The damage hit without warning. Of the 40 healthy volunteers who had enrolled in a Phase I trial for panadiplon, an experimental anxiety drug, 11 quickly showed liver injury, even though rat, dog and monkey studies hadn’t hinted at any toxicity issues. “That was really scary,” says Roger Ulrich, who worked on the study in the 1980s, “we had no idea what would happen to these people.” The individuals recovered, but the agent was killed. Fast forward to 2009, when liver toxicity popped up in a Phase I trial for Gilead’s cancer drug CAL-101. “It was the same boat — we didn’t know what was causing it, or whether these people were in dire straits or not,” says Ulrich, who has 30 years of drug development experience at big and small pharma companies. The elevated liver enzyme levels turned out to be asymptomatic, and the agent has since progressed into Phase III trials.

The incidents, decades apart, highlight an enormous challenge in drug development. “We’ve made some progress, but we still need a better understanding of what causes liver injury and who’s at risk, and better ways to predict patient outcomes,” says Ulrich. Drug-induced liver injury (DILI) is the primary reason why companies abandon compounds during development, and risks of idiopathic DILI even as low as 1 in 100,000 treated patients can lead companies to withdraw approved drugs.

As a result, academic and industry scientists have launched several toxicity-driven collaborations in recent years. In June, the Innovative Medicines Initiative (IMI) announced the most recent effort, a 5-year €32 million project called MIP-DILI. “These big initiatives allow us to lay our cards on the table and say, look, these are our problems, and these are some of the possible solutions, but we don’t have all of the answers,” says Scott Boyer, global head of molecular toxicology at AstraZeneca and MIP-DILI industry lead. “DILI is a huge problem. To solve it, we need scientists within industry and as many academic minds focused on this as possible so that we aren’t working in isolated pockets.”

The latest of many
In MIP-DILI, an interdisciplinary team from 17 biotech and pharma companies and 9 universities will take a combined physiological, pharmacological and toxicological approach. They’ll dig into the literature and proprietary pharma databases to compile what’s known about drugs associated with DILI and the mechanisms that cause it. Then they’ll conduct experiments with in vitro and in vivo models to assess their efficacy and physiological similarity to the human liver in order to determine each model’s ideal use — for instance, for testing focused responses such as reactive metabolite formation or cell stress responses, or complex responses such as inflammation and immune system activation.

“The idea is not to develop a single fit-for-purpose test but to understand how existing and novel test systems might predict or be useful for understanding different mechanisms of DILI,” says Kevin Park, MIP-DILI scientific leader at the MRC Centre for Drug Safety Science at the University of Liverpool, UK. “All of this will create a strong platform of mechanistic knowledge of DILI that will allow us to develop a stepwise process to go from relatively simple cell systems to more complex cell systems that can progressively answer more difficult questions with respect to safety.”

One of the outcomes Park and his colleagues are also shooting for is to reduce the need for animal models, which provide both false-negative and false-positive toxicity signals. “Animal models fall short, so a lot of people believe the solution is to humanize drug development,” says Paul Watkins, director of the Hamner-UNC Institute for Drug Safety Sciences and a member of the MIP-DILI external advisory committee.

But a single joint effort isn’t likely to solve the problem. “There hasn’t been any one collaboration that has given us an order-of-magnitude increase in knowledge. Each is incremental,” says Ulrich, who has participated in several such projects. “I think that we’ve learned that there is no one answer to what causes DILI.”

We might be able to crack the DILI problem in our lifetime.
Laura Suter-Dick, a scientific advisor at Roche, was a coordinator of the InnoMed PredTox Consortium, a 3-year public–private collaboration that wrapped up in 2008. The project assessed whether combining data from multiple ‘omics’ technologies with conventional rat-based toxicology methods can provide better screening of drug candidates. “We found that we can learn a lot of mechanistic information on why DILI is occurring using genomic analysis, and that was a shift in thinking,” says Suter-Dick. They found, for example, that the diabetes drug troglitazone — which was pulled from the market in 2000 because it induced idiosyncratic DILI — upregulated the expression of genes involved in peroxisomal fatty acid oxidation, suggesting that peroxisome abundance was likely to be the main mechanism of liver injury. “At the end we understood the need for new technologies to understand the biology behind DILI as well as to identify biomarkers.”

