

5C ONCOLOGY

O130 ENDOCRINE RESISTANT BREAST CANCER IS CHARACTERISED BY THE DEVELOPMENT OF A DIFFERENTIATION LESION WITHIN LUMINAL PROGENITOR CELLS

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Introduction: Breast cancer develops from mammary gland epithelia and is composed of hierarchically arranged cells. These cell types correlate to healthy cell types of the mammary gland. Here a developmental biology approach is utilised to understand endocrine resistance in breast cancer.

Method: Flow cytometry was used to assay expression of cell surface proteins (CSPs) defining the cell types of mammary epithelia. Sensitive and resistant models were compared to identify cell types that define resistance. The bioinformatics tool: GOBO, was used to assess steroid response and ER+ signatures in patient data. FACS isolated sub-populations paired with conditioned media were used to define the stem cell network.

Result: The CSP screen shows only the luminal arm of the mammary hierarchy is represented in endocrine resistant breast cancer models. The sensitive model has three cell types: FUT+MUC1- luminal progenitor cells (LPCs); FUT4-MUC1- and FUT4-MUC1+, both differentiated cells. The resistant model has one cell type: FUT4+MUC1- (LPCs). This is observed in two independently generated models of resistance. Furthermore, FUT4 and MUC1 expression respectively negatively and positively correlate with predicted steroid response in patients. A similar correlation is also seen with respect to ER+ status.

Conclusion: This study identified a novel marker: FUT4 that defines the same cells as the classic CD49f marker in a more robust fashion. Together with MUC1, FUT4 defines a three cell hierarchy whose dynamic remodelling defines endocrine resistance. Through characterising this stem cell network's transcriptome and secretome, this study is designed to identify actionable paracrine targets to complement traditional steroid therapies.

Take-home message:

Endocrine resistance in breast cancer is driven by a restructuring of the underlying cell hierarchy. Paracrine targeting should revert resistant tissue back to a sensitive state.

O131 CORRELATION OF 18-FDG PET-CT WITH ENDOSCOPIC ULTRASOUND IN OESOPHAGEAL CANCER

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Introduction: Initial staging and radiotherapy planning of oesophageal cancer, involves use of both endoscopic ultrasound (EUS) and 18-FDG PET-CT (PET). In some patients with impassable strictures, EUS can be challenging. Some operators would dilate the stricture to complete staging, however, there is a high risk of complications with this. PET can instead provide information with regards to the length and volume of tumours, yet there is little evidence available which demonstrates that PET-CT can replace EUS for this purpose. We have therefore conducted this study to assess the correlation between PET and EUS measurements.

Method: All patients who had both PET and EUS performed for oesophageal cancer staging at our centre between 2010 and 2016, were identified from a local database. Patients were excluded if they had FDG in-avid tumours or impassable strictures on EUS. PET data was analysed to establish the tumour volume and total length using various semi-quantitative parameters. The EUS data was also recorded for these patients and was correlated with PET using the Spearman's rank test.

Result: 70 patients were included in the analysis. Strong correlation was established between the length and volume of tumour on PET and EUS, above the critical value for Spearman's rho (0.278): Length- $r=0.562$, $p<0.01$ Volume- $r=0.667$, $p<0.01$.

Conclusion : Given the strong correlation between EUS and PET, FDG-PET can provide accurate information on the length and volume of tumour in patients who either cannot tolerate EUS or have impassable strictures obviating the need for dilatation.

Take-home message:

PET-CT can provide accurate information about the length and volume of oesophageal tumours and may potentially be used exclusively over EUS for staging and radiotherapy planning.

O132 A CLOSED LOOP AUDIT ON REDUCING COSTS ASSOCIATED WITH OVER-INVESTIGATION IN PRE-OPERATIVE BREAST SURGERY PATIENTS

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Introduction: There is evidence to suggest that over-investigation in pre-operative assessment clinics can lead to an increase in patient anxiety as well as unnecessary costs. The aim is to implement guidelines for pre-operative tests on breast surgery patients and reduce the costs associated with over-investigation.

