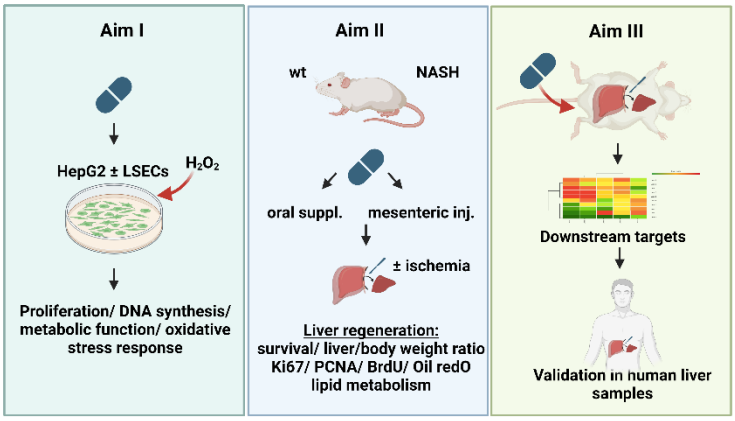
SUMMARY OF THE STUDY: The role of circulating metabolites in liver regeneration

Background: Post-hepatectomy liver failure (PHLF) as a consequence of impaired liver regeneration (LR) after extensive liver resections are afflicted with increased postoperative morbidity and mortality. Although desperately needed, by the time being, no single efficient therapeutic option could be identified to improve LR or even treat patients with PHLF, due to our still incomplete understanding of the complex mechanisms involved in LR. Underlying liver diseases, like non-alcoholic steatohepatitis (NASH) or chemotherapy-associated steatohepatitis (CASH), increasingly prevalent in patients with hepatic malignancies, featuring oxidative stress, mitochondrial dysfunction, abnormal lipid metabolism or gut dysbiosis, among others, are detrimentally associated with LR. A large body of evidence is already available, that the metabolites Carnosine and Spermidine render beneficial effects on liver metabolism and regeneration via their oxidative stress reducing and autophagy modulating effects in animal models of toxic liver damage. Indeed, within a panel of 180 metabolites, we identified Carnosine and Spermidine among the most relevant preoperatively decreased circulating metabolites in an established cohort of 96 matched patients, associated with PHLF. Additionally, 2 hours after induction of LR, patients with PHLF displayed an explicit decrease of plasma Carnosine and Spermidine level. Given these observations, we generated our **CENTRAL HYPOTHESIS that preoperative Carnosine/ Spermidine supplementation improve post-hepatectomy liver regeneration by modulating oxidative stress response, autophagy and hepatic lipid metabolism.** We formulated 3 specific aims to study metabolites mechanisms on LR in more detail:

Aim I: Carnosine/Spermidine rescues cell viability/ growth/ metabolism in oxidative stress conditions in vitro; In an *in vitro* cell model of HepG2 cells, we will study Carnosine/ Spermidine supplementation on metabolic activity, cell viability and oxidative stress response under normal and oxidative stress conditions. Additionally we will explore interactions with liver sinusoidal endothelial cells (LSECs) in co-cultural experiments, as they represent the primary cell line in the liver exposed to circulating metabolites beeing critically involved in post-hepatectomy LR.

Aim II: Carnosine/Spermidine supports liver regeneration after PHx in wt- and NASH mouse model; Further we will evaluate Carnosine and Spermidine effects in a 68% partial hepatectomy mouse model to boost LR and we will also mimic the clinical ischemia-reperfusion setting of temporarily inflow occlusion during Pringle manoeuvre via initial 40 min ischemia. We will evaluate effects of orally supplemented or portal vein injected Carnosine/ Spermidine supple-mentation on LR and metabolism via standard techniques (survival, liver-to-bodyweight ratio, Ki-67, PCNA, BrdU, Oil red O) in normal bred mice and a dietary induced NASH mouse model.



Aim III: identifying downstream targets of Carnosine/ Spermidine and validation of human data; Beside the pure regenerative aspect of this study, we are aiming to perform RNA sequencing in the liver of the strains of interest, to assess downstream targets of these metabolites, focusing on oxidative stress response, autophagy and lipid metabolism. We will further validate these experimental data in our human biorepository, consisting of liver biopsies from 60 patients, obtained prior as well as 2 hours after induction of LR, already at hand, for their relevance regarding development of PHLF.

If we are able to confirm our hypothesis that Carnosine or/and Spermidine are indeed supporting LR, as a next step, these results will lead to the development of a phase I clinical trial to use Carnosine or/and Spermidine as a preoperative treatment to support LR and therefore avoid impaired liver regeneration or even failure.

Timeframe: As models have already been established in our laboratory, and data from our human biorepository for validation are already at hand, we schedule experiments for a one-year period.