CO-CHAIR PAULS: We're going to go ahead and get started. I trust you had a really good night's sleep. Welcome to Washington at springtime, which is either cold or really hot, but nothing in between. For those of you who are staying, it is peak Cherry Blossom season, so if you get a chance, go down to the Tidal Basin because it is quite beautiful. That being said, I am going to turn over the program to Dr. Leonard Seeff and he will be the session chairperson for this morning.

DR. SEEFF: Okay. Well, good morning, everybody. As you heard, my name is Leonard Seeff. I'm sure many of you here don't know who I am and what I'm doing here. And I'm not sure what I'm doing here either. My only claim to fame, such as it is, is that I happen to work at the National Institutes of Health, specifically, in NIDDK, in the Liver Disease Research Branch. And my job there actually is, every Monday morning, to wash the windows on the 9th Floor of Building 31. And when I'm not doing that, I'm helping to oversee some studies. And one of them happens to be the NIH DILIN Network that I work on with Jose Serrano and Jay Hoofnagle an interesting ongoing study.

So having given you all that profoundly important information, we start today's session, which is billed as Research in Progress A. The first talk is about screening populations for transaminase elevations. Dr. Heide Stirnadel from GSK, UK. Sorry there about the pronunciation; I can hardly pronounce my own name, let alone others.

Link to Notes: Stirnadel Slides

DR. SEEFF: We will hold the questions until the discussion period at the end. The next talk is metabolic effects in subjects taking acetaminophen by Tom O'Connell from the home of this year's champion basketball players, UNC.

Link to Notes: O'Connell Slides

DR. SEEFF: Thank you, Tom. That was extremely interesting information and we
will hold off again for the questions until the end. So the next presentation is by my good friend, Will Lee, who is going to tell us all we need to know about acetaminophen hepatotoxicity. Will?

**Link to Note: Lee Slides**

DR. SEEFF: Well……I can tell you that NIDDK is absolutely delighted and proud to be supporting this amazing study by Will. And it only goes to show that starting with a single question, good investigators can expand the horizon and lead to even further outstanding information. So this is truly a terrific effort. The next talk is by Dr. Jussi Saukkonen, from Boston University, who is going to tell us about liver injury in patients on anti-TB therapy.

**Link to Notes: Saukkonen Slides**

DR. SEEFF: Can the other speakers come to the front? Unfortunately, we have a slightly shorter time than we had hoped for discussion, but why don't we get started now? Please, when you ask your question, give us your name and your affiliation, and use the microphone if you can.

CO-CHAIR PAULS: I have one right here.

DR. KAPLOWITZ: Neil Kaplowitz. I have a quick question for a few of the speakers, if that's okay.

DR. SEEFF: Yes, quick questions. We have a lot of other people with questions.

DR. KAPLOWITZ: Just to start with Heide. Do you have any information on -- I would assume you looked at the polymorphisms or you looked at SNP, ALT SNP itself, so there is no intrinsic control, genetic control of ALT expression, because -- due to the ALT gene. And in this ALT abnormalities, do we know anything about ALT-1 versus ALT-2?

DR. STIRNADEL: So I will have to check actually if this is actually on the gene, so, no, we haven't looked at it. But it didn't come up as genome significant.

DR. KAPLOWITZ: Thanks. From the metabonomics study, you didn't tell us what pathophysiological insight you had gotten from the pattern of urinary metabolites. Did you have an opportunity in the follow-up studies where your group has brought the patients back and given them acetaminophen a second time? Was the metabolome profile not reproducible when the ALT was
not reproducible and so on?

DR. O'CONNELL: We did include follow-up subjects in some of these studies, but, you know, with the inter-subject variability, it was difficult to and because the numbers of subjects that repeated, we didn't really get much, you know, conclusive data seeing, you know, responders transformed to non-responders. The numbers were a little bit low.

To address the first part, mapping, what we found back to pathways, this was done on a relatively low-field spectrometer, so we have now -- I think it was done on a 9 test and now we have a 14. So we're actually repeating large blocks of data now to pin down more metabolites. Our ability to make confident assignments is relatively modest. And the changes are quite subtle, so I think with higher resolution and sensitivity will address that question.