Method: Data collected consisted of the American Anaesthetic Society grade for each patient, grade of surgery, co-morbidities, pre-operative investigations requested and associated costs. Surveys were

completed by consultant anaesthetists and breast surgeons on pre-operative investigations. Interventions included local guidelines developed based on survey results and NICE guidelines (NICE 2015). Formal teaching sessions were also held and posters were displayed in pre-operative assessment clinics.

Results: The first and second cycle of the audit consisted of 88 and 58 patients, respectively. There was an improvement in the percentage of indicated investigations requested after implementation of the guidelines in all tests except for liver function, with the greatest improvements seen in requesting full blood count (17% improvement) and group and save (31% improvement). There was a statistically significant improvement in the number of indicated tests requested for full blood count and group and save. A small sample size may have prevented statistical significance for urea and electrolytes. There was a total savings of £377/100 patients by using the guidelines.

Conclusions: There remains a culture of investigating patients based on established practice. Implementation of pre-operative assessment guidelines can reduce costs associated with over-investigation. A further re-audit will be carried out to ensure continuing adherence to the guidelines.

Take-home message: Implementing national guidance may reduce the costs associated with over-investigation in pre-operative assessment clinics.

O133 CHARACTERISING TUMOUR ASSOCIATED MACROPHAGES IN THE GLIOBLASTOMA MICROENVIRONMENT USING FLOW CYTOMETRY

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Introduction: Glioblastoma is the most malignant primary brain tumour, with 12 month median survival. Efforts to identify new therapeutic targets have focussed on immune cells in particular Tumour associated macrophages and microglia (TAMM). TAMMs consist of tissue-resident brain microglia and peripheral bone marrow derived macrophages. Their roles are conflicting due to limitations of mouse models and cell-surface markers used. In normal rodent/human brain, the antibody marker-set CD11b+/CD45hi is said to represent peripheral macrophages, while CD11b+/CD45lo are microglia. We found this characterisation too broad and aimed to firstly identify a new marker-set using flow cytometry and then determine if peripheral macrophages and brain microglia have independent roles in glioblastoma.

Method: Using our non-myeloablative bone marrow transplant model we selectively replace the peripheral mouse immune system with congenic donor cells labelled with CD45.2 (pan-leucocyte marker) while retaining an intact host blood-brain barrier (labelled as CD45.1). Stereotaxic intracranial implantation of murine glioblastoma replicated normal glioblastoma development. Brain flow cytometry using a 15-marker panel was then used to characterise the immune cell populations.

Pilot Data: Our findings showed >90% bone marrow chimerism using CD45.1/CD45.2 model. Cerebral digestion and analysis of tumour-bearing hemispheres (n=6) demonstrated multiple tumour-tropic immune populations, but importantly identified a novel 4-marker set (Ly6C/MHCII/CD64/MeTK) to separate tumour associated macrophages and microglia with ≥95% confidence and purity. Forward plan: Our results highlighted a new approach to subcategorising TAMMs. Further work necessitates RNA extraction for transcriptional analysis of macrophage/microglia populations using our marker-set to identify novel markers for downstream functional analysis and immunotherapy development.

Take-home message:

Characterisation of the immune cell niche using a non-myeloablative conditioning transplant system alongside a detailed flow cytometry panel provides a powerful method to correctly identify tumour associated macrophage subpopulations in glioblastoma. Thereafter, the function of tumour-tropic macrophage cells can be determined with greater reliability for the development of future immunotherapies.

O134 THE AMOEBOID CELL IN CANCER

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Introduction: Metastasis and therapeutic failure may partly be due to cellular plasticity of cancer cells, as seen in epithelial-to-mesenchymal transition (EMT). In addition to the epithelial and mesenchymal tumour initiating cancer cells, we now describe in oral squamous cell carcinoma (OSCC) a third cell phenotype, the amoeboid, previously identified only in melanomas and sarcomas.

