Accelerated Compound Repurposing Using High Content Imaging: Application to Non-Alcoholic Fatty Liver Disease

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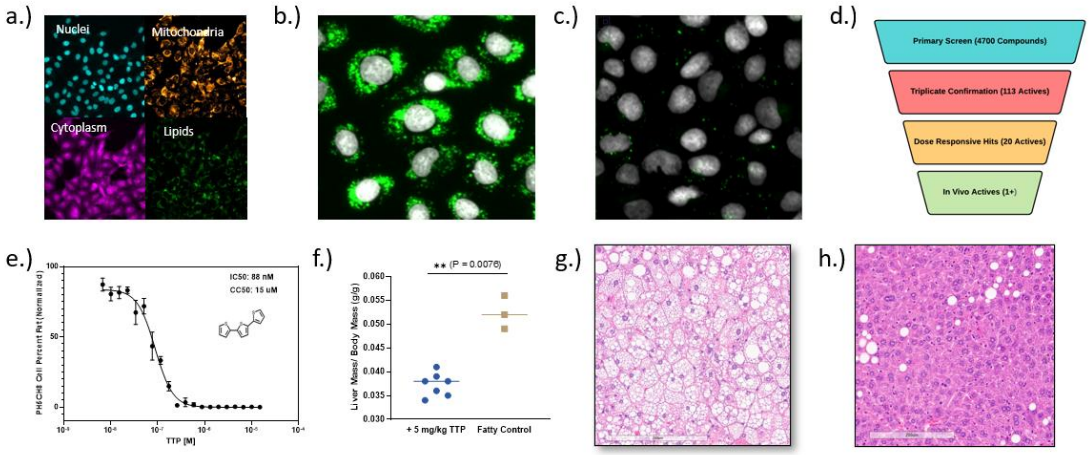
Extended Abstract

Non-Alcoholic Fatty Liver Disease (NAFLD) is a complex metabolic disorder characterized by excessive fat deposition in liver tissue. As of 2021, NAFLD is the most common cause of liver failure in the United States and is generally the result of a high-fat diet, insulin resistance and/or genetic factors. Despite the increasing frequency of this disease, there are currently no FDA approved drugs for the treatment of NAFLD, which represents an important gap within the medical community. Repurposing of bioactive compounds, including FDA approved drugs and clinical candidates, is a promising strategy for identifying rapidly translatable options for NAFLD. Benefits of repurposing include known safety profiles, robust supply chains and a short time-frame necessary for development. Additionally, these compounds serve as chemical probes that can illuminate novel molecular targets/pathways that influence the disease.

A complementary aproach to a standard *in vitro* NAFLD assay is high-content imaging-based morphological cell profiling. This technique provides significantly more information than single endpoint readouts and can help rapidly identify lead compounds for *in vivo* experimentation. This third year seminar highlights the development of a novel cell painting assay using an optimized model for lipid steatosis, followed by a high-content imaging-based drug repurposing screen. PH5CH8, a non-neoplastic immortalized human hepatocyte cell line, was used to model steatosis by “lipid loading” prior to treatment with a repurposing compound library. Cells were stained with an optimized multiplexed fluroescent probe set to identify various cellular compartments and take measurements relevant to the steatosis endpoint. Machine learning assisted image analysis was then used to generate a multivariate score for steatosis and identify compounds which reverse the steatosis phenotype.

From a a library of 5300+ FDA approved drugs, clinical candidates and bioactive molecules, 20 compounds were identified to have dose-responsive efficacy *in vitro*. While many of these compounds have reported anti-steatosis activity, several including periplocin (IC50 = 89 nM) and α-terthienyl (IC50 = 88 nM) are novel *in vitro* observations. Following the *in vitro* screen, α-terthienyl - a small molecule isolated from the root of african marigolds, was assessed for efficacy in an *in vivo* mouse model for NAFLD at a dosage of 5 mg/kg for 14 days. This compound showed strong *in vivo* efficacy as evident by a reduction of liver mass (P = 0.0076) and a remarkable improvement in liver histology. This result provides support for using morphological cell profiling as a strategy for rapidly translatable drug discovery.



Abstract Figure. High Content-Imaging Based Compound Repurposing for NAFLD. a.) PH5CH8 cells were used to model NAFLD via a novel cell painting assay to identify nuclei, mitochondria, cytoplasmic texture and neutral lipid droplets. b.) lipid loaded control showing lipids (green) overlayed with cell nuclei (gray) and c.) unloaded control. d.) Hit funnel from drug repurposing screen. e.) dose response curve for lead compound α-terthienyl, structure shown (IC50 = 88 nM) f.) which had strong efficacy in a mouse model for lipid steatosis. g.) H&E Liver histology for sham control mouse with NAFLD (lipid droplets appear white, nuclei in blue) and h.) representative liver histology for NAFLD mouse treated with 5 mg/kg α-terthienyl for 14 days via I.P. injection in DMSO.