**O140 DECISION THEORY AND COST-BENEFIT ANALYSIS OF RE-STAGING OESOPHAGEAL CANCER AFTER NEOADJUVANT CHEMOTHERAPY WITH PET-CT RATHER THAN CT**

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**Introduction:** Pre-operative progression of oesophageal cancer during neoadjuvant chemotherapy (NAC) to metastatic disease is common. There are currently no guidelines for the optimal re-staging modality. We previously found PET-CT to be more sensitive than contrast-enhanced CT. However, both differ in their risk (radiation dose and lifetime cancer risks) and financial cost. This study aimed to perform the first decision theory and cost-benefit analyses for restaging with PET-CT.

**Method:** Probability thresholds (Pts) were calculated using calculated risks and relative sensitivities using Pauker and Kassirer’s method, and used to inform existing logistic regression and decision tree models. Cost analysis was performed using 2013-2014 NHS tariffs.

**Result:** The Pt (probability of identifying metastases) for PET-CT was 0.037%. As this is considerably less than the likelihood of detecting progression (6.07%), routine re-staging with PET-CT is justified clinically. The number of PET-CT examinations (rather than CT) required to identify one additional patient with progression and prevent an inappropriate oesophagectomy was 23.3. This had a net cost £6,631.5, but a net 0.24% reduction in lifetime cancer risk. Although predictive models were 100% sensitive, sufficiently low risk patients could not be identified to forgo re-staging PET-CT.

**Conclusion:** Routine re-staging of oesophageal cancer after NAC with PET-CT rather than CT appears more effective and safer. Its minimal additional cost is more than offset by the avoidance of operative risk and impact upon quality of life resulting from an inappropriate resection. At present, predictive models cannot to reliably identify low risk patients to forgo re-staging PET-CT.

**Take-home message:** Routine re-staging of oesophageal cancer after neoadjuvant chemotherapy with PET-CT rather than CT appears more effective and safer. Its minimal additional cost is more than offset by the avoidance of operative risk and impact upon quality of life resulting from an inappropriate resection, and reduced radiation dose.

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**O141 TOLL-LIKE RECEPTOR 4 DEPENDENT TOLERISATION OF CANCER CELLS LEADS TO INCREASED ADHESION, INVASION AND VIABILITY**

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**Introduction:** Endotoxin tolerance, a well-known phenomenon, whereby cells stimulated with LPS show reduced responsiveness to repeated stimulations, has been extensively investigated in immune cells. Inflammation increases the incidence of tumour recurrence and metastases despite curative surgery in colorectal cancer. LPS, which binds to Toll-like receptor 4 (TLR4), plays an essential role in initiating the immune response and in subsequent tolerisation. This interaction between LPS and TLR4, act as a ‘double-edged sword’ due to effects on tumour cell adhesion, migration and invasion. Therefore in our study, we were investigated that, whether LPS pre-stimulation colorectal cancer cell lines induces tolerisation, thus leading to an altered metastatic potential in these cells.

**Method:** Human metastatic and non-metastatic colorectal cancer cell lines (SW620 and SW480) were pre-stimulated with different concentrations of LPS to induce tolerisation as determined by their cytokine profile. Non-tolerised and tolerised cell were further assessed for their viability or proliferation, adhesion to extracellular matrix components and invasion.

**Result:** Pre-stimulation of SW480 and SW620 cells with LPS resulted in reduced interleukin-8 (p=0.03) and vascular endothelial growth factor release (p=0.047) in response to LPS re-stimulation, confirming that LPS pre-stimulation induced tolerisation in these cells. Moreover, LPS-tolerised SW480 and SW620 cells displayed enhanced cell proliferation, attachment and invasion indicating an altered metastatic potential after the induction of LPS tolerisation.

**Conclusion:** These results suggest that endotoxin/LPS stimulation and tolerisation plays a significant role in colorectal cancer cell behaviour and survival in a septic environment, providing further evidence for the concept of immunogenic carcinogenesis.
Take-home message:
LPS pretreatment can induce tolerisation on cancer cell. Tolerisation in colorectal cancer cells leads to an enhanced metastatic potential.

