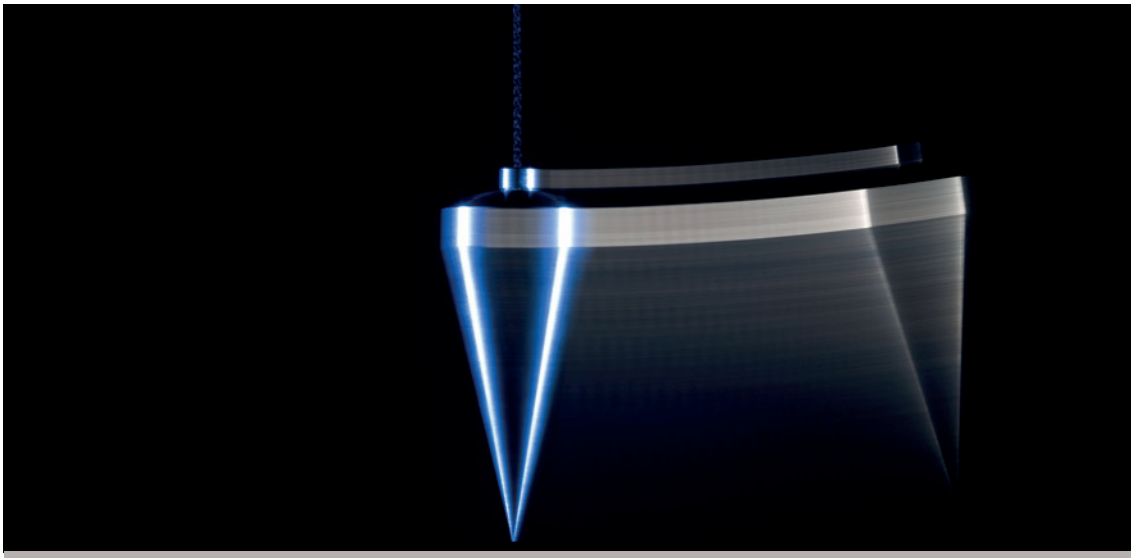

Ali Kabas/Alamy Stock Photo

The phenotypic screening pendulum swings

Industry and academic scientists are working together to figure out when and how best to use phenotypic screening in drug discovery.

Asher Mullard

Phenotypic screening is making a comeback. Rather than just searching for drug leads on the basis of a preconceived notion that a particular target is important, this means that drug companies are increasingly running unbiased screens to find compounds that induce disease-relevant changes in disease-relevant cells. Advocates of this approach hope that it could lower the clinical trial attrition rate and reduce herd mentality between companies who are all chasing the same targets. But because phenotypic screening is much more complicated than target-based screening, uncertainty remains over how to make the most of the phenotypic resurgence.

So, in October, 150 scientists from all the big pharmaceutical companies and from academia met at the New York Academy of Sciences to tackle the outstanding questions. What cells are worth using, and which phenotypes are worth measuring? How do you figure out which target a promising phenotypic screening lead binds to? And, how do you convince management to give the green light to phenotypic screening projects in the absence of target information?

"Do not expect a consensus," cautioned John Moffat, senior scientist at Genentech and one of the meeting organizers, as he wrapped up the one-day event.

Tick tock

By some accounts the pendulum started to swing back towards phenotypic screening in 2011 after a landmark study looked at the origins of 50 first-in-class small molecules that were approved between 1999 and 2008. The researchers found that 28 (56%) of first-in-class approvals had come from phenotypic screens, 17 (34%) had come from target-based approaches and 5 (10%) were synthetic or modified versions of natural substances, such as enzyme replacement therapies (*Nat. Rev. Drug Discov.* **10**, 507–519; 2011).

"There has been more success with the phenotypic discovery than people had appreciated," concluded David Swinney, CEO of the Institute for Rare and Neglected Diseases Drug Discovery and one of the authors of the study. He also argued that industry's focus on target-based discovery approaches since the 1990s could be contributing to the high attrition rates in clinical trials.

