HEPATOLOGY RESEARCH GROUP
INSTITUTE OF HEALTH AND CARE RESEARCH,
PENINSULA MEDICALSCHOOL, UNIVERSITY OF PLYMOUTH

Hepatology Research Group (HRG) structure:

Head
Prof Matthew Cramp, Professor of Hepatology and Consultant Hepatologist

Senior Clinical Academics:
Dr David Sheridan, Associate Professor and Honorary Consultant Hepatologist,
Dr Ashwin Dhanda, Consultant Hepatologist and Honorary Associate Professor

Senior Scientists:
Dr Daniel Felmlee, Lecturer,
Dr Leticia Scalioni, post-doctoral research scientist

Clinical Research Fellows:
Dr Ollie Rupar – PhD student (writing up)
Dr Huey Tan – PhD student

PhD Fellows:
Paula Boeira
Justyna Lopatecka
Hannah Windmill

MD Fellow:
Mr Andrei Tanese

NIHR Academic Clinical Fellows:
Dr Kris Bennett
Dr Keith Pohl

DClinSci fellow:
Kristen Lilly

Lab technician:
Euan Yates / Paula Boeira

Hepatology Research Group Annual Report 2020

Overview:
2020 was a very different and frequently difficult year for the Hepatology Research Group (HRG) due to the COVID-19 pandemic changing so many things. Despite the Derriford Research Facility laboratories temporarily closing, the absence of face to face team meetings, seminars and educational events and the move of all conferences to on-line meetings the group showed their resilience by rapidly adapting their skills to support work on understanding COVID immunology and contributing to clinical trials whilst still made progress on all areas of core work.

The clinical academics from the HRG were all involved in delivering care to in-patients with COVID and led recruitment to a number of interventional and cohort studies including RECOVERY, ISARIC, and the remdesevir clinical trials.

As a group we made an early decision to devote energy and expertise to contribute to the global push for a better understanding of the immunology that drives the variable outcomes from infection with SARS-CoV-2. Team members and lab equipment were moved across to a hospital facility suitable for this work.

After the first wave of infection settled the DRF re-opened and the work in the hospital facility had to stop as it was required to deliver the urgent public health studies including COVID vaccine studies and SIREN. The core activity in hepatology re-started and has continued through this second wave.

The group had 13 publications in 2020, many in high impact journals, as well as presentations at national and international meetings. The group remains focused on 4 main areas of hepatology research interest (1. hepatitis C virus infection and protection from infection, 2. non-alcoholic fatty liver disease and metabolic liver diseases, 3. alcohol related liver disease and 4. liver cancer development and its prevention) and a summary of the work undertaken and progress made in 2020 in each of these areas, plus the COVID related research, is included in this report.

At a national level a highlight of 2020 was expected to have been a joint British / American liver meeting (BASL and AASLD) being held in Plymouth to coincide with the Mayflower 400 celebrations - organised by Matthew Cramp when President of BASL. Sadly despite a lot of preparatory work this was not able to go ahead. The BASL special interest groups (SIGs) established in 2018 made further progress in facilitating collaborative work, research, grant applications and clinical developments at a national level in a wide range of liver conditions. Ashwin Dhanda was elected as Chair of the Alcohol SIG in late 2019 and is leading this group at a key time for national research initiatives.

The clinical trials program of the HRG and the wider clinical team of the South West Liver Unit continued but faced many challenges during the COVID pandemic. These included the difficulty of seeing patients face to face for monitoring visits during

lockdown and the research nurse team being diverted to delivering urgent public health studies. Despite this we have continued to recruit to in-patient studies on alcoholic hepatitis, follow up those already recruited and have set up one new interventional study in hepatitis B virus infection with a second study starting in January 2021.

The generosity of the Mary Kinross Trustees who provided funding to cover 18 months of salary allowed us to appoint Dr Leticia Scalioni as a post-doctoral scientist working on the HCV resistance program of work. Letitia joined us early in the year, just as COVID-19 was becoming a problem, but despite that difficult timing has settled in well.