O142  IRON CHELATION AS A NOVEL STRATEGY FOR THE TREATMENT OF COLORECTAL ADENOCARCINOMA
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Introduction: Colorectal adenocarcinoma is associated with iron excess, with evidence suggesting that malignant colonocytes are programmed to sequester and retain iron. This is hardly surprising, since iron is essential for DNA synthesis, ATP generation and cell cycle progression; all activities increased in cancer. Iron has also been shown to amplify Wnt signalling, the major oncogenic signalling pathway in the colon. The aim of this study, therefore, was to assess whether iron chelation represents a novel treatment strategy for colorectal cancer.

Method: The iron chelating agent ICL670A was administered to a panel of colorectal cell lines in-vitro. Cellular iron uptake and mobilisation were assessed by ferrozine assay. Cell cycle analysis was performed using FACS. Cellular viability and proliferation were assessed through MTT and BrdU assays respectively. A transgenic mouse model was then utilised to determine in-vivo efficacy. All animal work was performed under Home Office approved conditions.

Result: ICL670A decreased colorectal cellular iron uptake (by 49.6% vs. control, p<0.05) and intracellular iron levels (28.2% vs. control, p<0.05) leading to accumulation of cells in G1 of the cell cycle. The drug significantly reduced cellular viability and proliferation in a time and dose dependent manner. Interestingly, the drug demonstrated greater efficacy in adenocarcinoma lines than it did in adenomas. When administered to Villin-CreER+ Apcfl/fl mice, a significant increase in apoptotic activity (65.2% vs. control, p<0.05) was noted within intestinal crypts.

Conclusion: Iron chelation with ICL670A demonstrates significant efficacy in laboratory models of colorectal adenocarcinoma. Further in-vivo evaluation of the drug is underway before consideration of future human trials.

Take-home message: Iron chelation offers potential in the treatment of colorectal adenocarcinoma.

O143  MAPPING INTRA-TUMOUR HETEROGENEITY USING COPY NUMBER VARIATION ANALYSIS GENERATED WITH MASSIVELY PARALLEL SEQUENCING
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Introduction: Morphological intra-tumour heterogeneity (ITH) has long been recognised histopathologically. The theory of clonal subpopulations and evolutionary growth models of these populations is also widely accepted. Applying this knowledge clinically is a challenge as yet unanswered. We aimed to evaluate if low-coverage massively parallel sequencing could be used to analyse copy number variation (CNV) and demonstrate intra-tumour clonal heterogeneity in head and neck squamous cell carcinoma specimens.

Method: Human papilloma virus negative oral cancers were identified and tissue blocks obtained from the Leeds Pathology archive. A head and neck pathologist marked out geographically distinct areas of high tumour cell content. The DNA from these separate areas was extracted and processed to a sequencing library. These libraries were then multiplexed on the Illumina HiSeq 2000 and the subsequent data converted to a digital karyogram.

Result: Comparison of the karyograms from geographically distinct tumour areas demonstrates intra-tumour heterogeneity with differing dominant clonal populations. We are able to demonstrate the clonal evolution throughout the different samples. The CNV patterns also enable us to trace the specific clonal populations within a primary tumour responsible for a specific nodal metastasis.

Conclusion: Understanding ITH and incorporating it into personalised management strategies will be vital in the future. Our results show that low coverage sequencing provides an affordable tool to define relationships between clonal populations within primary tumours and their metastases. We can obtain data from standard diagnostic specimens within a time frame that could potentially be applicable in the context of a multi-disciplinary team meeting.