Subsequent analyses have muddied the waters somewhat. When a team from Novartis looked at 113 first-in-class drugs approved between 1999 and 2013,

they found that 78 (69%) originated from target-based approaches, compared with 33 (29%) that were discovered in the absence of a target hypothesis (*Nat. Rev. Drug Discov.* **13**, 577–587; 2014). And when Moffat and colleagues at Genentech analysed just the small-molecule cancer drugs approved in the same timeframe, they found that of the 15 first-in-class approvals, 11 (73%) originated from target-based programmes and 4 (27%) originated from phenotypic screens (*Nat. Rev. Drug Discov.* **13**, 588–602; 2014). However, the Novartis analysis included biologics, such as monoclonal antibodies, that were primarily discovered through target-based approaches. And oncology is a therapeutic area in which target-based small-molecule screening strategies have been particularly popular, driven by the success of kinase inhibitors.

These analyses — coupled with the growing appreciation of the limitations of target-based screening — are driving a comeback for phenotypic screening. For example, nearly 50% of Novartis's screens are now phenotypic, up from 20% 5 years ago.

Designing smaller, greyer boxes

The key to success with phenotypic screening lies in assay design, said Fabian Vincent in a short presentation at the meeting. An associate research fellow at Pfizer, Vincent has enumerated three key rules for phenotypic screeners (*Sci. Transl Med.* **7**, 293ps15; 2015).

First, the assay system needs to use cells that are as physiologically relevant as possible. Primary cell lines and induced pluripotent stem cells (iPSCs) trump generic cell lines such as HEK293 cells or lines that have been genetically modified to overexpress a single protein. Second, the stimulus that is used to induce a measurable phenotype needs to be selected carefully. The use of non-relevant stimuli, such as hydrogen peroxide to induce cellular injury, might not reflect disease biology, meaning that screeners will miss relevant hits. By using disease-like biological systems such as primary cells and iPSCs that inherently contain phenotype-inducing stimuli, researchers may be able to sidestep this problem. Third, the closer the assay's readout is to a clinically relevant end point, the better. The best assay end points are physical in nature, like muscle contraction rather than gene expression, said Vincent.

"Our goal should really be to miniaturize the disease in the well and to use the clinical end point in the assay," he summarized.

These efforts are helped by the availability of iPSCs, which have brought once inaccessible cells into the lab. For example, several groups are now using human neurons that carry disease-specific mutations to screen for drugs (*Nat. Rev. Drug Discov.* **14**, 589–591; 2015).

> "Our goal should really be to miniaturize the disease in the well and to use the clinical end point in the assay"

There has been a lot of scepticism about whether you can work with iPSCs in a reproducible, high-throughput manner, said Michael Jackson, senior vice president of drug discovery and development at Sanford Burnham Prebys Medical Discovery Institute. "We believe it is possible," he adds. "We can get highly reproducible data well to well to well, looking at multiple end points." Jackson showed in his presentation how iPSCs can be paired with high-content analytical systems to measure multiple end points at the same time.

Although phenotypic screening has historically been particularly fruitful for anti-infective and anticancer drugs, presenters discussed progress in cardiac regeneration, type 2 diabetes and psychiatric diseases. Researchers are pointed to rare genetic diseases as an area that is ripe with phenotypic screening opportunity.

As a case in point, scientists from Roche and Novartis independently presented promising progress with two small molecules for the treatment of spinal muscular atrophy (SMA), a splicing-defect disease. Friedrich Metzger, head of Discovery Rare Diseases at Roche, first described how his team used a phenotypic screen to identify an *SMN2*-expression-boosting small molecule (*Science* **345**, 688–693; 2014). Ongoing Phase II trials of this drug, RG7800, are tracking *SMN2* mRNA and protein levels as clinical end points, demonstrating the move towards assay end points that are clinically relevant.

Susanne Swalley, a biochemist at Novartis, then showed how her team set out to understand the mechanism of action of LMI070, their Phase II SMA candidate that came out of a phenotypic screen. After traditional target deconvolution tools failed, the team eventually used a tailored approach to trace the drug's efficacy as far as an interaction with the splicesome, the molecular machinery that removes introns from mRNA (*Nat. Chem. Biol.* **11**, 511–517; 2015).

Both Metzger and Swalley said that their companies were prepared to move their drugs into clinical trials even without insight into the mechanism of action of their hits.