DR. KAPLOWITZ: And one quick question will be about the adducts.

DR. O'CONNELL: Yes.

DR. KAPLOWITZ: The last time I heard about the acetaminophen adducts, there was talk about everyone who takes acetaminophen exhibiting evidence of certain leading adducts. Where do we stand on this? And is there a real clear-cut threshold or cutoff between, you know, appearance of adducts that are associated with toxicity versus presumably no toxicity?

DR. LEE: I think Paul Watkins' study may address this in part, but Laura James has been working with this for some time, as you know. And she came up with the figure that you saw on the slide, greater than one nanomole per mil as the threshold for significant toxicity. And most of the patients that have toxicity are way, way above and most of the patients that have therapeutic dose, even therapeutic dose with ALT elevations still are below the one nanomole level.

When we looked at the so-called Larson patients, so there were 275, for whom we had sera on 199. These were cases that were thought on clinical rounds to have taken acetaminophen. All but 11 were strongly positive for -- had levels above. So that's like 95 percent had -- of a group that you clinically thought had it. Now, what happened with those 11, I'm still trying to puzzle that out. Some of them looked like they didn't have the ALT elevations that one would have expected. Some of them were late presentations, but some were not. Some had a great history, should have had adducts and didn't. But again, 95 percent is pretty good. But Paul may want to comment on that.

DR. SEEFF: Follow-up? The mike is there.

DR. WATKINS: Yes, with the therapeutic dosing the programs today, we sent
the serum to Laura James and to her surprise at that time, she was able to detect circulating adducts, but the level was low. But the concern is what happens if obviously, those adducts are being formed and logically there would be much higher concentration of hepatocytes. I would assume that if you are taking therapeutic acetaminophen and then get acute hepatitis A or some other injury, could you then have adducts in a very high range and misdiagnose acetaminophen toxicity? In fact, to my knowledge, there is no data that directly addresses that.

DR. SEEFF: Arie, and then John.

DR. REGEV: It's a related question. It has been controversial now for many years whether we should run through -

DR. SEEFF: Can you speak louder. You're on, but speak up.

DR. REGEV: What's your current policy on liver testing monitoring when you treat a patient? Do you have age limits? Do you have different scenarios to geographical areas and so on?

DR. SAUKKONEN: The first line of defense that we take is we assess the risk benefit of treatment with -- for latent TB infection versus, you know, this other risk/ benefit, okay. And so if it's not optimal for -- you know, the risk is too high, then, you know, we may either offer rifampin instead of isoniazid.

If they have TB disease, well, the situation is different. But we may still modify the regiment. So for treatment of latent TB infection in terms of biochemical monitoring, first of all, everybody gets clinical monitoring. They only get one month's supply at a time. They have to show up in person, that's the standard. I know that sometimes clinics do different things, they will have a family member come pick something up, but that's not really the standard recommendation.

So biochemical monitoring is recommended if you have multiple medical problems, if you are on hepatotoxic medication, if you have a history of any kind of liver disease or if you drink alcohol, so there is no age-related recommendation from the ATS.

However, in the recent study we sort of said that you can have options for selecting individuals that you might think are so -- well, the original recommendation in 2001 was kind of a blanket, no biochemical monitoring. This sort of allowed a little more flexibility to individualize not only therapy, but monitoring. But most of that monitoring would be suggested by those risk factors that I identified.

PARTICIPANT: Good morning, four great talks. Thank you very much. A couple
questions for Dr. O'Connell, please. First, congratulations on having the decency to go over the data dysplasia confusion matrix. Others might want to learn –

Two questions. On that matrix, you had some predictabilities of 65, 75 percent and I took away -- I'm not going to speculate, what it actually does over time and what the rest of the predictability might be due to if it's not -- and how we might integrate, how we could use your data to find out what the rest of that predictability is.

And secondly, while it is disappointing it couldn't predict pre-dose who was going to respond, the next best thing, I guess, to that would be get a single test dose and see after that is that the predictable response, so that we have the opportunity to look at the single dosing of these people.