Method: The data is based upon in vitro study of normal, OSCC and fresh tumour specimens. Techniques employed include high-throughput invasion and migration assays, drug response assays, protein and differential gene expression profiling, fluorescence activated cell sorting, gene knockdowns and immunostaining.

Result: Amoeboid cells are smaller than other cells and migrate at greater speeds (x10 faster v other phenotypes), resembling leucocytes. They are significantly more invasive (x4-20 v mesenchymal cells) with greater chemoresistance profiles. Gene analysis produces a signature associated with angiogenesis,

chemoresistance and anti-apoptosis. Essentially lacking keratin, amoeboid cells may complicate histopathological assessment of tumour spread. However, they are plastic, allowing reversion to other phenotypes. They also have specific drug and signalling pathway responses that have produced potentially new drugs to specifically target amoeboid cells in vivo.

Conclusion: • We describe a new amoeboid OSCC phenotype that may confer on oral cancer a greater ability to invade, disseminate and resist therapy. • The amoeboid cell is faster and more invasive than other phenotypes and displays plasticity and enhanced chemoresistance • The cells appear to arise from mesenchymal-amoeboid transition (MAT) • Amoeboid markers and pathway analysis may allow identification in vivo and subsequent therapeutic targeting to improve patient outcomes

Take-home message:

Epithelial cancer cells can gain advantages in invasion and therapy evasion by changing into smaller, more motile amoeboid cancer cells, seen for the first time in oral cancer.

O135 AZD1775 INDUCES TOXICITY THROUGH DOUBLE-STRANDED DNA BREAKS INDEPENDENT OF 5-FU ACTIVITY IN P53 MUTATED COLORECTAL CANCER CELLS

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Introduction: Colorectal cancer is a global health problem with an estimated 700,000 deaths per year. AZD1775 is a small molecule WEE1 inhibitor used in combination with DNA-damaging agents to cause premature mitosis and cell death in p53 mutated cancer cells. Recently, AZD1775 has been shown to have monotherapeutic activity by causing double-stranded DNA (DS-DNA) breaks as a result of nucleotide exhaustion. We sought to determine the dominant mechanism of action of AZD1775 in combination with 5-FU in a p53-mutated colorectal cancer cell line.

Method: HT29 cells (p53 mutated) were treated with 5-FU (1 μ M) for 24 hours followed by AZD1775 (300 nM). Proliferation was assessed using a WST-1 assay. Flow cytometry was used to quantify levels of double-stranded DNA breaks and premature mitosis using the specific markers γ H2AX and pHH3. Caspase-3 dependent apoptosis was quantified using an Incucyte Imaging system.

Result: AZD1775 significantly improved the cytotoxicity of 5-FU decreasing the IC50 from 9.3 μ M to 3.5 μ M. It caused significantly more mitosis (3.8% vs 56.2%), DS-DNA breaks (5.1% vs. 60.5%) and caspase-3 dependent apoptosis (4% vs. 13%) compared to 5-FU alone. The addition of exogenous nucleosides significantly rescued the increased DS-DNA breaks (60.5% vs 6.9%) and caspase-3 dependent apoptosis (13% vs 4.8%) caused by AZD1775, suggesting this to be the dominant mechanism of action, not premature mitosis.

Conclusion: AZD1775 has independent cytotoxic effects from 5-FU in p53-mutated colorectal cancer cells. This finding is important for designers of future clinical trials when considering the timing and duration of AZD1775 treatment.

Take-home message:

AZD1775 causes DS-DNA breaks and has independent cytotoxic effects from 5-FU in p53-mutated colorectal cancer cells.