"It is impressive that two pharma companies found some interesting chemicals, and then conducted at-risk chemistry to optimize those leads without knowledge of their targets," said Jackson. This shows a willingness to pursue programmes when the phenotypic assays are 'rock solid', he added.

Target identification

New technologies and approaches are making it easier for drug hunters to figure out which targets their phenotypic hits are binding to.

Target deconvolution often relies on chemical proteomics strategies in which researchers chemically modify a lead compound so that it — and its target — can be purified or probed. But because modifications can change the properties of small molecules, these approaches often fail to find targets for phenotypic hits. Computational and label-free proteomics approaches are now emerging as ways to dissect the biological pathways at play.

Andras Bauer, a senior scientist at Boehringer Ingelheim, described how his team uses a chemical similarity search to figure out which targets a molecule will bind to on the basis of structural features that it shares with ligands that have known targets. Researchers at Novartis have also published their structure-similarity-based approach (*Proc. Natl Acad. Sci. USA* **109**, 11178–11183; 2012). With a hypothesis in hand, Bauer and colleagues then use a label-free mass spectrometry approach to check whether the ligand does indeed bind to a suspected target.

Aravind Subramanian, a computational biologist at the Broad Institute, presented the LINCS transcription-based approach to target deconvolution. His team has identified 1,000 genes that provide a signature of the transcriptional activity of a cell. By exposing cell lines to thousands of drugs and genetic modifications, his team is now in the process of capturing 10 million signatures. By comparing the signature that is induced by a phenotypic hit with the LINC signature library, the program can suggest other compounds, gene knockdowns or gain-of-function mutations that have similar effects. They currently have around 1.5 million signatures, which are already providing compelling matches. The three closest signature matches for pitavastatin, for example, are compounds in the same class — lovastatin, simvastatin and mevastatin.

The top genetic match for an mTOR inhibitor was a cell line in which mTOR expression had been knocked down.

"This is a hypothesis generation tool," said Subramanian. "The results give you interesting clues as to what your compound might do."

In a third new strategy, Gulio Superti-Furga, of the Austrian Academy of Sciences, makes use of the fact that targets tend to be more stable when they are bound to their ligands than when they are unbound. This principle is already used in cell-free assays to check when known targets are interacting with their ligands. But Superti-Furga has now shown that it holds in cell-based systems as well, and can be used in an unbiased manner to identify ligand–target interactions (*Nat. Methods*, published online 21 Sep 2015).

Because the assay takes place in a physiological context, and neither the ligand nor any of the cellular targets are labelled, the approach offers key benefits over other proteomics approaches. Although there are cases where the approach may not work — for instance, when a large protein complex is unlikely to be stabilized by a few extra interactions with a small molecule — the technology offers the potential to be transformative in industry, said Superti-Furga.

Genome-wide CRISPR knockdowns could also revolutionize target deconvolution, said Marco Prunotto, the phenotypic drug discovery and target identification lead at Roche and one of the organizers of the meeting. By using CRISPR technology to individually knock out each gene in the genome, and by then treating knocked out cells with an active compound of interest, researchers may be able to figure out which genes are key to a compound's activity (*Science* **343**, 80–84; 2014). "All the drug companies are already doing this," he said.

"The good news is that the technologies to deconvolute targets are now so powerful that it really should be feasible with some effort to figure out what the target is for most drugs," said Superti-Furga.

Others were more muted in their excitement for these technologies. "There are many drugs on the market that have been on the market for many years, and we still don't know how they work," said Jackson. "So why do we think we are going to necessarily have a lot of success with new deconvolution technologies?" He and others noted that it is still early days for all the new technologies, and their limitations and true potential remain to be established.

Jackson was also among a vocal majority of attendees who argued that target deconvolution is a luxury rather than a necessity anyway. Although it helps to have a target during lead optimization efforts, in toxicology testing, in dose finding and in trial design, the FDA regularly approves drugs without an understanding of their mechanism of action. "It's not that we shouldn't be trying to find the target," he said after the meeting, "but it is not an absolute need to know."

Despite clear optimism about the future of phenotypic screening, it remains to be seen just how far the pendulum will swing. "It is one thing to get phenotypic screeners to say that this is the way forward," said Swinney, who did not attend the meeting. The bigger challenge ahead will be to continue to get buy-in from everyone else involved in drug discovery.