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Human liver organoids can help identify hepatotoxic ingredients in herbal and dietary supplements associated with clinically significant liver injury: Results from the DILIN study

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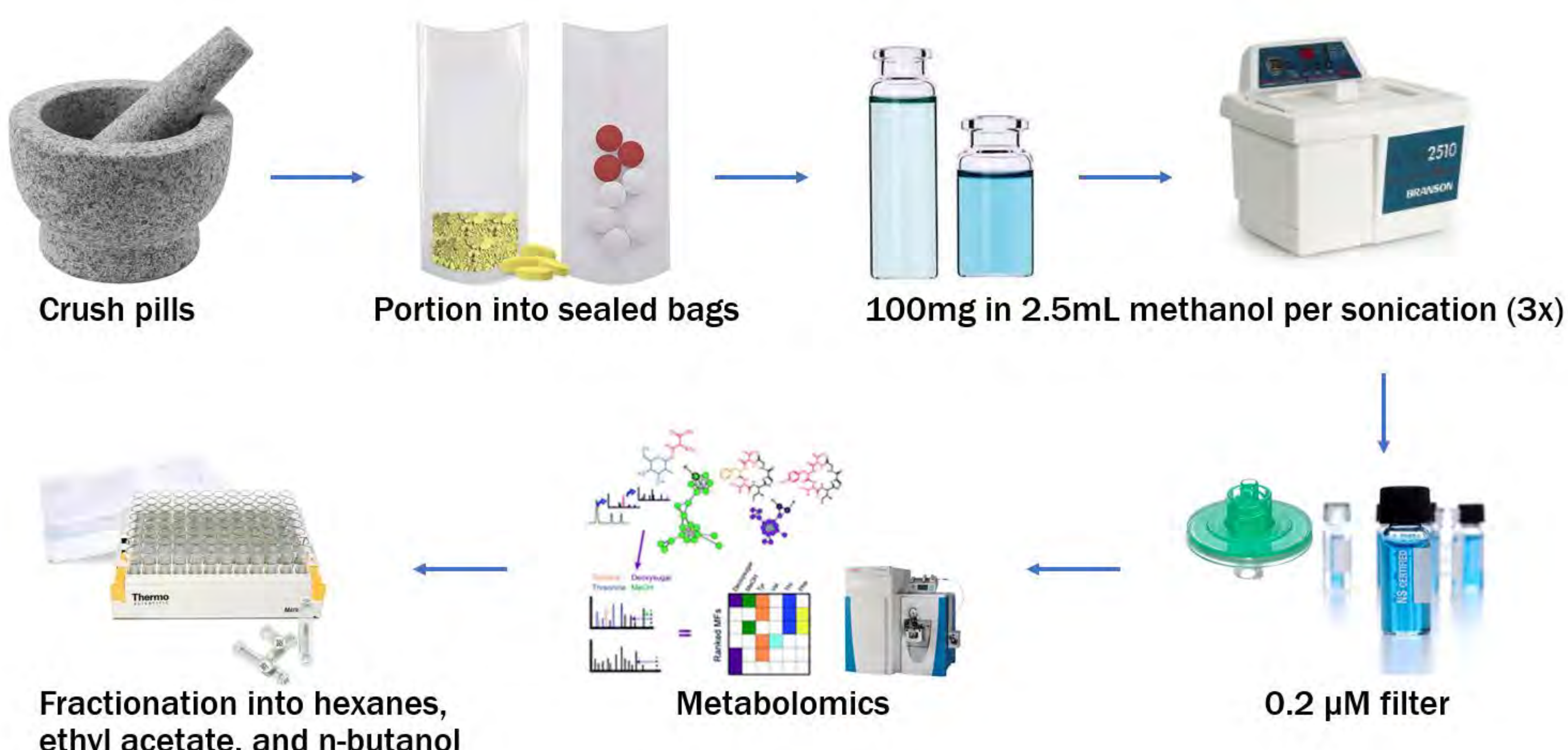
Introduction

