Project proposal: "Stage- and disease-specific signatures of immune dysfunction in decompensating advanced chronic liver disease"

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Background and preliminary data: The liver plays crucial roles in metabolism, detoxification and immunity. Advanced chronic liver disease (ACLD), the final stage of various hepatic diseases, encompasses functional and microarchitectural alterations to precipitate tissue dysfunction and clinical complications. As such, acute-on-chronic liver failure (ACLF), a frequent and devastating complication of ACLD, comprises hepatic and/or extrahepatic organ failure, associates with significant morbidity and mortality, and currently lacks targeted treatment options (1). The syndrome is most often triggered and/or complicated by bacterial infections, highlighting the central role of the liver in systemic immunity (2). Importantly, ACLF is reversible in a significant fraction of patients, indicating "molecular reprogramming" potentially amenable for targeted interventions. To study mechanisms of ACLF-associated immune dysfunction may thus not only deepen our understanding of the liver in host defense, but may also reveal targetable molecular pathways to stabilize or even revert ACLD manifestations, including susceptibility to infections.

Multi-omics approaches including transcriptomics, proteomics, and advanced imaging can be used for the generation of multidimensional signatures of disease states (3). Integration of these techniques constitutes a promising approach for a comprehensive characterization of molecular changes during ACLD, as well as for the development of strategies to prevent, delay or even revert the need for LT.

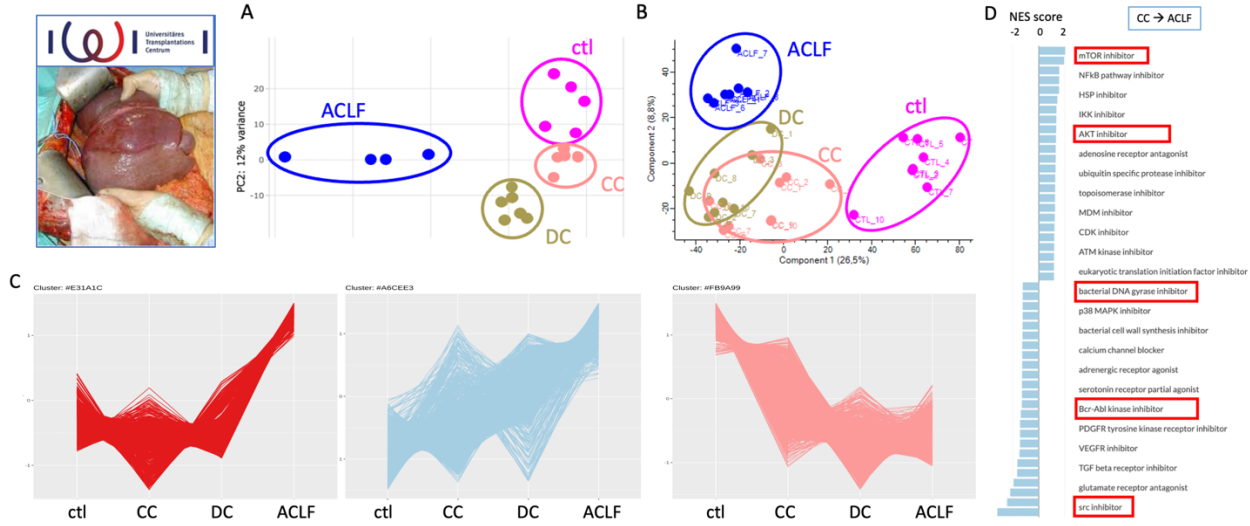

Figure 1: Principal component analyses (PCA) of bulk RNA sequencing (A) and whole liver tissue protein expression (B) in liver explants reveal transcriptional and translational clustering of different alcohol-related ACLD stages. C. Hepatic gene expression across ACLD stages can be grouped into characteristic expression patterns, three of which are delineated here. D. Mechanism of action (MOA) plot to identify potential pharmacologic modulation strategies aiming at the phase transition between ACLD stages, here: from CC to ACLF. ACLF – acute-on chronic liver failure, CC: compensated cirrhosis, DC: decompensated cirrhosis. NES – normalized enrichment score. Unpublished data (PH).

