to evaluate thermal effects, and the impact of novel voltage pulse packets on muscle contractions and tissue damage was studied.

**Results:** Mirai Medical's novel EP pulse protocols significantly reduced skeletal muscle contractions compared to traditional IRE. Temperature monitoring revealed lower heating around electrode sites. Histological analysis confirmed less thermal damage and reduced necrosis in surrounding tissues. Modifications to electrode design, replacing traditional needles with more user-friendly probes, further minimising superficial tissue damage.

**Discussion:** By optimising EP pulse parameters and electrode design, Mirai Medical's approach improves EP safety and efficacy profile. These innovations could enable EP therapy as a day procedure, reducing the need for anesthesia, shortening hospital stays, and improving patient recovery. This protocol has the potential to transform cancer treatment, offering a safer, more effective, and less invasive option for pre-malignant and malignant tumors.

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# Assessing the Mechanistic and Functional Role of Microbiome-Induced Transcriptional Alterations in Colorectal Cancer

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By 2030, early-onset colorectal cancer (EOCRC, <50 years) is projected to double, while late-onset CRC (LOCRC, >50 years) declines. These subtypes exhibit distinct molecular profiles—EOCRC is enriched for CMS1 (consensus molecular subtype), while LOCRC favors CMS4. Microbial dysbiosis is a key driver of CRC, with *Phocaeicola vulgatus (Pv)* strongly associated with EOCRC and *Fusobacterium nucleatum* subspecies *nucleatum (Fnn)* with LOCRC. *F. nucleatum* subspecies *animalis (Fna)* is the most prevalent subspecies in CRC, yet its mechanistic role remains unknown. Addressing this gap, we investigated microbiome-induced transcriptional reprogramming to uncover pathways that link microbial signatures to CRC biology.

HT29 (CMS1) and MDST8 (CMS4) cells were infected with *Fnn* and *Fna* at the optimized multiplicity of infection (MOI). Bacterial infection was assessed through MTT assay, gel electrophoresis, and qPCR. Transcriptome wide effects were further investigated by performing RNAseq analysis, Gene Set Enrichment analysis, KEGG analysis, and STRING analysis.

RNAseq analysis identified 18 differentially expressed genes (DEG) in *Fnn*-HT29 and 4 in *Fnn*-MDST8 (p-adj<0.05), with one, *DUSP1*, shared between both cell lines. "*Protein synthesis pathways*" were enriched in *Fnn*-HT29, while "*transcriptional pathways*" were enriched in *Fnn*-MDST8. RNAseq analysis identified 4,043 DEGs in *Fna*-HT29 and 1,034 in *Fna*-MDST8 (p-adj<0.05), with 354 common between both. "*Inflammatory pathways*" were enriched in *Fna*-HT29, while "*barrier remodeling pathways*" were in *Fna*-MDST8. In MDST8 samples, *DUSP1* was the only common DEG across *Fusobacterium* subspecies. Six DEGs were shared between subspecies in HT29 samples. The limited overlap between subspecies suggests divergent CRC disease mechanisms based on molecular subtypes.

Significant transcriptional differences between *Fusobacterium* species and cellular subtypes suggest that molecular subtypes strongly influence CRC progression in young patients. Ongoing work includes replicating previous steps for *Pv* and the control of bacteria, *F. varium*. Future experiments include assessing the potential role identified DEGs have on the CRC phenotype through siRNA knockdowns and patient derived organoids.

# Cannabinoid receptor 2 inverse agonism as a novel therapeutic strategy against metastatic uveal melanoma

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**Introduction:** Uveal melanoma (UM) is the most common primary intraocular malignancy, with Ireland reporting the highest incidence worldwide. Despite excellent local control, about 50% of patients develop metastases within 5–7 years of diagnosis, resulting in a median survival of only 13 months. Available systemic therapies have limited efficacy, as both chemotherapy and immune checkpoint inhibitors show poor response rates, and current immunotherapy options extend median survival only up to 22 months.

Cannabinoid receptors demonstrate a complex role in cancer biology. While their expression correlates with improved survival in some cancers (e.g., lung), overexpression is associated with poor prognosis in others (e.g., prostate, pancreatic, colorectal, and breast). This study investigates the role of cannabinoid receptors in UM and explores the therapeutic potential of their modulation in metastatic disease.

**Materials and Methods:** The bioactivity of CB1 and CB2 agonists and inverse agonists was assessed using long-term proliferation assays in primary and metastatic UM cell lines via the Incucyte Live Cell Analysis System. Toxicity was evaluated in zebrafish embryos by assessing vitality, development, and morphology.

**Results:** Targeting CB1 did not alter UM proliferation nor viability, whereas CB2 inverse agonism significantly (fold and p-value) reduced cell proliferation. Among the compounds tested, SR144528 (10μM) markedly inhibited proliferation without cytotoxicity, GP1a displayed moderate inhibition and toxicity at higher concentrations (20μM), and JTE-907 showed limited efficacy. In zebrafish assays, SR144528 caused no developmental abnormalities, while GP1a and JTE-907 induced toxicity and developmental delays, respectively.

**Discussion:** CB2 inverse agonism represents a promising therapeutic approach for metastatic UM. SR144528 demonstrated potent antiproliferative effects across multiple UM cell lines and was well tolerated in zebrafish embryos, supporting further preclinical evaluation in animal models.

# PINPOINT: Prediction and prevention of Venous Thrombosis during chemotherapy (NCT07196020)- a multicenter prospective observational study

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**Background:** Venous thromboembolism (VTE) is a lethal but preventable complication in cancer. Risk increases during chemotherapy where rates of up to 20% are reported. Guidelines recommend primary prophylaxis with direct oral anticoagulants (DOACs) in high-risk ambulatory patients undergoing chemotherapy. The Khorana score is the recommended validated risk score to guide prophylaxis. However, sensitivity of the score for certain cancers is low, presenting need for biomarker discovery and validation. Data from our group suggests that markers of procoagulant activity (Factor VIIIc, thrombin generation and thrombomodulin) can predict VTE in ovarian cancer.

**Study Aim:** To serially measure procoagulant markers (Factor VIIIc, thrombin generation, thrombomodulin) in patients undergoing chemotherapy as dynamic predictors of VTE during cancer treatment.

**Patients and Methods:** The study aims to recruit 380 patients with a diagnosis of ovarian, lung, gastric or pancreatic cancer receiving chemotherapy in three centres (St. James Hospital, Mater Hospital and Cork University Hospital). The primary outcome is VTE occurring during chemotherapy. Sample size calculations were based on previous data and an estimated rate of VTE during chemotherapy of 10-20%. Procoagulant markers will be measured at 4 timepoints (pre-treatment, after cycles 1, 3 and the end of current treatment regimen). Predictive ability of the biomarkers will be compared with the Khorana risk score.

**Results:** 121 patients have been screened with 56 consented for the study (17 ovarian, 24 lung and 15 gastric/oesophageal cancers). Main reasons for exclusion include routine use of DOACs and change of treatment center post screening. 25 patients have reached study endpoint, while 15 discontinued or died. To date, VTE has been reported in 6 patients. Khorana risk scores have been calculated to stratify patients enrolled at baseline.

**Conclusion:** PINPOINT aims to develop VTE risk assessment in cancer patients during chemotherapy to support tailored prophylaxis and potentially reduce VTE rates during therapy.

*Thank the funder of the study HRB, all study centers and the patients for donating samples.*

# Extracellular Vesicle Proteomic Signature for Minimally Invasive Diagnosis and Relapse Prediction in Multiple Myeloma

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**Background:** Multiple myeloma (MM) is an incurable haematological cancer characterised by clonal plasma cell proliferation within the bone marrow. MM diagnosis and disease monitoring rely on invasive bone marrow biopsy, limiting frequent assessment and early detection of progression or relapse. Minimally invasive blood-based liquid biopsy approaches capable of accurately distinguishing monoclonal gammopathy of undetermined significance (MGUS), the pre-symptomatic stage of MM, from newly diagnosed MM, and relapse status would address a critical unmet clinical need. Extracellular vesicles (EVs), are highly abundant in blood and carry protein cargo reflective of their cell of origin, capturing tumour and immune states and represent a promising, stable, and scalable liquid biopsy platform.

**Patients and Methods:** Plasma-derived EVs were isolated from peripheral blood samples of 33 patients across the MM disease spectrum (MGUS, newly diagnosed MM, relapsed MM) and 12 healthy donors recruited at the Mater Misericordiae Hospital, Dublin. EV protein cargo was profiled by mass spectrometry. Supervised machine learning was applied to develop blood EV-based diagnostic models for MGUS and MM, as well as a prognostic model for relapse. Model performance was assessed using receiver operating characteristic analysis and standard classification metrics.

**Results:** A novel six-protein EV-derived signature enabled the development of three clinically relevant models: (i) MGUS diagnosis, (ii) MM diagnosis, and (iii) relapse prediction. Peripheral blood EV-based logistic regression models achieved accuracies exceeding 85% with areas under the curve >0.91. The identified proteins demonstrated disease stage–specific expression patterns, reflecting progressive immune dysregulation.

**Conclusions:** This study demonstrates that peripheral blood EV proteomics can support accurate, minimally invasive diagnosis and relapse prediction across the MM disease continuum. Overall, these findings highlight the potential of EV-based liquid biopsy to reduce reliance on bone marrow biopsy and enable clinically scalable disease monitoring. Ongoing prospective validation in an independent clinical cohort will support future translational application of this approach.

*We thank all the patients who consented to the use of their samples in this study and the staff at the Mater (University and private) Hospital for collecting the samples. We are particularly indebted to the patient advocates and members of Multiple Myeloma Ireland (Mary Kelly, Ann Fleming, Dermot O'Leary, and Joe O'Brien) who were part of the PPI panel for RS PhD advisory group. We would also like to acknowledge the support provided by Dr. Alfonso Blanco at the UCD Flow Cytometry core facility for his expertise in flow cytometry and Kieran Wynne from Systems Biology Ireland for his contributions to mass spectrometry analysis.*

# Evaluation of the Genesis System for Circulating Tumour Cell (CTC) Isolation

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**Background:** Circulating tumor cell (CTC) enumeration/characterization may be used to identify patients who are at risk of disease recurrence. However, CTC analysis has not entered clinical practice for lung cancer due to their rarity, heterogeneity and low recovery using existing CTC enrichment systems. This study evaluated the novel Genesis System (Bio-Rad) which uses Celselect Slides™ to capture CTCs based on size.

**Materials and Methods:** The Genesis system has two workflows: 1) Enrichment (captured cells eluted) and 2) Enumeration (captured cells fixed and stained on slide). The recovery rate (RR) of the system was assessed using spike-in experiments. Healthy donor blood (5 mL) was spiked with 100 $EpCAM^{high}$ H1975 cells or $EpCAM^{low}$ H1299 cells and processed through each workflow. Peripheral blood samples were obtained from 9 patients with resectable non-small cell lung cancer and matched pulmonary vein (PV) samples were collected intraoperatively from 3 patients. These 12 samples were processed for Enumeration. Captured cells were fixed and stained with immunofluorescent antibodies (Pan Cytokeratin, EpCAM, Vimentin, CD45) and target cells were identified either on the Lionheart FX (Agilent) using the 'Rare Cell Analysis Software' or the Keyence BZ-X800 and its analysis software. Ascites and malignant pleural samples were also processed (enumeration workflow) to determine the system's capability to capture clusters.

**Results:** The enrichment RRs for H1975 and H1299 cells were 71.27 ± 7% and 66.69 ± 10% respectively. The enumeration RRs were higher, but this increase was not significant: H1975 (75.30 ± 8%) and H1299 (78.06 ± 5%). CTCs were detected in 2/9 peripheral and 3/3 PV samples. The system was also successfully able to isolate clusters in ascites and pleural samples.

**Conclusions:** Our findings demonstrate that the Genesis System has an efficient workflow and high recovery. We hypothesise that the system has potential for clinical applications in lung cancer.

*HEA's North-South funding of the All-Ireland Cancer Liquid Biopsies (CLuB).*

# Validation of Novel Circulating Tumour DNA Methylation Targets for Risk Stratification of Resectable Non-Small Cell Lung Cancer Patients

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Lung cancer is the leading cause of cancer-related mortality in Ireland and worldwide. The standard of care for early-stage non-small cell lung cancer (NSCLC) is curative-intent surgery, with or without systemic therapy, followed by regular surveillance imaging. Despite this treatment regimen, recurrence rates for NSCLC remain high at 24% for stage I disease and 50% overall, highlighting the need for improved surveillance strategies and on-treatment biomarkers. Changes in cell-free DNA (cfDNA) methylation has shown utility in predicting relapse/response to treatment in NSCLC. This project aims to identify a novel circulating tumour DNA (ctDNA) methylation signature capable of detecting recurrence and monitoring treatment response in resectable NSCLC patients as part of the Trans-Atlantic Cancer Alliance for Liquid Biopsy Research and Training (TALenT).