Herbal and dietary supplements (HDS) account for a significant proportion of patients diagnosed with drug induced liver injury (DILI). Prior studies have demonstrated that the labels of HDS products are frequently inaccurate limiting our ability to reliably identify the hepatotoxic ingredient(s).[1] The aim of this study is to develop an improved *in vitro* test system for DILI risk prediction using human liver organoids (HLO)[2,3] to elucidate the hepatotoxic ingredient(s) in various HDS products. **The aim of this study is to develop an improved in vitro test system for DILI risk prediction utilizing human liver organoids (HLO) to elucidate the hepatotoxic ingredient(s) in various HDS products.**

HDS Crude Extracts

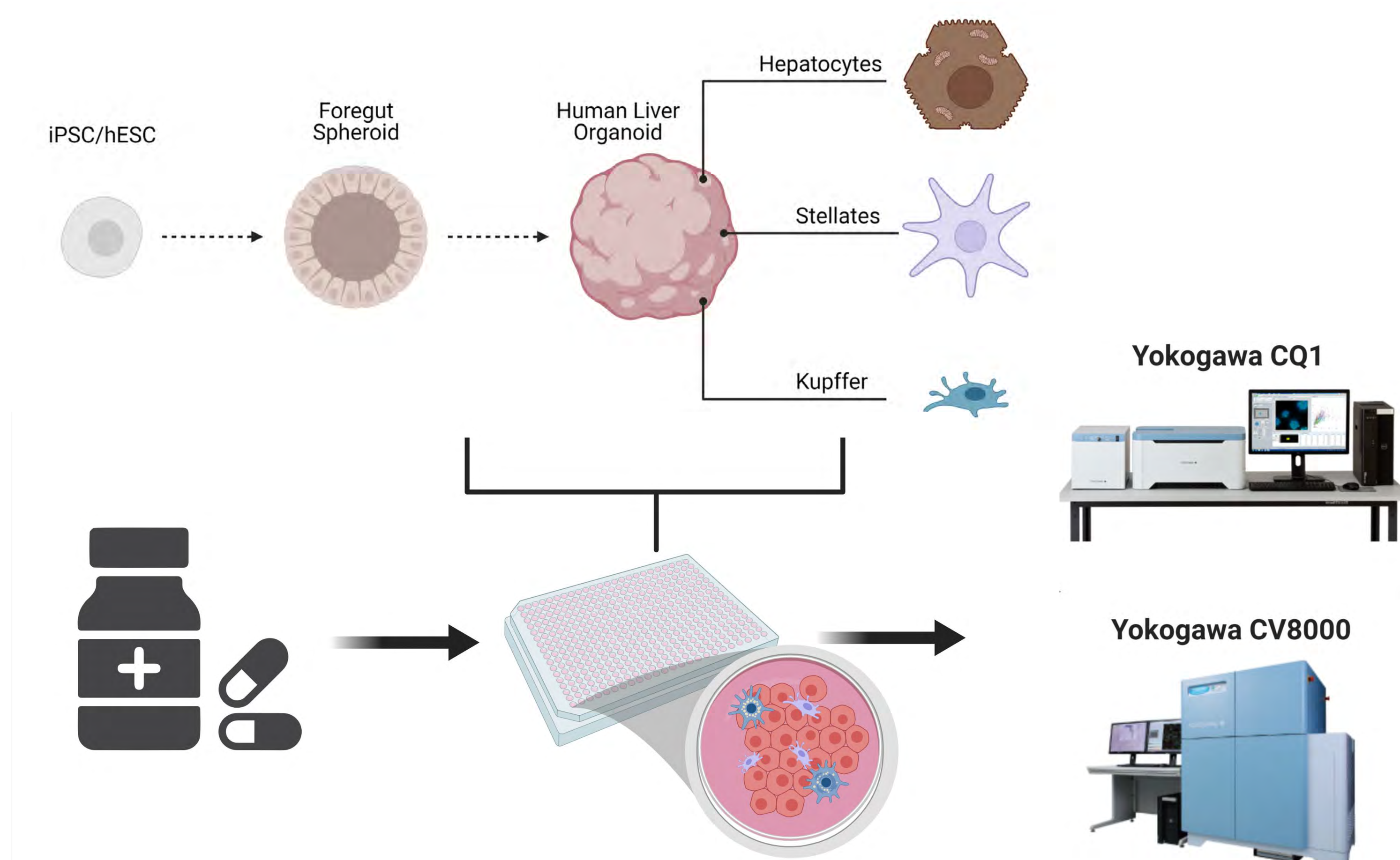
54 HDS products taken by DILI patients were available for testing from the DILIN HDS repository at the NCPR in Mississippi.



