**O15 FOXO3A EXPRESSION IN COLORECTAL CANCER: A PROMISING BIOMARKER OF MICROMETASTASES**

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**Introduction**
The Forkhead/winged helix box class O3a (FOXO3a) tumour suppressor belongs to a conserved family of transcription factors, and orchestrates critical programs of gene expression involved in differentiation, apoptosis, and DNA damage responses. The aim of the present study was to investigate the role of FOXO3a in human colorectal cancer (CRC).

**Methods**
Formalin fixed resection specimens from 20 benign, 164 primary, and 58 matched liver metastases were used to generate tissue microarrays. Triplicate cores of 1 mm diameter were taken from representative areas and immunohistochemical staining for FOXO3a conducted. Semi-quantitative scoring was carried out blindly by two investigators one of whom was a clinical pathologist.

**Results**
FOXO3a expression was significantly reduced with advancing cancer stage (p<0.0005) and when compared with normal and adenomatous tissue. 33 early stage (I/II) non-recurrent primary tumours showed significantly higher FOXO3a expression when compared with 33 stage-matched recurrent tumours. When stratified according to high and low FOXO3a expression, mean disease free survival in the Low FOXO3a expressing group was 28months (95% CI 15.8-50.6) compared to 64 months (95% CI 52.9-75.4) in the high FOXO3a expressing group (p=0.001). No difference between FOXO3a expression was noted between primary tumours with synchronous and metachronous recurrence and their matched liver metastases.

**Conclusions**
FOXO3a levels are reduced during CRC progression, and low levels in the primary tumour associated with recurrent disease. Low FOXO3a levels may represent a novel biomarker of nodal and distant disease spread.

**O16 EVALUATION OF THE ACUTE INFLAMMATORY RESPONSE TO OMEGA-3 FATTY ACIDS IN PATIENTS WITH SEVERE ACUTE PANCREATITIS: A RANDOMISED CONTROLLED TRIAL**

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**Introduction**
Polyunsaturated fatty acids (PUFA), especially omega-3 PUFA, like eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), which are major components of fish oil, have demonstrated potent anti-inflammatory and immunomodulatory activities in animal and human studies. We investigated the effects of omega-3 PUFA on inflammatory cytokine concentrations in patients with severe acute pancreatitis (SAP).

**Methods**
Forty-four patients, with predicted SAP, were randomly assigned to receive either a 50:50 mixture of medium-chain fatty acids and soybean oil (control group, n=22) or a 50:40:10 mixture of medium-chain fatty acids, soybean oil and fish oil (study group, n=22). Lipid mixtures were infused for a maximum of 7 days and circulating serum cytokine concentrations (IL-6,IL-8,TNF-α,IL-18,ICAM-1,IL-10) were measured at 6 time points from baseline to day 7 of the infusion using a multiplex cytokine array (MSD systems). Statistical analysis was performed using the Mann-Whitney test to compare means.

**Results**
Serum IL-6 concentration showed a significant reduction on days 2,3,5and7(p= 0.018, 0.004, 0.039, 0.039) in the fish oil group. This reduction in concentration had a significant correlation with ITU/HDU and hospital length of stay, p=0.029 and p=0.009 respectively. At each time point of analysis, IL-8,TNF-α,IL-18 and ICAM-1 serum concentrations displayed a tendency towards reduction (p>0.05). Results will be presented from a linear mixed effects regression model to explore further the association between the length of stay and the concentration of cytokines.

**Conclusion**
Omega-3 fish oil emulsion modifies the acute inflammatory response evidenced by reduction in pro-inflammatory cytokine concentrations in patients with SAP. These modifications are associated with a better and quicker recovery.

**Take-home message**
Omega-3 fish oil modulate the inflammatory response in patients with SAP, and results in a better and quicker recovery.
O17 GENOME ANALYSIS OF COLORECTAL CANCERS IN BRITISH BANGLADESHIS IDENTIFIES EARLY ONSET AND A HIGH PREVALENCE OF RBFOX1 DELETION COMPARED TO CAUCASIANS
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Introduction
Prevalence of colorectal cancer (CRC) in the British Bangladeshi population (BAN) is low compared to British Caucasians (CAU). Genetic background may influence mutations and disease features.