Twist cfDNA methylome profiling was performed on cfDNA extracted from the plasma of 10 treatment-naïve patients with resectable NSCLC (5 of whom had disease recurrence and 5 of whom remained disease-free at two years post-surgery) versus 10 healthy controls. Novel differential cfDNA methylation signatures were identified in the samples from NSCLC patients versus healthy controls, and in the samples from patients who relapsed compared to those who remained disease-free. Validation of a panel of 6-8 differentially methylated genes, including DOCK8, OSM, and NKX6-1 is currently underway using MSRE-qPCR on genomic and nucleosomal DNA extracted from lung cancer cell lines, buffy coat preparations, and matched FFPE normal and tumour tissues. The most promising candidate genes will then be tested in a large cohort of longitudinal plasma samples from both relapsed and disease-free patients using MSRE-ddPCR.

This ctDNA methylation signature will ultimately be translated into a blood-based clinical assay to stratify risk in patients with resectable NSCLC and guide personalised treatment, enabling timely delivery of optimal therapies.

*The HRB's funding of TALenT.*

# Establishment of small cell lung cancer patient-derived organoids from malignant pleural effusions for assessment of chemotherapy response in small cell lung cancer

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**Background:** Small cell lung cancer (SCLC) is an aggressive malignancy characterised by rapid progression, early metastasis, and overall survival below 10%. It is typically diagnosed at advanced stages, rendering most patients unsuitable for surgical resection, and frequently recurs, with relapsed disease almost universally refractory to chemotherapy. SCLC can give rise to malignant pleural effusions (MPEs), in which tumour cells accumulate in pleural fluid. MPEs represent an accessible source of viable tumour cells for generating patient-derived organoids (PDOs), which can recapitulate key tumour features and provide a physiologically relevant model for studying SCLC biology and therapeutic response.

**Methods:** Tumour cells were isolated from MPE fluid and initially expanded in 2D culture to enrich for tumour cells before seeding into 3D culture. Cells were embedded in either Cultrex Reduced Growth Factor BME or VitroGel® Hydrogel Matrix, and maintained in specialised lung PDO medium, with or without 10% MPE fluid supplementation. PDOs were validated for retention of tumour markers vimentin, NCAM1, and TTF-1 by immunofluorescence, and compared with diagnostic cytology samples by immunohistochemistry. Chemosensitivity to cisplatin and etoposide, alone and in combination, was assessed using CellTiter-Glo-3D assay.

**Results:** PDOs remained viable and expandable for over 10 passages and demonstrated enhanced growth in medium supplemented with 10% MPE fluid compared to medium alone. PDO growth rate and PDO size was enhanced in VitroGel, supporting its suitability as a xeno-free 3D matrix. PDOs retained tumour marker expression consistent with diagnostic samples. Chemotherapy treatment demonstrated measurable effects from single-agent and combination, and IC50 concentrations were determined, providing a foundation for future studies.

**Conclusion:** We establish a standardised protocol for generating long-term, tumour-representative SCLC PDOs from MPEs, and demonstrate their responsiveness to standard-of-care chemotherapy, providing a robust platform for studying SCLC biology and accelerating the development of personalised treatments in SCLC.

# Personalised Drug Discovery Approach for Multiple Myeloma Using an Ex Vivo Bone Marrow Microenvironment Model

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**Background:** Multiple Myeloma (MM) is a plasma cell malignancy with a strong dependence on the bone marrow microenvironment limiting the effectiveness of conventional ex vivo culture systems. We have developed a 3 dimensional (3D) co-culture model incorporating multipotent mesenchymal stromal cells (MSCs), endothelial progenitor cells (EPCs) and Matrigel, a solubilized basement membrane matrix hydrogel, to better mimic the MM tumour niche. We evaluated MM cell viability, proliferation, and drug response in this model compared with traditional two-dimensional (2D) cultures.

**Materials and Methods:** Bone marrow aspirates from MM patients were processed by density gradient centrifugation and CD138+ myeloma cells were isolated by magnetic bead isolation. Primary bone marrow-derived MSCs and cord blood-derived EPCs were obtained from StemBioSys. Cells were embedded in Matrigel and maintained in 3D coculture. Microscopy was performed using fluorescent and confocal microscopy.

**Results:** Within 3 days, MSCs and EPCs embedded in Matrigel formed interconnected cellular networks resembling prevascular structures. Myeloma cell lines and primary CD138+ cells demonstrated sustained viability and proliferation in 3D co-culture for up to 28 days. Notably, primary CD138+ MM cells exhibited increased resistance to bortezomib in 3D culture compared with 2D culture, indicating that the model more closely recapitulates the in vivo microenvironment.

**Conclusions:** This 3D co-culture system supports long-term survival of primary MM cells in a physiologically relevant ex vivo model. In addition, it requires less MM cells than conventional 2D cell culture. This model has multiple potential applications such as preclinical evaluation of novel anti-myeloma agents and in personalised medicine approaches to guide patient-specific treatment strategies.

# Targeting Hypoxia-Mediated Treatment Resistance: NANOX, an Oxygen-Carrying Nanoemulsion, Modulates Metabolism and Inflammation in 3D Ex Vivo Tumour Explants Under Hypoxia

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**Introduction:** Hypoxia is a common feature of solid malignancies and predicts poor prognosis regardless of treatment approach. Hypoxia synergises with several hallmarks of cancer, including dysregulated metabolism and immunosuppression in establishing a treatment-resistant tumour microenvironment. Previously we identified NanOx, a novel hypoxia-modifying perfluorocarbon nanoemulsion, which significantly enhances the radiosensitivity of hypoxic, radioresistant oesophageal adenocarcinoma cells [1]. However, the impact of NanOx on hallmarks of cancer in more complex models remains unexplored. This study uses 3D ex vivo gastrointestinal (GI) tumour explants to provide new insights into the multimodal action of NanOx across several hallmarks of cancer, with the overall goal of improving radiotherapy efficacy for these patients.

**Methodology:** 3D *ex vivo* tumour explants from consented GI cancer patients attending St James' Hospital were cultured for 4h under hypoxic conditions (0.5% $O_2$). A repeated-measures approach was used to assess metabolism, inflammation, and oxidative stress pre- and post- NanOx +/- 2Gy radiotherapy. Explants were treated with NanOx for 30 min prior to 2Gy under hypoxia (0.5% $O_2$). Metabolism was assessed via the Seahorse bioanalyser. Tumour-conditioned media was collected to assess inflammatory and oxidative stress mediators. Data were analysed using two-way ANOVA and pathway enrichment analyses were performed using STRING.

**Results:** GI tumour explants cultured under hypoxia have heterogeneous metabolic, oxidative stress, and inflammatory profiles, with oxidative stress significantly correlated with inflammation. NanOx significantly reduced oxidative phosphorylation in GI tumour explants. NanOx significantly modulated the inflammatory profile of GI tumour explants and distinct to that of the NanOx and radiotherapy combination treatment. NanOx also significantly increased the level of extracellular lactate dehydrogenase, an indirect marker of tissue damage.

**Conclusion:** Hypoxia is a common feature of solid malignancies and significantly impairs treatment efficacy. We have identified a novel hypoxia-modifying nanoemulsion that modulates the metabolism and secretome of GI tumour explants under hypoxia.

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# Classical Meets Targeted: A Novel Therapeutic Strategy for Chronic Lymphocytic Leukaemia

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**Background:** Chronic lymphocytic leukaemia (CLL) is an incurable B-cell malignancy, with Ireland reporting among the highest incidence rates globally. Aberrant activation of the B-cell receptor (BCR) signalling pathway in these cells has driven the development of therapies targeting a key downstream component - Bruton's tyrosine kinase (BTK).

Despite the significant advancements achieved with these therapies, challenges persist. Resistance, off-target effects, and low complete response rates underscore the need for novel combination treatment strategies. As CLL cells exhibit cytoskeletal abnormalities, with tubulin closely associated with BCR-mediated signalling molecules, one strategy is to investigate the use of microtubule-targeting-agents (MTA) in combination with BTK inhibitors.

This study explores a potential therapeutic approach combining the BTK inhibitor ibrutinib with the MTA vincristine, to leverage complementary mechanisms and improve outcomes in CLL.

**Materials and Methods:** The cytotoxicity of both drugs and the combination on a panel of CLL cell lines were determined using MTT proliferation assays. The levels of apoptosis were determined by annexin-V/propidium iodide staining and flow cytometry in CLL cell lines, patient-derived CLL cells cultured alone and in co-culture with the human stromal HS5 cells, and in healthy donor PBMC and platelets. Drug synergism was determined using CompuSyn software. The effect of this combination on primary CLL cell division was evaluated by CFSE staining and KI-67 expression in a specialised *ex-vivo* cell culture model.

**Results:** The ibrutinib/vincristine combination demonstrates a synergistic cytotoxic effect in a panel of CLL cell lines. Combination treatments resulted in significantly increased apoptosis of patient-derived CLL cells cultured alone and in co-culture with HS5 stromal cells. Primary CLL cells can be induced to proliferate in a specialised cell culture model and this proliferation is abrogated following treatment with this novel combination.

**Conclusions:** This work demonstrates that ibrutinib in combination with the MTA vincristine may show promise as a novel treatment strategy in CLL.

*All patients who donated their samples, clinical colleagues who orchestrated sample acquisition, all the staff at SJH for their ongoing work.*

# The TCD-SJH Haematology Biobank: Revolutionising Translational Blood Cancer Research

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**Background:** Haematological malignancies comprise a heterogeneous group of diseases, with incidence in Ireland projected to increase to 3000 new cases identified annually by 2030. Access to high quality patient biospecimens along with comprehensive clinical data is limited, but often a requirement to support translational research. Consequently, biobanks play a critical role in enabling this translational research, along with biomarker discovery and development of novel therapeutic strategies. We describe the continued growth and development, scope and patient sample methodology of the TCD-SJH Haematology Biobank which has supported blood cancer research for over a decade.

**Materials and Methods:** Peripheral blood and bone marrow aspirates are collected from patients with informed consent under institutional ethical approval. Samples are obtained at diagnosis and/during relevant clinical timepoints. Depending on disease type, plasma, serum and mononuclear cells are isolated using density gradient centrifugation and carefully harvested, immunophenotyped and prepared for cryopreservation before long-term storage in liquid nitrogen. Clinical and demographic data are recorded and linked to biospecimens in a secure database, readily available for approved research studies.

**Results:** As of 2026, approximately 444 patients have been consented into the Haematology Biobank. The diversity of the biobank is representative of the spectrum of haematological malignancies, including Acute Lymphoblastic Leukaemia (ALL), Acute Myeloid Leukaemia (AML), Promyelocytic Leukaemia (PML), Myelodysplastic Syndromes (MDS), Multiple Myeloma, Lymphoma and Chronic Lymphocytic Leukaemia (CLL). In 2025 alone, over 100 patients were successfully recruited, with biospecimens collected and cryopreserved.

**Conclusions:** This Haematology Biobank represents a robust and expanding resource of high quality blood and bone marrow patient samples with longitudinal clinical annotation. It provides a valuable platform to support current and future translational research in haematology, including but not limited to studies of disease biology, ex-vivo models for evaluating treatment response/precision medicine and novel therapeutic targets.

# Feasibility of Circulating Tumour Cell Detection and HER2 Characterisation in Advanced/Metastatic Oesophagogastric Cancer

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**Background:** Circulating tumour cells (CTCs) represent a potential non-invasive approach for tumour characterisation and disease monitoring in oesophagogastric cancers. However, detection and downstream phenotypic analysis of CTCs remain poorly explored in this context, particularly for the detection of clinically actionable biomarkers such as HER2. We present early findings from an ongoing pilot study evaluating CTC enrichment and HER2 characterisation as a proof of concept for wider biomarker testing using liquid biopsy based approaches.

**Methods:** Patients were prospectively enrolled as part of an ongoing translational study. Whole blood samples were obtained from peripheral venous access, with matched samples from peripherally inserted central catheters where available. CTC enrichment was performed using the ANGLE Parsortix microfluidic platform. Enriched cells underwent fluorescence-based imaging for CTC identification using epithelial and nuclear markers. HER2 status of the primary tumour was determined using standard immunohistochemistry(IHC), with fluorescence in-situ hybridisation(FISH) performed for equivocal (2+) cases in accordance with clinical guidelines. HER2 expression on detected CTCs was assessed using fluorescence-labelled antibodies. A subset of patients underwent longitudinal sampling during systemic chemotherapy to evaluate dynamic changes in CTC detection.