Take-home message: Intra-tumour heterogeneity is a vital concept in the development of cancer genomics and personalised medicine. We demonstrate how low-coverage massively parallel sequencing can be used to evaluate and trace intra-tumour heterogeneity in head and neck cancer specimens.
O144 INVESTIGATING THE ROLE OF MICRORNA-ASSOCIATED SINGLE NUCLEOTIDE POLYMORPHISMS IN BREAST CANCER
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Introduction: Micro (mi)RNAs are small non-coding RNA molecules that exert post-transcriptional effects on gene expression by binding to cis-regulatory regions in target messenger (m)RNA. miRNAs play a crucial role in regulation of a host of biological pathways. Functionally relevant Single nucleotide polymorphisms (SNPs) in miRNA precursors may alter miRNA function and may modify cancer risk. SNPs associated with mir146a and mir196a2 have been shown to modify breast cancer risk and age of onset. Aim: The aim of this study was to investigate the role of variants associated with mir196a2 (rs11614913) and mir146a (rs2910164) in breast cancer in an Irish sample.

Method: A case-control study was undertaken. Cases were recruited from symptomatic and screening breast units and cancer-free controls from the community. Patients with high-risk mutations in BRCA1/BRCA2 were excluded from analysis. DNA was extracted by crystallisation precipitation from blood/buccal swabs and genotyping was performed using a Taqman-based platform. Data was analysed using SPSS v22.

Result: A total of 719 (427 cases, 292 controls) patients were genotyped for mir146a-variant, and 635 (351 cases, 284 controls) for mir196a2-variant. Both variants were identified, with minor allele frequency of 0.2 for mir146a-variant, and 0.56 for mir196a2-variant. Expression of the variants did not differ significantly between cases and controls (p=0.189, mir-146a; p=0.931, mir-196-a-2, X2). Cases expressing the mir-146a-variant were affected at a significantly younger age than wild-type cases (57±12 vs 55 ±11, p=0.04, t-test).

Conclusion: A single nucleotide polymorphism in the mir-146a2 precursor is associated with younger age of onset of breast cancer in Irish patients.

Take-home message: microRNA single nucleotide polymorphisms can modify disease risk or phenotype, or age of onset.

O145 HYPOXIA RESPONSES IN 3-DIMENSIONAL BREAST CANCER CELLS
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Introduction: The application of 3-dimensional (3D) cell culture is rapidly replacing conventional 2D culture in cancer studies, particularly for drug screening, simulating in vivo tumour behaviour and comparing non-malignant with malignant cell responses. The aim of this primary study was to adapt established techniques for investigating subtle molecular changes associated with hypoxia as the cells proliferated.

Method: 3D spheroids from breast cancer cell lines MCF-7 and MDA-MB-231 were generated in ultra-low attachment (ULA) plates in the presence of 10% FBS-DMEM/F-12 supplemented with gentamycin and amphotericin. At different time points, spheroids were: (i) measured by confocal microscopy; (ii) counted following disaggregation; (iii) sectioned by cryostat-microtome for immunocytochemistry (ICC); (iv) examined for changes in mRNA expression; and (v) doxorubicin treated for IC50.

Result: Diameters of MCF-7 spheres were ~1.3 times larger than MB-231 spheres while MB-231 cells generated spheroids 3.1 times denser than MCF-7 spheroids (day 12). Vacuoles and markers of hypoxia (HIF-1α and VEGF) were observed in both cell lines and expression increased with time (MCF-7). Cell lines differed in their responses to doxorubicin: MB-231 spheroids were less sensitive than 2D cultures (IC50 6.7µM vs 1.5µM), while MCF-7 spheroids appeared stimulated.

Conclusion: As expected, spheres larger than 400µm in diameter demonstrated responses to hypoxia, evident from the increased expression of HIF-1α and VEGF. Additionally, sensitivity to doxorubicin decreases (MB-231) when cancer cells are cultured as spheroids instead of monolayers, concurring with clinical observations.

Take-home message: 3D spheroids more accurately simulates in vivo tumour behaviour, allowing laboratory research to be more clinically translatable and therefore more effective.

