Thank you for the introduction and thanks to the organizers for allowing me to give this talk, and for you all for skipping your coffee break so that we can get our talks in.

I am going to be discussing serum cytokeratin-18 and its role in the clinic as a biomarker, as an example. So, I will get to the questions that I want to raise towards the end. And unfortunately, I am going to be raising more questions than providing answers but really just starting the conversation on this.
This example comes out of the DILIsym Initiative, which is an effort by the pharmaceutical industry to support us in developing a tool for predicting, understanding, and decision-making with respect to DILI. So the goals are here on the right-hand side.
The problem I will discuss today, is just one of the many different applications that to which we have tried to apply DILIsym, such as extrapolating from in vitro data to get early clinical predictions, understanding variability and response across individuals, and so on.

Today I want to discuss a DILI dose response scenario where the question of whether there is or isn't DILI is not the question. The question is whether there is a risk mitigation strategy that can be taken forward.
And this is an example for a drug that is in development. I will be referring to Compound X. But just so you know, it is an actual example we are working on. The clinical concern with this novel compound is that is in development to address an important unmet medical need. Importantly, this is for the inpatient setting, patients in the ICU, more than likely, treated with the compound.

Clinical Concern with Compound X

- A novel compound (Compound X) is in development to address an important, unmet medical need
  - Target patient population would be treated in the in-patient setting
- Clinical concern
  - Dose dependent elevations in serum ALT and other biomarkers were observed in phase I clinical studies
  - No Hy’s Law cases observed
The concern is that ALT and other markers including cytokeratin 18 were elevated in some subjects in these studies. The question was whether there is any way forward for this. Some of the data that the company has given to us is shown here on the bottom left. You see ALT elevations in some of the subjects in one of the cohorts. The ALT time course showed three times and two times the upper limit of normal with no explanation. In this case, 4 out of 8 or so, 4 out of 7 were above three times the upper limit of normal and some well above. It has hard to see the green curve there at the bottom but that was actually the control.

But if we look at the data in a tabular format, you can see on the left-hand side in this table some numbers and words. So the numbers really refer to the dosing level, so of blinded the actual dose here but just think of 1x as the target dose, target daily dosing level. They did a number of small clinical studies with daily dosing levels below and above the targeted dose. This drug happens to be infused intravenously. So, they varied from long infusions to shorter infusions and in-between. You can see the DILI dose response on the right, with the ALT elevations they saw in the clinical study.

In general, their problem wasn’t correlated with infusion length but it was quite correlated with dose. So, as the dose went up, they saw more problems and more severity.

In addition, they also assessed, at our suggestion, some model biomarkers. For example, they assessed miR-122 or allowed us to measure. And miR-122 correlated on an individual patient level quite nicely with ALT and showed clearly for specificity. Cleaved cytokeratin 18 was also elevated and showed that this was a mode of cell death that was seen with both apoptosis and in some necrosis but predominately apoptosis. I will come back to these biomarkers at the end of the talk.
What were the goals for us with DILIsym? What were we trying to accomplish? First of all, to help understand what the potential mechanisms for this problem could be, in combination with some in vitro studies, and then also to help optimize the dose and monitoring protocols to find, if possible, an adequate liver safety margin for the compound.

Compound X Simulation Project Objectives

- Primary objectives for DILIsym® analysis:
  - Identify/substantiate potential mechanisms related to the hepatic effects
  - Optimize the dosing and monitoring protocols to achieve an adequate liver safety margin
To give you a very brief snapshot of DILIsym, it is a computational tool made up of ordinary differential equations and parameters that represent several species and humans, but they are focused on humans.

The liver in this model is represented by three distinct zones, rather than continuously. They are lumped and assumptions are made, but you can see some of the key processes that we have been working on, including PK, oxidative stress, intracellular bile acids, and their homeostasis throughout the body, as well as mitochondrial dysfunction and disruption. For this particular project, we focused on a few areas within DILIsym: pharmacokinetics, and of course oxidative stress were key mechanisms, and of course the turnover and potential death of cells and the relationship to biomarkers that would come out. To do this project, we went through different steps that are not atypical for a DILIsym application.
First, was gathering of laboratory data and experiments to understand the mechanisms. In this case, the key mechanisms that came out of that data were electron transport chain inhibition and oxidative stress being caused by the compound. And those endpoints were assessed in hepG2 cells.