Methods
We interrogated BAN CRC genomes using mutation profiling and high-density single nucleotide polymorphism arrays and compared findings to CAU CRCs.

Results
Age of onset of BAN CRC was significantly lower than for sporadic CAU patients (p=3.0 x 10^-5) and not due to Lynch syndrome or polyposis. KRAS and BRAF mutations were comparatively rare with significantly fewer KRAS mutations (5.4%) compared to CAU MSS CRCs (25%; p=0.04) and no BRAF mutations (CAU CRCs, 12%). Array data revealed patterns of gains (chromosome 7 and 8q), losses (8p, 17p and 18q) and LOH (4q, 17p and 18q) similar to CAU CRCs. A small deletion on chromosome 16p13.2 involving the RNA-binding alternative splicing factor RBFOX1 only was found in significantly more BAN (50%) than CAU CRCs (15%) cases (p=0.04). Novel RBFOX1 mutations were identified in CRC cell lines and tumours; mRNA and protein expression was also reduced. Conclusions Mutations of KRAS and BRAF were rare in BAN CRC. Loss of RBFOX1 may explain the anomalous splicing activity associated with CRC and warrants further investigations.

Take-home message
A small deletion on Chromosome 16 is significantly more in Bangladeshi patients with colorectal cancer compared to Caucasian patients

O18 EVALUATION OF ONE STEP NUCLEIC ACID AMPLIFICATION (OSNA) MOLECULAR ASSAY FOR INTRAOPERATIVE DIAGNOSIS OF SENTINEL LYMPH NODE METASTASIS
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Introduction
One step nucleic acid amplification (OSNA) is a recently developed molecular diagnostic assay to detect lymph node metastases. Aim of this study was to assess OSNA for intra-operative detection of sentinel node metastases in breast cancer with use of standard histopathology as the “gold standard”. Discordant cases were reviewed and further investigated to assess their impact on patients’ management.

Methods
170 breast cancer patients with clinically and ultrasonographically negative axilla underwent axillary staging with sentinel node biopsy. 270 sentinel lymph nodes were intra-operatively evaluated by OSNA and subsequently with standard histopathology undertaken on alternate slices of the excised nodes. Axillary node clearance was performed if the sentinel node was positive for OSNA or histopathology. The time taken for an OSNA result was recorded.

Results
Sensitivity and specificity of OSNA were calculated at 95.5% and 96% respectively. Concordance between OSNA and Histopathology was 95.5% with positive predictive value and negative predictive value of OSNA 80% and 99% respectively. Discordant nodes were identified in 12 patients. In 7 patients axillary node clearance was performed on the basis of the OSNA result alone. 2 patients underwent delayed axillary node clearance
when histopathology showed metastases after a negative OSNA result. Turn-around time for an OSNA result for 1 node was 44 minutes (range 37-50) and 2 nodes 60 minutes (range 50-79).

**Conclusion**
OSNA is a reliable, highly accurate standardised method for the intra-operative evaluation of axillary lymph node metastasis with very few patients requiring delayed axillary node clearance in our study.

**Take-home message**
OSNA is a reliable, highly accurate standardised method for the intraoperative evaluation of axillary lymph node metastasis with very few patients requiring delayed axillary node clearance.

**O19 RESPONSE OF COLORECTAL CANCER CELLS TO DIFFERENT MEK INHIBITORS**
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**Introduction**
The MAP kinase pathway is essential in most cell types and its aberrant activation in many cancers leads to enhanced proliferation and survival, among other effects. New chemotherapeutics directed against the kinase MEK successfully interrupt its signalling cascade; however, their efficacies vary among cancer subtypes.

**Methods**
Two colorectal cancer cell lines, HT29 and HCT116, bearing mutations in BRAF and KRAS respectively (upstream of MEK), were treated with the MEK inhibitors AZD6244 (currently in clinical trials), PD98059 and U0126. Their effects on cell proliferation were measured by metabolic activity (MTT assay) and by total DNA quantification. Findings were complimented by western blotting of phosphorylated ERK (pERK) after treatment with the inhibitors and activation of the pathway with EGF. Quantitative RT-PCR was performed to validate MEK as a target.