There are also several biomarker projects underway. On the heels of PredTox, the IMI funded the 5-year SAFE-T project to develop strategies for assessing whether clinical urine and blood biomarkers can highlight impending DILI in trial volunteers. “SAFE-T is entirely complementary to what we’re trying to do with MIP-DILI,” says Park. “It will provide us with really useful biomarkers that are human-relevant and that we can use as read-outs for our experimental systems.” (SAFE-T is not exclusively focused on DILI, and is also looking for cardiovascular and kidney toxicity biomarkers).

Other collaborations are taking another tack, working on predictive in silico models. The German government is funding Virtual Liver, a multidisciplinary research programme aimed at developing a whole-organ model of the human liver. And the IMI’s eTox, which is halfway through its 5-year programme, is building a database of toxicity data from across companies, and using it to build models to predict toxicological profiles of small molecules in early stages of drug development. The group demonstrated its prototype in April. “This prototype is already populated with several predictive models, ranging from the prediction of receptor binding, to phospholipidosis, to complex cardiotoxicity based on a combination of ion channel interference, and more,” Francois Pognan, executive director in preclinical safety at the Novartis Institutes for BioMedical Research, and eTox coordinator, wrote in an e-mail. eTox is now in the process of validating the prototype and refining the models and the final user interface.

In the United States, Watkins is heading up the DILI-Sim initiative, another in silico effort with ten industry partners. “It is our intent to have a model that will be a routine part of the preclinical safety testing toolbox,” says Watkins. Part of the drive behind the programme, says Boyer, is that current in vivo and in vitro models don’t sufficiently reflect variability in human hepatotoxicity. “We’ve seen from some of our cardiac modelling work that computational models can give us a better handle on population variability and the variability of the expected outcomes,” says Boyer. The hope is that DILI-Sim will help do the same for the liver.

Test-tube livers

Even as computational scientists are advancing the in silico front, cell-based systems are also making waves. Traditionally, compounds have been tested in primary cells — hepatocytes from humans or animals. But these cells are difficult to obtain and die within a day or so in culture. Since 2005, several companies have therefore been growing cells on chips: hepatocytes, the most common liver cells (and sometimes other cell types, depending on the platform), grow over scaffolding to simulate tissue. The result is a three-dimensional engineered liver with cells that last a month or more.

This approach, although more work-intensive than cells in flat dishes, still has limitations. “It’s the Model-T,” says Boyer, “but at least it has four wheels and it rolls.” Park adds that these bioreactors can run for weeks or months and so may be better than primary cells at predicting DILI, especially when it comes to idiosyncratic DILI, which can take several weeks to develop. “There are a lot of challenges, but it’s moving closer to understanding what happens in humans rather than simply putting a drug into a hepatocyte,” he says.

There’s also a lot of interest in moving beyond primary hepatocytes by harnessing stem cell technology to provide a potentially cheaper and more standardized source of new liver cells. And a Japanese team recently coaxed induced pluripotent stem cells (iPSCs) — created by reprogramming human skin cells — into hepatocytes, added two more types of cells and ended up with a three-dimensional liver bud. “It is too early to tell, but it is extremely promising as a model to assess the effects of compounds on the liver,” says Suter-Dick.

Watkins is also pursuing this approach, with a twist: he wants to make the iPSCs from people who have suffered from rare liver injury. Watkins is head of the steering committee for the US National Institutes of Health’s Drug Induced Liver Injury Network (DILIN), a registry of people who have experienced idiosyncratic liver injury. Having started with blood cells drawn from four people in the registry, Watkins and collaborators at Duke University and Cellular Dynamics International are making iPSCs and trying to coax these into mature liver cells. “It’s highly experimental,” Watkins cautions, but if it works these cells could be used to screen candidates “and get read-outs that will say whether a 1 in 20,000 liability exists in your drug”.

Chimeric mice, whose hepatocytes are partly replaced with human liver cells, are another tactic for humanizing preclinical testing. Suter-Dick sees this as a “scientifically interesting approach” to study toxicity, but one Roche isn’t broadly pursuing. “I believe its use as a screen may be limited as it involves costly animal strains and is quite work-intensive,” she says.

Ultimately, though, dealing with DILI is going to require better data collection and analysis, better modelling and experimentation around mechanisms and better tests, says Boyer. “I think it’s going to take all the weapons in the arsenal, but we might be able to crack the DILI problem in our lifetime.”