O146 MORPHINE POTENTIATES THE CHEMOTHERAPEUTIC ACTION OF 5-FLOUROURACIL, POSSIBLY THROUGH INHIBITION OF AUTOPHAGY
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Conclusion: As expected, spheres larger than 400µm in diameter demonstrated responses to hypoxia, evident from the increased expression of HIF-1α and VEGF. Additionally, sensitivity to doxorubicin decreases (MB-231) when cancer cells are cultured as spheroids instead of monolayers, concurring with clinical observations.
**Introduction:** Morphine is used regularly in clinical practice for pain management. Its action on the growth of various cancers has been researched with varying results. 5-fluorouracil (5-FU) is commonly used in chemotherapeutic regimens for colon cancer, and the combination of morphine and 5-FU can be given in clinical practice. Autophagy is a cell survival mechanism that occurs when stress is applied: by digesting non-essential organelles, the cell can continue to fuel essential processes. We aimed to investigate the effect of the combination of these two drugs on tumour growth and to determine whether this effect is due to a change in autophagic flux.

**Method:** Human colon cancer cell lines SW480 and SW620 (primary and metastatic, respectively) were treated with varying doses of 5-FU & morphine alone and in combination for various time points in vitro. Morphology by cytospin, proliferation by viable cell count, apoptosis by propidium iodide staining, autophagic flux by “Cyto-ID” assay and recovery from treatment by modified clonogenic assay were assessed.

**Result:** Morphine alone had little effect on SW480 and SW620 compared to control, increasing proliferation and decreasing apoptosis slightly but not significantly. 5-FU alone decreased proliferation, increased apoptosis and autophagic flux, and substantially reduced the colony count of SW480 and SW620. Morphine and 5-FU in combination significantly suppressed tumour colony growth/recovery (p<0.05 versus 5-FU alone), with decreased proliferation, apoptosis, and autophagic flux.

**Conclusion:** Morphine potentiates the chemotherapeutic effect of 5-FU. Further study is required to clarify whether this is due to the suppressed survival mechanism, autophagy.

**Take-home message:** Morphine potentiates the chemotherapeutic effect of 5-FU. Interestingly, autophagy is decreased in the combination of these drugs compared to 5-FU alone but more needs to be done in order to show this is the reason for decreased cellular recovery from treatment.

**O147 A NEW USE FOR AN OLD DRUG: ESTABLISHED CHEMOTHERAPY AGENTS CAN REDUCE CELLULAR OXYGEN CONSUMPTION AND DECREASE TUMOUR HYPOXIA IN COLORECTAL CANCER INDEPENDENT OF TOXICITY**

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**Introduction:** Tumour hypoxia describes the phenomenon of reduced oxygen tension within the core of solid tumours. The consequences of this include increasing morbidity, mortality and metastasis. We aim to identify agents from established chemotherapeutics that may reduce oxygen consumption and hence, improve tumour hypoxia and response to radiation therapy in vivo.

**Method:** Four colorectal cancer cell lines (COLO320DM, DLD1, HCT116, HT29) and a non-transformed cell line (MRC5) were investigated. Clonogenic and cytotoxicity assays of a range of agents were used to determine sub-lethal concentrations. The oxygen consumption of treated cells, as well as mitochondrial function measuring basal respiration, ATP turnover, proton leak and spare respiratory capacity were assessed with the XF96 Analyser. The most responsive cell lines were tested in spheroids for hypoxia by staining with EF5, Ki-67 and Hoechst.

**Result:** The oxygen consumption of all cancer cell lines were markedly reduced with a number of the agents compared with the non-transformed cell line. DLD1 and HCT116 cell lines, in particular, showed the greatest balance of resistance to toxicity and reduction in oxygen consumption. Hypoxia imaging of the spheroids from these cell lines further demonstrated a reduction in hypoxia consistent with drug induced decrease in oxygen consumption.

**Conclusion:** Despite decades of clinical use these known chemotherapeutics have not been implicated in alterations in oxidative metabolism. These results raise the prospect of using them in an alternative way. Improving tumour hypoxia will potentially improve clinical outcome and may lead to a better understanding of the optimal timing of radiotherapy and surgery.

**Take-home message:** Manipulating cancer cell metabolism to use less oxygen improves tumour hypoxia. This is a new, exciting and potentially significant alternative approach to chemotherapy.