We built a compound profile for this compound in DILIsym and simulated some of their early clinical studies. So, these were studies they had already run. We ran the simulations and we got, for the most part, very good qualitative agreement with their studies. We had issues at the higher dose levels in the simulations, but no issues at the lower levels. But the simulations didn't correlate spot on. As we typically do, if we have clinical outcomes data, we combine that with our in vitro data to get the dose response as close as possible to what they saw in the clinic. And then we move forward to look at what might be safe for future studies to extrapolate to unanswered questions, really. So, we went through this process. In addition to that, we also wanted to apply this to a number of different simulated individuals, not just sort of an average person, which we know doesn't truly exist. And do to this we used what we call our SimPops or our populations.
There are a number of different parameters that are varied in the population we used. They include areas such as oxidative stress production and how the body handles that stress, apoptosis, mitochondrial dysfunction pathways, and others. For each of these parameters, imagine there is a distribution, based on the literature. And when we pull that parameter out from these distributions and put them altogether, you have a simulated virtual human.
What you will see in this table here across the top, to the right-hand side of the table are our simulated ALT elevations. And then the overall minimum percent of hepatocytes that were viable. To interpret that, it is the worst case scenario that we saw out of the 900 people we simulated. The lower that number, the more liver that was lost in that worst case person. The little circle in blue denotes that we incorporated, if you like, an in-silico physician. A component in these simulations was that when we hit stopping criteria that they had defined in their clinical studies, we stopped dosing just like they did in their clinical studies. What you see here are the results that I showed before on the left for the left two columns, which is their data. But then on the right you see our simulated dose response. And so we see, by and large, fairly good agreement between the simulations and the data. We saw increasing ALT elevations as dose went up and increasing severity. And we predicted a severe liver injury event at the highest dose level, if they had dosed out to 900 people. In addition to this, we did see within the simulations apoptosis and necrosis present based no oxidative stress as a mechanism. This fit well with the cleaved cytokeratin 18 levels that were measured before.

We have 300 distinct simulated humans for this project and we actually ran each simulated human at three dosing levels or three exposure levels to incorporate sort of PK variability in sort of an estimated way. So, we ended up with 900 distinct simulations for what I am going to show.
In terms of dynamics for the time course we were predicting, we saw changes that were very similar to what they saw in patients. This is one example of a particular infusion length and dosing time. And you can see the black arrow at the bottom shows when they had to stop dosing, and then we had to stop dosing in the simulated study. So, the dynamics were fairly similar as well.

First we looked at seven or so subjects per group in these phase 1 studies, and we had 900 simulations per group. So, as you can imagine, our tails are a little larger. So, just keep that in mind.
The first question they asked was what was the margin safety above their predicted efficacy level of a predicted dosing level. So, the part of the table highlighted in black shows their target dosing level, which was 1x and the medium infusion length. And in their early clinical study, they saw no ALT elevations, no issues. We saw a very few number of ALT elevations and no significant DILI events. Within the simulations increased that and looked for the margin. We saw serious liver injury at three times the dosing level. So, it seemed like the simulations would at least suggest that there was a three-fold margin of safety for the compound. However, without monitoring, there was a lower margin of safety. So, that was one key component of this is that we sort of reinforced or quantified, I guess you would say, the importance of monitoring in this scenario.
We then went on to look at these individuals and to isolate the effects of why some simulated humans were responding and some weren’t to this treatment. And some of the things that fell out of that were their ability to respond to oxidative stress, their propensity for caspase activation but also body weight or exposure. And so that is pretty intuitive. You have a dose response or a dose-dependent DILI event exposure would be an important component.

And so one of the things that we then went on to do for this simulation project was to help them assess, quantify the importance of potentially dosing on a body weight basis. In the same patient setting, you could imagine that you could give smaller individuals less drug and larger individuals more drug, and actually adjust your dose for the individuals. And because this is infused, it is certainly not as complicated as if it was in oral form.
So, we went on to do those simulations prior to them having conduct the clinical study. So, we first suggested the weight, the dosing for the weights of the individuals that we were simulating. We normalized it at a 78 kilogram individual and then we extrapolated out with that weight-adjusted strategy. So, again, smaller individuals getting less, larger individuals getting more, and the margin of safety went up to 4.5-fold.