Dr Keith Pohl was appointed as an NIHR funded Academic Clinical Fellow joining the HRG in Sept 2020 and will be working on his academic development and a PhD proposal over the next 3 years.

Immune dysfunction in COVID-19: investigation of mechanisms and identification of immune biomarkers of clinical outcome

- **Immune Biomarkers of Outcome from COVID-19 (IBOC)**

Leads: Ashwin Dhanda and Matthew Cramp

The virus, SARS-CoV2, leads to coronavirus disease 2019 (COVID-19) and has resulted in a global pandemic with millions of deaths. The outcome and severity of illness from COVID-19 is very variable, but severe disease is more common in older people and those with underlying health conditions.

In May 2020, we opened an observational clinical trial at UHP to test biomarkers of outcome of patients hospitalised with COVID-19, which was featured on BBC Spotlight. Recruitment was supported by the Clinical Research Nursing team from the Research, Development and Innovation Department at UHP as well as our clinical research fellows and lab work was performed by all members of our group in a great example of collaboration between UHP and University of Plymouth.

In this study we have investigated markers of immune function when a person first attends hospital with confirmed COVID-19 to try and identify those who will go on to have a more severe infection. Biomarkers able to identify those most at risk have potential to help treat patients more effectively, for example, by starting drug treatments early or moving high risk patients to an intensive care setting at an early stage.

IBOC had 3 objectives:

1. To define immune phenotype in peripheral blood of patients with COVID-19 and correlate with clinical course and outcome

2. To measure global immune function in patients with COVID-19 and correlate with clinical course and outcome

3. To measure markers of immune senescence and correlate with clinical course and outcome

As part of this study we have tested a novel assay looking at interferon gamma release in response to the SARS-CoV-2 spike protein (a virus specific T cell response) that has the potential to be used as a widely available marker of cellular immunity to infection and are currently studying immune senescence markers to better understand the role of age in disease severity.

Our numbers studied to date have been small due to logistical and regulatory challenges in undertaking this work. For this reason the IBOC study outputs are limited thus far, but the long awaited and much delayed opening of the containment level 3 laboratory at the DRF will address much of this.

Hepatitis C Virus infection and Protection from Infection
Leads: Dan Felmlee, Matthew Cramp.

The importance of new strategies to fight viral infections has rarely ever been so brought to awareness as the past year. We have continued in our aims to utilise genetic data from individuals who are highly resistant to hepatitis C virus (HCV) infection, as identified from the UK Hepatitis C Lookback Programme. Using next generation sequencing, we found 6 genes expressed in the liver that had rare genetic signatures in the small number of people who appear resistant to HCV. The next step to scientifically test that these genes contribute to viral resistance, is to functionally validate these genes as playing a critical role in infection. We are enthusiastic that identification and validation of these genes may reveal pan-viral targets. Our gene targets revealed two biological pathways that have cross-over to other screens for viral infections, including SARS-CoV2. These type of studies are important in preparation for future coronavirus "spill-over" events.

Leticia Scalioni a post-doctoral researcher with background in hepatitis C virus and host genetics joined the lab early in 2020 to contribute to this project. We have progressed in using cutting edge CRISPR/Cas9 gene-editing technology to develop an HCV-permissive cell line with each of these genes knocked out for expression. Access to the research labs has been limited in the past year to keep safe distancing, but we have managed to develop these lines and are currently testing them for HCV entry experiments. Leticia has applied for a competitive Branco-Weiss fellowship and we are excited for this work to come to fruition.

A key component for this study is the implementation of a containment level 3 laboratory (CL3 lab); a sophisticated negative pressure room, where all potential pathogens exit only through multiple HEPA filters. Implementing such safety equipment is often rife with complications and setbacks, and unfortunately we have not proven to be an exception to this trend. After many delays we have recently been able to utilise this lab for COVID-19 studies and engineers are scheduled to complete the work to allow us to commission the CL3 lab in April, 2021.

