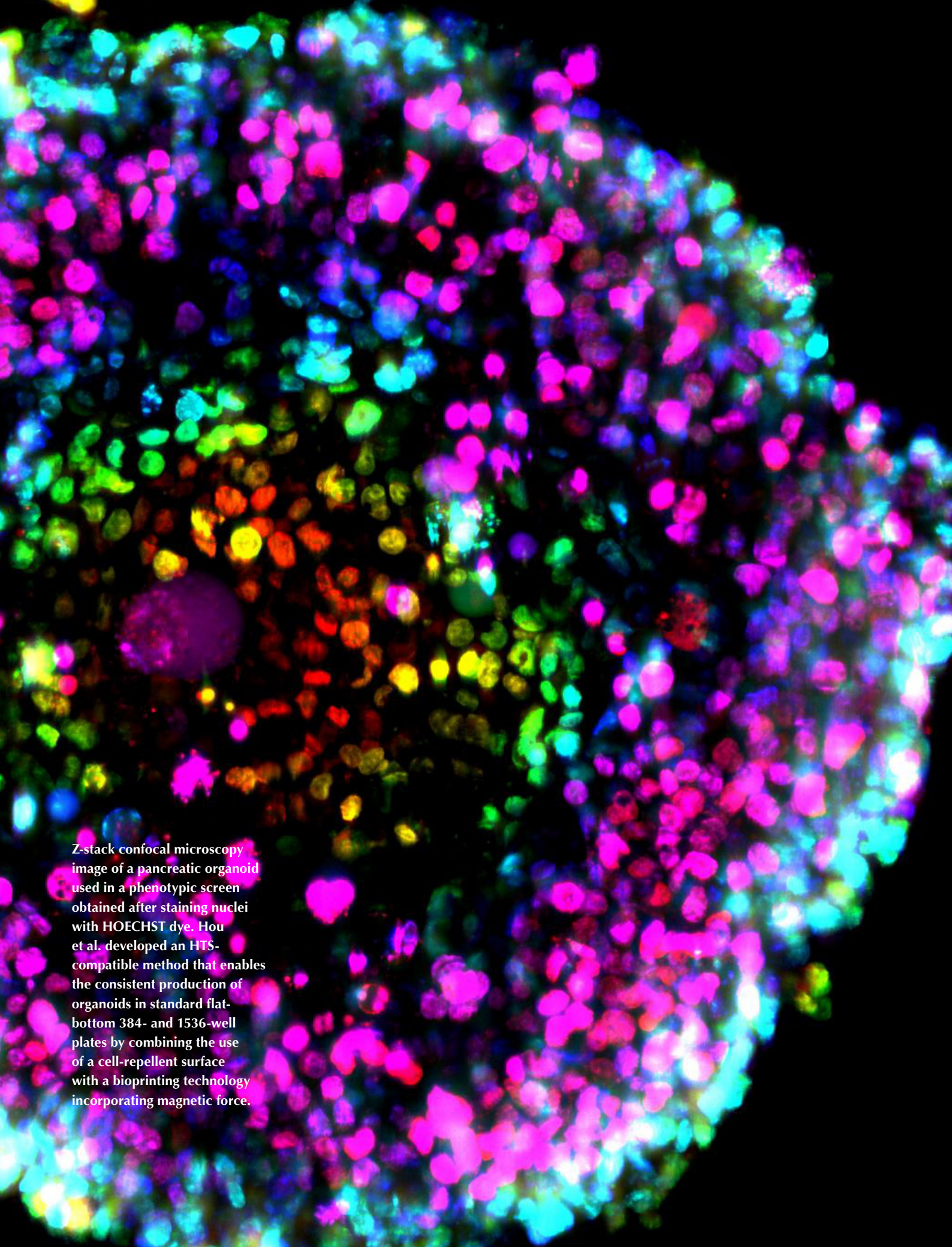


Z-stack confocal microscopy image of a pancreatic organoid used in a phenotypic screen obtained after staining nuclei with HOECHST dye. Hou et al. developed an HTS-compatible method that enables the consistent production of organoids in standard flat-bottom 384- and 1536-well plates by combining the use of a cell-repellent surface with a bioprinting technology incorporating magnetic force.