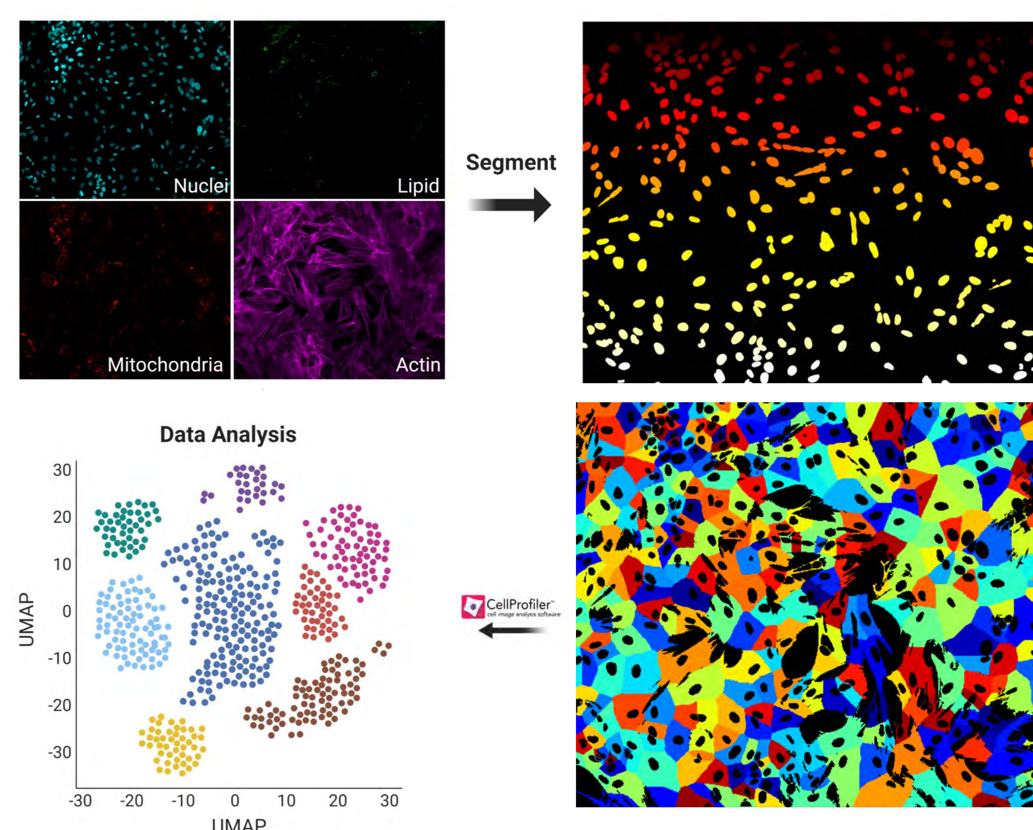
HDS products were first crushed and dissolved in methanol by sonication and then extracted with hexanes, ethyl acetate, and n-butanol to partition crude extracts by polarity. This resulted in a total of 162 unique extracts that were formatted into 384-well plates for high throughput screening. LC-MS-MS metabolomics data were collected for each sample to aid in hepatotoxicity analysis.

Initial qHTS Screen

HLOs derived from iPSC 72.3 (a healthy male donor) were dispersed into a single cell suspension and seeded onto 384-well plates. Cells were treated with the extract library and a list of known hepatotoxic pure compounds at concentrations of 0.5, 1, 5, and 10 µg/mL along with negative control (DMSO vehicle) and positive controls APAP and fialuridine. After a 5-day incubation period, the cells were fixed and stained with fluorescent probes labeling nuclei, neutral lipid droplets, mitochondria, and the cytoskeleton. High content automated confocal fluorescence imaging was performed with either a Yokogawa CQ1 or CV8000 high-content microscope.



