Introduction Alcohol induced liver disease is the predominant cause of alcohol-related mortality in the UK. Therefore abstinence-based treatments are essential. Upto 70% of patients receiving alcohol treatment relapse within 6 months,[1] NICE attribute much of this failure of treatment to underutilisation of pharmacotherapy and recommend this be made available.[2] However, current licensed pharmacotherapies are contraindicated for patients with ALD. Baclofen has shown efficacy in the promotion of abstinence in patients with severe alcohol dependence[3,4] including those with ALD,[5] without exhibiting any of the complications or side effects elicited by current pharmacotherapies. Therefore the primary aim of this study was to measure the effectiveness of Baclofen in maintaining abstinence in this difficult to treat group.
Methods An observational prospective clinical audit was performed. Patients with liver disease and concomitant alcohol use were commenced on Baclofen at 10 mg three times daily (TDS), and titrated according to tolerability and response up to 30 mg TDS. Primary outcome measures were severity of physical dependence, as determined by SADQ score, and weekly alcohol consumption. These were compared at baseline, and 6 months.
Setting Acute Hospital Trust
Participants 149 patients referred to Hepatology for investigation of abnormal liver function and heavy drinking
Results Of the 149 patients commenced on Baclofen 100 (67.1%) remained engaged in treatment for 6 months. There was a significant reduction in alcohol consumption ($P < 0.0001$ 95% CI for difference 18 to 20) with 81 of the 149 patients (54.3%) maintaining total abstinence, 20 (13.4%) continued to drink and 48 (32.2%) were lost to follow-up and assumed to have returned to drinking. There was a significant reduction in the presence of physical dependence ($c^2 = 77.4$ $P < 0.0001$) as categorised by SADQ, and a non-significant improvement of liver biochemistry.
Conclusion Baclofen has a positive impact on alcohol consumption in this very difficult to treat, high risk patient group. A RCT is needed to confirm the benefit of baclofen in this patient group.

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Disclosure of Interest None Declared.

PTU-119 PHENOTYPIC CHARACTERISTICS AND LOCALISATION OF NOVEL HUMAN LIVER INFILTRATING NKP46 SUBSETS

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Introduction $CD56^+$Natural killer cells are the principal effector cells of the innate immune system and have a well-established role in tumour surveillance and anti-viral immunity. Expression of NKp46 has been shown to correlate closely with the severity of liver inflammation, viral resistance to IFN treatment and the attenuation of liver fibrosis. $CD56^+$NKp46 cells expressing IL-17 and IL-22 have also been described as a family of innate lymphoid cells in humans. Although the role of intrahepatic NK cells has been well described, little is known about the function and phenotype of intrahepatic NKp46 subsets. Thus, We aim to investigate the phenotypic characteristics of $CD56^+$ NKp46 cells in the inflamed human liver, with a view to exploring their functional role.
Methods Liver infiltrating lymphocytes were freshly isolated from explanted human liver tissue from our transplant program and phenotyped with multicolor flow cytometry. Cellular localization was investigated by immunohistochemistry and confocal microscopy
Results Human liver infiltrating NK cells reside predominantly around biliary epithelial cells at the portal tract close to regulatory T cells. We observed two populations of liver-infiltrating $CD3^{neg}$ $CD19^{neg}$ $CD56^{pos}$ cells distinguished by different levels of NKp46, $NKp46^{mid}$ (15% ±4.8 SD) and $NKp46^{high}$ (11% ±1.2 SD) neither subset expressed NKp44. The chemokine receptor expression of $NKp46^{mid}$ and $NKp46^{high}$ populations was: CCR6 (12% ± 3 vs. 7%± 2.4), CCR9 (20% ± 5.6 vs. 9% ± 0.9), CX3CR1 (18% ± 14 vs. 10% ± 1) CXCR3 (47%±14.4 vs 38%±11.0) and CXCR6 19% ± 4.0 vs. 14% ± 4.6). Both populations expressed IL-18R (42% ± 5.4 vs 7% ± 1.0), IL-23R (19% ± 6.0 vs. 11% ± 2.5), surface receptor CD161 (61% ± 12.1 vs 85% ±4.8) and the integrin receptor CD103 (4% ± 1.35 vs. 16% ± 1.7). The $NKp46^{high}$ population was highly enriched with the activation marker CD69 (77%±18%). NKp46 cells were also shown to express TNF-α (29% ± 7.5), IFN-γ (70% ± 7.0), Granzyme B (23% ± 11.0) and Perforin (23% ±11.1) along with transcription factor Tbet (19% ± 9.1).
Conclusion We hereby report novel subsets of liver infiltrating $CD56^+$NKp46 cells, which localise around the portal tract biliary epithelium in the inflamed human liver. These populations have distinct cytokine, chemokine and CD103 expression, which may explain their recruitment, positioning and effector functions in the inflamed hepatic microenvironment.
Disclosure of Interest None Declared.