**Results:** To date, 11 patients have been included; two (18%) had locally advanced, node-positive disease treated with curative-intent neoadjuvant chemotherapy followed by surgery, and nine (82%) had stage IV metastatic disease receiving palliative systemic therapy. Primary tumour HER2 assessment demonstrated HER2 3+ expression in 3 of 11 patients (27%), HER2 2+ in 1 patient (9%), and HER2 1+ in the remainder. CTCs were detected in 3 of 11 patients (27%).Among patients with detectable CTCs, HER2-positive CTCs were identified in 1 patient In 1 patient sampled longitudinally, clearance of detectable CTCs following administration of chemotherapy was observed.

**Conclusion:** CTC detection is feasible in advanced/metastatic oesophagogastric cancer, although detection rates were modest in this small pilot cohort, recruitment is ongoing within a prospective pilot study.HER2 characterisation from CTCs provides proof of concept for this approach and supports ongoing expansion of the study to evaluate additional biomarkers and longitudinal disease dynamics.

# Enhancing Radiotherapy Efficacy in Gastrointestinal Tumours – Development of a Lipid-based Nanoparticle Delivery System for Locoregional Administration of microRNA and Cancer Therapy

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Many solid malignancies are inherently resistant to radiotherapy. Developments in RNA interference as a therapeutic avenue in cancer treatment may offer a novel anti-tumour approach; with our lab previously demonstrating enhanced radiotherapy sensitivity of gastrointestinal tumours in response to microRNA modulation. Here, we develop and optimise the formulation of lipid-based nanoparticles for the delivery of microRNA to solid tumours. An optimal size range of 100nm to 200nm has been reported to improve efficacy and safety of nanoparticle systems; achieved through varying lipid composition ratios, or downstream techniques, such as sonication.

Lipid-based nanoparticles were prepared via the thin-film hydration method, containing the ionizable cationic lipid 6-((2-hexyldecanoyl)oxy)-N-(6-((2-hexyldecanoyl)oxy)hexyl)-N-(4-hydroxybutyl)hexan-1-aminium (ALC-0135), with 18:1 (Δ9-Cis) PC (DOPC), cholesteroland 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG(2000)). Lipids were dissolved in ethanol and solvent removed using a rotary evaporator. Lipid films were then rehydrated with phosphate-buffered saline. To examine the effect of lipid composition on nanoparticle size, lipid ratios were varied. Following rehydration, the mixture was disrupted by probe sonication at 50% amplitude, for varying time intervals. Material characterisation of resultant nanoparticles, including particle size distribution (DH) and polydispersity index (PDI), were measured via dynamic light scattering using a Zetasizer Nano ZS.

Results from both DH and PDI testing indicate that varying phospholipid:cholesterol ratio affected the efficiency of lipid film rehydration and resulted in changes to average particle size. Sonication resulted in improvements in size and homogeneity, causing a pronounced reduction in both measures after 2 minutes sonication; with longer durations producing modest further improvements.

Our results indicate the phospholipid:cholesterol ratio affects average nanoparticle size, affecting the lipid rehydration process. Sonication offers a suitable down-sizing technique, confirmed with improvements across DH and PDI. This work will inform future formulation studies to encapsulate radio-sensitising microRNA within the nanoparticle system. Further work will assess the radiosensitising effects under varying hypoxia concentrations; and if microRNA-mediated modification of cell biology can enhance tumour radiosensitivity.

## Proteomic profiling of extracellular vesicles cargo identifies key proteins that could have diagnostic and prognostic relevance as liquid biopsies in non-small cell lung cancer

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**Background:** Lung cancer is the leading cause of cancer-related death in men and the second leading cause in women. CT scans are currently used for lung cancer screening, but are limited by cost and a high rate of false-positive findings, hence there is a need for biomarkers for detection and surveillance of lung cancer. Extracellular vesicles (EVs) represent a promising source of biomarkers, as they circulate in the blood carrying cargo (such as proteins and nucleic acids) that reflect the tumour cells from which they originate.

**Methods:** Plasma samples were collected from patients with non-small cell lung cancer (NSCLC, $n$=30) prior to surgery, as well as from healthy donors (HD, $n$=10). Samples were processed by size-exclusion chromatography to collect EVs. The EVs were characterised by immunoblotting, transmission electron microscopy, and nanoparticle tracking analysis. EVs from all 40 individuals were analysed by mass-spectrometry to determine their proteomic profiles. The proteomics data was analysed using Perseus, and key proteins were validated by ELISA.

**Results:** Proteomic analysis identified 5 proteins significantly up-regulated and 17 proteins down-regulated in EVs from patients with NSCLC compared to HDs. Two upregulated proteins, lipopolysaccharide-binding protein (LBP) and quiescin sulfhydryl oxidase 1 (QSOX1), were selected for validation. Both proteins were found to discriminate between NSCLC and HDs, based on ROC curve analysis (LBP – AUC: 0.9700, $p<0.0001$, QSOX1 – AUC: 0.8767, $p=0.0004$). LBP quantities appeared to decrease after surgery (2 days post-op), but not in a statistically significant manner. However, QSOX1 quantities dropped significantly between the pre-op and post-op samples ($p=0.0262$).

**Conclusion:** Blood-based EVs-carried LBP and QSOX1 can discriminate between healthy people and those with NSCLC. Quantities of both proteins decrease post-surgery, supporting our hypothesis that they are released from the tumour.

*Acknowledgements: HEA's North-South funding of the All-Ireland Cancer Liquid Biopsies (CLuB)*

# Large extracellular vesicles in blood liquid biopsies: potential diagnostic and prognostic biomarkers across ovarian cancer subtypes

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**Background:** Epithelial ovarian cancer (EOC) is the most common type of ovarian cancer. As its symptoms can be non-specific, the disease often goes undiagnosed until it is too late. Biomarkers for diagnosing and subtyping EOC are urgently needed. Thus, this study aimed to investigate if blood-based extracellular vesicles (EVs) might have relevance as minimally-invasive biomarkers for this purpose.

**Methods:** Plasma samples from patients with benign ($n=20$) and malignant ($n=42$, mixed histology) ovarian tumours were processed by size-exclusion chromatography to collect EVs, which were characterised by immunoblotting, transmission electron microscopy, and nanoparticle tracking analysis.

**Results:** EVs from women with benign ovarian lesions were significantly smaller ($p<0.0001$) yet more abundant ($p<0.0001$) compared to those from malignant ovarian tumours. Particle distribution parameters were used to assess differences between patient groups. NTA distribution parameters (D10, D50, D90) represent the points in the size distribution under which 10%, 50% and 90% of the sample is found. EVs collected from patients with malignant tumours had significantly higher D10, D50, and D90 thresholds ($p<0.0001$ for each) compared to those coming from patients with benign lesions. This shows that particles are bigger in samples collected from patients with cancer. Interestingly, when comparing early-stage (stage I+II, $n=20$) with advanced-stage disease (stage III+IV, $n=22$), total particle amounts did not differ. However, early-stage patients had significantly lower D10 ($p=0.0013$), D50 ($p=0.0029$), and D90 ($p=0.0036$) compared to those with more advanced stage disease. This shows that the patients with more advanced disease have significantly bigger particles overall than those with early-stage disease.

**Conclusion:** Our research indicates that there are differences in the sizes of EVs produced by malignant tumours compared to benign lesions. It also suggests that the amounts of blood-based particles of the larger size correlate with more advanced disease.

*Acknowledgements: HEA's North-South funding of the All-Ireland Cancer Liquid Biopsies (CLuB).*

# Investigating NKAPL, a Potential Marker for Platinum Resistance, in High-Grade Serous Ovarian Cancer

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**Background:** Understanding mechanisms of acquired chemoresistance in high-grade serous ovarian cancer (HGSOC) is a critical unmet question, with potential to improve patient outcomes. Previously, we found *NKAPL* (NF-KB activating protein-like) to be hypermethylated and gene expression reduced in chemoresistant HGSOC tumours. We hypothesise that increased *NKAPL* methylation and low expression may be an indicator of resistance in HGSOC and aim to investigate its suitability as a prognostic/predictive biomarker.

**Material and Methods:** A panel of HGSOC cell lines were characterised for chemosensitivity, *NKAPL* methylation and gene expression. OVCAR4, CAOV3, PEO1, PEO4, UWB1.289-Parental, COV318, Kuramochi and UWB1.289-BRCA1 were used and *NKAPL* gene expression and methylation was determined through RT-qPCR and qMSP. Since *NKAPL* is poorly characterised, overexpression was performed in HEK293T cells to gain mechanistic insights and since *NKAPL* may regulate NOTCH signalling, downstream gene expression was analysed. *NKAPL* protein was assessed by Western Blot.

**Results:** Low levels of *NKAPL* gene expression were seen in all cell lines apart from the platinum-resistant COV318. No correlation between chemosensitivity, DNA methylation and gene expression was seen. In cell lines from the same patient before and after platinum treatment (PEO1 & PEO4) chemosensitivity was reduced and *NKAPL* methylation significantly increased post-treatment. Western blotting revealed a molecular weight of *NKAPL* of ~65kDa, higher than the predicted ~46kDa suggesting post-translational modifications. No changes in *NOTCH* target gene expression were seen in HEK293T cells after *NKAPL* overexpression.

**Conclusions:** DNA methylation results in the paired cell lines PEO1 and PEO4 support the hypothesis that *NKAPL* methylation may affect platinum sensitivity. However overall, chemosensitivity did not correlate with methylation or expression of *NKAPL* across the HGSOC cell line panel. Further analysis clarifying the function and structure of *NKAPL* is needed and its clinical relevance needs to be evaluated in ovarian specific models.

# A Cell Atlas of Malignant Pleural Effusion

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**Background:** Malignant Pleural effusion (MPE) is the over-accumulation of fluid in the pleural cavity which may present as a complication of lung, breast or other cancers. MPE has a devastating effect on patient survival, reducing median survival to 3-12 months. Current diagnostic cytology techniques attain 46-51% sensitivity in detecting malignant cells in pleural fluid. Recent pilot studies have shown that Single Cell RNA-sequencing (scRNA-seq) achieves 91% sensitivity in detecting malignant cells, but this requires tissue-specific reference data. Utilising scRNA-seq datasets we constructed a Cell Atlas of Pleural Effusion (CAPE) which may be used as a reference to detect cancer cells in pleural fluid scRNA-seq samples.

**Methods:** We created a reference database by carrying out a systematic literature review to identify publicly available scRNA-seq datasets from PE patient samples. We performed scRNA-seq experiments to generate novel datasets from PE samples isolated from patients at St James's Hospital. These datasets were computationally integrated using mutiomic techniques to create CAPE. Clinical metadata was also collated and harmonised to the standards of Human Cell Atlas consortium.

**Results:** To date we identified 35 public scRNA-seq datasets. We computationally integrated 14 of these in our current version of CAPE, which consists of 420,000 cells from 81 individuals, including 25 individuals with lung or breast malignancies. Comparing these 25 samples to 36 control samples we found that CEACAM6+ cell percentage to be a promising diagnostic biomarker, achieving 92% sensitivity and 97.2% specificity (ROC AUC 0.947, $p = 5.84\ 10^{-10}$).

**Conclusion:** To validate CEACAM6 as a diagnostic biomarker we need a larger and more diverse cohort. To realize this we will generate novel datasets, including 39 MPE from lung, breast and other cancers, with 29 matched controls. Our final CAPE resource will contain over 1 million cells from over 150 patients.

# PRC2 deficiency is associated with targetable metabolic vulnerabilities in high-risk AML

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**Background:** Acute Myeloid Leukaemia (AML) is the most frequent cause of blood cancer mortality in young people. We and others have previously reported that loss of function of Polycomb Repressive Complex 2 (PRC2) is associated with chemoresistance and poorer prognosis in AML, identifying a patient cohort requiring more effective treatment strategies. Given increasing evidence for the importance of metabolic phenotype in AML cells, and the role of EZH2-mediated PRC2 activity in regulating energy metabolism, our aim is to understand how PRC2 loss-of-function alters the energetic profile of AML cells to identify potential novel treatments.