# Side effects - a thing of the past....?

**DRUG DEVELOPMENT** Despite huge investments into drug safety, the pharmaceutical industry still loose hundreds of billions of dollars by safety-related attrition. For both, patients and pharma R&D productivity an improvement would add significant benefit. Novel biomarkers designed to detect drug induced organ injury preclinically and in Phase I trials could improve the situation.

Did you know that 500,000 patients per year are hosptialised after having taken a virtually harmless drug such as Paracetamol? 500 of them die from liver-toxic adverse effects – and that's just the figure for the United Kingdom. With estimated 2,500 deaths per week, taking properly prescribed drugs is the fourth leading cause of death in the US.

However, drug safety issues mostly occur rarely so that drug developers can recognise them not until late-stage clinical testing or not before market authorisation. Because currently there is no means, to monitor i.e. drug-induced organ damage, in worst case potentially life-saving drugs must be withdrawn from market ruining the development work of 13 years – the average time a drug needs from bench to bedsite – along with huge development costs.

"Even a Phase III failure could mean a waste of €1bn or more", says Dr. Michael Merz from ETH Zurich. Merz, an industry expert in preclinical and clinical drug safety, coordinates a 5-year project (see interview p. xx-xx) that aims to identify drug-induded safety issues much earlier in the drug development process – during preclinical or Phase I development. If successful, the impact of TransBioLine, the name of the €28m project kicked-off by the Innovative Medicines Initiative (IMI) in April 2019, could be huge. Experts estimate the financial burden of safety issues for industry to range in the three-digit billion dollar range per year.

Overall, 20% of clinical trail failures and more than 65% of post-launch withdrawls have been attributed to clincal safety issues such as organ-based, mechanism-based or off-target toxicity. A recent analysis of the attrition of 812 drug candidates from AstraZeneca (AZ), GlaxoSmithKline (GSK), Pfizer, and Takeda, revealed that "non-clinical toxicology was by far the highest cause of attrition, accounting for 40% of drug failures, while Phase I safety issues contributed to further 25% in failure. The industry experts stress that "although minimising safety-related attrition has been a significant area of investment across the industry in the past decade, it remains a key area for improvement that could only be addressed by collaboration and development of new assays tackling the complex problem". In 2014, the FDA estimated that just a 10% improvement in the ability to predict drug failures before clinical trials could save $100M in development costs per drug.

**TORSTEN HOFFMANN, PHD**
**Senior VP Drug Discovery, Taros Chemicals GmbH, Dortmund**

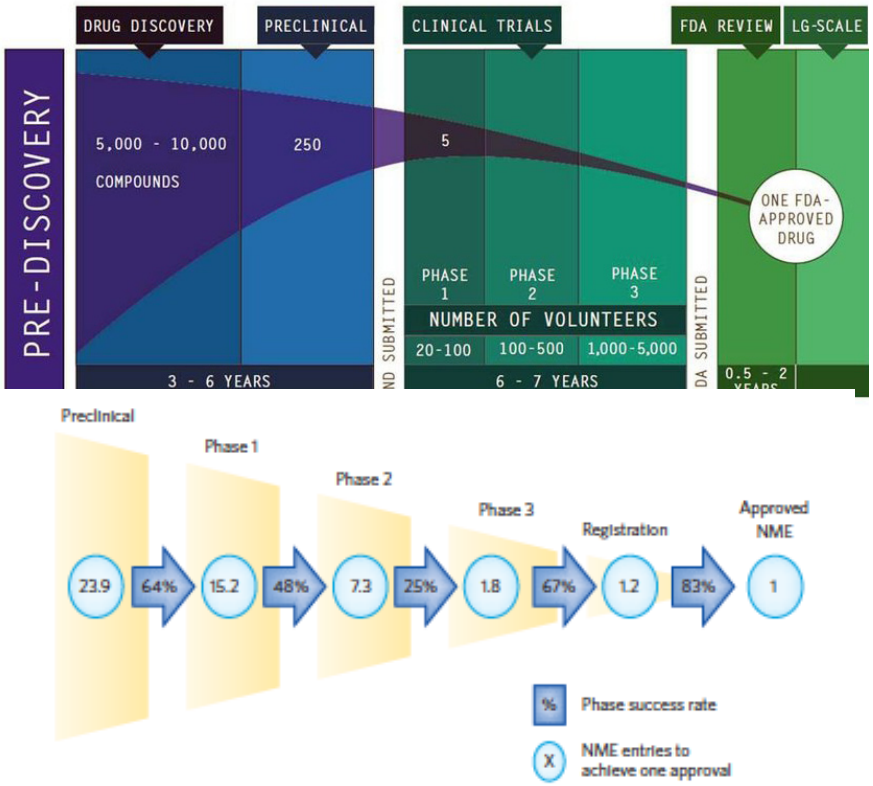
? What value do the industry loose annually because of drug safety problems?