PTU-120 EFFECTIVENESS OF NURSE LED HEPATITIS C TREATMENT; A LARGE DISTRICT GENERAL HOSPITAL AUDIT

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Introduction Hepatitis C is the third most common risk factor for liver diseases in the UK. Updated estimates suggest that around 216,000 individuals are chronically infected with hepatitis C. Treatment with combination of pegylated Interferon and ribavarin is well established. Specialist viral hepatitis nurses working collabaratively with clinicians play a major role in delivering excellent clinical outcomes.
Methods Evaluate the safety and clinical effectiveness of chronic hepatitis C treatment that was led by the specialist viral hepatitis nurses under the supervision of gastroenterologists.

Data was obtained from a prospectively maintained hepatitis C database over a 5-year period from September 2008 to date. A retrospective analysis of the database was carried out looking at the treatment outcomes. Patients with liver transplant and/or co-infection with hepatitis B or human immunodeficiency virus (HIV) were excluded. The dedicated viral hepatitis specialist nurses closely followed up all patients.
Results A large database of 437 patients who underwent treatment was analysed. There were 128 (29.2%) females and 309 (70.7%) males ranging between 23–84yrs old (mean age of 42).

Majority of patients were treated with combination therapy of pegylated interferon α2a and ribavarin whilst a small proportion (28) have received (triple therapy) protease inhibitors. The total number of patients who achieved sustained virologic response (SVR) at the end of treatment were 264 (60.41%). 196 (74.42%) of those were genotype 3a, 57 (21.60%) were genotype 1a/1b and 11 (4.17%) were genotype 2b/2a. 43 (9.84%) were considered non-responders. 49 (11.21%) patients were unable to complete treatment due to critical physical or mental illness with 12 of those (24.50%) have achieved SVR. Patients' feedback for this nurse-led service has been very positive.

Conclusion Specialist nurse-led and clinicians supported hepatitis C service has delivered a high quality of care. Our dedicated specialist nurses working closely with clinicians have achieved high successful treatment rates in such a large cohort of patients.

Disclosure of Interest None Declared.

PTU-121 LLT1 IS UPREGULATED IN HEPATOCELLULAR CARCINOMA AND INHIBITS NK CELL CYTOTOXICITY

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Introduction Liver cancer is on the rise and prognosis is poor partly due to late diagnosis and a lack of systemic chemotherapy. Identification of novel markers allowing early diagnosis of hepatocellular carcinoma (HCC) and to act as targets for immunotherapies is essential to kerb rising mortality. Lectin-like transcript 1(LLT1) interacts with CD161,[1] a receptor expressed on almost all NK cells. For the first time, we investigate the expression of LLT1 in the liver and HCC, both *in vivo* and *in vitro*, and determine the effect of LLT1 on NK cell function.

Methods LLT1 expression *in vivo* was determined by immunohistochemistry comparing 10 HCC specimens (resections and biopsies) to 15 normal liver controls. *In vitro* expression was demonstrated using flow cytometry to review HCC cell lines (Huh7, HepG2). Peripheral blood mononuclear cells (PBMCs), including NK cells, from healthy donors were incubated with target cells expressing different levels of LLT1:Huh7 cells, Huh7 cells expressing increased levels of LLT1(achieved by transfection), and Jurkat cells lacking LLT1. The effect of these incubations on $CD161^+$ and CD161-NK cell cytotoxicity was assessed by measuring CD107a expression using flow cytometry. This assay was performed in triplicate using 2 different donors on 3 separate occasions. Statistical analysis of variance (ANOVA) was performed to assess differences in NK cell cytotoxicity between each incubation condition.