**Materials and Methods:** To study the effects of PRC2 loss in AML, we generated isogenic cell line models with deficient PRC2 activity by CRISPR-Cas9–mediated deletion of EZH2 in three AML cell lines. We performed metabolic profiling using Seahorse and drug sensitivity assays to identify altered metabolic pathways in PRC2-deficient AML, while RNA-seq and proteomics were used to define network changes underpinning these alterations. Further orthogonal validation using pharmaceutical PRC2 inhibition was also performed.

**Results:** We found that PRC2-depleted cells exhibit significantly altered metabolism. Specifically, EZH2-deficient cells show increased ATP production via oxidative phosphorylation (OXPHOS) and, accordingly, altered sensitivity to pharmacological inhibition of glycolysis (2DG) and OXPHOS (IACS-010759), with reduced sensitivity to glycolysis inhibition alone and requiring dual inhibition to effectively induce cell death. Supporting this, proteomic analysis suggests upregulation of fatty acid oxidation, which may sustain increased OXPHOS and represents a potential therapeutic target.

**Conclusions:** Our data identifies that PRC2 loss of function is associated with targetable altered metabolic activity in AML. We are currently investigating the contribution of specific nutrients, including amino acids and fatty acids to the increased mitochondrial respiration in these cells to identify novel therapeutic targets for this form of AML, and further study the precise epigenetic mechanisms underlying this altered metabolic phenotype.

# Elucidating Malignant Prostate Cell Interactions with Nerves: A Potential New Target for Cancer Therapy

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**Background:** Prostate cancer (PCa) is the most common malignancy among men in western countries. Its progression is influenced by hypogastric and pelvic nerves, which regulate prostate function. Tumour-induced peripheral nerve hypertrophy, tumour invasion, and neurotransmitter signalling promote cancer growth, metastasis, and pain. This study aims to identify factors secreted by PCa cells with neuromodulatory effects using bioinformatics and a PC12 differentiation model.

**Materials and Methods:** A cohort of 467 PCa patients was analysed using the cBioPortal platform to examine the role of neuronal signalling and nerve fibre hypertrophy in PCa progression. Gene expression profiles across disease stages (localised vs. advanced/metastatic) were investigated using the GEO database. Secreted neurotrophic factors from three PCa cell lines (LNCaP, DU145, PC3) of varying aggressiveness were assessed by applying conditioned media (CM) to PC-12 cells, followed by neurite outgrowth quantification using phase-contrast microscopy and ImageJ. The CM was then concentrated and re-tested to determine whether enrichment of secreted neurotrophic factors enhanced neurite outgrowth. The contribution of NGF was assessed by repeating the assay with CM containing an NGF-neutralising antibody.

**Results:** Bioinformatic analysis identified NGF, TGFβ1, and STX1A as genes associated with PCa aggressiveness. NGF and TGFβ1 are implicated in neuronal differentiation, whereas STX1A is essential for neurotransmitter release. PC-3 and DU145 CM significantly promoted PC-12 cell differentiation, with DU145 CM exhibiting the strongest effect. Concentrated CM increased neurite length and the number of neurite outgrowths. Neutralisation with an anti-NGF antibody partially attenuated DU145 CM-induced differentiation; however, responses remained significantly elevated compared to controls, indicating the involvement of additional secreted factors.

**Conclusions:** These findings suggest that PCa cells produce neuro-neoplastic signalling factors correlated with disease aggressiveness. Further studies will characterise molecules secreted by DU145 and their effects on peripheral neurons. Inhibitors of neurotransmission warrant investigation as potential therapeutic interventions.

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# Epigenetic Modifier GSK-J4 Enhances Sensitivity to Venetoclax in Multiple Myeloma

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**Introduction:** Multiple myeloma (MM) is an incurable haematological cancer with increasing prevalence. MM cells are heterogeneous relying on diverse anti-apoptotic BCL-2 family proteins (BCL-2, MCL-1, and BCL-XL), for survival. Venetoclax, an FDA-approved BCL-2 inhibitor, has shown promise in a subset of MM patients that are BCL-2-dependent t(11;14) cells. However, approximately only 20% of MM patients rely on BCL-2 for survival. To expand the therapeutic potential of venetoclax, we performed an epigenetic modifier screen and identified that GSK-J4 enhanced the sensitivity to venetoclax.

**Methods:** A panel of non-t(11;14), MCL-1–dependent MM cell lines and patient samples were treated with a combination of GSK-J4 and venetoclax. Cell death was assessed using Annexin V/PI. mRNA and protein expression were accessed by RNA sequencing, qRT-PCR, and western blotting, following GSK-J4 treatment. Mature MM surface markers were assessed after GSK-J4 treatment by flow cytometry. The SeahorseXFe96 assay was used to assess oxygen consumption rate, after GSK-J4 treatment.

**Results:** First, we confirmed that the combination of GSK-J4 and venetoclax enhanced cell killing non-t (11;14) cell lines and in primary MM patient samples. Next, we assessed the transcriptional changes induced by GSK-J4 using RNA sequencing. GSK-J4 treatment induced an upregulation of BCL-2 mRNA and downregulation of MCL-1 mRNA, which was confirmed by qRT-PCR and Western blotting. Pathway analysis of the RNA sequencing revealed a downregulation of genes involved in OXPHOS. Next, we confirmed that GSK-J4 altered OXPHOS metabolism using the Seahorse assay. We also identified an altered mitochondrial network using confocal microscopy and TMRE staining. Lastly, GSK-J4 decreased the expression of B-cell maturation antigen BCMA and CD138 on the surface of MM cells, suggesting that the epigenetic modifier may be inducing a more immature phenotype.

**Conclusion:** Our findings suggest that the epigenetic modifier GSK-J4 is driving mature non-t(11;14) MM cells to a more immature phenotype. Thereby inducing a reliance on BCL-2 and an altered metabolic phenotype. This combination could expand the use of venetoclax into both t(11;14) and non-t(11;14) MM patients.

# Investigating the Role of FKBPL in Tumour-Associated Macrophages in Hepatocellular Carcinoma

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**Background:** Hepatocellular carcinoma (HCC) is a major cause of cancer mortality, requiring better therapies. FKBPL, an FKBP family member, shows anti-cancer roles in breast/ovarian cancer but pro-tumour effects in HCC; its mechanism remains unclear. Tumour-associated macrophages (TAMs) influence tumour progression via M1 (anti-tumour) or M2 (pro-tumour) phenotypes. FKBPL regulates ER-phagy under stress, suggesting a link to macrophage polarization.

**Materials and Methods:** THP-1 cells were polarized to M1/M2 using LPS+IFN-$\gamma$ or IL-4+IL-13. qPCR assessed M1 (TNF-$\alpha$, IL-1$\beta$), M2 (IL-10, CD206), and FKBPL expression. HepG2 cells were FKBPL-overexpressed (OE) or knocked down (KD), and their conditioned media (CM) tested on macrophage polarization. Cytokines in CM were measured by ELISA; ER stress markers (BiP, CHOP) analyzed after FKBPL KD.

**Results:** FKBPL was differentially expressed in M0, M1, and M2 macrophages, with highest levels in M1. CM from HepG2, HepG2OE and HepG2KD cells modulated the macrophage phenotype, potentially suppressing M1 polarization. FKBPL knockdown in M0 macrophages disrupted polarization dynamics. Cytokine assays showed reduced RANTES, MIP-1$\delta$, and IL-6sR in CM from HepG2OE cells, which may induce immune-tolerant TAM formation. FKBPL knockdown enhanced ER stress gene expression ($p<0.05$) following treatment with tunicamycin, a known ER stressor.

**Conclusions:** These data suggest that FKBPL may promote HCC progression by modulating ER stress and shifting macrophage polarisation toward an immunosuppressive M2 phenotype. Targeting FKBPL could offer a novel strategy to reprogram the TME and enhance anti-tumour immunity.

# Investigating the role of FK506-Binding Protein-Like (FKBPL) in treatment-resistant oesophageal adenocarcinoma

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**Background:** The incidence rate of oesophageal cancer is predicted to rise sharply by 2045. The standard of care for oesophageal adenocarcinoma (OAC) is neoadjuvant chemotherapy with or without radiation. However, only ~30% of patients achieve a complete pathological response. The resistant patients face therapy-related toxicities, often worsening their prognosis. Currently, no reliable biomarkers predict treatment response, highlighting the urgent need to elucidate potential mechanisms of treatment-resistance.

**Materials and Methods:** FKBPL levels were assessed in the isogenic radioresistant OAC model OE33 parental (OE33P) and OE33 radioresistant (OE33R) cells at baseline and following a clinically-relevant dose of 1.8Gy radiation. mRNA and protein expression of FKBPL were measured by qPCR and Western Blot. Serum FKBPL levels were measured by ELISA in 112 pre-treatment OAC patients and FKBPL mRNA levels were assessed in OAC biopsies by qPCR and correlated with treatment response.

**Results:** FKBPL mRNA levels were not altered in OE33P or OE33R cells at baseline or following 1.8Gy radiation. FKBPL protein expression was higher in OE33R cells compared to OE33P cells ($p=0.0320$). Following 1.8Gy radiation, FKBPL protein levels increased in the OE33P cells ($p=0.0298$) but not the OE33R cells. Higher FKBPL mRNA expression was observed in OAC patient biopsies that had a poor response to neoadjuvant treatment ($p=0.036$). There was no significant difference in serum FKBPL levels between good and poor responders to neoadjuvant treatment.

**Conclusions:** FKBPL is elevated in treatment resistant OAC cells and patient biopsies suggesting FKBPL may be implicated in treatment resistance in OAC and has potential as a biomarker for treatment response.

# Remodelling of the Tumour Glycome in Oesophageal Adenocarcinoma: Implications for Tumour Microenvironmental Immune Suppression

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Oesophageal adenocarcinoma (OAC) remains a lethal malignancy with an incidence that continues to rise worldwide. Although recent regulatory approvals have enabled the use of immune checkpoint inhibitors (ICIs), response rates in OAC and most solid tumours remain low, typically ranging from 13–29%. Current clinical strategies largely focus on combining ICIs with chemotherapy or radiotherapy, yet relatively few approaches directly target the immune-evasion mechanisms that contribute to resistance. One emerging axis of interest is cancer-associated glycosylation—the enzymatic modification of proteins and lipids with sugar structures or glycans. Aberrant glycosylation, particularly the upregulation of a specific sugar, sialic acid, on tumour cells, can suppress anti-tumour immunity through binding to SIGLECs (Sialic acid-binding ImmunoGlobulin-like LECtins) receptors expressed on specific immune cell populations. This mechanism is increasingly recognised as a contributor to tumour infiltrating T-cell dysfunction and poor ICI responsiveness.

We hypothesised that tumour progression and exposure to tumour microenvironmental and therapy-induced stress drive enhanced tumour cell sialylation during OAC development. To investigate this, a panel of well-characterised oesophageal epithelial cell models representing normal epithelium, Barrett's oesophagus, dysplasia and oesophageal adenocarcinoma was assessed. Basal glycosylation was profiled using lectin-based flow cytometry, to assess specific glycan structures. OAC cell glycosylation was further examined under hypoxic conditions and following exposure to clinically relevant radiation doses.

Distinct alterations in glycosylation and sialylation were observed across disease progression, with OAC cells demonstrating increased sialic acids compared with non-malignant models. Hypoxia further modified glycan and sialylation profiles in a cell line-dependent manner, while irradiation was also associated with altered tumour sialylation.

This data indicates that disease associated sialylation alterations are established early in malignant oesophageal disease progression and is further shaped by tumour microenvironmental and treatment-related stress.

Previous work from our group has shown that irradiation induces increased expression of immune checkpoint molecules, including PD-1 and its ligand, consistent with treatment-induced immunosuppression. As sialic acids are a prerequisite for SIGLEC engagement, treatment-associated increases in tumour sialylation may create a permissive landscape for SIGLEC-mediated immune inhibition.

# Proteomic Analysis Reveals Impaired Activation and Mitochondrial Defects in NK Cells from Metastatic Breast Cancer Patients

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**Background:** Natural killer (NK) cells are critical mediators of anti-tumour immunity. Previous work from our group has demonstrated that NK cells from metastatic breast cancer (MetBC) patients exhibit metabolic and functional defects. However, the molecular mechanisms underpinning this dysfunction remain poorly understood. Therefore, we applied quantitative proteomics to identify pathways associated with NK cell dysfunction in MetBC patients.

**Materials and Methods:** Mass spectrometry–based quantitative proteomics was performed on unstimulated and IL-2–activated NK cells from healthy donors and MetBC patients. Flow cytometry was used to assess mitochondrial parameters, functional outputs and global histone H3 acetylation.