! *Viewing the fact that since 2014 pharmaceutical revenues exceeded US1tn and that in Phase III studies alone a failure rate of 30% is observed, the failure rate due to safety during clinical development can be estimated to amount to globally unrealised revenues of a 3-digit billion dollars figure.*

## Drug safety: a complex task

According to Dr Joanne Bowes, Global Safety Assessment, AstraZeneca, the problem is complex as types of adverse drug reactions (ADRs) vary broadly: about 75% are dose-dependent and principally predictable from primary, secondary and safety pharmacology. However, inpredictable idiosyncratic responses; dose-related ADEs; long term adaptive changes and delayed rebound effects following discon-

Pictures: Shurong Hou, SLAS Discovery, doi: 10.1177/2472555218766842 (left), Raros Chemicals GmbH, Dortmund (right)




**Stages (A) and attrition rates (B) within the drug development process. Attrition rates vary with the medical field addressed: with respect to cardiovascular diseases, only 20% of agents earn FDA approval; for anticancer drugs, the approval rate falls to 5%.**

tinuation of therapy make up the 25% of mostly lethal ADEs.

Roughly two decades ago, Big pharma companies realised that datasets from individual companies were too small and thus insufficient to solve the drug safety issue. For the first time within decades, they began to share data and to collaborate precompetitively. Two FP6 pilot projects coordinated by the European Federation of Pharmaceutical Industries and Associations (EFPIA) – termed InnoMed AddNeuroMed and InnoMed PredTox – ended in the formation of the Innovative Medicines Initiative (IMI) in 2008, which has attracted €5bn in funding so far from the EFPIA and the European Commission.

## From target selection …

Current industry strategies to improve the management of safety issues are two-tiered: On the one hand, drug developers try to sort out drug candidates in the early drug discovery process that might show mechanism-based or off-target safety problems.

> An overreliance on animal models of disease has in part led to the poor levels of Phase II survival."

Companies such as Pfizer, Takeda or GSK and CROs reportedly apply so-called pharmacological promiscuity indices to exclude problematic compounds that activate safety relevant targets such as the cannabinoid receptor CB1, a well-documented inducer of suicidality. With new tools such CRISPR editing, RNAi or knock-outs they have identified dozens of such safety-averse targets.

"In the end, successful pharmaceutical R&D comes down to careful target selection", says Torsten Hoffmann, Vice President Drug Discovery at Taros Chemicals GmbH, which coordinates the European Lead Factory (ELF), an EU drug discovery resource launched in 2013 by the IMI. In May ELF got €36.5m funding for the ESCulab project, which aims to find new targets and compounds by high throughput and phenotypic screens against the Joint European Compound Library including 550,000 compounds.

Machine learning has also been adopted by drug discovery companies, predicting a functional impact score from gene ontology annotations, transcriptional profiles and off-target sites across different species. Drug discovery CROs such as Hamburg-headquartered Evotec SE or pharma companies also benefit from MetaMapTox, the largest metabolomics database globally, which was developed by Bayer subsidiary metanomics. The database represents more than 100 validated toxicological modes of action in several rat organs.

## … to translational biomarkers

The other white hope of drug development companies is identification and validation of translational biomarkers that are sensitive and specific enought to assess drug-incuded organ injury in animal models, and healthy volunteers enroled in early-stage clinical trails.

"An important task of preclinical safety research is to identify the doses at which new compounds cause adverse effects. Therefore, biomarkers with well-established performance characteristics are required, " says Jan Hengstler, Head of system toxicology at IFADO, Dortmund. "Numerous useful biomarkers of toxicity have been established in the past. Therefore, the challenge of current research is to clearly demonstrate whether new biomarkers show superior performance metrics in a specific context."

