Engineering Functional Recovery of Hyperproliferative Hepatocytes for Implantable Liver Grafts

Team 16: **Veronica Hui, Chi (Jeffrey) Li**
Advisors: **Amy Stoddard, Delaney Gray-Scherr, Christopher Chen**

Engineered liver grafts have emerged as a promising new therapy that could bolster liver function and improve the survival of patients awaiting a life-saving organ transplant. To reach a clinically relevant size for humans, previous work drove the implanted hepatocytes into a hyperproliferative state by using a constitutively active mutant Yes-associated protein (YAP5SA) in the YAP signaling pathway. However, while YAP5SA hepatocytes proliferate robustly, they lose differentiated liver functions, thereby limiting their clinical utility. We hypothesize the loss in function occurs when YAP5SA competes with hepatocyte nuclear factor 4 alpha (HNF4a), a protein associated with hepatocyte function, for mutual transcriptional cofactors. Therefore, we aim to genetically engineer hepatocytes that can modulate between a hyperproliferative and functional phenotype. To do so, we evaluated the effect of active YAP5SA signaling duration and protein stability on functional recovery. Then, we designed and validated a construct consisting of a ligand-induced degron (LID) attached to the YAP5SA protein for an inducible and accelerated degradation response. Lastly, we assessed the functional response of hepatocytes by measuring HNF4a, urea, and albumin levels. Our findings show that longer durations of YAP5SA exposure reduce hepatocyte function and its ability to recover function, indicating that the extended half-life of YAP5SA may hinder its functional capabilities. Our engineered YAP5SA-LID construct reduces half-life, showing potential for improved functional recovery. Based on these results, modulating protein kinetics may become a promising addition to regulating liver graft growth and function for implantable therapies.