**Results**
AZD6244 (1nM-10µM) and PD98059 (10-100µM) showed a consistent dose-dependent inhibition of proliferation in both cell lines; [AZD6244: p<0.001 at 100nM (HT29) and at 1µM (HT116), PD98059: p<0.05 at 100µM (HCT116) and p<0.01 at 50nM (HT29)]. Conversely, U0126 (10nM-1µM) induced proliferation in HT29 (p<0.001 at 50nM) and inhibited proliferation in HCT116 (p<0.001 at 1µM). Increasing concentrations of AZD6244 reduced the levels of pERK, demonstrating that proliferation inhibition is due to impairment of MEK activity. There was also a positive correlation between increased proliferation and pERK in U0126 treated HT29 cells.

**Conclusion**
AZD6244 may be a promising therapeutic molecule against certain colorectal cancers, while the contradictory effect of U0126 on proliferation is consistent with reports on various anti-cancer drugs in vitro.

**Take-home message**
AZD6244 may be a promising therapeutic molecule for certain colorectal cancers.

**O20 MYELOID CELLS ARE RECRUITED BY LIVER METASTASES AND AID THEIR DEVELOPMENT**
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**Introduction**
Myeloid cells aid the progression of various cancers. We identified a murine myeloid population recruited to the liver during the development of colorectal hepatic metastasis. Inhibition of myeloid recruitment through inhibition of the tumour-derived chemokine CCL2 delayed murine metastases and thus implicated a potential therapeutic target for patients with metastatic colorectal cancer.

**Methods**
Murine hepatic metastases were induced by intrasplenic injection of MC38 colon cancer cells. Myeloid populations were identified by flow cytometry. Tumour-derived chemokines were detected in cell-conditioned medium by protein array. MC38-derived CCL2 was inhibited using shRNA. Serum CCL2 expression was quantified by ELISA in 130 colon cancer patients and metastatic colon cancer tissues were analysed immunohistochemically.

**Results**
Cells co-expressing the myeloid markers Gr1 and CD11b and the chemokine receptor CCR2 (CD11b+/CCR2+) increased 6-fold in mouse liver bearing hepatic metastases compared with controls. MC38 cells expressed CCL2 and murine serum CCL2 concentration correlated with tumour burden and hepatic CD11b+/CCR2+ count suggesting a role for CCL2 in their recruitment. Consistent with this, inhibition of MC38-derived CCL2 reduced hepatic CD11b+/CCR2+ recruitment, delaying metastatic development. Serum CCL2 was elevated in patients
with colon cancer compared to healthy controls. Furthermore, CCR2 and CD11b co-localised within human hepatic metastases indicating the presence of a myeloid population similar to that seen in metastasis-bearing mice.

**Conclusions**

Recruitment of CD11b+/CCR2+ myeloid cells through the tumour-derived chemokine CCL2 aids the development of murine hepatic metastases. The development of therapeutics aimed at inhibiting myeloid cell trafficking may improve operability and survival for patients with metastatic colorectal cancer.

**Take-home message**

A specific sub-set of myeloid cells are recruited to colon cancer metastases via the action of the tumour-derived chemokine CCL2. Inhibition of this process via manipulation of the chemokine pathway delays the development of hepatic metastasis.

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**O21 METHYLATION OF THE NOTCH PATHWAY IN PATIENTS AT HIGHER RISK OF COLORECTAL CANCER AND EFFECTS OF SUPPLENTATION WITH RESISTANT STARCH IN A RANDOMISED CONTROLLED TRIAL**

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**Introduction**

Colorectal cancer (CRC) arises from genetic defects in stem cells. Notch signalling plays a key role in stem cell replication. NOTCH-related genes are overexpressed in CRC. The mechanism for this is not known but could include epigenetic activation of NOTCH oncogenes via promoter hypomethylation. Methylation can be modulated by environmental stimuli including dietary factors such as butyrate, produced by bacterial fermentation of non-digestible carbohydrates in the colon. Butyrate exerts potent anti-oncogenic effects in the colorectal mucosa.