According to Merz, existing biomarkers such as creatinine or ALT are often mostly not sensitive enough to detect drug-induced organ injury before the organ has already been significantly damaged. Thus, the search is ongoing for novel biomarkers that work both, in animal models and

Pictures: Cancer Cell, doi: 10.1016/j.ccr.2013.09.007

humans. If toxicity issues are seen preclinically, usually, a programme is killed, though there is no proof that the ADEs will also occur in humans. Technologies that emerged in the past few years now offer new options to establish such translational biomarkers or brigde the safety predictability gap by screenings on human cells.

According to Hoffmann, "the most promising emerging and bridging technology relate to human organ-on-a-chip systems in which biomarkers are used to to establish a therapeutic index." Such systems use reprogrammed human induced pluripotent stem cells (hiPSCs) or organoids embedded in a matrix that mimics the physico-chemical conditions of organs (such as beating cardiomyocytes) and connect compartments through microfluidic channels.

Within FP7 and Horizon 2020, IMI consortia led by Roche and Pfizer invested €90m to establish quality-controlled hiPSC cell banks StemCellBanc (2012, Basel) and EBiSC (2014, Cambridge, UK/ Saarbrücken, Germany) along with differentiation and manufacturing protocols into human cells sufficent for secondary drug screenings. Consortia of EFPIA member companies have also played a prominent role in validation studies aimed at establishing novel biomarkers for drug-induced organ damage.

A decade ago, the situation was described by the FDA as follows: "Three organs needed better clinical monitoring of drug- induced injuries"

- Kidney: current standard biomarkers increase only once 50-60% of kidney-function is lost.
- Liver: standards are not sensitive and specific enough and do not adequately discriminate adaptors from patients at high risk to develop liver failure.
- Vascular System: currently no biomarkers available for drug-induced vascular injury in humans.

## Prominent role of IMI

Within the IMI pilot Innomed Predtox, 15 pharma companies and two SMEs identified damage markers such as KIM-1, CLU or TIMP-1 for drug-induced kidney (DIKI) injury by means of transcriptome and proteome analysis. New biomarkers were added by the Predicted Safety Testing Consortium (PSCT) established in 2006 under supervision of the FDA's Critical Path Institute. In 2008 first candidate

# The art of biomarker validation

**DIAGNOSTICS** It's not easy to find predictive biomarkers that specifically reflect organ function. European Biotechnology spoke with biomarker expert Andreas Bergmann, how to find the needle in the haystack.

**EuroBiotech**_There is a 20-year history of failure in developing biomarkers that specifically reflect renal function. Why?
**Bergmann**_*There are many blood or urine biomarkers that look good on first sight but do not take the multimorbidity of the real patient into account. While working well in cell culture or under the artificial conditions of an animal model, all biomarkers supposed to reflect kidney injury failed to specifically do under real-life conditions. The reason is, biomarkers such as KIM-1, NGAL etc. are sensitive enough to do their job; however, their test results are massively affected by co-morbidities, first of all inflammation. So, elevated values may not reflect kidney damage or renal function but inflammation. The consequence is, these biomarkers are unsuitable for the large group of patients who have inflammatory comorbidities, for example sepsis or diabetes. There is a unmet need of biomarkers that independently and specifically display kidney function – not only in drug safety assessment but also in critical care.*