We enthusiastically look forward to completing early milestones of these studies, and work toward developing potential therapies, derived from clinical data, that could impact protection from viral infection in the future.

Non-alcoholic fatty liver disease and metabolic liver diseases
Lead: David Sheridan, Dan Felmlee

COVID has posed many challenges to the metabolic programme of research in 2020. Dr Sheridan took on the role of Clinical Service Director for the South West Liver Unit just prior to the pandemic. NASH trials were suspended, but several participants previously randomised to investigational medical products continued to be monitored for safety throughout the pandemic. Several new phase 3 studies for NASH and PBC are in set up to start in 2021.
PhD student Paul Boeira's project was interrupted by the DRF closure for COVID, but her results are providing novel insights into the intricate role that metabolites derived from the gut microbiome may play in the development of hepatic steatosis by mediating changes in mitochondrial bioenergetics. Investigation of mitochondrial dysfunction in the context of metabolic liver disease and frailty in cirrhosis remains an ongoing theme for grants and collaboration between Dr Charlie Affourtit in the Mitochondrial biology group. Building on collaboration and critical mass in metabolic health as a research theme in PIHR is a priority for 2021.
The Inflammatory Liver Disease Biobank has been granted refreshed ethical approval for the next
5 years, aiming to be a resource of clinical material to support ongoing and future translational studies for the HRG and external collaborators, and is registered with the UK CRC tissue directory. Biobanking Directory - Collection Search Results in Inflammatory Liver Diseases Biobank (biobankinguk.org)
Dr Sheridan supported the virtual BASL annual meeting in 2020, co-chairing the free papers section. Clinical education has continued with the Healthcare Environments Special Study Unit 'Introduction to Hepatology' delivered virtually to year 2 BMBS students, hoping to inspire the next generation of budding liver doctors and researchers.

Alcohol related liver disease
Lead: Ashwin Dhanda

Unsurprisingly, 2020 has been a challenging year. However, Ashwin has achieved a number of notable successes. In January, he secured funding from the Jon Moulton Charity Trust for a pilot trial of a novel psychological therapy to reduce alcohol-related harm in patients with alcohol dependence and liver disease (https://www.plymouth.ac.uk/news/study-to-explore-whether-new-therapy-can-help-address-problem-drinking). Functional Imagery Training (FIT) is a technique which uses visualisation to enhance motivation for behaviour change. As Chief Investigator, Ashwin is working closely with FIT co-inventor, Prof Jackie Andrade from the University of Plymouth and is leading a multicentre randomised trial with the support of the Peninsula Clinical Trials Unit. It is due to start recruitment in April 2021 with results available by the end of 2022. If successful, it will pave the way for a larger definitive trial to test the clinical and cost-effectiveness of FIT in this patient group.

Ashwin continues to lead clinical trials in alcohol-related liver disease in Plymouth. As member of the Medical Research Council Precision Medicine Consortium on Minimising Mortality from Alcohol Hepatitis (MIMAH; http://mimah.org), he has led recruitment to two clinical trials this year. Firstly, he was the third highest recruiter nationally to the ISAIAH clinical trial which has now been completed. Secondly, he was the first site to recruit a patient to the MICAH cohort study of patients with alcoholic hepatitis with UHP currently the top recruiter, a real accolade for the hepatology clinical research team in the face of the pandemic. He is leading a workstream investigating mechanisms of resistance to steroid treatment in patients with alcoholic hepatitis. As part of this Dr Huey (Queenie) Tan is evaluating the role of oxidative stress in steroid resistance and Paula Boeira is supporting the validation of the BLISS assay, a novel bioassay to measure steroid responsiveness.

In his role as Lead for the BASL Alcohol-related Liver Disease Special Interest Group, Ashwin noted concerns from the liver community on the effect of COVID-19 lockdown on patients with liver disease with anecdotes of higher number and acuity of admissions of patients with alcohol-related liver disease. He established a working group and has led a national service evaluation to collect detailed data on this patient group in August 2020 compared to 2019. He has linked with colleagues from Public Health England to combine this with an analysis of the national data. Findings are due to be published in early 2021.