**Results:** Global proteomic structure was comparable between healthy donors and MetBC patients. However, following IL-2 stimulation, MetBC NK cells exhibited an attenuated response, upregulating substantially fewer proteins. This indicates a failure to appropriately remodel the NK cell proteome in response to cytokine activation.

Pathway enrichment analysis revealed substantial mitochondrial dysfunction in MetBC patients. Despite having a higher mitochondrial mass, components of the electron transport chain, particularly complexes III–V, were deficient under both basal and activated conditions. Functionally, this was associated with reduced mitochondrial membrane potential, consistent with impaired capacity to establish and maintain mitochondrial polarization.

In parallel, IL-2–activated NK cells from MetBC patients failed to induce global histone H3 acetylation. Notably, this defect persisted following treatment with the pan-histone deacetylase inhibitor panobinostat, suggesting a constrained capacity for histone acetylation in metabolically dysfunctional NK cells.

**Conclusions:** These data demonstrate that NK cell dysfunction in metastatic breast cancer is characterised by impaired activation-induced proteomic remodelling, selective deficiencies in mitochondrial respiratory chain components, and defective induction of histone H3 acetylation. Together, these findings provide new mechanistic insight into NK cell dysfunction in MetBC and identify metabolic and epigenetic constraints that may limit effective anti-tumour immunity.

## Turning Up the Voltage on Anti-Cancer Immunity

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**Introduction:** Electroporation therapy employs pulsed electric fields to transiently permeabilize cancer cell membranes, enabling intracellular delivery of otherwise impermeant agents such bleomycin (electrochemotherapy) or calcium ions (calcium electroporation; CaEP). CaEP has induced tumour regression in clinical settings of metastatic melanoma and gastrointestinal cancers. A small subset of patients experience systemic anti-cancer responses potentially induced by strong immunogenic cell death (ICD)[1].

The metal ions manganese ($Mn^{2+}$), zinc ($Zn^{2+}$) and selenium ($Se^{4+}$) have been noted for their ability to induce mitochondrial dysfunction and ICD[2,3]. In this study, the ability of these ions to induce ICD and anti-tumour immune responses by electroporation-mediated delivery was investigated by comparison against the established CaEP protocol.

**Methods:** MC38 cells were electroporated in the presence of selected ions using standard clinical electroporation parameters. $IC_{50}$ concentrations were determined for each ion using real-time imaging. ICD was determined by cell-surface expression of calreticulin and caspase-1 activation by flow cytometry. Gasdermin-D cleavage was assessed by immunoblot.

**Results:** Each ion induced cell death in a dose-dependent manner. At 24 hours post-electroporation, SeEP and ZnEP displayed increased cell surface CALR expression relative to CaEP (~20% and ~30%, respectively). ZnEP displayed the greatest increase in caspase-1 activation relative to electroporation alone.

**Conclusion:** Emerging evidence suggests that robust induction of ICD is essential to drive adaptive anti-cancer responses, and may improve the outcomes of immunotherapies. These data combined show that delivery of zinc ions enhances the immunogenicity of electroporation-based treatments compared to calcium.

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# Investigating the applicability of cytokine-induced memory-like NK cell therapy for obesity-associated cancer

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**Background:** Oesophageal adenocarcinoma (OAC) is a poor-prognosis cancer with a 5-year survival rate below 25% and limited responses to chemo-radiotherapy and immunotherapies. Natural Killer (NK) cell–based adoptive therapies are constrained by poor in vivo persistence and ineffective tumour homing and infiltration. Cytokine-induced memory-like (CIML) NK cells are an emerging subset with potential for enhanced persistence and function within the solid tumour microenvironment. Here, we propose to investigate the therapeutic potential of CIML NK cells for OAC.

**Methods:** To generate CIML NK cells, healthy-donor NK cells were treated with high-dose IL-12, IL-15 and IL-18 for 16 hours, then expanded in the presence of low-dose IL-15 for 7 days. Control cells were maintained in low-dose IL-15 throughout. On day 7, cells were phenotyped using flow cytometry. NK cell cytotoxicity was tested using a K562 killing assay.

**Results:** Our CIML NK cell generation protocol yields high frequencies of reprogrammed memory-like NK cells with a highly activated phenotype, expressing higher levels of CD69, CD25, NKp30, NKp46 and TRAIL and lower levels of PD-1. CIML NK cells expressed significantly higher levels of CCR5 at baseline, and decreased expression of CX3CR1 following restimulation. Upon restimulation, CIML NK cells exhibited increased Granzyme B, TNF-$\alpha$ and IFN-$\gamma$ production while retaining cytotoxicity against K562 cells, indicating resistance to functional exhaustion. Further, CIML NK cells were more resistant to suppression of IFN-$\gamma$ production induced by soluble factors from OAC tumours.

**Conclusions:** Our data indicate that CIML NK cells are highly activated and do not become functionally exhausted following culture and re-stimulation. The distinct chemokine signature of CIML NK cells provides a potential target for chemokine directed modulation of migration to improve tumour homing. Future studies will further interrogate CIML NK cell potency within the OAC tumour microenvironment and identify whether specific pathways can be targeted to maximise infiltration and killing of OAC tumours.

# Evaluation of the Genesis System for Circulating Tumour Cell (CTC) Isolation

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**Background:** Circulating tumor cell (CTC) enumeration/characterization may be used to identify patients who are at risk of disease recurrence. However, CTC analysis has not entered clinical practice for lung cancer due to their rarity, heterogeneity and low recovery using existing CTC enrichment systems. This study evaluated the novel Genesis System (Bio-Rad) which uses Celselect Slides™ to capture CTCs based on size.

**Materials and Methods:** The Genesis system has two workflows: 1) Enrichment (captured cells eluted) and 2) Enumeration (captured cells fixed and stained on slide). The recovery rate (RR) of the system was assessed using spike-in experiments. Healthy donor blood (5 mL) was spiked with 100 $EpCAM^{high}$ H1975 cells or $EpCAM^{low}$ H1299 cells and processed through each workflow. Peripheral blood samples were obtained from 9 patients with resectable non-small cell lung cancer and matched pulmonary vein (PV) samples were collected intraoperatively from 3 patients. These 12 samples were processed for Enumeration. Captured cells were fixed and stained with immunofluorescent antibodies (Pan Cytokeratin, EpCAM, Vimentin, CD45) and target cells were identified either on the Lionheart FX (Agilent) using the 'Rare Cell Analysis Software' or the Keyence BZ-X800 and its analysis software. Ascites and malignant pleural samples were also processed (enumeration workflow) to determine the system's capability to capture clusters.

**Results:** The enrichment RRs for H1975 and H1299 cells were 71.27 ± 7% and 66.69 ± 10% respectively. The enumeration RRs were higher, but this increase was not significant: H1975 (75.30 ± 8%) and H1299 (78.06 ± 5%). CTCs were detected in 2/9 peripheral and 3/3 PV samples. The system was also successfully able to isolate clusters in ascites and pleural samples.

**Conclusions:** Our findings demonstrate that the Genesis System has an efficient workflow and high recovery. We hypothesise that the system has potential for clinical applications in lung cancer.

*HEA's North-South funding of the All-Ireland Cancer Liquid Biopsies (CLuB).*

# Validation of Novel Circulating Tumour DNA Methylation Targets for Risk Stratification of Resectable Non-Small Cell Lung Cancer Patients

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Lung cancer is the leading cause of cancer-related mortality in Ireland and worldwide. The standard of care for early-stage non-small cell lung cancer (NSCLC) is curative-intent surgery, with or without systemic therapy, followed by regular surveillance imaging. Despite this treatment regimen, recurrence rates for NSCLC remain high at 24% for stage I disease and 50% overall, highlighting the need for improved surveillance strategies and on-treatment biomarkers. Changes in cell-free DNA (cfDNA) methylation has shown utility in predicting relapse/response to treatment in NSCLC. This project aims to identify a novel circulating tumour DNA (ctDNA) methylation signature capable of detecting recurrence and monitoring treatment response in resectable NSCLC patients as part of the Trans-Atlantic Cancer Alliance for Liquid Biopsy Research and Training (TALenT).

Twist cfDNA methylome profiling was performed on cfDNA extracted from the plasma of 10 treatment-naïve patients with resectable NSCLC (5 of whom had disease recurrence and 5 of whom remained disease-free at two years post-surgery) versus 10 healthy controls. Novel differential cfDNA methylation signatures were identified in the samples from NSCLC patients versus healthy controls, and in the samples from patients who relapsed compared to those who remained disease-free. Validation of a panel of 6-8 differentially methylated genes, including DOCK8, OSM, and NKX6-1 is currently underway using MSRE-qPCR on genomic and nucleosomal DNA extracted from lung cancer cell lines, buffy coat preparations, and matched FFPE normal and tumour tissues. The most promising candidate genes will then be tested in a large cohort of longitudinal plasma samples from both relapsed and disease-free patients using MSRE-ddPCR.

This ctDNA methylation signature will ultimately be translated into a blood-based clinical assay to stratify risk in patients with resectable NSCLC and guide personalised treatment, enabling timely delivery of optimal therapies.

*The HRB's funding of TALenT.*

# Establishment of small cell lung cancer patient-derived organoids from malignant pleural effusions for assessment of chemotherapy response in small cell lung cancer

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**Background:** Small cell lung cancer (SCLC) is an aggressive malignancy characterised by rapid progression, early metastasis, and overall survival below 10%. It is typically diagnosed at advanced stages, rendering most patients unsuitable for surgical resection, and frequently recurs, with relapsed disease almost universally refractory to chemotherapy. SCLC can give rise to malignant pleural effusions (MPEs), in which tumour cells accumulate in pleural fluid. MPEs represent an accessible source of viable tumour cells for generating patient-derived organoids (PDOs), which can recapitulate key tumour features and provide a physiologically relevant model for studying SCLC biology and therapeutic response.

**Methods:** Tumour cells were isolated from MPE fluid and initially expanded in 2D culture to enrich for tumour cells before seeding into 3D culture. Cells were embedded in either Cultrex Reduced Growth Factor BME or VitroGel® Hydrogel Matrix, and maintained in specialised lung PDO medium, with or without 10% MPE fluid supplementation. PDOs were validated for retention of tumour markers vimentin, NCAM1, and TTF-1 by immunofluorescence, and compared with diagnostic cytology samples by immunohistochemistry. Chemosensitivity to cisplatin and etoposide, alone and in combination, was assessed using CellTiter-Glo-3D assay.

**Results:** PDOs remained viable and expandable for over 10 passages and demonstrated enhanced growth in medium supplemented with 10% MPE fluid compared to medium alone. PDO growth rate and PDO size was enhanced in VitroGel, supporting its suitability as a xeno-free 3D matrix. PDOs retained tumour marker expression consistent with diagnostic samples. Chemotherapy treatment demonstrated measurable effects from single-agent and combination, and IC50 concentrations were determined, providing a foundation for future studies.

**Conclusion:** We establish a standardised protocol for generating long-term, tumour-representative SCLC PDOs from MPEs, and demonstrate their responsiveness to standard-of-care chemotherapy, providing a robust platform for studying SCLC biology and accelerating the development of personalised treatments in SCLC.

# Personalised Drug Discovery Approach for Multiple Myeloma Using an Ex Vivo Bone Marrow Microenvironment Model

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**Background:** Multiple Myeloma (MM) is a plasma cell malignancy with a strong dependence on the bone marrow microenvironment limiting the effectiveness of conventional ex vivo culture systems. We have developed a 3 dimensional (3D) co-culture model incorporating multipotent mesenchymal stromal cells (MSCs), endothelial progenitor cells (EPCs) and Matrigel, a solubilized basement membrane matrix hydrogel, to better mimic the MM tumour niche. We evaluated MM cell viability, proliferation, and drug response in this model compared with traditional two-dimensional (2D) cultures.

**Materials and Methods:** Bone marrow aspirates from MM patients were processed by density gradient centrifugation and CD138+ myeloma cells were isolated by magnetic bead isolation. Primary bone marrow-derived MSCs and cord blood-derived EPCs were obtained from StemBioSys. Cells were embedded in Matrigel and maintained in 3D coculture. Microscopy was performed using fluorescent and confocal microscopy.

**Results:** Within 3 days, MSCs and EPCs embedded in Matrigel formed interconnected cellular networks resembling prevascular structures. Myeloma cell lines and primary CD138+ cells demonstrated sustained viability and proliferation in 3D co-culture for up to 28 days. Notably, primary CD138+ MM cells exhibited increased resistance to bortezomib in 3D culture compared with 2D culture, indicating that the model more closely recapitulates the *in vivo* microenvironment.

