MuSIC by design

By Tracey Baas, Senior Editor

A Harvard Medical School and Tsinghua University team has developed a technology, dubbed MuSIC, that identifies synergistic drug pairs.¹ The group showed proof of concept by finding new combinations of therapeutics for HIV, but weeding out false negatives could prove challenging.

Finding synergistic drug combinations typically involves either a hypothesis-driven approach or an unbiased pairwise combinatorial screen that uses two compounds per well. The former approach can miss unanticipated interactions between targets not thought to be mechanistically linked, whereas the latter can be too unwieldy for most academic labs.

The HMS-Tsinghua team opted for a different approach called multiplex screening for interacting compounds (MuSIC), which was able to evaluate 10 compounds per well.

The team used MuSIC to examine about 500,000 drug pairs from 1,000 FDA-approved or clinically tested drugs and showed that the technology covered all 2-drug combinations using <3% of the number of wells needed in a standard unbiased screen.

A key strategy that makes MuSIC so efficient is an algorithm that guarantees each drug pair occurs in at least one well, minimizes the number of redundant pairs and provides an arrayed compound library made up of 13,106 wells—or pools—distributed in multiple 384-well plates (see Figure 1, “MuSIC strategy”).

Thus, the researchers could evaluate 13,106 drug pools rather than 500,000 drug pairs.

The team then used a two-part cellular assay to evaluate the pools with HIV-infected cells during early and late stages of the virus’ lifecycle. Part one of the assay monitored viral infection from entry to protein translation. Part two reinforced part one and also monitored viral infection during assembly, budding and infectivity.

Based on low infection rates and low cytotoxicity, 288 pools were selected and 12,904 unique drug pairs were identified. The researchers then constructed a secondary arrayed compound library and used it to home in on the top 116 drug pairs. The pairs were further validated using concentration titrations of the two drugs in each well (see Figure 1, “MuSIC strategy”).

Many of the hits belonged to a small set of drug classes including nonsteroidal anti-inflammatory drugs (NSAIDs), anticholinergics and glucocorticoids. Indeed, four glucocorticoids appeared most frequently within the top drug pairs.

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To validate these results, the team did a pairwise screen of a generic glucocorticoid, prednisolone, with the same drugs used in the MuSIC screen and showed that 7 of the top 15 hits of the pairwise screen had also been identified using MuSIC, providing an estimated discovery rate of about 47%.

The researchers next looked more closely at the molecular pair with the highest efficacy: prednisolone and the antiprotozoal drug nitazoxanide.

In cell-based assays, nitazoxanide affected the viral lifecycle after entry but before, or at, reverse transcription. Prednisolone affected the virus lifecycle after reverse transcription. The researchers hypothesized that the synergy between the drugs resulted from their targeting different steps in the HIV lifecycle.

Interestingly, the cell-based assay that focused on the early HIV lifecycle revealed enrichment for many drugs with known anti-HIV activity and anti-inflammatory functions, whereas the cell-based assay that focused on the late HIV lifecycle revealed only one drug with known anti-HIV activity and others that were new targets for HIV therapies.

Although chronic inflammation is known to contribute to infection-associated pathology,² the new results suggest that anti-inflammatory

Figure 1. MuSIC strategy. Multiplex screening for interacting compounds (MuSIC) technology is able to identify synergistic drug pairs. The technology involves distributing 1,000 approved or clinically tested drugs [a] into an arrayed compound library. An algorithm [b] ensures each of the possible 500,000 interactions between two given molecules is represented in 13,106 pools [c]. The pools in the primary arrayed compound library are evaluated for activity against HIV-infected cells [d]. Hits (red wells) from the primary screen are evaluated, and identified synergistic pairs are used to construct a secondary arrayed compound library, which also is evaluated for antiviral activity [e]. Hits (red wells) from the secondary library are validated using concentration titrations of the two drugs [f]. The analyses of antiviral efficacy for steps [d] and [e] involved a two-part cell-based assay that measured effects of molecules on early and later stages of the virus’ lifecycle.

therapies can actually inhibit virus propagation.

Results were reported in *Nature Biotechnology*.

Mixing the MuSIC

“Compared to the direct combination screen that is used widely in industry, MuSIC affords higher efficiency from screening a large number of combinations by multiplexing,” said team leader Stephen Elledge, professor of genetics and medicine at Harvard Medical School.

“This work is a great example of combining clever computational and experimental approaches to screen for undiscovered and effective combinations on a vast scale,” added Brent Stockwell, associate professor of biological sciences and chemistry at **Columbia University**. “The disadvantage is that with the complexity of the pool, there could be a high rate of false positives and false negatives.”

“While the method will miss unusual combinations that aren’t strong hits, I like the MuSIC method because it allows for very efficient screening through many combinations, and I would be excited to see this methodology applied across a large panel of microbial assays,” noted Joseph Lehar, associate director of bioinformatics at the **Novartis Institutes for BioMedical Research** and adjunct assistant professor at **Boston University**.

“Any pooled screening method will miss possible combinations—these techniques cannot find all interactions,” said Alexis Borisy, a partner at **Third Rock Ventures**. “I think more studies are necessary to understand the true false-positive and false-negative rates. The team shows one validation set and says they find 47% of all possible combinations, but this of course will vary between the experiment types selected for each disease indication.”

Prior to joining Third Rock, Borisy was president and CEO of CombinatoRx Inc. (now **Zalicus Inc.**), which developed combinations of approved drugs using combination high throughput screening (cHTS) technology.

Chen Yu Zong, professor of pharmacy and lead of the Bioinformatics and Drug Design Group at the **National University of Singapore**, had another caveat. In the study, the researchers excluded highly active antiretroviral therapy (HAART) drugs and other antivirals to avoid the screen being dominated by these drugs. By doing so, said Zong, the researchers are precluding the discovery of potentially new or more

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potent drug combinations.

Birgit Schoeberl, VP of discovery at **Merrimack Pharmaceuticals Inc.**, said it would be interesting to compare the drug combinations identified by the screen with the standard-of-care HAART regimen or to add prednisolone and nitazoxanide to HAART in animal models of HIV infection.

Going forward, Schoeberl could see MuSIC applied to identify new drug combinations in other infectious diseases, such as hepatitis C or malaria, or in oncology.

“Cancer drug screens could be done using a malignant cell line and a benign cell line to look for drug combinations that specifically kill the malignant cells, and the readout could be any cell viability assay,” said Xu Tan, postdoctoral researcher in the Elledge lab and lead author of the paper describing the findings. “Any disease with a cell culture model could be screened using the MuSIC method.”

Ongoing work includes testing combinations identified in the study to inhibit HIV and pathogenic viruses and using MuSIC to evaluate combinations that could be used to treat other infectious diseases, cancer or asthma.

The Harvard Medical School team’s work is not patented or licensed.

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