In October, Ashwin welcomed Hannah Windmill to the group as a PhD student investigating functional and structural changes in the brain in patients with minimal hepatic encephalopathy. Ashwin is Hannah's second supervisor and is working closely with Prof Stephen Hall in the Brain Imaging Centre (BRIC) and Dr Alastair Smith in the School of Psychology to test novel MRI techniques in patients before and after liver transplantation.

Liver cancer development and its prevention
Lead: Matthew Cramp

Dr Ollie Rupar's 3 years of funded research ended in March 2020 and having completed work on universal tumour associated antigens (U-TAA) and their possible role in primary liver tumours he moved back into clinical training as a pathologist. He is currently writing up his PhD thesis reporting on Survivin and Telomerase using a combination of histological and genetic methods. His work on telomerase and survivin sequencing (from both tumour and non-tumour tissue) in formalin fixed, paraffin embedded, archived tissue samples is complete and has highlighted clear differences both between tumour and non-tumour tissues and between primary liver tumour types. His immunohistochemistry work was completed in early 2020 and findings presented at the annual BASL meeting. There is a plan to complete further work looking for serum biomarkers of liver tumour development in the exosomal fraction of sera stored in the biobank. Ollie will submit his PhD thesis in 2021.

Current and future plans

A key goal for 2021 is to have the containment level 3 (CL3) laboratory at the Derriford Research Facility commissioned and active. Unfortunately, the COVID pandemic and resulting lockdown restrictions delayed this but we are now very close. The CL3 lab will facilitate both our viral hepatitis work and also the immunological work we have started on SARS-CoV-2. The CL3 lab requires a sophisticated negative pressure room, where all potential pathogens exit only through multiple HEPA filters. The initial lab build did not meet the requirements, but engineers are scheduled to complete building the one-way air systems and capability to airlock them for the capacity for emergency fumigation in early 2021. It is anticipated that the England Health Safety Executive will be able to commission the CL3 lab in April, 2021. This will represent important infrastructure for Plymouth and Southwest England that impacts viral studies in the region and we enthusiastically look forward to progress on the viral resistance work.

Collaborations, both within University of Plymouth and outside the university, remain a major part of the groups long term viability strategy. Work continues with our main external collaborators Imperial College London (MRC personalised medicine bid in alcoholic hepatitis), the MRC Centre for Virology in Glasgow (HCV exposed uninfected work) and with the Institute of Hepatology, Kings College London (lipidomics in HCV resistance) and we hope to strengthen these links further in 2021. For 2021 we plan to establish a University of Plymouth multi-morbidity group focused on obesity, its causes and consequences and treatment approaches. This will

be a multi-disciplinary group aiming to foster and develop internal collaborations with a specific remit seeking NIHR grant support.

Our involvement in clinical trials continues. The HRG and SWLU are the largest recruiter to trials in liver disease in the southwest and have brought a range of new treatments to patients from across Devon Cornwall and Somerset. After a downturn in activity as a consequence of COVID, we expect to see an upturn in 2021. In-patient studies have continued throughout, but the out-patient NAFLD studies are now re-starting with new studies investigating novel treatments for HBV infection, primary biliary cholangitis, primary sclerosing cholangitis all in set up for 2021.

Ensuring the financial sustainability of the group at a time of increasing pressure on university and academic funding remains a key priority. The next round of university funding (REF2021) is underway, with the HRG submitting a case of impact study for HCV infection and contributing to other areas that we hope will secure medium term support to the wider group.

The financial support provided by the Mary Kinross Trust has again been instrumental in keeping the HRG viable with their support funding our new post-doctoral post (Dr Scalioni) for much of 2021. Our challenge for 2021 will be to continue working towards greater financial stability from external grant support.

We remain indebted to all the patients and their families who have supported us by their involvement in clinical trials and the research work, to the wider clinical team at the SWLU and to all our collaborators both in the UK and abroad.

