**Rapidly Translational Drug Discovery Using High Content Imaging: Applications to Non-Alcoholic Fatty Liver Disease and the COVID-19 Pandemic**

By

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 High content screening (HCS) is an extremely versatile imaging approach to drug discovery which combines high throughput assay technology and morphological profiling with cell-based models for diseases. Sometimes referred to as “Cell Painting”, this approach uses multiplexed fluorescence images of cells, which are automatically segmented and analyzed to extract >500 unique phenotypic features including measurements of texture, intensity, radial distribution, and area for each labelled cellular compartment. This morphological information, in the context of drug treatment, can be used to efficiently identify therapeutic leads with high clinical translational potential. As will be shown, HCS can be effectively applied to drug screening, mechanism of action (MOA) elucidation for lead compounds, medicinal chemistry optimization, and toxicology. This thesis defense highlights the application of HCS to two unrelated diseases: non-alcoholic fatty liver disease (NAFLD) and coronavirus disease 2019 (COVID-19).

 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. To address this gap, we applied HCS and morphological cell profiling to a drug repurposing screen in a cell-based model for NAFLD. Hits were selected using a machine learning assisted approach, and ultimately led to the identification of α-terthienyl, a plant natural product with potent efficacy in an *in vivo* mouse model for NAFLD.

 COVID-19, in contrast, is a respiratory illness caused by the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since its emergence in China in December 2019, SARS-CoV-2 has caused a global pandemic, resulting in the death of over 6.5 million people worldwide as of November 2022. At the beginning of the pandemic, there were not any treatments available for treating COVID-19, representing an extremely urgent medical need. To address this need, we were among the first groups in the world to develop an HCS based quantitative high-throughput assay to identify efficacious agents against live SARS-CoV-2 virus. From a library of 1,425 FDA approved compounds and clinical candidates, we identified 17 hits that inhibited infection and analyzed their antiviral activity in multiple cell lines. Notably, we discovered that lactoferrin, a glycoprotein found in secretory fluids including mammalian milk, inhibits SARS-CoV-2 infection in the nanomolar range in all evaluated cell models with multiple modes of action, including blockage of virus attachment to cellular heparan sulfate proteoglycans and enhancement of interferon responses.

 Followup work from SARS-CoV-2 screening included an evaluation of two different antiviral leads, lactoferrin and niclosamide, against emerging variants of concern (VOCs), as well as mechanistic elucidation for these compounds using morphological cell profiling. As a cheap and widely available dietary supplement with potent multimodal efficacy against multiple VOCs, lactoferrin represented a promising option for the treatment or prevention of COVID-19. Ultimately, a human clinical trial for lactoferrin was initiated, demonstrating the rapid translational potential for HCS. Additional work towards novel COVID-19 therapeutics included a structure activity relationship (SAR) campaign for niclosamide, and the development of potent antisense oligonucleotides (ASOs) targeting viral and human host factor genes.

Overall, the results presented in this thesis defense highlight the power of HCS, which can lead to the rapid identification and characterization of clinically translatable leads for both emergent and existing diseases.