DR. O'CONNELL: Well, we just didn't have the samples to follow-up on the single dose study. As far as the confusion matrix, I think they are aptly named. I didn't make that up. As far as the predictability of that, the p-values were really sort of a take-home message on that 65, you know, low 60s percentage, that they were really -- it was really statistical over-fitting. So for the other days where the p-values were reasonable, if the question is what other factors come into play, that it's pretty hard to address. What I would say is that the information contained in the urinary metabolome can only do so much and it turns out that peaks out around 60 or 70 percent.

That being said, as I mentioned to Dr. Kaplowitz' question, we are redoing this study with higher sensitivity, higher resolution instrumentation, so it's likely that we -- or it's hopeful that we will be able to improve those numbers with more identified signals.

And the next step of that would be to go in and not just look at spectral features, but to do a quantitative fitting of the key metabolites. And then once you look at metabolite levels as opposed to spectral features, you will eliminate a lot of noise.

So that's the direction we're going to hopefully improve that predictive capacity.

DR. SEEFF: John Senior?

DR. SENIOR: I'm John Senior, FDA. Congratulations to Heide and Chris for their excellent work on transaminases. I think we need to be very clear. The NHANES story, which is based on one measurement in a lot of people, you are going to pick up imostly people who have chronically elevated transaminases.

And it's not surprising that if you have chronic liver disease, you are more likely to
die of liver failure. But the prevalence of transaminase elevation may not be what we are really after. What we are really after is not just transaminase elevations, most of which go away spontaneously even if you use drugs. What we are interested in is those that will progress to more serious injury, which cannot be reliably predicted by the transaminase elevations.

And that carries over to what Jussi Saukkonen talked about in the worldwide huge array of studies going on where they are still using the old NCI criteria of severity based purely on transaminase activity levels. We wouldn't want to stop treatment for tuberculosis or prevention for tuberculosis to avoid severe liver disease that is never going to happen. We wouldn't want to kill people by withholding treatment. I might ask this: how many of the people in whom the transaminase elevations caused treatment to be stopped then went on to die of tuberculosis?

DR. SAUKKONEN: That's not really known. Well, in that -- in Malaysia in the study they were actually able to reintroduce drugs, so I don't think -- you know, the sample size is not high enough to have any deaths. So I don't think we really know, you know, what the incidence is. I think it's probably pretty low, I mean, if you stop it three times the upper limit of normal.

DR. SEEFF: Unfortunately, we have to curb the discussion and I know John has a question. And I actually have a question also, a brief question for Dr. Saukkonen when you're all done.

PARTICIPANT: Dr. Stirnadel, I was wondering in your analysis have you had any opportunity to look at the delta in the individual patients from the baseline during the study? In other words, those that are achieving three or greater multiples of the upper limit of normal in the transferase, it is important, obviously, from the standpoint of toxicity and the clinical outcomes. But how about the change while on study from a base or level, as we saw from several of the graphs, the individuals tend to maintain a relatively constant level of ALT under normal conditions and have we deterred the acetaminophen or other things? And I'm just wondering whether or not there are sub-clinical signals in the ALT in individuals?

DR. STIRNADEL: Yes, I think this a very good question. And we looked actually at the kind of change from baseline itself. Obviously the number of people in population is very, very limited. And what you can see as well you look longitudinal, I mean they pop up, some of course drop out when high elevation and then the others sustain the trial as the ALT goes down again. I couldn't make any assurances on these populations. But I think if you have an underlying population of oncology where you have an amount of liver injury, you might be able actually to
model how many have to stay on the plateau and how many go down. Thank you.

PARTICIPANT: Neil Kaplowitz asked a question for Bill. To allow a less toxic delivery of a great therapeutic like pyrazinamide, why not try a pill that combines NAC with blood delivering growth to the liver at the same time? I mean, has anybody tried it?

DR. SAUKKONEN: No. I mean, you know, I've proposed it. And for treatment of active TBZs in which the two most hepatotoxic drugs would be isoniazid and pyrazinamide, so I would propose co-administering it. And as I say, it's a study looking for a funder. We don't know exactly what the mechanism of pyrazinamide is. Presumably, it's similar.

