HIROSHI WATANABE/DIGITALVISION/GETTY IMAGES

Drug induced liver injury is one of the major reasons for late stage clinical trial failures and post-approval drug withdrawals.

TRANSCRIPTOMICS BRINGS NEW ERA OF TOXICOLOGY PREDICTION

HIGH-THROUGHPUT METHODS of measuring gene activity will play a major role in delivering safer drugs to the market.

In 2013, fasiglifam, a promising diabetes drug candidate, was withdrawn from late-stage clinical trials by its developer, following signs of liver damage in the trial participants. Like all drugs tested in humans, fasiglifam had been investigated for toxicity in animals. Neither those preclinical studies, nor the small, early phase clinical trials, revealed issues serious enough to stop development before the large and costly phase III stage began.

Fasiglifam is far from being the only example. As the main site for drug metabolism, the liver is particularly vulnerable to compounds that alter its function, and drug-induced liver injury is one of the most common reasons for failure in drug development. Many liver issues are noticed only during late-stage clinical trials, or even after market approval: between 1953 and 2013, approximately 18% of drug withdrawals from the market were due to liver damage[1].

Today, fasiglifam is proving useful in another context. It is one of thousands of compounds that Cyprotex, a company specializing in toxicology, is including in its reference library to help predict drug-induced liver injury before clinical trials. "The idea is to make better informed decisions earlier in your discovery campaign when you can select potentially safer compounds, rather than finding a safety liability later on," says Paul Walker, Vice President Head of Toxicology at Cyprotex, in Cheshire, UK.

Cyprotex has joined forces with parent company, Evotec, a leader in the field of transcriptomics. Understanding the role of transcriptomics in drug-induced toxicity is a key focus for the team. This technique sequences thousands of molecules of messenger RNA to identify which processes are active in the cell. Any compound that affects the biology of the cell will alter the pattern of

messenger RNA activity.

Transcriptomics was able to identify problems in liver cells treated with fasiglifam that could have been a red flag earlier in the drug's development. "Our studies have found potential effects on mitochondrial function, which were previously missed in preclinical studies," says Walker. As such, transcriptomics has the potential to supplement or reduce *in vivo* toxicology studies by effectively identifying safety signals early in drug development, saving time and money — and animal testing.

High-throughput screens

The big advantage of transcriptomics is its use of human cells. And not just individual cells in a dish, which can never recapitulate the complex systems within the body, but 3D 'organoids' that have advanced the translational relevance considerably. These spheres of thousands of cells, only 200 microns in size, allow the creation of heart tissue that beats, or liver tissue that secretes enzymes for days. "On top of that, a 3D system allows repeat dosing, mimicking dosing regimens *in vivo* and potentially helps to detect effects due to toxic metabolites," says Walker.

"OUR STUDIES HAVE FOUND POTENTIAL EFFECTS ON MITOCHONDRIAL FUNCTION, WHICH WERE PREVIOUSLY MISSED IN PRECLINICAL STUDIES."

In addition, these organoids can be placed in 384-well plates and individually molecular barcoded for simultaneous sequencing. It's this high-throughput process that opens up transcriptomics to wider adoption.

"People have thought about using transcriptomics for toxicology before, but it was always a numbers game," explains Rüdiger Fritsch, Principal Scientist and Project Lead for EVO*panOmics* at Evotec. Toxicology studies typically require testing of several doses, at different times, and in multiple organs. Performing that for dozens or even hundreds of compounds requires a robust high-throughput approach.

"For any compound that's a real troublemaker, the evidence will show up in the transcriptomics data if you profile it in a relevant model," adds Fritsch. "You just need to test appropriate dosing-scenarios with the breadth of genome-wide off-target effects so that you have a chance to find it."

Learning from reference databases

Evotec offers transcriptomics services to drug developers and carries out the entire process in-house, from growing the organoids to sequencing and analysis. This pipeline allows its researchers to screen hundreds of compounds a day, each delivering tens of thousands of data points on RNA levels. To analyse all of these data, Evotec has developed a software platform called EVO*panHunter* that interactively visualizes changes.

"We want to democratize data analysis for the biologists who know the biological pathways and processes, without them needing to rely on additional experts from the bioinformatics department for routine tasks," says Carla Tameling, Head of Sales and Application for EVO*panHunter* at Evotec.

Machine learning helps to trawl through this immense amount of data. "The more data we get, the harder it is for a human to dig through it all," explains Tameling. The software is there to find patterns and alert the researchers to dig deeper. "Transcriptomics is an unbiased view," she adds. "You don't need to define what to look at prior to your studies — you get all the data, and you might see things that you didn't think would be relevant initially."

With support from large, well-characterized reference databases, as Cyprotex has for drug-induced liver injury, machine learning can also compare the pattern of gene activity to known toxic molecules and make its own prediction of whether a compound is likely to have issues. The team is also building databases for understanding toxicity in other organs, such as heart, kidney and brain, using publicly available drug development trial results. "We're running reference compounds from all kinds of sources where we know there are either late-stage clinical findings or withdrawals from the market," states Walker.

The pressure to replace

The difference in metabolism between species is one of the major reasons why toxicology predictions can be inaccurate. It is estimated that only half of compounds that cause liver toxicity in humans are picked up by animal studies[2].

"Animal toxicity testing has proven to be useful, but there are gaps," says Fritsch. One of the key aspects with the liver is species-specific drug metabolism. Due to this "the concordance with humans is one of the lowest of any organ toxicity. That was one of the reasons we prioritized liver injury prediction using the transcriptomics tools together with human liver *in vitro* assays".

Currently, all pharmaceutical candidates are legally required to be tested in animals before entering clinical trials. However, regulators and lawmakers are increasingly looking to replace these tests with non-animal methods. The US Environmental Protection Authority announced in 2019 that it would stop conducting or funding chemical safety testing in mammals by 2035. And in September 2021, the European parliament passed a non-legally binding resolution to phase out the use of animal testing for research and toxicology purposes.

"IT'S ONLY A MATTER OF TIME BEFORE PANOMICS BECOMES A REGULATORY STANDARD APPROACH."

"We're a long way from getting a drug to market based only on *in vitro* data," says Walker. "However, we're increasingly seeing these data being presented as part of regulatory submissions, and we're working with regulators to help them understand these assays."

"The technology is advancing rapidly, and we will have even more tools at our disposal in five years from now," adds Fritsch. "As various industries move towards human-relevant alternatives to animal testing, it's only a matter of time before panomics becomes a regulatory standard approach for preclinical toxicity testing." ■

REFERENCES

1. Onakpoya, I.J., Heneghan, C.J. & Aronson, J.K. *BMC Med* **14**, 10 (2016).
2. Olsen, H. *et al. Regul Tox Pharmacol* **32**, 56–67 (2000).

nature research CUSTOM MEDIA

evotec cyprotex AN EVOTEC COMPANY