signalling, in regulating intestinal epithelial apoptosis in vivo have not previously been investigated.

Aims To assess susceptibility to intestinal apoptosis and the associated molecular changes in mice with germline deletions of three individual NFκB family members.

Methods Intestinal apoptosis was induced in male c-Rel-null, NFκB1-null and NFκB2-null mice and their wild-type (C57BL/6) counterparts by 8Gy γ-irradiation (n=6 per group). Apoptosis was assessed on a cell positional basis from H/E stained sections. The mRNA expression of 10 key apoptosis regulating genes in the small intestine and colon (TRAIL, Caspase12, BAK, FAS-L, FAS, p53, BCL2, BCL-XL, c-IAP2 and XIAP) was assessed by real time PCR (n=4 per group). Statistical comparisons were by ANOVA with Bonferroni post-hoc tests.

Results Basal small intestinal crypt apoptosis was significantly increased in NFκB2-null relative to C57BL/6 mice. In addition, small intestinal and colonic crypt apoptotic indices were both significantly increased (up to threefold) in NFκB1-null and NFκB2-null mice 4.5 h after 8Gy γ-irradiation relative to wild-type and c-Rel-null mice. Untreated NFκB1-null and NFκB2-null small intestine showed reduced mRNA expression of the anti-apoptotic genes BCL2, BCL-XL, c-IAP2 and XIAP. Following irradiation, NFκB1-null mice showed significant increases in the mRNA of the pro-apoptotic genes TRAIL, Caspase12 (in both small intestine and colon) and BAK (small intestine only) compared to wild-type mice. Significant increases in the mRNA of the pro-apoptotic genes Caspase12 and FAS-L were also seen in irradiated NFκB2-null small intestine and colon relative to wild-type mice.

Conclusion c-Rel expression does not appear to regulate susceptibility to intestinal epithelial apoptosis in vivo. NFκB1 and NFκB2 deletion both caused increased susceptibility to intestinal apoptosis and this was associated with altered expression of TRAIL, Caspase12, BAK and FAS-L. These NFκB family members may therefore also regulate the susceptibility of intestinal epithelia to other consequences of DNA damage such as cancer.

Competing interests None declared.

OC-020 RECURRENCE AFTER RADIOFREQUENCY ABLATION FOR BARRETT'S RELATED HIGH GRADE DYSPLASIA IS DUE TO PERSISTENCE OF EPITHELIAL MUTATIONS

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Introduction Radiofrequency ablation (RFA) is a relatively new endoscopic method for ablation of high grade dysplasia (HGD) and intramucosal adenocarcinoma (IMC) in Barrett's oesophagus. Clinical trials have shown enduring eradication of these pathologies.[1] However, some patients develop recurrent dysplasia or cancer.[1] The reason for this is unknown. The aim of the study was to investigate whether this is related to the persistence of known cancer driving mutations after radiofrequency ablation.

Methods Patient records were searched for patients with recurrent HGD or IMC after RFA. Biopsies and endoscopic mucosal resection (EMR) specimens were available before and after RFA for each case. Whole biopsies underwent nested polymerase chain reaction (PCR) sequencing for mutations commonly implicated in progression to adenocarcinoma (*TP53, CDKN2A, K-ras*).

Results Tissue was obtained for six patients before and after RFA. All patients were male (mean age 67 SD±2). Samples from five patients contained detectable mutations. In 3/5 patients, the same mutation was found in material taken before RFA as after (all TP53 mutations). The indication for RFA in all three patients was HGD; two of the patients developed IMC after RFA. In these two cases laser microdissection was performed on the post-RFA EMR samples. PCR revealed the pre RFA mutation to also be present throughout the IMC specimen. Dysplastic tissue adjacent to the IMC contained a mixture of crypts that were either wild type for the mutation in the IMC or contained the mutation indicating that the IMC was a monoclonal outgrowth and that the persistent mutation was driving the development of the recurrence. Furthermore, in all three cases, PCR of a further specimens (performed at a later time point to the first post-RFA EMR), revealed the same mutation as the initial biopsy before RFA, and the first post-RFA EMR.

Conclusion The recurrence of dysplasia and cancer after RFA is likely to be due to failure to remove persistent, cancer driving mutations. Further work will need to be done to assess whether this is because of technical errors, or related to specific problems such as "buried Barrett's".