Research Outputs for 2020:

Publications:

Assessment of hepatic steatosis by controlled attenuation parameter using the M and XL probes: an individual patient data meta-analysis.
Petroff, D Valentin Newsome, P; Shalimar Voican, Thiele, M; de Lédinghen, V; Baumeler, S; Chan, W:; Perlemuter, G; Cardoso, AC; Aggarwal, S; Sasso, M; Eddowes, PJ; Allison, M; Tsochatzis, E; Anstee, QM; **Sheridan D**, Cobbold, JF; e Naveau, Lupsor-Platon, Mueller, S; Krag, A; Irles-Depe, M; Semela, D; Lai-Hung Wong, G; Vincent Wai-Sun Wong; Villela-Nogueira, C; Garg, H; Chazouillères, O; Wiegand; Karlas, T.
Lancet Gastroenterology and Hepatology January 15, 2021
DOI:https://doi.org/10.1016/S2468-1253(20)30357-5

Editorial: can quantitative fibrosis assessment be used to enhance prediction of outcomes in patients with alcohol-related liver disease?
Moodley P, **Dhanda AD**.
Alimentary Pharmacology and Therapeutics. 2021 Jan;53(1):183-184. doi: 10.1111/apt.16134.PMID: 33333610

Multi-ancestry fine mapping of interferon lambda and the outcome of acute hepatitis C virus infection
Candelaria Vergara, Priya Duggal, Chloe L. Thio, Ana Valencia, Thomas R. O'Brien, Rachel Latanich, Winston Timp, Eric O. Johnson, Alex H. Kral, Alessandra Mangia, James J. Goedert, Valeria Piazzola, Shruti H. Mehta, Gregory D. Kirk, Marion G. Peters, Sharyne M. Donfield, Brian R. Edlin, Michael P. Busch, Graeme Alexander, Edward L. Murphy, Arthur Y. Kim, Georg M. Lauer, Raymond T. Chung, **Matthew E. Cramp**, Andrea L. Cox, Salim I. Khakoo, Hugo R. Rosen, Laurent Alric, Sarah J. Wheelan, Genevieve L. Wojcik, David L. Thomas & Margaret A. Taub
Genes & Immunity 2020; 21, (5) 348-359 DOI

Glucocorticoid treatment in patients with newly diagnosed immune thrombocytopenia switches CD14++ CD16+ intermediate monocytes from a pro-inflammatory to an anti-inflammatory phenotype.
Williams EL, Stimpson ML, Lait PJP, Schewitz-Bowers LP, Jones LV, **Dhanda AD**, Lee RWJ, Bradbury CA.
British Journal of Haematology. 2020 Dec 18. doi: 10.1111/bjh.17205. PMID: 33338291

Effect of zinc treatment on clinical outcomes in patients with liver cirrhosis: A systematic review and meta-analysis
Tan HK, Streeter A, Cramp ME & Dhanda AD
World Journal of Hepatology 2020; 12, (7) 389-398 DOI

Trace element deficiency is highly prevalent and associated with infection and mortality in patients with alcoholic hepatitis
Dhanda A, Atkinson S, Vergis N, Enki D, Fisher A, Clough R, **Cramp M** & Thursz M
Alimentary Pharmacology & Therapeutics 2020; 52(3):537-544. DOI Open access

Adult liver transplantation: A UK clinical guideline - part 1: pre-operation
Millson C, Considine A, **Cramp ME**, Holt A, Hubscher S, Hutchinson J, Jones K, Leithead J, Masson S & Menon K
Frontline Gastroenterology 2020; 11, (*5) 375-384* , DOI

Adult liver transplantation: UK clinical guideline - part 2: surgery and post-operation
Millson C, Considine A, **Cramp ME**, Holt A, Hubscher S, Hutchinson J, Jones K, Leithead J, Masson S & Menon K
Frontline Gastroenterology 2020; 11, (5) 385-396 , DOI

Applicability, safety, and biological activity of regulatory T cell therapy in liver transplantation
Sánchez-Fueyo A, Whitehouse G, Grageda N, **Cramp ME**, Lim TY, Romano M, Thirkell S, Lowe K, Fry L & Heward J
American Journal of Transplantation 2020; 20, (4) 1125-1136 , DOI