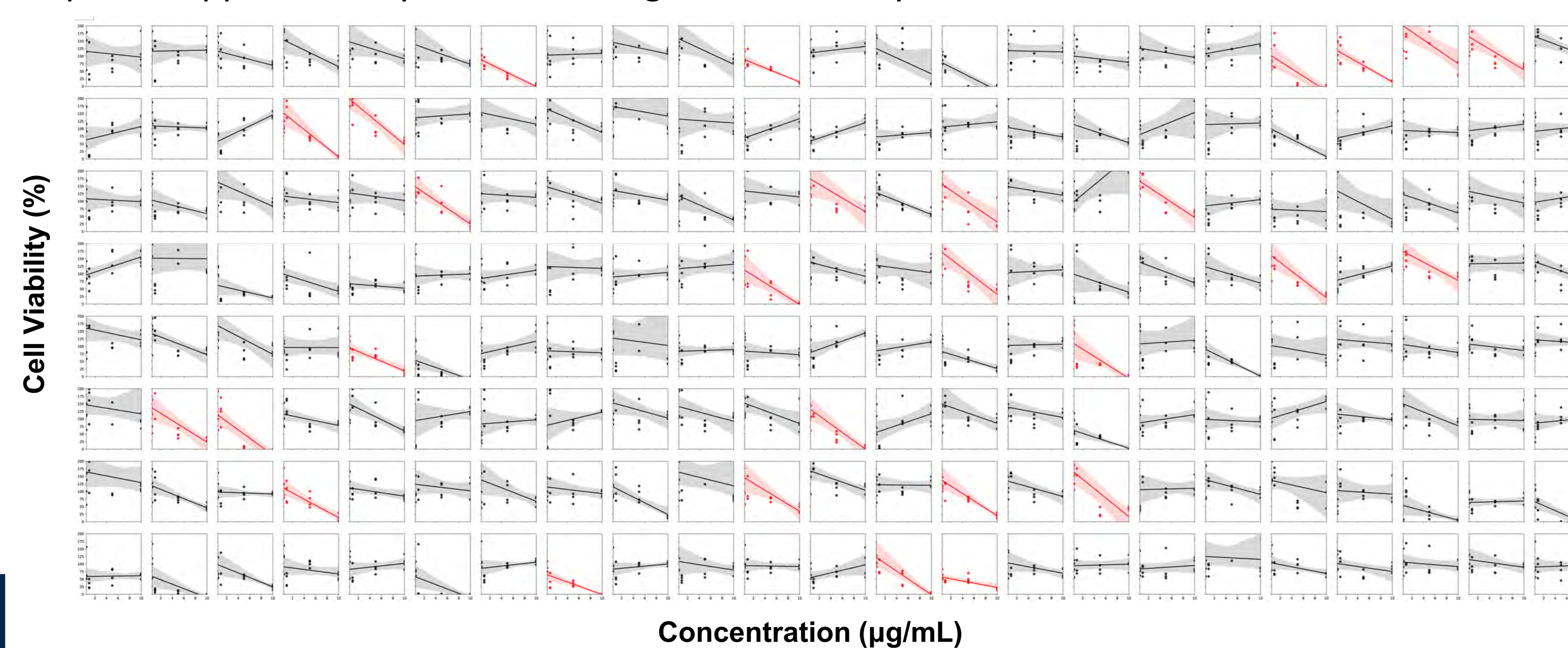
Cell Segmentation and Feature Extraction



