Phenotypic screening, take two

By Joanne Kotz, Senior Editor

Phenotypic screening is making a comeback in drug discovery, as some researchers have concluded that reductionist approaches such as target-based screening are useful but may also limit the breadth of new findings. Although companies such as **Novartis AG** and **GlaxoSmithKline plc** are embracing this resurgence, others such as **Roche's Genentech Inc.** unit remain focused on target-based screening.

Two broad types of screens have sequentially dominated early stage drug development over the last hundred years or so—phenotypic screens and target-based screens. The former looks at the effects, or phenotypes, that compounds induce in cells, tissues or whole organisms, whereas the latter measures the effect of compounds on a purified target protein via *in vitro* assays.

Phenotypic screens used to be the mainstay of drug development. Such screens can potentially lead to the identification of a molecule that modifies a disease phenotype by acting on a previously undescribed target or by acting simultaneously on more than one target. However, subsequently determining the relevant target or targets of molecules identified by phenotypic screening has often proven slow or impossible.

Beginning in the 1980s, advances in molecular biology and genomics led to phenotypic screens largely being replaced by screens against defined targets implicated in disease.

Over the last decade, however, some drug developers have questioned whether an over-reliance on genetic approaches to validating targets for subsequent target-based drug discovery has resulted in reduced success in discovering first-in-class medicines.¹

The topic of phenotypic versus target-based screening was a focus of discussion at the “Addressing the Challenges of Drug Discovery” Keystone Symposium in Lake Tahoe in March, which was co-organized by Stephen Frye, Michael Varney and James Wells.

Frye is director of the Center for Integrative Chemical Biology and Drug Discovery at **The University of North Carolina at Chapel Hill**. Varney is SVP of small molecule drug discovery at Genentech. Wells is chair of the Department of Pharmaceutical Chemistry at the **University of California, San Francisco**.

The meeting brought together drug developers from academia and industry for sessions on emerging biological targets and how to access new chemical space. Two of the topics most discussed between sessions were the rise of academic drug discovery, highlighted in an interview with Frye in *Nature Reviews Drug Discovery*, and the increasing impact of phenotypic screening on drug discovery.

SciBX caught up with scientists in academia and at companies after the meeting to discuss the re-emerging role of phenotypic screening in drug discovery.

A number of talks at the meeting discussed phenotypic screens and candidate molecules whose pharmacology “isn’t emergent until you are in a complex system,” Wells told *SciBX*. He added that “diseases often don’t converge on a single target.” In these cases, or if the biology of the disease is poorly understood, “you are better off doing a phenotypic screen.”

Nonetheless, Wells added that “the challenge with phenotypic screening is being able to find out the target or targets that are being hit” by the candidate molecule when a disease-modifying effect is observed. Indeed, that basic challenge has discouraged some drug developers from using phenotypic screens in their early stage discovery efforts.

However, Wells believes the situation has recently changed, placing phenotypic screens in a more favorable light. “Our ability to identify the target is improving, and there will be additional technologies that come out to identify the target, which will make people much more comfortable” with using phenotypic screens, he said.

A discovery channel

Some biotechs and pharmas have a significant focus on phenotypic screening.

Mark Fishman, president of the **Novartis Institutes for BioMedical Research (NIBR)**, initiated a phenotypic screening effort when he moved to Novartis 10 years ago. “For me it’s a discovery tool. The single biggest impediment to drug discovery is the small number of new, validated targets that we have. Phenotypic screening is one way of moving beyond well-defined targets from the literature to discover new therapeutic targets and new disease biology,” he told *SciBX*.

NIBR runs a substantial number of phenotypic screens. “It’s not necessarily a majority, but it’s a lot,” Fishman said. NIBR’s phenotypic screens range from single cell types to highly complex tissues that incorporate many different cell types.

The cell-based assays sometimes have relatively straightforward readouts. “For example, in oncology we look at what we now call the Cancer Cell Line Encyclopedia (CCLE)² at relatively simple phenotypes such as inducing apoptosis.”

CCLE is a collection of nearly 1,000 cell lines derived from human cancer patients that have been genetically characterized and can be used to identify molecules that selectively kill cancer cells with particular genomic alterations.

“Even more revelations, though, will come from screens that consist of more complex tissues, for instance organotypic screens,” said Fishman. Organotypic cultures use tissues that retain features of a multicellular organ. For example, NIBR scientists use a multicellular system reconstituting many features of the gut to study regeneration of epithelial cells of the intestine after injury, Fishman noted.

“These types of assays are not trivial to set up, to maintain or to screen. But they give you a lot of information on cell-autonomous and non-cell-autonomous effects,” said Fishman.

Fishman said NIBR has discovered molecules acting via new mechanisms through phenotypic screening, although none is yet in the clinic.

He also noted that discovering molecules that modify disease-associated phenotypes can be relatively rapid. “What becomes slower is discovery of the target and target validation,” he added. In most cases, “We would prefer to have the target in hand” to do better validation and to enable structure-based drug discovery. “We’re somewhere in the 40% range for successfully finding the target.”