**Conclusions:** This 3D co-culture system supports long-term survival of primary MM cells in a physiologically relevant *ex vivo* model. In addition, it requires less MM cells than conventional 2D cell culture. This model has multiple potential applications such as preclinical evaluation of novel anti-myeloma agents and in personalised medicine approaches to guide patient-specific treatment strategies.

# Targeting Hypoxia-Mediated Treatment Resistance: NANOX, an Oxygen-Carrying Nanoemulsion, Modulates Metabolism and Inflammation in 3D Ex Vivo Tumour Explants Under Hypoxia

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**Introduction:** Hypoxia is a common feature of solid malignancies and predicts poor prognosis regardless of treatment approach. Hypoxia synergises with several hallmarks of cancer, including dysregulated metabolism and immunosuppression in establishing a treatment-resistant tumour microenvironment. Previously we identified NanOx, a novel hypoxia-modifying perfluorocarbon nanoemulsion, which significantly enhances the radiosensitivity of hypoxic, radioresistant oesophageal adenocarcinoma cells [1]. However, the impact of NanOx on hallmarks of cancer in more complex models remains unexplored. This study uses 3D ex vivo gastrointestinal (GI) tumour explants to provide new insights into the multimodal action of NanOx across several hallmarks of cancer, with the overall goal of improving radiotherapy efficacy for these patients.

**Methodology:** 3D *ex vivo* tumour explants from consented GI cancer patients attending St James' Hospital were cultured for 4h under hypoxic conditions (0.5% $O_2$). A repeated-measures approach was used to assess metabolism, inflammation, and oxidative stress pre- and post- NanOx +/- 2Gy radiotherapy. Explants were treated with NanOx for 30 min prior to 2Gy under hypoxia (0.5% $O_2$). Metabolism was assessed via the Seahorse bioanalyser. Tumour-conditioned media was collected to assess inflammatory and oxidative stress mediators. Data were analysed using two-way ANOVA and pathway enrichment analyses were performed using STRING.

**Results:** GI tumour explants cultured under hypoxia have heterogeneous metabolic, oxidative stress, and inflammatory profiles, with oxidative stress significantly correlated with inflammation. NanOx significantly reduced oxidative phosphorylation in GI tumour explants. NanOx significantly modulated the inflammatory profile of GI tumour explants and distinct to that of the NanOx and radiotherapy combination treatment. NanOx also significantly increased the level of extracellular lactate dehydrogenase, an indirect marker of tissue damage.

**Conclusion:** Hypoxia is a common feature of solid malignancies and significantly impairs treatment efficacy. We have identified a novel hypoxia-modifying nanoemulsion that modulates the metabolism and secretome of GI tumour explants under hypoxia.

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# Classical Meets Targeted: A Novel Therapeutic Strategy for Chronic Lymphocytic Leukaemia

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**Background:** Chronic lymphocytic leukaemia (CLL) is an incurable B-cell malignancy, with Ireland reporting among the highest incidence rates globally. Aberrant activation of the B-cell receptor (BCR) signalling pathway in these cells has driven the development of therapies targeting a key downstream component - Bruton's tyrosine kinase (BTK).

Despite the significant advancements achieved with these therapies, challenges persist. Resistance, off-target effects, and low complete response rates underscore the need for novel combination treatment strategies. As CLL cells exhibit cytoskeletal abnormalities, with tubulin closely associated with BCR-mediated signalling molecules, one strategy is to investigate the use of microtubule-targeting-agents (MTA) in combination with BTK inhibitors.

This study explores a potential therapeutic approach combining the BTK inhibitor ibrutinib with the MTA vincristine, to leverage complementary mechanisms and improve outcomes in CLL.

**Materials and Methods:** The cytotoxicity of both drugs and the combination on a panel of CLL cell lines were determined using MTT proliferation assays. The levels of apoptosis were determined by annexin-V/propidium iodide staining and flow cytometry in CLL cell lines, patient-derived CLL cells cultured alone and in co-culture with the human stromal HS5 cells, and in healthy donor PBMC and platelets. Drug synergism was determined using CompuSyn software. The effect of this combination on primary CLL cell division was evaluated by CFSE staining and KI-67 expression in a specialised *ex-vivo* cell culture model.

**Results:** The ibrutinib/vincristine combination demonstrates a synergistic cytotoxic effect in a panel of CLL cell lines. Combination treatments resulted in significantly increased apoptosis of patient-derived CLL cells cultured alone and in co-culture with HS5 stromal cells. Primary CLL cells can be induced to proliferate in a specialised cell culture model and this proliferation is abrogated following treatment with this novel combination.

**Conclusions:** This work demonstrates that ibrutinib in combination with the MTA vincristine may show promise as a novel treatment strategy in CLL.

*All patients who donated their samples, clinical colleagues who orchestrated sample acquisition, all the staff at SJH for their ongoing work.*

# The TCD-SJH Haematology Biobank: Revolutionising Translational Blood Cancer Research

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**Background:** Haematological malignancies comprise a heterogeneous group of diseases, with incidence in Ireland projected to increase to 3000 new cases identified annually by 2030. Access to high quality patient biospecimens along with comprehensive clinical data is limited, but often a requirement to support translational research. Consequently, biobanks play a critical role in enabling this translational research, along with biomarker discovery and development of novel therapeutic strategies. We describe the continued growth and development, scope and patient sample methodology of the TCD-SJH Haematology Biobank which has supported blood cancer research for over a decade.

**Materials and Methods:** Peripheral blood and bone marrow aspirates are collected from patients with informed consent under institutional ethical approval. Samples are obtained at diagnosis and/during relevant clinical timepoints. Depending on disease type, plasma, serum and mononuclear cells are isolated using density gradient centrifugation and carefully harvested, immunophenotyped and prepared for cryopreservation before long-term storage in liquid nitrogen. Clinical and demographic data are recorded and linked to biospecimens in a secure database, readily available for approved research studies.

**Results:** As of 2026, approximately 444 patients have been consented into the Haematology Biobank. The diversity of the biobank is representative of the spectrum of haematological malignancies, including Acute Lymphoblastic Leukaemia (ALL), Acute Myeloid Leukaemia (AML), Promyelocytic Leukaemia (PML), Myelodysplastic Syndromes (MDS), Multiple Myeloma, Lymphoma and Chronic Lymphocytic Leukaemia (CLL). In 2025 alone, over 100 patients were successfully recruited, with biospecimens collected and cryopreserved.

**Conclusions:** This Haematology Biobank represents a robust and expanding resource of high quality blood and bone marrow patient samples with longitudinal clinical annotation. It provides a valuable platform to support current and future translational research in haematology, including but not limited to studies of disease biology, ex-vivo models for evaluating treatment response/precision medicine and novel therapeutic targets.

# Feasibility of Circulating Tumour Cell Detection and HER2 Characterisation in Advanced/Metastatic Oesophagogastric Cancer

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**Background:** Circulating tumour cells (CTCs) represent a potential non-invasive approach for tumour characterisation and disease monitoring in oesophagogastric cancers. However, detection and downstream phenotypic analysis of CTCs remain poorly explored in this context, particularly for the detection of clinically actionable biomarkers such as HER2. We present early findings from an ongoing pilot study evaluating CTC enrichment and HER2 characterisation as a proof of concept for wider biomarker testing using liquid biopsy based approaches.

**Methods:** Patients were prospectively enrolled as part of an ongoing translational study. Whole blood samples were obtained from peripheral venous access, with matched samples from peripherally inserted central catheters where available. CTC enrichment was performed using the ANGLE Parsortix microfluidic platform. Enriched cells underwent fluorescence-based imaging for CTC identification using epithelial and nuclear markers. HER2 status of the primary tumour was determined using standard immunohistochemistry(IHC), with fluorescence in-situ hybridisation(FISH) performed for equivocal (2+) cases in accordance with clinical guidelines. HER2 expression on detected CTCs was assessed using fluorescence-labelled antibodies. A subset of patients underwent longitudinal sampling during systemic chemotherapy to evaluate dynamic changes in CTC detection.

**Results:** To date, 11 patients have been included; two (18%) had locally advanced, node-positive disease treated with curative-intent neoadjuvant chemotherapy followed by surgery, and nine(82%) had stage IV metastatic disease receiving palliative systemic therapy. Primary tumour HER2 assessment demonstrated HER2 3+ expression in 3 of 11 patients(27%), HER2 2+ in 1 patient(9%), and HER2 1+ in the remainder. CTCs were detected in 3 of 11 patients(27%).Among patients with detectable CTCs, HER2-positive CTCs were identified in 1 patient In 1 patient sampled longitudinally, clearance of detectable CTCs following administration of chemotherapy was observed.

**Conclusion:** CTC detection is feasible in advanced/metastatic oesophagogastric cancer, although detection rates were modest in this small pilot cohort, recruitment is ongoing within a prospective pilot study.HER2 characterisation from CTCs provides proof of concept for this approach and supports ongoing expansion of the study to evaluate additional biomarkers and longitudinal disease dynamics.

# Enhancing Radiotherapy Efficacy in Gastrointestinal Tumours – Development of a Lipid-based Nanoparticle Delivery System for Locoregional Administration of microRNA and Cancer Therapy

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Many solid malignancies are inherently resistant to radiotherapy. Developments in RNA interference as a therapeutic avenue in cancer treatment may offer a novel anti-tumour approach; with our lab previously demonstrating enhanced radiotherapy sensitivity of gastrointestinal tumours in response to microRNA modulation. Here, we develop and optimise the formulation of lipid-based nanoparticles for the delivery of microRNA to solid tumours. An optimal size range of 100nm to 200nm has been reported to improve efficacy and safety of nanoparticle systems; achieved through varying lipid composition ratios, or downstream techniques, such as sonication.

Lipid-based nanoparticles were prepared via the thin-film hydration method, containing the ionizable cationic lipid 6-((2-hexyldecanoyl)oxy)-N-(6-((2-hexyldecanoyl)oxy)hexyl)-N-(4-hydroxybutyl)hexan-1-aminium (ALC-0135), with 18:1 (Δ9-Cis) PC (DOPC), cholesteroland 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG(2000)). Lipids were dissolved in ethanol and solvent removed using a rotary evaporator. Lipid films were then rehydrated with phosphate-buffered saline. To examine the effect of lipid composition on nanoparticle size, lipid ratios were varied. Following rehydration, the mixture was disrupted by probe sonication at 50% amplitude, for varying time intervals. Material characterisation of resultant nanoparticles, including particle size distribution (DH) and polydispersity index (PDI), were measured via dynamic light scattering using a Zetasizer Nano ZS.

Results from both DH and PDI testing indicate that varying phospholipid:cholesterol ratio affected the efficiency of lipid film rehydration and resulted in changes to average particle size. Sonication resulted in improvements in size and homogeneity, causing a pronounced reduction in both measures after 2 minutes sonication; with longer durations producing modest further improvements.

Our results indicate the phospholipid:cholesterol ratio affects average nanoparticle size, affecting the lipid rehydration process. Sonication offers a suitable down-sizing technique, confirmed with improvements across DH and PDI. This work will inform future formulation studies to encapsulate radio-sensitising microRNA within the nanoparticle system. Further work will assess the radiosensitising effects under varying hypoxia concentrations; and if microRNA-mediated modification of cell biology can enhance tumour radiosensitivity.

# Proteomic profiling of extracellular vesicles cargo identifies key proteins that could have diagnostic and prognostic relevance as liquid biopsies in non-small cell lung cancer

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**Background:** Lung cancer is the leading cause of cancer-related death in men and the second leading cause in women. CT scans are currently used for lung cancer screening, but are limited by cost and a high rate of false-positive findings, hence there is a need for biomarkers for detection and surveillance of lung cancer. Extracellular vesicles (EVs) represent a promising source of biomarkers, as they circulate in the blood carrying cargo (such as proteins and nucleic acids) that reflect the tumour cells from which they originate.

**Methods:** Plasma samples were collected from patients with non-small cell lung cancer (NSCLC, $n=30$) prior to surgery, as well as from healthy donors (HD, $n=10$). Samples were processed by size-exclusion chromatography to collect EVs. The EVs were characterised by immunoblotting, transmission electron microscopy, and nanoparticle tracking analysis. EVs from all 40 individuals were analysed by mass-spectrometry to determine their proteomic profiles. The proteomics data was analysed using Perseus, and key proteins were validated by ELISA.

