

Feature Articles

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Repositioning Idle Drugs via Systematic Serendipity **Systems-based Approaches for Boosting Stalled Drugs**

Gail Dutton

When it comes to drug repurposing, companies may be searching for a systematic approach to serendipity. The emphasis, however, still seems to be on the serendipity part of the equation.

In this regard, companies are turning back the clock, eschewing targeted drug discovery for the more systems-oriented approaches that were used decades ago. The feeling on this side of the industry seems to be that the human system is so complex that targeted approaches invariably miss things.

“As an industry, our work is only as good as the knowledge base we work from, and the knowledge base is incomplete,” notes Andrew G. Reaume, Ph.D., president, CEO, and cofounder of **Melior Discovery** (www.meliordiscovery.com). Or, as another executive says, “you can’t find what you’re not looking for.” Those unexpected features oftentimes make the difference between a new blockbuster drug and a failed compound.

“These drugs are assets to which a lot of time and money have already been invested, but they are sitting idle now,” elaborates Scott Turner, Ph.D., vp of research for **KineMed** (www.kinemed.com). “Typically, they have been advanced to a stage in preclinical or clinical work that shows they are bioavailable and have cleared the basic requirements for drugs in terms of chemistry and safety. But, they failed for the indications for which they were developed.” The quest, then, is to find what these compounds do effectively.

Shotgun Approach

A shotgun approach would be an apt description of Melior’s repurposing strategy. Dr. Reaume calls it a “blue sky approach that cuts to the end result.” Whatever you call it, this method doesn’t rely on knowing targets’ linkages, pathways, or even the method of action.

Instead, Melior has developed a multiplexing platform of 40 different animal models through which druggable compounds are taken indiscriminately, without compromising the quality of the models. To do that, Dr. Reaume explains, “you need to know something about all the permutations of assays used with the model, and you need a way to ensure they don’t interfere with one another.”

The basic process was used in the 1970s, but not multiplexed, Dr. Reaume says. The approach, he adds, “is very effective. In 18 months we’ve developed a robust pipeline. Of the first nine discontinued drugs we researched, we found three therapeutic candidates.”

One, originally a lead for diabetes, was pursued through Phase III trials in the late 1970s and developed in the 1980s for gastric ulcers, but showed a lack of efficacy when compared to the new H1 antagonists that were entering the market. “We ran it through the TheraTrace platform,” Dr. Reaume says, “where it showed effectiveness in treating metabolic disorders.”

“People are surprised by the high success rates,” Dr. Reaume continues. Melior achieves them by carefully selecting the type of compounds investigated. “Biology is more complex than people predict,” he says, affecting many receptors outside the scope of the original studies. “We don’t try to predict the full biology. We just go test it.”

“There’s a huge cost benefit,” Dr. Reaume emphasizes. “One-third of the compounds we tested became therapeutic targets meriting further investigation, at a cost of less than \$2 million.”

Stalled Compounds

KineMed's approach looks at stalled compounds, searching out new indications. Thus, the complexity of late-stage failures offers an opportunity to uncover novel activities by identifying both on-target and off-target effects. "The idea is to get early proof of concept in man," Dr. Turner says.

To gain that evidence, KineMed screens the agents with its technology in a small number of animals in studies lasting one to three weeks. Follow-on studies are again conducted in animals and quickly confirm hits and dose response. Early success is then demonstrated with small, rapid clinical studies.

The process relies upon assays for some specific metabolic pathways, including de novo lipogenesis, reverse cholesterol transport, insulin resistance, and neurodegeneration. "These assays are unique," Dr. Turner adds, "in that they measure flux or kinetics of a pathway in vivo, rather than measuring the concentrations of metabolites. The targets identified by measuring flux effectively predict functional response in the whole animal."

KineMed analyzes biological samples with highly sensitive mass spec technology to determine the dose-response modulation of the specific metabolic pathways affected, thus providing a broad systems biology perspective that isn't available in vitro. "When we look in animals, the outcomes we see often aren't predictable," he says.

Of the more than 300 pathways involved in common diseases, KineMed measures about half using stable isotope/mass spectrometry, and considers about 50 of critical importance. Thirty of those are being currently pursued by KineMed, which has validated its ability to precisely measure flux changes in 16 of those critical pathways.

"One of those, a pathway that forms part of the etiological basis for axonal dysfunction, has led KineMed to 'discover' a compound that is active in certain central nervous system disorders," Dr. Turner says. The compound originally was developed as an antitussin—a cough suppressant—and is now advancing to the IND stage.

Characterizing Compounds

Caliper Life Sciences (www.caliperls.com) approaches drug repositioning both as a contract research organization—through Caliper Discovery Alliances and Services—and as a solutions provider. A platform initially designed to phenotypically characterize genetically modified animals, which began as a **Pfizer** collaboration, was leveraged to create a broader enterprise for characterizing compounds.