The approach that has proven most successful for target identification following a successful phenotypic screen is a combination of genetics and chemical proteomics, said Fishman. Chemical proteomics is a method in which an active ligand is attached to a bead and used to pull down interacting proteins from cell or tissue lysates, which are then identified by mass spectrometry.

If the company cannot identify the target, Novartis will sometimes proceed anyway, because knowledge of the target is not always a regulatory requirement for drug approval. “For instance, we are pursuing a very early candidate in spinal muscular atrophy because the disease is so desperate,” Fishman said.

But he noted that preclinical and clinical development is substantially more challenging with an unknown target, particularly because it is more difficult to predict mechanism-based toxicities.

From phenotype to target

A commitment to phenotypic screening requires complementary methods to subsequently identify the targets of active molecules. For example, GSK is returning to phenotypic screens now that the company has built up a chemical proteomic platform that provides a means to do that.

GSK had nearly abandoned cell-based screens 10 years ago in favor of target-based screening, said Timothy Willson, director of chemical biology at GSK. Now “a significant percentage” of the screens being run at GSK are cell based, he said.

What turned the tide, according to Willson, is chemical proteomics, which can often identify the targets of phenotypic screening hits.

To illustrate the change, Willson said that four years ago GSK

ran five cell-based screens focusing on pathways relevant to metabolic diseases, such as insulin secretion. In every case, “we found nice, chemically tractable molecules.” But at the time “we didn’t have a robust way of finding the molecular targets. We had nice chemical leads and they looked good in our phenotypic assays, but all lost out on prioritization to target-based screens because we couldn’t tell someone what the target of the molecule was.”

Willson has since led the establishment of a chemical proteomics group within GSK. “In the last few years there has been almost a revolution in using chemical proteomics to find the targets of

molecules. In a month to six weeks we can go from asking the question to getting the answer in approximately 70% of the cases,” said Willson, although he noted that the success rate may depend on the phenotypic screen being run and the targets involved.

“This is really driving the resurgence of interest within GSK. We think we’ll be able to find tractable molecules and their targets,” he said.

Staying on target

In contrast to Novartis and GSK, Genentech remains focused on target-based screens, believing that too much time and resources are needed to identify the targets of a phenotypic screen.

The company does not typically screen large chemical libraries in cell-based assays, according to Varney. Although there may be exceptions, he said that “it is not a common occurrence—there is a high bar.”

The key hurdle, as Varney sees it, is the difficulty in identifying the targets of active molecules. “What I feel going on in the industry is that phenotypic screens are starting to make the turn—becoming trendy, in vogue. But without some fundamental advance in target deconvolution such as easily conducted interaction or affinity screens, I see this as a big effort with relatively little value,” said Varney.

Varney told *SciBX* that knowing the target is critical because the company’s pipeline portfolio committee would always favor advancing a compound with a known target into development. “In the end, if you don’t know what the target is that you are pursuing, even with the greatest phenotypic readout the project won’t compete in the portfolio,” he said.

Additionally, Varney noted that even if researchers can find the target, there is no guarantee that it will be a tractable target for lead optimization or that the target will have the right toxicity profile or even elicit the amount of efficacy needed. “The overall effort relative to the likelihood of success is too low” with phenotypic screening, said Varney.

Changing the equation

Additional tools and approaches are being developed by academia and pharma that could more rapidly or more successfully identify the targets of active molecules identified in phenotypic screens.

For example, Brian Shoichet, professor in the Department of Pharmaceutical Chemistry at UCSF, has been developing computational approaches for identifying small molecule targets.³ The approach he is using can identify putative targets more rapidly and less expensively than current experimental approaches.

“When you get a molecule that lights up the right biological circuits, you can potentially figure out why very quickly,” said Shoichet. He noted that currently a compound inhibits the predicted target, at least *in vitro*, about half of the time.

In an alternate strategy, **Medicines for Malaria Venture (MMV)** has joined up with three independent teams led by GSK, the **Novartis Institute for Tropical Diseases (NITD)** and **St. Jude Children’s Research Hospital** to test an open-source approach to target identification.

The GSK, NITD and St. Jude teams independently conducted phenotypic screens looking for antimalarial compounds^{4–6} and are now pursuing preclinical and clinical development of the top hits from their screens, said R. Kiplin Guy, chair of the Department of

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Chemical Biology & Therapeutics at St. Jude. Guy led one of the three teams.

MMV is making other high-quality, active molecules identified in the screens available to the research community. MMV is freely distributing a “malaria box”—a collection of 400 diverse antimalarial compounds—with only the request that any resulting data be published.

The hope is that crowd-sourcing phenotypic screening follow-up in a precompetitive space will speed up the identification and validation of new antimalarial targets, said Guy.

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COMPANIES AND INSTITUTIONS MENTIONED

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