Hypothesis: Clinical ACLD stages are characterized by disease- and stage-specific alterations in liver microarchitecture as well as transcriptional and translational signatures, which affect immune networks to facilitate infections and dysregulate host responses to pathogens. Investigations into the molecular foundations of ACLD stages may aid in the identification of signaling pathways associated with disease progression and loss of liver immunologic functionality. The anticipated findings may inform strategies to reprogram the failing liver into an at least temporarily stable state, and to revert cirrhosis-associated immune dysfunction to prevent infections and infection-related complications.

Aims and Work Programme:

Aim 1: To characterize stage-specific tissue architectural, transcriptional and translational alterations in human liver tissues across a spectrum of etiologically diverse chronic liver diseases (i.e., alcoholic liver disease "ALD", chronic hepatitis C infection "HCV", primary sclerosing cholangitis "PSC") at different stages of decompensation (ACLD to ACLF) with a predominant focus on immune cell architecture and immune signaling networks.

Work programme: We will use 8-10 samples per disease stage (healthy resection margins from liver metastasectomies, "HRM" as controls, CC, DC, ACLF) each from three etiologically diverse chronic liver diseases (ALD, HCV, PSC) to decipher commonalities and disease-specific ACLD-stage signatures. Samples will processed via bulk transcriptomics and whole tissue proteomics.

In preparation of this proposal, we have identified above mentioned samples in our tissue biobanks (patient consent available), which may be used upon funding approval. Selected representative samples will additionally be used for single cell sequencing, but these will need to be prospectively harvested during LT in a protocol-based manner. A cooperation with the Department of Visceral Transplant Surgery (UKE) has been informally established in preparation of this proposal.

Aim 2: To characterize immune microarchitectural changes associated with ACLD progression.

Work programme: samples from the above mentioned explants will be processed for spatial transcriptomics and multiplex immunofluorescence imaging, using established cell and liver zonation markers, as well as known or newly described (Aim 1) functional markers relevant in regulating key (patho-)physiological pathways in liver parenchyma. Multiplex-IF will aid in the mapping of signaling alterations to liver parenchymal and stromal cells as well as to liver-resident immune cells including Kupfer cells, hepatocytes, stellate cells and others. Bioinformatic analysis will aid in qualitative and quantitative assessment of disturbances in lobular and sublobular liver architecture and functionality.

Cellular composition/ distribution	Arginase-1	hepatocytes
	CD11b	BMDM
	CD11c	dendritic cells
	CD68	macrophages
	CD20	B cells
	CD36	LSEC
	CD8	cytotoxic T cells
	CD4	T helper cells
	IL-17	Th17
	CD34	vasculature
	CK7	biliary epithelial cells
Liver zonation	GLUL1	Glutamine synthetase, pericentral
	ASS1	Argininosuccinat synthetase, periportal
	aSMA	stellate cells
	INOS	M1 polarization
	CD206	M2 polarization
	ß-Catenin	Wnt signalling (liver zonation)
IC	PDL-1	immune checkpoint
	PD-1	immune checkpoint

Table 1: Antibodies used for multiplex-IF (already available in our working group)

Aim 3: To assess the functional and phenotypical validity of current and newly developed rodent models of ACLD as scientific tools for the identification of relevant biological pathways and pre-testing of medical treatments for different stages of ACLD and particularly infectious complications.

Work program: Liver tissues from rodent models, including the "chronic CCl_4 model", the Mdr2-/- model of progressive cholestatic liver disease as well as the Tak1Δhep model of chronic cell death-dependent hepatopathy will be compared to human liver tissues samples from aims 1 and 2 with regards to their phenotypic and (dys-)functional similarities to human ACLD and particularly ACLF. In subsequent studies, LPS and infections with live bacteria will elicit functional consequences of ACLD-associated dysregulated host responses to pathogens.

References

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