Drug Discovery Turns to Smart Throughput

High-Throughput Screens That Count Pings as Hits May Give Way to More Definite Approaches

By Caroline Seydel
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Oncodesign recently presented results from a Phase I clinical study assessing a radiotracer’s potential as a companion biomarker for patients suffering from non-small cell lung cancer. The radiotracer, 18F-ODS2004436, uses the company’s Nanocyclix technology and targets a mutation of the EGFR kinase. Left: A positron emission tomography (PET) image showing how the radiotracer labeled the mutated EGFR present in a patient. Notice the primary tumor and bone metastases. Right: PET scan showing the same patient upon treatment with an EGFR inhibitor. Notice the absence of labeling, which is due to competition for the EGFR binding site. These images exemplify the high specificity of the Nanocyclix PET tracer.

For every new drug that hits the market, hundreds of other promising leads fizzle out during the long journey from initial library screening to Phase III clinical trials. Over the past few decades, advances in screening techniques have focused on increasing throughput. The goal? Speed the screening of huge libraries to help researchers score more hits in less time.

This goal wasn’t always realized. Often, the time and effort that was spent pursuing ostensible hits that turned out to be duds ended up increasing the cost of drug development and the average time to produce a new, marketable drug.

Some researchers and drug developers started to look beyond large-scale screening. Eventually, they caught glimpses of a better route to generating novel medicines. When these glimpses are pieced together, as they are in this article, they amount to a visionary new approach. It could, in contrast with high-throughput screening (HTS), be called smart-throughput screening.

Mechanistic Pharmacology
“I believe that early drug discovery has reached a point where there’s diminishing returns from a focus on HTS throughput,” says Peter Tummino, Ph.D., vice president and global head, lead discovery, Janssen Research & Development. “A different approach, an approach that we’ve taken, is to focus on mechanistic pharmacology, to think more about disease relevance, and even to consider that in some cases, lower throughput assays are going to end up ultimately being more effective.”

Dr. Tummino gave the keynote presentation at the Discovery on Target meeting, held recently in Boston, where he and others discussed novel approaches to more efficient drug lead discovery and optimization.

“We’re looking for molecular and cellular assays that are more relevant to human disease,” Dr. Tummino states. To find compounds whose pharmacological activity will translate to human disease, it’s necessary to mimic as closely as possible those human disease conditions. “In the past, assay and instrument technology were much more limited,” he notes. More relevant cellular systems and technologies that detect powerful multiparametric readouts have allowed researchers to focus less on technical limitations and more on recreating the native biology.

“Even in molecular systems, we give consideration to what may be more disease relevant, and often employ things like protein complexes and full-length proteins for screening,” Dr. Tummino continues.

Advances in cellular assays allow collection of more sophisticated data, and these multiparametric readouts are key to more robust lead identification. Employing a 30-gene “gene signature” as the detection method in HTS, for instance, provides an information-rich readout of compound activity. To analyze these detailed datasets presents its own kind of challenge.

“The analytics are quite daunting,” Dr. Tummino admits. Accordingly, Janssen employs a dedicated computational sciences team to explore cutting-edge technologies, including machine learning, for both data analysis and generating activity predictions.

**Fewer Compounds, More Hits**

Domainex, a contract research organization based in the United Kingdom, similarly winnows the pool of compounds that enter the screening pipeline. The company suggests that a pre-winnnowing approach can result in more selective screening.

“We need to make sure that everything we screen against is something we would be happy to take forward into drug discovery,” says Trevor Perrior, Ph.D., CEO of Domainex.

Domainex uses a proprietary virtual screening system called LeadBuilder to improve hit-finding efficiency. Departing from the conventional approach of screening more compounds faster, then ruling many of them out, Domainex narrows the field before starting the screen.

“The compounds that we screen have been carefully chosen to make sure they are high-quality lead-like molecules,” emphasizes Dr. Perrior. Compounds are rejected based on
physical or chemical characteristics that make them unattractive candidates, leading to poor bioavailability or likely toxicity. From this carefully curated starting library, LeadBuilder virtually tests the compounds against the target molecule, using Target Site Pharmacophore Models based on known ligands or X-ray crystallography data. Hit rates can exceed those of random screening by a factor of 10, ranging from about 1 to 10%.

This approach successfully reduces the time and cost associated with the hit-to-lead development phase. “Most of our clients are biotech companies for whom the cost of running high-throughput screening may be prohibitive, or take too long,” Dr. Perrior notes. “They are looking for much more efficient and cost-effective ways of getting hit compounds, and virtual screening is one of those technologies.”