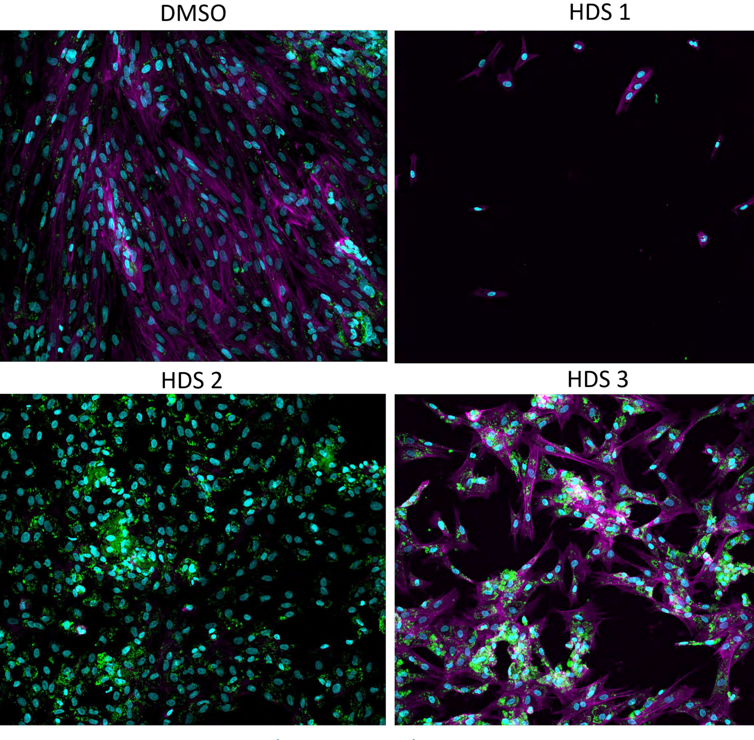
Confocal images of cells stained for nuclei, lipid, mitochondria, and actin undergo an automated image analysis pipeline to extract cell level features. Cell segmentation to identify individual nuclei is done with Cellpose 2.0[4] followed by feature extraction with CellProfiler 4.2.4.[5] The resulting feature matrix is then normalized, and features are filtered out based on high correlation with other features and low variability. This processed feature matrix is then reduced into 2D using the UMAP method to visualize cell clusters based on morphological similarity.