So, it shows that perhaps this strategy combined with monitoring could help, given a little bit more safety margin and a little more comfortable. The things that we did really here were help identify the mechanism for injury, which we think is oxidative stress, or at least that is what we would suggest, and also help optimize the right dosing level with the right monitoring strategy and dosing strategy, in this case, a weight-adjusted dosing strategy.

But some of the things that came out along the way for this project really relate back to the biomarker issue. In this project and some others as well, we are seeing really early assessments of some of these novel biomarkers in phase 1 studies. So these cleaved cytokeratin 18 and full-length keratin 18, miR-122, HMGB1, the things that have been discussed today. And you can see our simulated values for these biomarkers here.
One interesting thing, first of all, as I pointed out, the cleaved cytokeratin 18 supported the mode of cell death, which was important, I think, for the company to understand the mechanism. But also you may have noticed that there were scenarios in our simulations where hepatocytes were lost but no ALT elevations were predicted. And this is because the mode of cell death at those low levels of hepatocyte loss were primarily apoptotic.

The hypothesis is that perhaps there are levels of cell death that are so low with apoptosis that you wouldn't see ALT rise, and cleaved cytokeratin 18 might be more sensitive in that scenario. We found ourselves addressing questions and asking questions, such as how should markers like cleaved cytokeratin 18 be applied clinically. First of all, is apoptosis a good thing or a bad thing? I think these have presented some interesting data that suggest that at least in low dose acetaminophen scenario apoptosis is a better outcome than necrosis. But by and large, there are arguments or discussions you could have on both sides of that coin.

Are there any stop-rule applications to be implemented for some of these new biomarkers? There was a question earlier about special populations in miR-122. And then also what might be the clinically relevant levels of these markers? We know with ALT and AST there is a lot of empirical clinical experience that is brought to the table for those questions but not with these newer markers. And sometimes in these early phase 1 studies, decisions are being made and these questions are on the table.

The only point here I am going to address today briefly is the last one, and put forth a strategy to think about for how we are trying to perhaps address this issue of clinically
relevant levels.
To do that, I am going to show this schematic here, where we have on the top a number of different gray shapes, representing hepatocytes. And just imagine that the baseline ALT in an individual, at least in our model, is 30 U/L. If we induce the process in a simulated environment to raise the ALT from 30 to 60, a two-fold change, we can then count the exact number of hepatocytes in the simulation that it took to get that change. And then we can go and kill the same number of hepatocytes via apoptosis and determine how much cleaved cytokeratin 18 was released in that scenario. By doing that, we can assess a number of different cell death levels and determine sort of "equivalent" fold changes for cleaved cytokeratin 18 on the right in the blue table here, as a corollary to the ALT fold changes on the left. You can take the exact numbers with a grain of salt, because we are still working through this cytokeratine-18 model within DILIsym and pulling together datasets like this from clinical studies where we can get really nice datasets. But the concept is that we can use this simulation tool to help draw parallels between what an ALT level might look like and what at least a cell death-relevant level of cleaved cytokeratin 18 might look like.

With the understanding the ALT is an imperfect marker, should we correlate with ALT? That is another question. But at least it is a starting place for how a group developing a drug, a physician might think about an ALT or cK18 level and what it means for cell death and for the liver. Of course, fold-changes aren't going to correlate properly because the baseline levels are totally different for these markers.
Some of the questions that we have been left with in several of these projects is should emerging biomarkers be assessed in the clinical trial setting as early as phase 1 and how should data be interpreted when considering the different modes of cell death; and the inactivation with respect to the patients in these studies and at these study sites; and then what levels of cK18 should be flagged as significant. And we have tried to address this within the DILIsym Consortium early on but we are still just starting out.
I want to thank the conference organizers for the chance to give this talk, the sponsor here who graciously let us present this while they are still working through this problem, and our members who continue to support our work. So, thanks a lot.