Sequential Cohort Analysis After Liver Transplantation Shows de Novo Extended Release Tacrolimus Is Safe, Efficacious, and Minimizes Renal Dysfunction
Lim TY, McPhail MJ, Shah A, Mahgoub S, Nayagam J, **Cramp M**, Bernal W, Menon K, Jassem W, Joshi D , Heneghan MA, Agarwal K, Heaton ND, Suddle A, O'Grady JG, Aluvihare VR
Transplantation Direct 2020; 6, (2) e528-e528 , DOI

Characterisation of the Serum Metabolic Signature of Cholangiocarcinoma in a United Kingdom Cohort Alsaleh M, Leftley Z, Barbera TA, Koomson LK, Zabron A, Crossey MME, Reeves HL, **Cramp M**, Ryder S & Greer S
Journal of Clinical and Experimental Hepatology 2020; 10, (1) 17-29 , DOI

Unacceptable failures: the final report of the Lancet Commission into liver disease in the UK
Williams R, Aithal G, Alexander GJ, Allison M, Armstrong I, Aspinall R, Baker A, Batterham R, Brown K, Burton R, **Cramp ME** et al.
The Lancet 2020; 395, (10219) 226-239 , DOI

Risk-adjusted survival in liver transplant patients assessed and managed by a non-transplanting centre: South West Liver Unit experience
Norton BC, Srivastava A, Ramos K, Vine L, Taylor R, Aluvihare V, Heaton N, **Cramp ME.**
Frontline Gastroenterol. 2020; 11(3): 202–208

Oxidative stress in alcohol-related liver disease.
Tan HK, Yates E, Lilly K, Dhanda AD.
World Journal of Hepatology. 2020 Jul 27;12(7):332-349. doi: 10.4254/wjh.v12.i7.332.PMID: 32821333

High prevalence of hepatitis A in indigenous population in north Brazil.
de Paula VS, Milagres FAP, Oliveira GM, Miguel JC, Cruz HM**, Scalioni LP**, Marques VA, Magalhães MAFM, Romão AR, Gracie R, Villar LM. *BMC Res Notes.* 2020 Sep 29;13(1):458. doi: 10.1186/s13104-020-05303-y.

Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial.
Younossi ZM, Ratziu V, Loomba R, Rinella M, Anstee QM, Goodman Z, Bedossa P, Geier A, Beckebaum S, Newsome PN, **Sheridan D et al.** REGENERATE Study Investigators. *Lancet. 2019 Dec 14; 394(10215):2184-2196.* doi: 10.1016/S0140-6736(19)33041-7.

Conference Presentations:

International Meetings:

European Association for the Study of the Liver meeting - August 2020:

Yates E, Tan HK, Cramp M, Dhanda AD. A novel lymphocyte proliferation assay accurately predicts 90-day survival in severe alcoholic hepatitis patients
Journal of Hepatology: vol 73 Suppl. 1, Aug 2020

Boeira P, Affourtit C, Felmlee D, Sheridan D. Investigation of microbiome metabolites and mitochondrial function in non-alcoholic fatty liver disease
Journal of Hepatology: vol 73 Suppl. 1, Aug 2020

National meetings:

BASL annual meeting - September 2020

Tan HK, Yates E, Cramp M, Dhanda A. Patients with alcohol-related liver disease have high levels of oxidative stress.
Gut: vol 69 – suppl 1; Sept 2020

Rupar O, Denson J, Cramp M Survivin expressing primary liver cancers have lower survival and adverse clinical features – a digital pathology experience using QuPath.
Gut: vol 69 – suppl 1; Sept 2020

BSG Annual meeting 2020 (deferred and held in Jan 2021)

Rupar O, Denson J, Cramp M Promoter sequences of Universal Tumour Antigens in Primary Liver Neoplasms: Cholangiocarcinoma and Hepatocellular carcinomas differ.