Dr. Perrior points out that platforms like LeadBuider have grown out of advances in computational power, as well as powerful modern X-ray sources, which allow better acquisition of structural data on the target molecule. “There’s a whole slew of different technologies,” he observes. “These technologies are getting better all the time, and they are making novel screening approaches more practical.”

**Run Rings around Kinase Inhibitors**

Oncodesign, a biopharmaceutical company headquartered in Dijon, France, has developed what it calls Nanocyclix compounds. They are designed to improve on existing kinase inhibitors.

Kinases, which represent a huge, important family of molecules, are already targeted by dozens of approved kinase inhibitors that are used to fight cancer and other diseases. Because kinases regulate so many biochemical pathways, any inhibitor must be highly specific or risk creating serious side effects. The challenge, then, is designing an inhibitor that binds tightly to only one target kinase, while retaining favorable drug-like properties, such as low immunogenicity and good bioavailability. A good kinase inhibitor candidate should also be practical to manufacture.

“You need to make your molecule quite big if you want it to be very specific, and if you do that, you lose drug-like properties,” says Jan Hoflack, Ph.D., CSO of Oncodesign. Because of their rigid 3D shape, macrocycle molecules sidestep this problem, achieving high affinity and selectivity in a much smaller molecule.

The “macro” in the name refers only to the molecule’s large ring structure, not the overall size of the compound, Dr. Hoflack points out: “The molecule is actually nothing more than that single big cycle—what we call a kinase scaffold, an area in the molecule that is recognized by the kinase. Surprisingly to many people, this allows us to get this potency and selectivity in molecules that are about half the size of a typical linear kinase inhibitor.” To emphasize this point, Oncodesign dubbed the platform Nanocyclix.

Compact Nanocyclix molecules have even crossed the blood-brain barrier, Dr. Hoflack says, and work is underway on a LRRK2 inhibitor, for Parkinson’s disease. Currently, Oncodesign has about 10,000 molecules in its Nanocyclix library, and the company is searching for novel
targets by testing its molecules against hundreds of kinases, especially those that have been
difficult to inhibit or whose function is not yet fully understood.

“We are able to address what people call the ‘unexplored human kinome,’” Dr. Hoflack
asserts. “First we do the chemistry, and then the chemistry takes us to novel targets.” It’s an
unusual strategy, Dr. Hoflack admits, but “it leads to high levels of innovation, by definition.”

Dispose of Disease-Driving Proteins

After millions of years of evolution, complex yet elegant cellular systems have arisen. Taking
advantage of these ready-made pathways, C4 Therapeutics seeks to fight disease by hacking
the cell’s protein recycling machinery. The company’s platform of novel molecules harness the
cell’s native ubiquitin ligases, allowing researchers to tag a protein of their choosing for
targeted degradation.

“We’re doing something quite different from simple inhibition,” says Stewart Fisher, Ph.D.,
CSO of C4 Therapeutics. “Our goal is to direct with small molecules the destruction of the
protein that is the target, through the cell’s normal machinery.”

To mark proteins for degradation, the ubiquitin-proteasome system attaches ubiquitin
molecules in long chains to the protein to be destroyed. Three classes of enzymes make this
happen: the E1 and E2 ligases, which tee up the ubiquitin, and the E3 ligases, which hook
ubiquitin onto the doomed protein.

To engage the UPS ubiquitin-proteasome system and direct it toward proteins of interest, C4
Therapeutics deploys Degronimid™ molecules. Each such molecule consists of a pair of binding
domains connected by a linker. The molecule binds an E3 ligase on one side, while the other
domain is customized to a selected target protein.

The catch, Dr. Fisher says, is getting the linker just right. “Binding is required, but not
sufficient,” he explains. “You have to have all that machinery lined up so the ubiquitin can
transfer.” This added 3D structure requirement allows for greater specificity, and also enables
targeting of proteins previously thought to be “undruggable,” or that develop resistance to
direct inhibitors.

A degrader molecule can catalyze multiple ubiquitin ligations until the target protein is
completely wiped out. This means the molecule can eliminate the target at much lower
concentrations than would be needed for an inhibitor. Plus, it’s irreversible. “When these
molecules work really well, you can get very rapid protein ubiquitination and degradation,”
Dr. Fisher asserts. “You can watch the protein band disappear in minutes.”

“That’s the magic of these molecules,” he continues. “They hijack the E3 ligase to become a
specific catalytic degrader of the protein, and that means they turn over many, many cycles,
until the protein is gone.” The push forward now, he says, is finding ways to optimize not just
the binding but also the catalytic activity.