Project title: ***Oxysterols and liver fibrosis***

Supervision:

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- Prof Ellie Barnes, Professor of Hepatology, Nuffield Department of Medicine
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Summary:

Context: The clinical burden of advanced liver disease continues to escalate predominantly driven by steatotic liver disease (SLD) comprising alcohol-related liver disease (ALD) and metabolic dysfunction-associated steatotic liver disease (MASLD). ALD is associated with a substantial increase in premature deaths, >60% in the last 20 years [1]. The high prevalence of MASLD, affecting >30% of the population (>10 million adults in UK), drives significant morbidity and healthcare associated costs, >£5billion/year in the UK [2-4]. Advanced fibrosis is associated with a >4-fold increase in mortality and morbidity including liver decompensation and hepatocellular carcinoma, (HCC) and ALD and MASLD account for the top two indications for liver transplant.

Challenges and hypotheses: Despite the magnitude of the clinical problem there are many significant challenges and unmet needs facing clinicians managing patients with advanced SLD. There is a lack of robust and validated clinical biomarkers that can accurately predict liver outcomes including hepatic decompensation. Current assessments of 'liver function', are insensitive and only identify patients where their liver disease has progressed to severe impairment. In addition, the molecular processes that drive deteriorations in liver function are poorly understood and a detailed understanding of these process my open avenues to specific therapeutic targeting.

Cholesterol metabolism, bile acid synthesis and steroid hormone metabolism are critical hepatic functions Oxysterols are oxidised metabolites of cholesterol and have distinct roles as signalling molecules regulating multiple cellular phenotypes, through (at least in part) Liver X receptor (LXR) activation. 5β-reductase (AKR1D1) generates all 5β-reduced dihydrosteroid metabolites, but also has critical role in bile acid synthesis. AKR1D1 utilises multiple oxysterol substrates including, 7α-hydroxycholest-4-en-3-one (7α-HCO), 7α,12α-dihydroxycholest-4-en-3-one (7α,12α-diHCO) and 7α-hydroxy-3-oxocholest-4-enoic acid (7α-HOCA). 7α-HCO levels are elevated in patients with steatohepatitis and fibrosis and levels correlate with disease severity [5-7]. In addition, published data suggest oxysterols have profibrogenic effects on hepatic stellate cell models [8]. Our published data has shown progressive down regulation of AKR1D1 with increasing fibrosis, alongside concomitant increases in AKR1D1 substrates [9]. The biological impact of 7α-HCO, 7α,12α-diHCO and 7α-HOCA (and other oxysterols) on the liver remain unknown and we propose that they may have a primary pathogenic, pro-fibrotic role in SLD progression. Our data in human hepatoma cells demonstrate that they potently activate LXRα, fuelling lipid accumulation, promote cycle arrest, apoptosis and DNA damage.

We therefore hypothesise that these (and other) oxysterols drive SLD progression. We will delineate their mechanisms of action in human models and test their utility as biomarkers to assess severity and predict liver outcomes. Finally, we will begin to test the hypothesis that enhanced oxysterol clearance may improve liver phenotype.

Aims and objectives:

1. To use targeted oxysterol profiling in cohorts of patients with advanced fibrotic SLD to define biomarker profiles.
2. To use longitudinal samples collected from patients with advanced SLD to determine the ability of oxysterol profiles to predict liver outcomes.
3. To define cell mechanisms by which oxysterols cause hepatic dysfunction and fibrosis
4. To determine whether enhancing oxysterol clearance improves cellular phenotype.

Potential applications and benefits: This proposal has the potential to develop robust and reproducible biomarkers to assess stage and severity of SLD, alongside predicting liver-related adverse outcomes. In addition, it will define mew mechanisms underpinning the

pathogenesis of liver disease progression and begin to explore an entirely novel therapeutic strategy to clear toxic oxysterol accumulation.

Status of ethical approvals for data sharing with industry partners: Samples from the Steroids&HCC, TrUSt-NAFLD and DeLIVER cohorts (see below) have already been collected and stored. Ethics approvals covers sharing sample and data with industrial partners.

Alignment with therapeutic area and key scientific theme(s):

The proposal focuses on ***Liver fibrosis across the SLD spectrum,*** combining clinical sample analysis, development of biomarkers and cell models to probe mechanisms of action.

Project delivery:

Aim1 (months 1-12): We have established cohorts of patients with detailed clinical data in whom samples (plasma, serum, urine) have been already been collected and stored (Steroids&HCC cohort: Cirrhosis, including MASLD n=67, ALD n=140; TrUSt-NAFLD: F0 n=9, F1 n=47, F2 n=58, F3 n=112, F4 n=50, healthy control n=150) which can be used for oxysterol profiling (blood, urine). In addition, we have access to cirrhotic samples (compensated and decompensated) from the DeLIVER consortium (https://deliver.cancer.ox.ac.uk, Prof. Ellie Barnes). Oxysterol profiles (>25 oxysterols) will be profiled using mass spectrometry through collaboration with University of Swansea (Profs. W. Griffiths, Y. Wang) and incorporating machine learning (with Prof. M. Biehl, University of Groningen, NL). Our preliminary data has shown the utility of this approach, distinguishing patients with cirrhosis from controls***.***

Aim 2 (months 12-24): Longitudinal oxysterol analysis of samples from the DeLIVER cohort in individuals who have developed liver-related complications. The utility of baseline oxysterol profiles to predict liver outcomes will be assessed.

Aim 3 (months 1-18): Using combinations of human hepatocyte models (Huh, HepG2), precision cut liver slices (local hepatobiliary surgeons providing 2 preps / month) and human stellate cells (LX-2), the cellular impact of individual (and combinations of) oxysterols will be assessed using RNA-seq, cell cycle analysis, apoptosis and DNA damage assays, cell activation and fibrogenic markers (LX-2). Co-culture protocols will be used to determine the impact of altering hepatocyte derived oxysterols on human hepatic stellate cells.

Aim 4 (months 18-36): We will use genetic over-expression of AKR1D1 to drive oxysterol clearance in human cellular models (including co-culture models). Analytical methods as described in aim 3 will be used.

This proposal will provide training in the development and analysis of biomarkers strategies (including mass spec methodology) incorporating machine learning approaches. In addition, there will be training in molecular biology techniques including the analysis of RNA-seq data.

Research environment:

This work builds on an existing program of biomarker work stratifying stage and severity of liver disease (notably MASLD) [10]. The collaborations the proposal are already established and opportunities for the fellow to spend time through laboratory visits at collaborating laboratories will form part of the proposal. The investigators and laboratory facilities embedded within OCDEM are supported through the NIHR Oxford Biomedical Research Centre (BRC), and Prof Tomlinson leads the Metabolic Experimental Medicine BRC theme. The appointed fellow will have the opportunity to attend relevant NHS clinics (as part of their training, max. 1 clinic per week), formal timetabled clinical commitments are not anticipated.

The clinical fellow will have at-least weekly formal interactions with supervisors, presenting at meetings and departmental seminars. OCDEM has a successful in-house graduate mentoring scheme for personal and professional development. The University of Oxford Medical Sciences Division's Skills Training Programme provides transferable and structured courses in communication, research and teaching skills.

References

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