**Methods**

Participants were recruited at endoscopy and included those at normal risk of CRC (n=75), or higher risk of CRC because of previous adenomatous polyps (n=28) or ulcerative colitis (n=12). Participants provided 9 rectal biopsies. Normal risk participants were randomised to resistant starch (Hi-maize260) or polydextrose in a 2x2 factorial placebo controlled trial for 50 days. Methylation of several CpG sites in the promoters of JAG1 (NOTCH pathway ligand) and RBP-J (NOTCH intracellular activator) was quantified using pyrosequencing.

**Results**

For JAG1 there was trend towards lower methylation at all CpG sites in those at higher CRC risk. Methylation at RBP-J CpG 11 was lower in polyp patients than in controls (18.0 (1.5) vs. 23.6% (0.8), p=0.011). At JAG1 CpG 4, methylation increased following polydextrose supplementation compared to placebo (3.1 (0.4) vs. 1.7% (0.4), p=0.009). A similar, but non-significant, trend was observed at other CpG sites for JAG1.

**Conclusions**

DNA methylation of NOTCH signalling genes is altered in macroscopically normal colorectal mucosa of patients at higher CRC risk. The observed changes in JAG1 methylation after polydextrose supplementation are consistent with a protective effect against carcinogenesis.

**Take-home message**

Notch signalling activation is a key process in colorectal carcinogenesis for which no mechanism has yet been established. This study suggests that methylation alterations of the genome may result in epigenetic activation of the Notch pathway and that dietary intervention may modulate this change.

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**O22 PROGRESSIVE ALTERATIONS IN CD4+ T CELL PHENOTYPES IN BREAST CANCER PATIENTS FOLLOWING CHEMOTHERAPY**

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**Introduction**

Initial immune toxicities of chemotherapy have been reported but longer-term effects are less clear. Our aims were to study the time-course and determinants of changes in T lymphocytes during and after breast cancer chemotherapy, and to define characteristics of repopulating cells.

**Methods**

Ethical approval was obtained. Peripheral blood was collected from 88 patients during/after primary breast cancer treatment. Lymphocyte numbers/phenotypes were assessed by flow-cytometry.
Results
Chemotherapy resulted in significant reductions in numbers of circulating CD4+ (median 39.2% of pre-chemotherapy levels, p<0.001) and CD8+ T cells (51.6%, p<0.001). At 9 months post-chemotherapy there was almost complete recovery of CD8+ cells (94.0% of pre-chemotherapy level), but only partial recovery in CD4+ cells (54.8%, p=0.013). The CD4+ T cells present during recovery also differed in phenotype from pre-chemotherapy and showed little sign of normalizing. Indeed, phenotypes continued to diverge progressively from pre-chemotherapy levels. CD45RO+ memory CD4+ T cells progressively expanded from 62.8% of the CD4+ compartment pre-chemotherapy to 67.8% at 2 weeks, and 73.8% at 9 months post-chemotherapy (p<0.006). Similarly, at the same time-points naive CD4+RA+CD62L+ cells decreased from 46.6% to 34.0% and 33.7% (p<0.03). CD4+ CD45RO- CD31+ recent thymic emigrants also decreased from 23.0% to 18.3% and 13.7% (p<0.001). Finally, CD25hiFoxP3+ regulatory cells expanded from 3.0% to 3.4% and 5.26% (p<0.001), increases that were particularly prevalent in patients presenting with larger tumours (p=0.012) or undergoing herceptin therapy (p=0.028).

Conclusion
T cell levels and phenotypes remain altered even 9 months after chemotherapy, hinting at potential long-term immune defects.