O136 CDX2 AS A PROGNOSTIC BIOMARKER IN STAGE II AND STAGE III MISMATCH REPAIR DEFICIENT COLORECTAL CANCER

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Introduction: Stage II mismatch repair deficient (MMRd) colorectal cancer (CRC) derives no benefit from adjuvant chemotherapy. Absent expression of Caudal-related homeobox transcription factor 2 (CDX2) identifies a subgroup of patients with high-risk stage II disease who may benefit from adjuvant chemotherapy. This study sought to determine if absent CDX2 expression was of prognostic significance in Stage II and III MMRd CRC.

Method: MMRd tumours were identified from an institutional database of all CRC. CDX2 expression was determined by immunohistochemistry. Receiver operating characteristic analysis was used to identify a level of CDX2 expression that had a high specificity for recurrence. The kappa statistic was used to test interrater reliability. Survival analysis was performed using Kaplan-Meier curves, the log-rank test and Cox regression.

Result: Of 238 CRCs in 223 patients absent CDX2 expression was noted in 13.4% (n=32) of tumours. Absent CDX2 expression was associated with pTstage, grade and lymphovascular invasion ($p \leq 0.005$). Absent CDX2 was a predictor of nodal metastases ($p=0.036$) and was associated with worse overall survival (HR; 1.877, 95% CI; 1.062- 3.316, $p=0.030$). Absence of CDX2 expression identified high-risk stage III MMRd, with a worse disease free survival (DFS $p=0.045$), but was not of prognostic significance in Stage II MMRd CRC. DFS was the same within stage III MMRd once adjusted for adjuvant chemotherapy.

Conclusion: Absent CDX2 expression identified high-risk stage III MMRd CRC that appeared to benefit from adjuvant chemotherapy. There was no observed benefit with adjuvant chemotherapy in stage II MMRd tumours.

Take-home message:

Absent CDX2 expression identified high-risk stage III MMRd CRC that were at high risk of recurrence and that appeared to benefit from adjuvant chemotherapy. There was no prognostic stratification or observed benefit with adjuvant chemotherapy in stage II MMRd tumours.

O137 A COMPARISON OF SURVIVAL RATES IN ELDERLY BREAST CANCER PATIENTS TREATED WITH SURGERY OR PRIMARY ENDOCRINE THERAPY WITH AROMATASE INHIBITORS

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Introduction: Elderly patients diagnosed with breast cancer may be frail with multiple comorbidities. Primary endocrine therapy (PET), usually in the form of an aromatase inhibitor, offers an alternative to surgery. We analysed a cohort of patients to compare the survival of those treated with surgery or PET alone.

Method: Patients aged 70 and over treated with PET or surgery for breast cancer between 2009 and 2013, were identified from a prospectively collected database through the regional Managed Cancer Network. Date, cause of death, and comorbidity data used to calculate the Charlson Comorbidity Index, were obtained from each patient's electronic clinical record. Survivors were censored. Patients with DCIS or metastases at diagnosis were excluded. Overall and cancer specific survival were compared using Kaplan Meier curves.

Result: 834 elderly patients were treated, of which 199 had PET and 612 had surgery. Median follow up time was 49 months. 288 deaths occurred. Overall 5 year survival was 30% in the endocrine group and 78% in the surgery group ($p=0$). Breast cancer specific survival was 81% in the endocrine group and 92% in the surgery group ($p=0$). Patients treated with endocrine treatment only had a significantly higher Charlson score (mean 5, range 3-12) compared to patients with surgical treatment (mean 4, range 3-13) ($p=0$).

Conclusion: Surgery is superior to primary endocrine therapy as treatment for breast cancer in elderly patients, showing superior breast cancer specific survival. However, endocrine therapy is a reasonable alternative for those with multiple comorbidities as a significant proportion seem to die of their comorbidities within 5 years.

Take-home message:

Surgery is superior to primary endocrine therapy as treatment for breast cancer in elderly patients, showing superior breast cancer specific survival. However, endocrine therapy is a reasonable alternative for those with multiple comorbidities as a significant proportion seem to die of their comorbidities within 5 years.