**Results:** Proteomic analysis identified 5 proteins significantly up-regulated and 17 proteins down-regulated in EVs from patients with NSCLC compared to HDs. Two upregulated proteins, lipopolysaccharide-binding protein (LBP) and quiescin sulfhydryl oxidase 1 (QSOX1), were selected for validation. Both proteins were found to discriminate between NSCLC and HDs, based on ROC curve analysis (LBP – AUC: 0.9700, $p<0.0001$, QSOX1 – AUC: 0.8767, $p=0.0004$). LBP quantities appeared to decrease after surgery (2 days post-op), but not in a statistically significant manner. However, QSOX1 quantities dropped significantly between the pre-op and post-op samples ($p=0.0262$).

**Conclusion:** Blood-based EVs-carried LBP and QSOX1 can discriminate between healthy people and those with NSCLC. Quantities of both proteins decrease post-surgery, supporting our hypothesis that they are released from the tumour.

*Acknowledgements: HEA's North-South funding of the All-Ireland Cancer Liquid Biopsies (CLuB)*

# Large extracellular vesicles in blood liquid biopsies: potential diagnostic and prognostic biomarkers across ovarian cancer subtypes

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**Background:** Epithelial ovarian cancer (EOC) is the most common type of ovarian cancer. As its symptoms can be non-specific, the disease often goes undiagnosed until it is too late. Biomarkers for diagnosing and subtyping EOC are urgently needed. Thus, this study aimed to investigate if blood-based extracellular vesicles (EVs) might have relevance as minimally-invasive biomarkers for this purpose.

**Methods:** Plasma samples from patients with benign ($n=20$) and malignant ($n=42$, mixed histology) ovarian tumours were processed by size-exclusion chromatography to collect EVs, which were characterised by immunoblotting, transmission electron microscopy, and nanoparticle tracking analysis.

**Results:** EVs from women with benign ovarian lesions were significantly smaller ($p<0.0001$) yet more abundant ($p<0.0001$) compared to those from malignant ovarian tumours. Particle distribution parameters were used to assess differences between patient groups. NTA distribution parameters (D10, D50, D90) represent the points in the size distribution under which 10%, 50% and 90% of the sample is found. EVs collected from patients with malignant tumours had significantly higher D10, D50, and D90 thresholds ($p<0.0001$ for each) compared to those coming from patients with benign lesions. This shows that particles are bigger in samples collected from patients with cancer. Interestingly, when comparing early-stage (stage I+II, $n=20$) with advanced-stage disease (stage III+IV, $n=22$), total particle amounts did not differ. However, early-stage patients had significantly lower D10 ($p=0.0013$), D50 ($p=0.0029$), and D90 ($p=0.0036$) compared to those with more advanced stage disease. This shows that the patients with more advanced disease have significantly bigger particles overall than those with early-stage disease.

**Conclusion:** Our research indicates that there are differences in the sizes of EVs produced by malignant tumours compared to benign lesions. It also suggests that the amounts of blood-based particles of the larger size correlate with more advanced disease.

*Acknowledgements: HEA's North-South funding of the All-Ireland Cancer Liquid Biopsies (CLuB).*

# Investigating NKAPL, a Potential Marker for Platinum Resistance, in High-Grade Serous Ovarian Cancer

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**Background:** Understanding mechanisms of acquired chemoresistance in high-grade serous ovarian cancer (HGSOC) is a critical unmet question, with potential to improve patient outcomes. Previously, we found *NKAPL* (NF-KB activating protein-like) to be hypermethylated and gene expression reduced in chemoresistant HGSOC tumours. We hypothesise that increased *NKAPL* methylation and low expression may be an indicator of resistance in HGSOC and aim to investigate its suitability as a prognostic/predictive biomarker.

**Material and Methods:** A panel of HGSOC cell lines were characterised for chemosensitivity, *NKAPL* methylation and gene expression. OVCAR4, CAOV3, PEO1, PEO4, UWB1.289-Parental, COV318, Kuramochi and UWB1.289-BRCA1 were used and NKAPL gene expression and methylation was determined through RT-qPCR and qMSP. Since *NKAPL* is poorly characterised, overexpression was performed in HEK293T cells to gain mechanistic insights and since *NKAPL* may regulate NOTCH signalling, downstream gene expression was analysed. *NKAPL* protein was assessed by Western Blot.

**Results:** Low levels of *NKAPL* gene expression were seen in all cell lines apart from the platinum-resistant COV318. No correlation between chemosensitivity, DNA methylation and gene expression was seen. In cell lines from the same patient before and after platinum treatment (PEO1 & PEO4) chemosensitivity was reduced and *NKAPL* methylation significantly increased post-treatment. Western blotting revealed a molecular weight of *NKAPL* of ~65kDa, higher than the predicted ~46kDa suggesting post-translational modifications. No changes in *NOTCH* target gene expression were seen in HEK293T cells after *NKAPL* overexpression.

**Conclusions:** DNA methylation results in the paired cell lines PEO1 and PEO4 support the hypothesis that *NKAPL* methylation may affect platinum sensitivity. However overall, chemosensitivity did not correlate with methylation or expression of *NKAPL* across the HGSOC cell line panel. Further analysis clarifying the function and structure of *NKAPL* is needed and its clinical relevance needs to be evaluated in ovarian specific models.

# A Cell Atlas of Malignant Pleural Effusion

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**Background:** Malignant Pleural effusion (MPE) is the over-accumulation of fluid in the pleural cavity which may present as a complication of lung, breast or other cancers. MPE has a devastating effect on patient survival, reducing median survival to 3-12 months. Current diagnostic cytology techniques attain 46-51% sensitivity in detecting malignant cells in pleural fluid. Recent pilot studies have shown that Single Cell RNA-sequencing (scRNA-seq) achieves 91% sensitivity in detecting malignant cells, but this requires tissue-specific reference data. Utilising scRNA-seq datasets we constructed a Cell Atlas of Pleural Effusion (CAPE) which may be used as a reference to detect cancer cells in pleural fluid scRNA-seq samples.

**Methods:** We created a reference database by carrying out a systematic literature review to identify publicly available scRNA-seq datasets from PE patient samples. We performed scRNA-seq experiments to generate novel datasets from PE samples isolated from patients at St James's Hospital. These datasets were computationally integrated using mutiomic techniques to create CAPE. Clinical metadata was also collated and harmonised to the standards of Human Cell Atlas consortium.

**Results:** To date we identified 35 public scRNA-seq datasets. We computationally integrated 14 of these in our current version of CAPE, which consists of 420,000 cells from 81 individuals, including 25 individuals with lung or breast malignancies. Comparing these 25 samples to 36 control samples we found that CEACAM6+ cell percentage to be a promising diagnostic biomarker, achieving 92% sensitivity and 97.2% specificity (ROC AUC 0.947, $p = 5.84\ 10^{-10}$).

**Conclusion:** To validate CEACAM6 as a diagnostic biomarker we need a larger and more diverse cohort. To realize this we will generate novel datasets, including 39 MPE from lung, breast and other cancers, with 29 matched controls. Our final CAPE resource will contain over 1 million cells from over 150 patients.

# PRC2 deficiency is associated with targetable metabolic vulnerabilities in high-risk AML

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**Background:** Acute Myeloid Leukaemia (AML) is the most frequent cause of blood cancer mortality in young people. We and others have previously reported that loss of function of Polycomb Repressive Complex 2 (PRC2) is associated with chemoresistance and poorer prognosis in AML, identifying a patient cohort requiring more effective treatment strategies. Given increasing evidence for the importance of metabolic phenotype in AML cells, and the role of EZH2-mediated PRC2 activity in regulating energy metabolism, our aim is to understand how PRC2 loss-of-function alters the energetic profile of AML cells to identify potential novel treatments.

**Materials and Methods:** To study the effects of PRC2 loss in AML, we generated isogenic cell line models with deficient PRC2 activity by CRISPR-Cas9–mediated deletion of EZH2 in three AML cell lines. We performed metabolic profiling using Seahorse and drug sensitivity assays to identify altered metabolic pathways in PRC2-deficient AML, while RNA-seq and proteomics were used to define network changes underpinning these alterations. Further orthogonal validation using pharmaceutical PRC2 inhibition was also performed.

**Results:** We found that PRC2-depleted cells exhibit significantly altered metabolism. Specifically, EZH2-deficient cells show increased ATP production via oxidative phosphorylation (OXPHOS) and, accordingly, altered sensitivity to pharmacological inhibition of glycolysis (2DG) and OXPHOS (IACS-010759), with reduced sensitivity to glycolysis inhibition alone and requiring dual inhibition to effectively induce cell death. Supporting this, proteomic analysis suggests upregulation of fatty acid oxidation, which may sustain increased OXPHOS and represents a potential therapeutic target.

**Conclusions:** Our data identifies that PRC2 loss of function is associated with targetable altered metabolic activity in AML. We are currently investigating the contribution of specific nutrients, including amino acids and fatty acids to the increased mitochondrial respiration in these cells to identify novel therapeutic targets for this form of AML, and further study the precise epigenetic mechanisms underlying this altered metabolic phenotype.

# Elucidating Malignant Prostate Cell Interactions with Nerves: A Potential New Target for Cancer Therapy

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**Background:** Prostate cancer (PCa) is the most common malignancy among men in western countries. Its progression is influenced by hypogastric and pelvic nerves, which regulate prostate function. Tumour-induced peripheral nerve hypertrophy, tumour invasion, and neurotransmitter signalling promote cancer growth, metastasis, and pain. This study aims to identify factors secreted by PCa cells with neuromodulatory effects using bioinformatics and a PC12 differentiation model.

**Materials and Methods:** A cohort of 467 PCa patients was analysed using the cBioPortal platform to examine the role of neuronal signalling and nerve fibre hypertrophy in PCa progression. Gene expression profiles across disease stages (localised vs. advanced/metastatic) were investigated using the GEO database. Secreted neurotrophic factors from three PCa cell lines (LNCaP, DU145, PC3) of varying aggressiveness were assessed by applying conditioned media (CM) to PC-12 cells, followed by neurite outgrowth quantification using phase-contrast microscopy and ImageJ. The CM was then concentrated and re-tested to determine whether enrichment of secreted neurotrophic factors enhanced neurite outgrowth. The contribution of NGF was assessed by repeating the assay with CM containing an NGF-neutralising antibody.

**Results:** Bioinformatic analysis identified NGF, TGFβ1, and STX1A as genes associated with PCa aggressiveness. NGF and TGFβ1 are implicated in neuronal differentiation, whereas STX1A is essential for neurotransmitter release. PC-3 and DU145 CM significantly promoted PC-12 cell differentiation, with DU145 CM exhibiting the strongest effect. Concentrated CM increased neurite length and the number of neurite outgrowths. Neutralisation with an anti-NGF antibody partially attenuated DU145 CM-induced differentiation; however, responses remained significantly elevated compared to controls, indicating the involvement of additional secreted factors.

**Conclusions:** These findings suggest that PCa cells produce neuro-neoplastic signalling factors correlated with disease aggressiveness. Further studies will characterise molecules secreted by DU145 and their effects on peripheral neurons. Inhibitors of neurotransmission warrant investigation as potential therapeutic interventions.

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# Epigenetic Modifier GSK-J4 Enhances Sensitivity to Venetoclax in Multiple Myeloma

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**Introduction:** Multiple myeloma (MM) is an incurable haematological cancer with increasing prevalence. MM cells are heterogeneous relying on diverse anti-apoptotic BCL-2 family proteins (BCL-2, MCL-1, and BCL-XL), for survival. Venetoclax, an FDA-approved BCL-2 inhibitor, has shown promise in a subset of MM patients that are BCL-2-dependent t(11;14) cells. However, approximately only 20% of MM patients rely on BCL-2 for survival. To expand the therapeutic potential of venetoclax, we performed an epigenetic modifier screen and identified that GSK-J4 enhanced the sensitivity to venetoclax.

**Methods:** A panel of non-t(11;14), MCL-1–dependent MM cell lines and patient samples were treated with a combination of GSK-J4 and venetoclax. Cell death was assessed using Annexin V/PI. mRNA and protein expression were accessed by RNA sequencing, qRT-PCR, and western blotting, following GSK-J4 treatment. Mature MM surface markers were assessed after GSK-J4 treatment by flow cytometry. The SeahorseXFe96 assay was used to assess oxygen consumption rate, after GSK-J4 treatment.

**Results:** First, we confirmed that the combination of GSK-J4 and venetoclax enhanced cell killing non-t (11;14) cell lines and in primary MM patient samples. Next, we assessed the transcriptional changes induced by GSK-J4 using RNA sequencing. GSK-J4 treatment induced an upregulation of BCL-2 mRNA and downregulation of MCL-1 mRNA, which was confirmed by qRT-PCR and Western blotting. Pathway analysis of the RNA sequencing revealed a downregulation of genes involved in OXPHOS. Next, we confirmed that GSK-J4 altered OXPHOS metabolism using the Seahorse assay. We also identified an altered mitochondrial network using confocal microscopy and TMRE staining. Lastly, GSK-J4 decreased the expression of B-cell maturation antigen BCMA and CD138 on the surface of MM cells, suggesting that the epigenetic modifier may be inducing a more immature phenotype.