Competing interests None declared.

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OC-021 A NOVEL SUBSET OF FUNCTIONAL IL-10 SECRETING CD8 REGULATORY T CELLS INFILTRATE HUMAN HEPATOCELLULAR CARCINOMA

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Introduction Tumour specific effector T-cells can be detected in the blood and tumours of patients with hepatocellular carcinoma but fail to mount effective immune responses. Attempts to amplify anti-tumour immune responses using immunotherapy show promise, but are hampered by the presence of suppressive regulatory T-cells (Tregs) that inhibit anti-tumour immune responses. Tregs are crucial in the maintenance of immune homeostasis and in the prevention of auto-reactive immune response but in the context of cancer they can suppress beneficial anti-tumour immunity leading to tumour progression. A novel subset of CD8 expressing Tregs has recently been described and we now report the presence of such cells in human hepatocellular carcinoma and define their functional and homing properties.

Methods Fresh tissue from hepatocellular carcinoma and matched distal non-involved tissue was obtained from patients undergoing liver resection or transplantation at the Queen Elizabeth Hospital, Birmingham after informed consent. Liver-derived T-cells were isolated and phenotyped using multi-colour flow cytometry including intracellular cytokine staining. $CD8^+$Tregs were isolated using a Mo-Flow cell sorter for functional assays. Distribution of $CD8^+$Tregs was investigated by immunohistochemistry and immunofluorescence.

Results The percentage of $CD8^+$Tregs (defined as $CD8^+CD25^{high}CD127^{low}FoxP3^+$) infiltrating hepatocellular carcinoma tumours was significantly greater compared with matched non-involved liver. Tumour-derived $CD8^+$Treg isolated by Mo-Flo sorting suppressed allogeneic effectors cells in vitro and secreted interleukin-10 (IL-10). In contrast T-cell interferon-γ (IFN-γ) production was decreased within the tumour compared with matched non-involved liver. The chemokine receptor CXCR3 which is involved in T-cell recruitment to the inflamed liver was highly expressed on tumour-derived $CD8^+$Tregs.

Conclusion A novel subset of functional IL-10 secreting $CD8^+$Tregs may suppress anti-tumour immunity in hepatocellular carcinoma.

Their expression of CXCR3 provides a potential mechanism for recruitment into the tumour environment.

Competing interests None declared.

BASL plenary session

OC-022 EMBOLISATION OF INFLOW TO ALLOW SAFER LIVER RESECTION—IS MORE, BETTER?

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Introduction Portal vein embolisation (PVE) is now an established technique to increase the future liver volume/remnant (FLR) prior to liver resection. For those patients where hypertrophy is still considered insufficient complete uni-lateral embolisation incorporating both portal and hepatic artery embolisation (HAE) has been less frequently reported. The aim of this study was to evaluate the feasibility of sequential PV/HA embolisation to increase the FLR prior to liver resection.

Methods All HPB patients are discussed at a weekly MDT meeting to decide on appropriate management decisions including the necessity for FLR augmentation. PVE is performed by initially obtaining a portogram by percutaneous trans-hepatic puncture. Selective embolisation of the necessary portal veins are then performed using a combination of coils and glue etc. Embolisation of Segment 4 PV branches are performed on a selective basis. HA embolisation is performed by mapping arterial inflow and selectively embolising the desired segments planned for resection while carefully preserving the FLR. The aim of this study was to evaluate the feasibility/safety of PVE with sequential HAE over a 5-year period (January 2006–May 2011).

Results 50 patients (M:F = 38:12) underwent a right PVE; 33 (66%) progressed to liver resection. Reasons for inoperability (34%) following PVE (n=17) were (1) Small FLR, (n=6) all underwent HAE (with five proceeding to liver resection) (2) extra-hepatic disease (n=7) (3) progression of hepatic disease (n=4). The median FLR of those who progressed to resection following PVE, by CT volumetry, was 384.5 cc (330–490), significantly more than those who did not 237 cc (110–280) p=0.03. HAE increased the FLR by a further 99.8 cc (range 80.5–130 cc). An R0 resection was achieved in 25 patients (76%), including 4/5 (80%) of sequential patients. Following PVE and sequential embolisation; 9/33 (27%) and 3/5 (60%) suffered serious complications (Clavien-Dindo 3 or 4). There were six post operative deaths including 5/33 (15%) after PVE and 1 (20%) following sequential embolisation respectively.