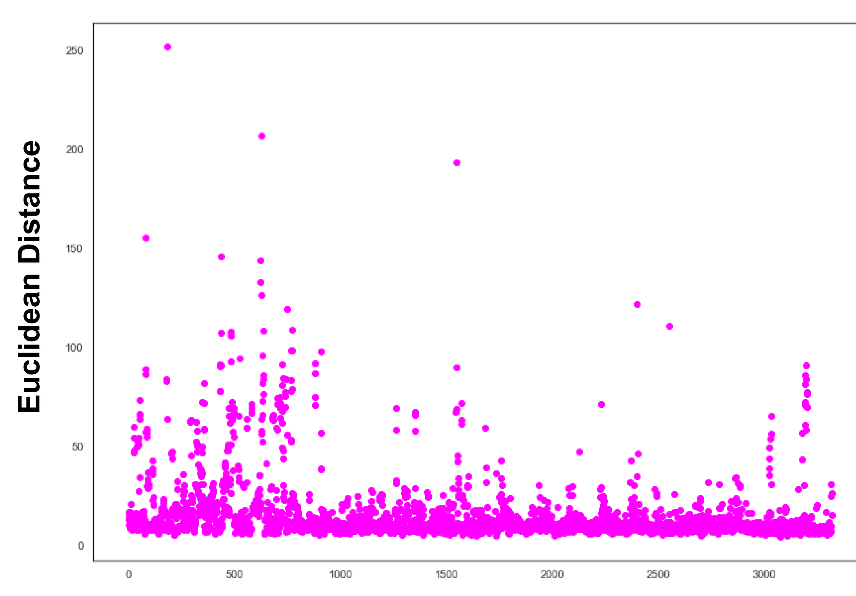
Hit Selection

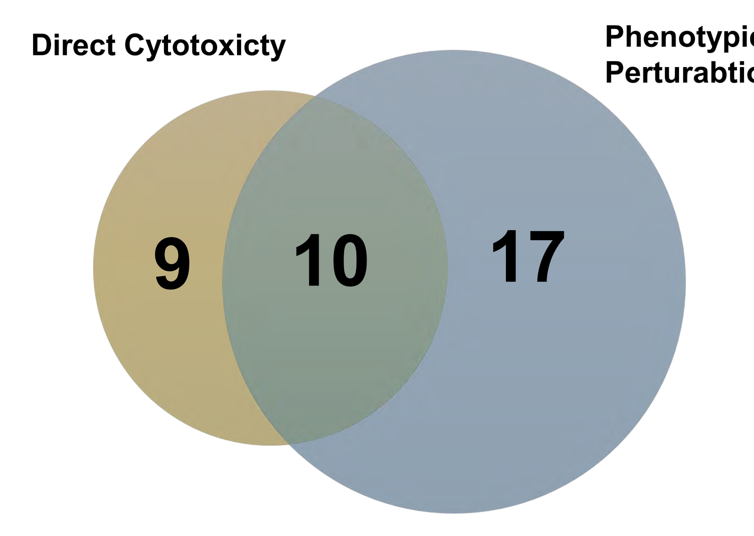
Linear regressions were fitted for each extract based on cell viability vs concentration. Initial round of hits were determined based on a slope of < -5 and CI < 10 OR slope < -3.5 and CI < 3. Of the 162 extracts, 27 were selected as hits for follow-up hepatotoxicity studies. These 27 extracts implicate approximately half of the original HDS library.



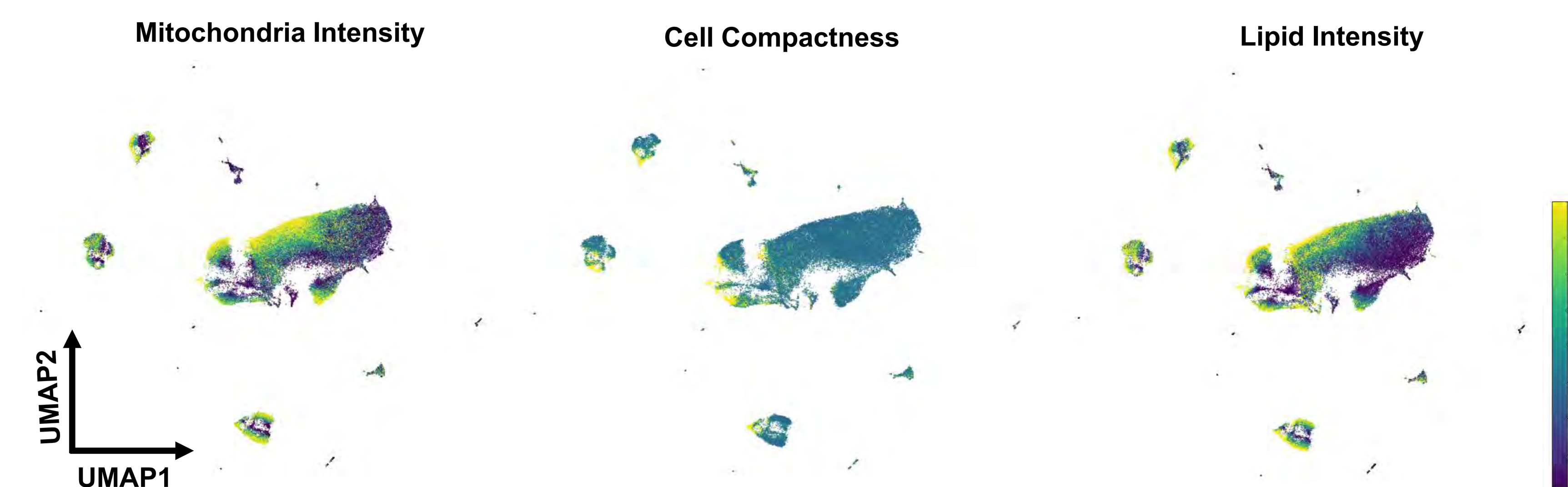
An additional round of bioactivity analysis was done using morphological distance scoring based on single-cell measurements of treated HLOs. Euclidean distance for each cell was computed and compared to the vehicle control to identify compounds causing a significant phenotypic perturbation without a direct loss of viability. Linear regression analysis was performed based on distance score vs concentration and hits were chosen based on positive slope (increasing distance) and small confidence intervals. This method identified 10 of the original hits with an additional 9 that caused significant phenotypic perturbation.