**Conclusion:** Our findings suggest that the epigenetic modifier GSK-J4 is driving mature non-t(11;14) MM cells to a more immature phenotype. Thereby inducing a reliance on BCL-2 and an altered metabolic phenotype. This combination could expand the use of venetoclax into both t(11;14) and non-t(11;14) MM patients.

# Investigating the Role of FKBPL in Tumour-Associated Macrophages in Hepatocellular Carcinoma

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**Background:** Hepatocellular carcinoma (HCC) is a major cause of cancer mortality, requiring better therapies. FKBPL, an FKBP family member, shows anti-cancer roles in breast/ovarian cancer but pro-tumour effects in HCC; its mechanism remains unclear. Tumour-associated macrophages (TAMs) influence tumour progression via M1 (anti-tumour) or M2 (pro-tumour) phenotypes. FKBPL regulates ER-phagy under stress, suggesting a link to macrophage polarization.

**Materials and Methods:** THP-1 cells were polarized to M1/M2 using LPS+IFN-$\gamma$ or IL-4+IL-13. qPCR assessed M1 (TNF-$\alpha$, IL-1$\beta$), M2 (IL-10, CD206), and FKBPL expression. HepG2 cells were FKBPL-overexpressed (OE) or knocked down (KD), and their conditioned media (CM) tested on macrophage polarization. Cytokines in CM were measured by ELISA; ER stress markers (BiP, CHOP) analyzed after FKBPL KD.

**Results:** FKBPL was differentially expressed in M0, M1, and M2 macrophages, with highest levels in M1. CM from HepG2, HepG2OE and HepG2KD cells modulated the macrophage phenotype, potentially suppressing M1 polarization. FKBPL knockdown in M0 macrophages disrupted polarization dynamics. Cytokine assays showed reduced RANTES, MIP-1$\delta$, and IL-6sR in CM from HepG2OE cells, which may induce immune-tolerant TAM formation. FKBPL knockdown enhanced ER stress gene expression ($p<0.05$) following treatment with tunicamycin, a known ER stressor.

**Conclusions:** These data suggest that FKBPL may promote HCC progression by modulating ER stress and shifting macrophage polarisation toward an immunosuppressive M2 phenotype. Targeting FKBPL could offer a novel strategy to reprogram the TME and enhance anti-tumour immunity.

# Investigating the role of FK506-Binding Protein-Like (FKBPL) in treatment-resistant oesophageal adenocarcinoma

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**Background:** The incidence rate of oesophageal cancer is predicted to rise sharply by 2045. The standard of care for oesophageal adenocarcinoma (OAC) is neoadjuvant chemotherapy with or without radiation. However, only ~30% of patients achieve a complete pathological response. The resistant patients face therapy-related toxicities, often worsening their prognosis. Currently, no reliable biomarkers predict treatment response, highlighting the urgent need to elucidate potential mechanisms of treatment-resistance.

**Materials and Methods:** FKBPL levels were assessed in the isogenic radioresistant OAC model OE33 parental (OE33P) and OE33 radioresistant (OE33R) cells at baseline and following a clinically-relevant dose of 1.8Gy radiation. mRNA and protein expression of FKBPL were measured by qPCR and Western Blot. Serum FKBPL levels were measured by ELISA in 112 pre-treatment OAC patients and FKBPL mRNA levels were assessed in OAC biopsies by qPCR and correlated with treatment response.

**Results:** FKBPL mRNA levels were not altered in OE33P or OE33R cells at baseline or following 1.8Gy radiation. FKBPL protein expression was higher in OE33R cells compared to OE33P cells ($p=0.0320$). Following 1.8Gy radiation, FKBPL protein levels increased in the OE33P cells ($p=0.0298$) but not the OE33R cells. Higher FKBPL mRNA expression was observed in OAC patient biopsies that had a poor response to neoadjuvant treatment ($p=0.036$). There was no significant difference in serum FKBPL levels between good and poor responders to neoadjuvant treatment.

**Conclusions:** FKBPL is elevated in treatment resistant OAC cells and patient biopsies suggesting FKBPL may be implicated in treatment resistance in OAC and has potential as a biomarker for treatment response.

# Remodelling of the Tumour Glycome in Oesophageal Adenocarcinoma: Implications for Tumour Microenvironmental Immune Suppression

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Oesophageal adenocarcinoma (OAC) remains a lethal malignancy with an incidence that continues to rise worldwide. Although recent regulatory approvals have enabled the use of immune checkpoint inhibitors (ICIs), response rates in OAC and most solid tumours remain low, typically ranging from 13–29%. Current clinical strategies largely focus on combining ICIs with chemotherapy or radiotherapy, yet relatively few approaches directly target the immune-evasion mechanisms that contribute to resistance. One emerging axis of interest is cancer-associated glycosylation—the enzymatic modification of proteins and lipids with sugar structures or glycans. Aberrant glycosylation, particularly the upregulation of a specific sugar, sialic acid, on tumour cells, can suppress anti-tumour immunity through binding to SIGLECs (Sialic acid-binding ImmunoGlobulin-like LECtins) receptors expressed on specific immune cell populations. This mechanism is increasingly recognised as a contributor to tumour infiltrating T-cell dysfunction and poor ICI responsiveness.

We hypothesised that tumour progression and exposure to tumour microenvironmental and therapy-induced stress drive enhanced tumour cell sialylation during OAC development. To investigate this, a panel of well-characterised oesophageal epithelial cell models representing normal epithelium, Barrett's oesophagus, dysplasia and oesophageal adenocarcinoma was assessed. Basal glycosylation was profiled using lectin-based flow cytometry, to assess specific glycan structures. OAC cell glycosylation was further examined under hypoxic conditions and following exposure to clinically relevant radiation doses.

Distinct alterations in glycosylation and sialylation were observed across disease progression, with OAC cells demonstrating increased sialic acids compared with non-malignant models. Hypoxia further modified glycan and sialylation profiles in a cell line-dependent manner, while irradiation was also associated with altered tumour sialylation.

This data indicates that disease associated sialylation alterations are established early in malignant oesophageal disease progression and is further shaped by tumour microenvironmental and treatment-related stress.

Previous work from our group has shown that irradiation induces increased expression of immune checkpoint molecules, including PD-1 and its ligand, consistent with treatment-induced immunosuppression. As sialic acids are a prerequisite for SIGLEC engagement, treatment-associated increases in tumour sialylation may create a permissive landscape for SIGLEC-mediated immune inhibition.

# Proteomic Analysis Reveals Impaired Activation and Mitochondrial Defects in NK Cells from Metastatic Breast Cancer Patients

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**Background:** Natural killer (NK) cells are critical mediators of anti-tumour immunity. Previous work from our group has demonstrated that NK cells from metastatic breast cancer (MetBC) patients exhibit metabolic and functional defects. However, the molecular mechanisms underpinning this dysfunction remain poorly understood. Therefore, we applied quantitative proteomics to identify pathways associated with NK cell dysfunction in MetBC patients.

**Materials and Methods:** Mass spectrometry–based quantitative proteomics was performed on unstimulated and IL-2–activated NK cells from healthy donors and MetBC patients. Flow cytometry was used to assess mitochondrial parameters, functional outputs and global histone H3 acetylation.

**Results:** Global proteomic structure was comparable between healthy donors and MetBC patients. However, following IL-2 stimulation, MetBC NK cells exhibited an attenuated response, upregulating substantially fewer proteins. This indicates a failure to appropriately remodel the NK cell proteome in response to cytokine activation.

Pathway enrichment analysis revealed substantial mitochondrial dysfunction in MetBC patients. Despite having a higher mitochondrial mass, components of the electron transport chain, particularly complexes III–V, were deficient under both basal and activated conditions. Functionally, this was associated with reduced mitochondrial membrane potential, consistent with impaired capacity to establish and maintain mitochondrial polarization.

In parallel, IL-2–activated NK cells from MetBC patients failed to induce global histone H3 acetylation. Notably, this defect persisted following treatment with the pan-histone deacetylase inhibitor panobinostat, suggesting a constrained capacity for histone acetylation in metabolically dysfunctional NK cells.

**Conclusions:** These data demonstrate that NK cell dysfunction in metastatic breast cancer is characterised by impaired activation-induced proteomic remodelling, selective deficiencies in mitochondrial respiratory chain components, and defective induction of histone H3 acetylation. Together, these findings provide new mechanistic insight into NK cell dysfunction in MetBC and identify metabolic and epigenetic constraints that may limit effective anti-tumour immunity.

# Turning Up the Voltage on Anti-Cancer Immunity

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**Introduction:** Electroporation therapy employs pulsed electric fields to transiently permeabilize cancer cell membranes, enabling intracellular delivery of otherwise impermeant agents such bleomycin (electrochemotherapy) or calcium ions (calcium electroporation; CaEP). CaEP has induced tumour regression in clinical settings of metastatic melanoma and gastrointestinal cancers. A small subset of patients experience systemic anti-cancer responses potentially induced by strong immunogenic cell death (ICD)[1].

The metal ions manganese ($Mn^{2+}$), zinc ($Zn^{2+}$) and selenium ($Se^{4+}$) have been noted for their ability to induce mitochondrial dysfunction and ICD[2,3]. In this study, the ability of these ions to induce ICD and anti-tumour immune responses by electroporation-mediated delivery was investigated by comparison against the established CaEP protocol.

**Methods:** MC38 cells were electroporated in the presence of selected ions using standard clinical electroporation parameters. IC50 concentrations were determined for each ion using real-time imaging. ICD was determined by cell-surface expression of calreticulin and caspase-1 activation by flow cytometry. Gasdermin-D cleavage was assessed by immunoblot.

**Results:** Each ion induced cell death in a dose-dependent manner. At 24 hours post-electroporation, SeEP and ZnEP displayed increased cell surface CALR expression relative to CaEP (~20% and ~30%, respectively). ZnEP displayed the greatest increase in caspase-1 activation relative to electroporation alone.

**Conclusion:** Emerging evidence suggests that robust induction of ICD is essential to drive adaptive anti-cancer responses, and may improve the outcomes of immunotherapies. These data combined show that delivery of zinc ions enhances the immunogenicity of electroporation-based treatments compared to calcium.

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# Investigating the applicability of cytokine-induced memory-like NK cell therapy for obesity-associated cancer

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**Background:** Oesophageal adenocarcinoma (OAC) is a poor-prognosis cancer with a 5-year survival rate below 25% and limited responses to chemo-radiotherapy and immunotherapies. Natural Killer (NK) cell–based adoptive therapies are constrained by poor in vivo persistence and ineffective tumour homing and infiltration. Cytokine-induced memory-like (CIML) NK cells are an emerging subset with potential for enhanced persistence and function within the solid tumour microenvironment. Here, we propose to investigate the therapeutic potential of CIML NK cells for OAC.

**Methods:** To generate CIML NK cells, healthy-donor NK cells were treated with high-dose IL-12, IL-15 and IL-18 for 16 hours, then expanded in the presence of low-dose IL-15 for 7 days. Control cells were maintained in low-dose IL-15 throughout. On day 7, cells were phenotyped using flow cytometry. NK cell cytotoxicity was tested using a K562 killing assay.

**Results:** Our CIML NK cell generation protocol yields high frequencies of reprogrammed memory-like NK cells with a highly activated phenotype, expressing higher levels of CD69, CD25, NKp30, NKp46 and TRAIL and lower levels of PD-1. CIML NK cells expressed significantly higher levels of CCR5 at baseline, and decreased expression of CX3CR1 following restimulation. Upon restimulation, CIML NK cells exhibited increased Granzyme B, TNF-$\alpha$ and IFN-$\gamma$ production while retaining cytotoxicity against K562 cells, indicating resistance to functional exhaustion. Further, CIML NK cells were more resistant to suppression of IFN-$\gamma$ production induced by soluble factors from OAC tumours.

**Conclusions:** Our data indicate that CIML NK cells are highly activated and do not become functionally exhausted following culture and re-stimulation. The distinct chemokine signature of CIML NK cells provides a potential target for chemokine directed modulation of migration to improve tumour homing. Future studies will further interrogate CIML NK cell potency within the OAC tumour microenvironment and identify whether specific pathways can be targeted to maximise infiltration and killing of OAC tumours.

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