Conclusion PVE is an increasingly used technique to increase the FLR allowing a significant proportion of patients an R0 resection despite initially being considered inoperable. Nevertheless at least 20% of patients will also have progression of disease. Patients who do not achieve adequate hypertrophy can potentially have HA embolisation to increase the FLR by a further 100 cc but perhaps at the expense of increasing post-operative complications.

Competing interests None declared.

OC-023 EXTRACORPOREAL LIVER SUPPORT USING UCL-ARSENEL REDUCES INFLAMMATION, IMPROVES HAEMODYNAMIC FUNCTION AND INCREASE SURVIVAL TIME IN A PORCINE PARACETAMOL-INDUCED ACUTE LIVER FAILURE MODEL

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Introduction Though the incidence of liver disease continues to increase, an effective liver support device remains an unmet clinical need. We have demonstrated that in liver failure, albumin function is irreversibly damaged, preventing detoxification processes, and that bacterial endotoxins induce systemic inflammation and neutrophil dysfunction. To date, toxin removal devices have failed to demonstrate clinical efficacy, which may be due to an inability to address albumin damage and/or inflammation. An albumin replacement system with a novel endotoxin ligation (ARSeNEL) component was developed to selectively to adsorb endotoxin and replace damaged albumin in patients' plasma.

Methods We tested the device in an acetominophen model of acute liver failure (ALF). 16 female landrace pigs (eight ALF, five ALF + UCL-ARSeNEL) were studied. Irreversible ALF was induced by acetaminophen administration via a jejunel catheter, confirmed by deranged clotting function (PT >30% normal). Treatment was with UCL-ARSeNEL or CVVH control within 2 h of ALF confirmation. The ARSeNEL device consists of three components; plasmapheresis, endotoxin and high cut-off (100 kDa) filters; with fresh frozen plasma replacing ultrafiltered plasma. Endpoints were: survival; ICP; heamodynamic parameters, standard biochemistry; cytokines; albumin damage; and plasma endotoxin levels.

Results UCL-ARSeNEL significantly increased survival post ALF (ALF 15.8±2.4 h vs UCL-ARSeNEL 23.8±1.9 h; p=0.02). Endotoxin reduced by a quarter (1.99±0.18 Eu/ml vs 1.42±0.21 Eu/ml) in the device group at 16 h. The changes in ICP index (1.7±0.07 vs 1.4±1.58), INR (16.6±6.6 vs 6.8±0.5), ischaemia-modified albumin ratio (0.45±0.166 vs 0.35±0.108), noradrenaline requirement (61.11±15.4 vs 28.7±15.2 μg/Kg), and mean arterial pressure (71±7.6 vs 87±6.0 mm Hg) showed marked improvement in the UCL-ARSeNEL group. Measured inflammatory cytokines IL8, IL6, IL1b, TNFa and neutrophil activation (spontaneous burst p=0.03) were all found to be reduced in the ARSeNEL treated group compared with ALF control.

Conclusion These results confirm that UCL-ARSeNEL improves survival in ALF by addressing key pathophysiological derangements such as albumin dysfunction and endotoxinaemia; which impact upon systemic inflammation and end-organ function. The reduction in inflammation is associated with improved vascular function and reduced inotropic support requirements.

Competing interests None declared.

OC-024 DEVELOPMENT AND VALIDATION OF A NOVEL CAPTURE-FUSION MODEL FOR HCV REPLICATION

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Introduction HCV replicates poorly in vitro, so testing of novel antiviral therapies currently relies on modified viral replicons, based on genotype (G)1, or the G2 JFH-1 virus. A model allowing patient virions to be cultured would facilitate drug discovery and allow direct sensitivity testing. Here we describe the development of a novel HCV replication assay, its validation using the antiviral agents alisporivir and telaprevir and its value in identifying responses to interferon and ribavirin.

Methods CD14 (+) monocytes derived from patients with chronic HCV infection, or pre-stimulated THP-1 cells infected with serum from G1 and G3 HCV infected donors, were fused with HuH7 cells and treated with antiviral agents at various concentrations. The fused cells were maintained in tissue culture for up to 5 days, before