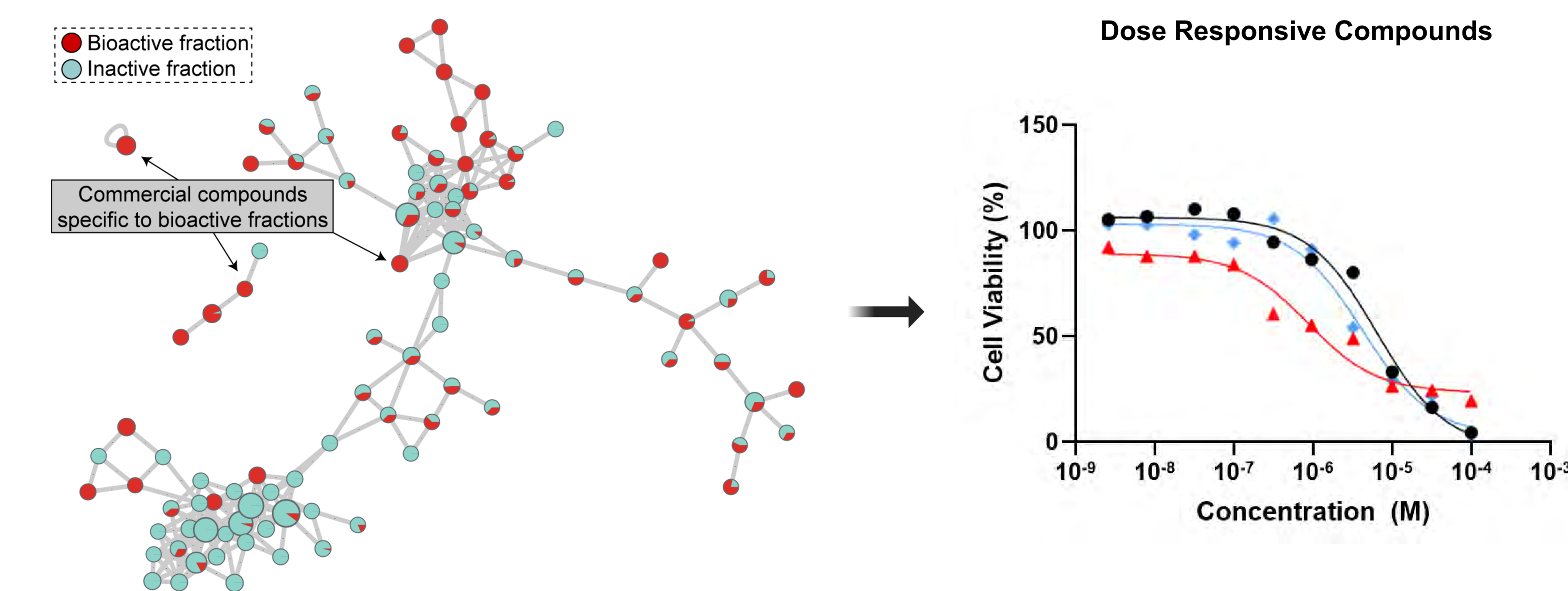


Morphological Characterization of HDS



The morphology feature matrix of our hits are reduced using the UMAP method. Clusters in the UMAP represent cells with similar appearance. The majority of cells reside in the center cluster representing a normal, healthy morphology. Satellite clusters represent cells perturbed as compared to control and are of highest interest in the context of DILI.

Single Agent Compound Confirmation



HDS fractions identified as hits were analyzed by liquid-chromatography-tandem mass spectrometry (LC-MS-MS). MS-MS spectra were grouped into "bioactive" and "inactive" fractions based on bioassays and analyzed by GNPS-molecular networking for matches to the GNPS spectral library. Natural products corresponding to the GNPS spectral library hits were purchased and are being evaluated for single compound hepatotoxicity.

Future Directions

Single agent compounds confirmed to result in dose-responsive hepatotoxicity are chosen to move forward into follow-up assays. Follow-up assays include screening in additional cell lines and testing in advanced cell culture assays, including organ-on-chip, multicellular co-culture, and animal models.

Conclusion

- No reliable and high-throughput platform exists for large scale liver safety screening
- HLOs dispersed into 384-well plates show promise in compound DILI screening and is compatible with high-content imaging
- DILI risk of HDS extracts are scored by either direct cell viability or phenotypic perturbation
- Single-agent DILI culprits from HDS extracts are being identified

References and Acknowledgement

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