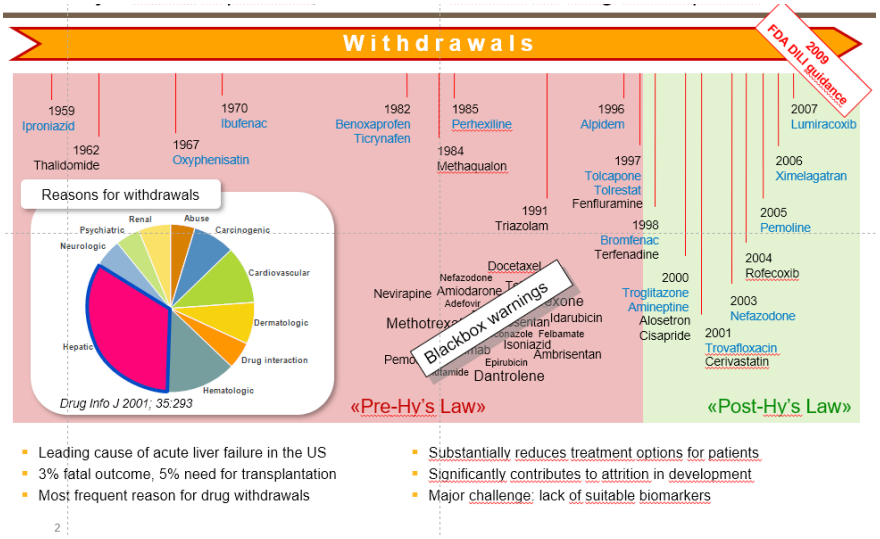
**EuroBiotech**_What about existing standard or reference biomarkers?
**Bergmann**_ *Creatinin values are not dependent from inflammation but are not sensitive enough. At earliest you see elevated values two days after renal injury occured. There is an in vivo reference method, "true" glomerulal filtration rate (GFR), that reflects acute kidney function but costs $1,000 per measurements and requires prior injection of a radioactive or fluorscent labels.*

**EuroBiotech**_What has to be done to have better markers?
**Bergmann**_*Identify a biomarker that independently reflects kidney function, i.e. true GFR, in blood biobanks of long-term epidemiological cohorts, and then validate it under real-life conditions in a large population of patients with and without inflammatory co-morbidities*

**EuroBiotech**_Do marker panels may improve the situation?
**Bergmann**_*They increase sensitivity, what is good in safety assessment, but they also multiply false-positive results.*

**Dr Andreas Bergmann**, CEO Sphingotec GmbH, co-founder of Brahms AG, Adrenomed AG, and Sphingotec Therapeutics, developed the $600m/ year sepsis biomarker PCT and a series of acute care biomarkers predicting kidney and vascular function.

Pictures: Dewpoint Therapeutics Inc




**The IMI's TransBioLine project's work includes the two major threats to patients: drug-induces liver injury and drug-induced vascular injury contribute 25% each to safety-releated clincial attrition rate.**

biomarkers were passed to the FDA for qualification. The qualification work was continued and extended to biomarkers for drug-induced liver injury (DILI) and cardiovascular injury (DIVI), which contribute to 50% of attrition due to safety problems, by the IMI's SAFE-T consortium (2009, budget €36.5m) under lead of Pfizer and Merz, who led preclinical and clinical safety at Novartis at that time.

In 2018, the FDA qualified six renal injury biomarkers for use in Phase I studies: albumin, β2 microglobulin, clusterin, cystatin C, KIM-1, total protein, and trefoil factor-3. Qualification will be completed for DILI and DIVI markers and started for pancreatic and CNS injury markers within the new IMI project TransBioLine (see interview p. xx).

Jürgen Wnendt, CEO of MLM Medical Labs, a CRO participating in TransBioLine, stresses it would be crucial to continue TransBioLine activities even after the end of the funding period as a continuous stream of novel biomarkers should fuel the qualification pipeline in order to validate new and more sensitive biomarkers.

In fact, head-to-head studies have recently identified novel biomarkers that reflect kidney and vascular function, independently from inflammation, which is connected to organ injury. In studies on 30,000 patients, proenkephalin (penKid®) was non-inferior to glomerular filtration rate, the *in vivo* gold standard for kidney function assessment. A marker called adrenomedullin (bio-ADM®) has been shown to reflect endothelial dysfunction in humans. Another kidney-specific early injury biomarker, uromodulin, has just been opened for licensing.

## Latest approach: miRNA

Since research teams across the workld recently discovered that tissue-specifically expressed micro-RNAs can give a hint to organ injury at earlierst stage, research teams have tried to establish circulating miRNA profiles from blood samples as tissue- and mechanism- specific diagnostic tools. The hope of the TransBioLine contributors is to find profiles in banked blood samples that can be used to monitor ADEs and diseasessuch as NASH. According to Merz this would not only allow to kill unsuitable drug canidates early in drug development but also allow improved risk assessment and therapy management of effective drugs with proven safety issue. ■

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Artikeltext endet auf der Linie