Elucidating the role of immune cell interactions during human liver regeneration using single nuclear sequencing

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Background: Posthepatectomy liver failure (PHLF) is responsible for approximately 50% of short-term mortality after liver resection. Accumulating evidence suggests a central relevance of inflammatory processes in human liver regeneration and a disbalanced inflammatory response in patients with PHLF. In fact, intrahepatic neutrophil accumulation is significantly increased in PHLF patients. Liver sinusoidal endothelial cells (LSECs) are the first responder during liver regeneration and critically affecting intrahepatic immune cell infiltration, as suggested by our most recent bulk sequencing evidence.

Aims (Figure 1): The primary aim (**AIM 1**) of this project is to characterize the gradual phenotypical dynamics of LSECs during progressing stages of liver disease and in patients with PHLF. We will further aim to recapitulate these changes in well characterized mouse models of chronic liver disease (**AIM 2**) to ultimately explore targetability of these processes (**AIM 3**).

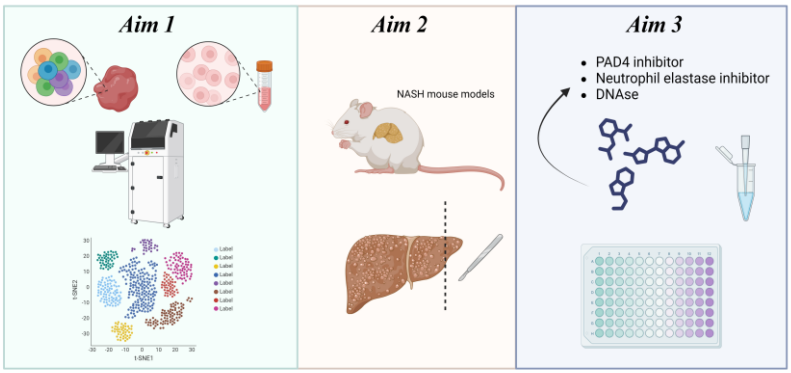

Figure 1

Methodology: We will use single nuclear sequencing of liver biopsies from our biorepository to further characterize LSECs during human liver regeneration in different types of liver disease (Table 1). Samples were collected intraoperatively at baseline as well as 2 hours after induction of liver regeneration. We will also use single nuclear sequencing to explore cell to cell communication with a particular focus on LSECs and immune cell communication. We will further validate single nuclear sequencing results in a diet induced NASH mouse model and compare them to age matched controls, to document causality of our findings in a highly standardized model of liver disease. We will further use our well established 70% partial hepatectomy mouse model in NASH mice to evaluate targetability of this process. Standard regeneration markers will be utilized in this setting: liver-to-bodyweight ratio, Ki-67 and survival. We will start to assess direct targetability of inflammatory processes in this model. As our previous results have suggested that neutrophil accumulation and activation are critically important in PHLF development, we will initially directly target these processes with neutrophil activation inhibitors (PAD4 inhibitor, neutrophil elastase inhibitor, DNAse). We will further explore the possibility of modulating LSEC responsiveness to inflammatory stimuli. Our previous bulk sequencing results suggested that LSEC DUSP4 reduction is critical in LSEC immune cell communication. As DUSP4 is negatively regulated by miRNA 122, we will explore the effects of a miRNA 122 inhibitor on liver regeneration in this model.

	Baseline	Regeneration
Group 1	Normal Liver (NL)	Normal Liver (NL)
Group 2	NAFLD	NAFLD
Group 3	NASH	NASH
Group 4	PHLF – with NL	PHLF – with NL
Group 5	PHLF – with NAFLD	PHLF – with NAFLD

Table 1

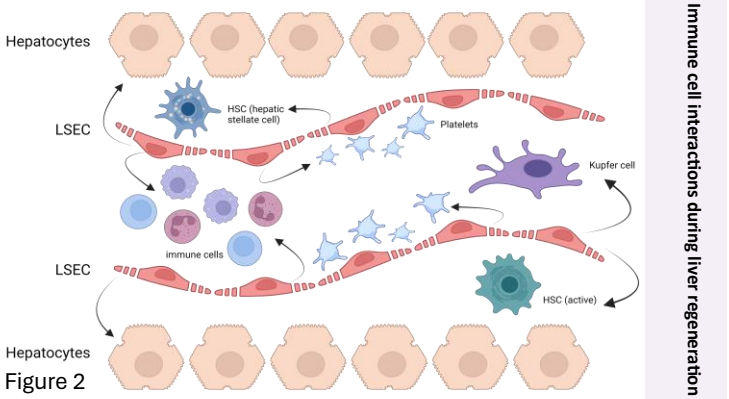

Figure 2

Innovation: With the aid of our innovative project combining basic and translational science, we will shed light into the changes of LSECs in progressing stages of chronic liver disease, their response during the early phase of liver regeneration as well as the consequences of these changes on intrahepatic immune cell accumulation and consequently postoperative liver dysfunction (Figure 2). As we will also explore the targetability of these processes, the results of our proposal could be potentially paradigm-changing, since to date no therapeutic interventions have been translated into human relevance. **Timeframe**: The estimated timeframe for our project is expected to be one year.