Results Hepatocytes in normal and cirrhotic liver do not express LLT1. However, LLT1 is extensively upregulated in HCC *in vivo* with strong, diffuse staining in 9 out of 10 cases, and more focal staining seen in the remaining case. LLT1 is expressed *in vitro* on HCC cell lines (Huh7 and HepG2). $CD161^+$NK cells show reduced cytotoxicity, compared to CD161-NK cells, when incubated with Huh7 cells expressing LLT1. Incubation with Huh7 cells correlates with significantly reduced activity by $CD161^+$NK cells ($p \leq 0.001$) when compared to $CD161^+$NK cell activity against non-LLT1 expressing Jurkat cells. $CD161^+$NK cell cytotoxicity is further reduced when incubated with Huh7 cells expressing increased LLT1 levels (as achieved by transfection; $p \leq 0.05$ when compared to $CD161^+$NK cell activity when incubated with non-transfected Huh7 cells). In contrast, the activity of $CD161^+$ and CD161-NK cells is not significantly different when the target cell does not express LLT1(Jurkat cell).

Conclusion We demonstrate for the first time that LLT1 is not expressed by normal liver tissue, but upregulated in HCC. LLT1 inhibits NK cell cytotoxicity, representing a possible mechanism for HCC to evade the immune response to cancer. Therefore, not only may LLT1 be used as a diagnostic marker for HCC, it represents a novel immunotherapy target.

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Disclosure of Interest None Declared.

PTU-122 A GMP TREG EXPANSION PROTOCOL RESTORES TREG SUPPRESSOR FUNCTION IN END-STAGE LIVER DISEASE; IMPLICATIONS FOR ADOPTIVE TRANSFER THERAPY

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Introduction Long-term survival in liver transplant recipients remains suboptimal because of the morbidity and mortality associated with the long-term use of immunosuppression (IS). However, IS weaning early post liver transplantation (LT) has largely been unsuccessful, supporting the need for active tolerance induction strategies. $CD4^+CD25^+FOXP3+$ (Tregs) play an important role in immunoregulation and have been shown in animal models to promote transplantation tolerance. Phase I trials in bone marrow transplantation have shown that *ex vivo* expanded Tregs have an excellent safety profile, which is encouraging for the broader application of these cells. The clinical trial, ThRIL, soon to be initiated at King's College London, aims to investigate the therapeutic potential of Tregs in the setting of LT.

We have devised a GMP compatible protocol that ensures the successful isolation and expansion of a functional and stable human Treg population in preparation for this trial.

Methods Tregs were isolated from 150ml of blood from patients with end-stage liver disease by a CliniMACS-based GMP isolation technique and expanded using anti-CD3/CD28 beads, IL-2 and rapamycin.

Results A 580-fold expansion of pure Tregs was achieved (97.4% $CD4^+CD25^+$ and 0.008% $CD8^+$ cells) and the cells maintained FoxP3 expression (99.6% of the $CD4^+CD25^+$ cells express FoxP3). The populations of Tregs obtained were also stable and did not convert to Th17 cells when cultured in the presence of pro-inflammatory stimuli.

This protocol further proved to be ideal for the expansion of Tregs from patients with liver disease in view of restoring the Tregs' suppressive function (1:1 ratio – expanded Tregs 91.1% vs. freshly isolated Tregs 28.6% suppression, 1:10 ratio – 80.7% vs. 20.8% respectively). Based on these findings, we subsequently conducted an in-depth phenotypic characterisation of freshly isolated Tregs in order to delineate a population responsible for the apparent lack of suppressive function. An investigation into the possible mechanisms is currently ongoing.

Conclusion The feasibility of Treg based therapy is now widely accepted, provided that tailor-made clinical grade procedures for isolation and ex-vivo cell handling are available. Our rapamycin-based protocol is ideal in this setting as it not only satisfies the rigours of GMP manufacturing standards, but also