In the current use, mice are used to comprehensively characterize genetic targets and compounds. This helps Caliper efficiently validate genetic targets and position potential therapeutics much earlier in the drug discovery process, as well as uncover secondary indications opportunities for early-stage compounds and reposition existing therapeutics and compounds, according to David S. Grass, Ph.D., vp, scientific operations.

The strategy to characterize genetic targets is called serial phenotyping comparison technology (SPCT). Using a small group of animals, he says, researchers can see how, or whether, genetic modifications affect the mouse response in 60 different bioassays and challenge assays covering more than 450 different parameters, relevant to 15 therapeutic areas.

For example, one program involves 50 knock-out and 50 wild-type mice, on which 60 assays were conducted without compromising the average number of animals per assay. Conclusions are reached in 17 weeks for each cohort of mice. Competing, nonmultiplexed methods would require a total of 900 mice and three months to get comparable data, according to Dr. Grass. (The funds spent on breeding the mice accounts for about 25% of the research testing budget using SPCT, versus 75% of the research testing budget using nonmultiplexed methods.)

Because this approach covers most of the major therapeutic areas, researchers are more likely to uncover unanticipated results and, therefore, may better focus their compounds. They may even start new projects with different concepts or potential indications.

Caliper is working on augmenting its platform to also capture pharmacodynamics readouts by following the expression of genes in key pathways, using its in vivo biophotonic imaging technology, Dr. Grass adds. This technology is able to noninvasively detect light emitted through mammalian tissue, and can be applied to oncology and infectious disease studies.

"For example," Dr. Grass says, "tumors genetically modified to express the luciferase gene can be tracked or

monitored noninvasively through the body of the animal, allowing real-time, in vivo measurement of tumors, as well as their growth or regression. This approach enhances data quality and efficiency in orthotopic and metastatic tumor models, as well as subcutaneous tumor models.”

In one case involving the evaluation of a clinical development candidate in a subcutaneous tumor model, caliper measurements indicated the tumor wasn't regressing in response to the compound. Yet, measuring the light emitted by the firefly luciferase-expressing tumor cells revealed a necrotic core, proving that the compound was, in fact, efficacious.

When it comes to targets, “GPCRs are still the most addressed target class in the drug development industry,” notes Keith Olson, Ph.D., vp of R&D for **DiscoverX** (www.discoverx.com).

DiscoverX has leveraged that situation by developing a panel of more than 120 GPCR assays for high-throughput screening and profiling. The panel consists of 80 high-value GPCRs and more than 40 uncharacterized GPCRs. Although GPCRs have many different signaling pathways, and some use multiple channels, virtually all bind with arrestin. “Consequently,” Dr. Olson says, “the PathHunter™ Arrestin Assay is about as universal a platform as you can get.”

This panel's broad assay windows and robust performance make it possible to use as few as 1,000 cells in a 1,536-well format, making it particularly useful for GPCRs with poor signaling or limited cell quantities. Another benefit, Dr. Olson says, is that it measures direct interactions rather than detecting secondary messengers. The PathHunter Arrestin assay is based on DiscoverX' enzyme fragment complementation and generally yields readouts in about one hour.

“The single-addition assay is ideally suited for screening, without force coupling, against GI and orphan receptors,” Dr. Olson notes. With a growing list of GPCRs included in the panel, the PathHunter Arrestin platform identifies agonists, antagonists, and inverse agonists while providing information about signaling pathways.”

In Silico Method

Not all repositioning efforts rely strongly on serendipity, though. Ruben Abagyan, Ph.D., professor, molecular biology, Scripps Research Institute (www.scripps.edu), is using in silico research to identify which existing drugs can be repurposed into promising compounds for prostate cancer therapy. “The in silico approach allows us to rationally recognize a short list of candidates or activities for repositioning,” he says.

Working in silico, Dr. Abagyan and his colleagues screened about 2,000 marketed drugs to identify new nonsteroidal antagonists against the human androgen receptor. Each drug molecule was converted into a flexible, 3-D model docked into the two best-performing models of antagonists-bound receptors. The best compounds from that stage, including the phenothiazine antipsychotics, were tested in vitro for their anti-androgen activity. Of the 11 most likely candidates, 4 worked, he says.

The computational approach lets researchers focus on specific receptors that need to be targeted for a specific condition. “It's hard to test each drug for every condition, and is expensive too,” Dr. Abagyan elaborates. Because most of this work is conducted on the computer, costs are relatively low. “This approach is just the first step that identifies a specific candidate,” he cautions. “Careful in vitro and in vivo work still must be conducted.”

The candidates were further chemically altered to reduce their original antipsychotic activity, he adds. It will then be tested in xenograft model mice, and later, in people.

The next step in this methodology, Dr. Abagyan says, is to expand the procedure to make it faster, better, and more accurate as well as to expand the database of safe compounds. The purpose of this in silico research, he adds, is to find new chemical starting points for prostate cancer therapies. “The existing inhibitors are never sufficient,” he explains, “because the target proteins develop escape mutations. Therapeutics from new chemical types are needed.”