Using Contingent AI™ to Discover Targets and Mechanism of Action from High-Content Imaging Screen

BioSymetrics

Summary

Application of the Mechanism of Action (MOA) machine learning tool to the high-content imaging (HCI) screening dataset (RxRx-19 COVID data set) allowed us to:

- **Set an optimal "hit" cut-off for the dataset.** Defining any cut-off to an experimental dataset, above which results will be seen as "hits", too often is done manually or based on previous approaches. Here, by iteratively applying our MOA framework using a ContingentAI™ approach, we were able to objectively define a cutoff that provided maximal insight for this dataset.
- **Identify novel COVID-relevant disease mechanisms.** Running the MOA prediction framework identified novel 22 molecular mechanisms as novel targets to pursue in COVID therapeutic design.

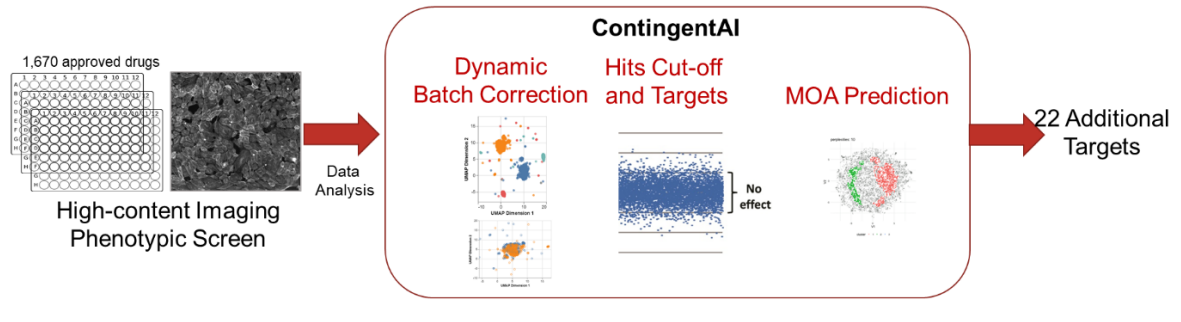
The approach and results are described in more detail below, please contact Dr. Mikalai Malinouski (mikalai@biosymetrics.com) for more details.

Overview and Results

The dataset examined here evaluates a set of 1,670 clinically available drugs as potential COVID-19 therapuetics by evaluating their effect on SARS-CoV-2 infected human cells using high-content imaging (HCI). HCI identifies a range of complex morphological features that correspond to specific patterns of response for infected cells in the presence of these drugs. At BioSymetrics, we apply a two-step process: 1) A dynamic de-noising of the dataset to remove potential batch effects or other sources of experimental noise, 2) A mechanism of action (MOA) prediction framework used to identify target proteins or pathways responsible for the observed response.

By integration and repeated running of the MOA prediction process, we can identify an optimal cut-off for what are considered "hits" in the HCI assay, and as a result we highlight 22 novel mechanisms as avenues for experimental study of relevance to COVID.

Table 1Figure 1. Contingent AI™ analysis approach. It allowed to uncover 22 additional targets in the screen.



The first step in this analysis was to apply dynamic batch correction to the RxRx-19 COVID data, a process we describe in detail here. In short, we applied a method to merge samples from the authors' experimental settings to evaluate all drugs tested in a single context. Once batch

corrected, we apply our Mechanism of Action (MOA) prediction framework, which is designed to predict MOA for small molecules given phenotypic screening data (chemical structures alongside binary annotations of hits).

To fit the RxRx-19 data into this process, we had to determine a cut-off according to both the overall therapeutic efficacy and consistency across a dose-response curve. To do so, we repeated the MOA prediction process six times in an application of our Contingent AI framework, varying the selected threshold and the use of batch correction, comparing the efficacy of the results after each run.

We immediately observed that batch correction both improved the total number of targets enriched and increased the overlap with targets observed in the original publication (4/7 highlighted COVID-relevant mechanisms identified following manual examination of the results). The three we were not able to recapitulate were cysteine protease, SARS-CoV-2 viral protease (MPro), and vitamin D receptors. Cysteine proteases were weakly annotated in our database, so these were filtered before target enrichment. Since MPro is a novel target, our platform is not able to correlate chemical information with this target. Vitamin D receptors were not identified because of a strong disparity between various agonists (e.g. compounds like maxacalcito showed very weak activity). This analysis identified the "strict thresholding" method as being optimal, and as a result, prompted us to further investigate the novel mechanisms identified by MOA Miner (Fig.1).

Among the 20 novel mechanisms highlighted by the MOA prediction process were two specific examples that have now entered clinical examination. First are Syk inhibitors, which although not directly screened in the assay, we identified through mechanistic guilt-by-association. The Syk inhibitor fostamatinib is currently being evaluated for treatment of COVID-19 related pneumonia. Next, we identified inhibitors of the TUBA complex, such as Colchicine as hits of interest, despite what was initially thought of as a moderate response in the assay. Colchicine is currently in clinical trial for COVID-19 and was found to significantly improve time to deterioration. The remainder of the implicated mechanisms are currently being evaluated using appropriate experimental models (Fig.2).

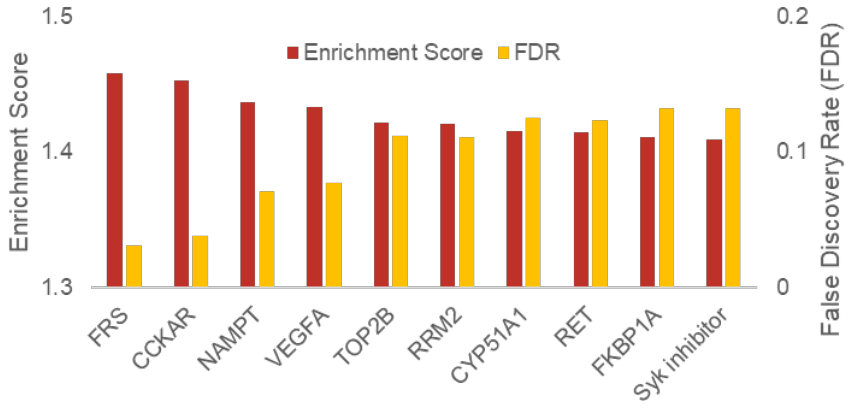


Figure 2. *Top 10 targets discovered using Contingent AI with enrichment score and false discovery rate (FDR).*