O23 LONG TERM FOLLOW-UP OF THE MRC CONVENTIONAL VERSUS LAPAROSCOPICALLY ASSISTED RESECTION IN COLORECTAL CANCER TRIAL
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Introduction
Laparoscopic resection is widely used in the management of colorectal cancer; however there is limited data regarding long-term outcomes, particularly regarding rectal cancer. This study presents the long-term follow-up of the MRC-CLASICC trial of conventional versus laparoscopic surgery in the treatment of colorectal cancer.

Methods
A total of 794 patients from 27 UK centres were randomised to laparoscopic or open surgery in a 2:1 ratio between 1996 and 2002. Long-term follow-up data involving intention-to-treat and actual treatment groups was analysed to determine differences in survival outcomes and recurrences. Survival and recurrence data were compared using Kaplan-Meier and cumulative incidence function curves respectively, and tested using log-rank and Wilcoxon rank-sum tests.

Results
Median follow-up of all patients was 62.9 (IQR: 22.9-92.8) months. For all patients median OS was 85.1 (95% CI 72.7-105.7) and 73.6 (95% CI 64.3-89.5) months, whereas DFS was 94.8 (95% CI 74.2-108.7) and 70.6 (95% CI 55.0-85.5) months for colon and rectal cancer respectively, with no difference by randomised procedure for either OS (P=0.78) or DFS (P=0.59). Colon cancer patients experienced significantly worse OS (P=0.0005) and DFS (P=0.0068) following conversion. No significant difference in recurrences were observed by randomised procedure; however at 10 years right-sided local recurrence was increased (14.7%) compared to left-sided (5.2%), P=0.019. Rectal cancer patients experienced significantly improved early survival with laparoscopic surgery, Wilcoxon P=0.0071.

Conclusions
Long-term results continue to support the use of laparoscopic surgery for colon and rectal cancer. Trends in local recurrence warrant further investigation.

Take-home message
Laparoscopic surgery is a safe alternative to conventional open surgery in the treatment of both colon and rectal cancer in suitable patients.

O24 GLOBAL ANALYSIS OF THE SRC-1 AND HOXC11 TRANSCRIPTOMES IDENTIFIES A NOVEL ROLE FOR SRC-1 AND HOXC11 IN SUPPRESSING LUMINAL A MARKERS IN BREAST CANCER
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Introduction
Recurrent breast cancer may offer a different phenotype than the primary tumour. Cellular plasticity may drive a more aggressive phenotype in response to environmental stimuli. We describe crosstalk between steroid (SRC-1) and developmental (HOXC-11) pathways, identifying novel common targets PAWR and CD24 and examine their influence at cellular and patient level.

**Methods**
Common SRC-1 and HOXC-11 targets were identified following ChIPseq analysis of endocrine-resistant breast cancer cell lines. Regulation was confirmed by RT-PCR, Western blotting and flow cytometry using siRNA and in an SRC-1 knockout mouse. Gene methylation status was confirmed using methylation-specific arrays and methylation-specific DNA sequencing. A TMA comprising 560 patients was stained for SRC-1, PAWR and CD24 as were three patient samples of primary and recurrent tissue.

**Results**
Bioinformatic analysis identified 92 common targets. Luminal A markers PAWR and CD24, were down-regulated by SRC-1/HOXC11. PAWR/CD24 down-regulation was confirmed in cell lines. Methylation-specific arrays showed significant de-methylation of PAWR promoter with HOXC11 knockdown. CD24 promoter was de-methylated following SRC-1 knockdown. In our TMA, PAWR and CD24 associated with good disease-free survival (p<0.01 and 0.001), luminal A status (p<0.001) and inversely with SRC-1 (p<0.001). In endocrine-treated recurrences, primary tumours were luminal A (PAWR+, CD24+, SRC-1-). In recurrent tissue SRC-1 was up-regulated, while PAWR and CD24 were suppressed.

**Conclusions**
We describe a novel role for SRC-1 as a co-repressor. PAWR and CD24 may be suppressed by SRC-1 and HOXC11 via methylation. In endocrine-treated patients who recur, SRC-1 may drive an aggressive phenotype and suppress luminal A markers.

**Take-home message**
This work identifies a novel mechanism for SRC-1 to operate as a co-repressor and uncovers novel therapeutic potential.