DR. LEE: Could I ask one question? If you go putting that into Tylenol, then I don't have much to offer except to say that yes, there were a bunch of isoniazid cases in our study. I don't know what FDA thinks of these combinations to protect and how that would work out in terms of compounding and so forth. I mean, it's a complicated issue. It has never been addressed even in the acetaminophen situation. Why couldn't we put NAC in acetaminophen is the question I did ask or why not put Tagamet in acetaminophen since that seems to block the two-way lung pathway.

DR. SEEFF: I was struck with the data that you showed us about the high rate of liver failure in the one study of patients with hepatitis B who were being treated. In our DILIN study, we're struggling with this issue. How do you identify drug-induced liver injury in individuals who have pre-existing liver disease, such as hepatitis, that could flare?

So how do we know, for example, that that was not a flare of hepatitis B rather than drug-induced liver injury? Of course, testing for hepatitis B biomarkers can be helpful. It is much more difficult with hepatitis C, because we don't really have a good way of identifying if there is a flare from hepatitis C. But could the liver injury have been a result of a hepatitis B flare rather than drug-induced liver injury?

DR. SAUKKONEN: Yes, it certainly could have been. I think that those results were probably pretty atypical. So but nevertheless, it raises concern. I think what it does sort of say is that we have inadequate tools to identify individuals who are at risk that are purely relying on serologies and even baseline transaminases are inadequate. I mean, we are kind of mired in very old ways of identifying people. TB treatment actually represents an opportunity to not only generate hypothesis developing studies, but also validate larger previously identified biomarker studies.

DR. SEEFF: Well, I'm afraid that -- I'm sure we have other questions, but we're going to have to cutoff now. And we will all get together again at 10:30. Thank you.
Dr. Seeff: So we'll begin the second session on Research in Progress B. And the first speaker is Dr. Michael Aleo from Pfizer who will be telling us about some interesting stuff on cellular imaging of hepatocytes to predict clinical DILI.

Link to Notes: Aleo Slides

Dr. Seeff: So we will hold the questions again until the end of this session. Thank you very much for that interesting presentation. Now, Dr. Rick Paules from the NIEHS is going to tell us about blood transcriptomic findings and acute liver injury.

Link to Notes: Paules Slides

Dr. Seeff: So thank you for that presentation, Dr. Paules. We now come to the third presentation, Dr. Sangeeta Bhatia who will be telling us about human hepatocyte cultures for study of DILI.

Link to Notes: Bhatia Slides

Dr. Seeff: Wow, that's exciting. That really is something. I'm sure you will be hearing from a lot of people. The next talk then is by Arie Regev, who is going to tell us about drug-induced liver injury leading to liver transplantation. So, Arie, go for it.

Link to Notes: Regev Slides

Dr. Seeff: Well, that was very solid data. We thank you for that. And can we get the other speakers to come up? Surprise, surprise, Neil. Do you want to start with the first speaker who was out here? Okay. All right. You need a microphone.

Dr. Kaplowitz: Three questions. In terms of the African-American distribution
with respect to acetaminophen, do we know from data from poison control centers and so on that there is a difference in -- you know, that suicidal overdose, you know, per million African-Americans versus --

DR. REGEV: If there is some --

DR. KAPLOWITZ: And did you break this down to a perspective intentional and unintentional acetaminophen induced-liver failure?

DR. REGEV: Well, those are some good questions. We -- there is no such information in the UNOS database. So we don't know if it was intentional or not. And we don't know if there is a difference in patterns of use of acetaminophen or use for suicide or not for suicide. So it's completely -- this is a face value observation.

DR. KAPLOWITZ: And with respect to the INH, I was kind of struck by the fact that almost all your anti-tuberculosis cases were INH alone. Does this reflect the fact that people who are on, you know, right or multiple anti-tuberculosis drugs are unlikely to be transplanted, because of active TB? I mean, what --

DR. REGEV: It could be. This is also -- it was very interesting to us as well. But there is no explanation for the decision made by the transplant surgeons there. But it is clear that the vast majority, except for two patients, all the patients were just -- were on INH only. And your guess is as good as mine what was the reason for that.

DR. KAPLOWITZ: Okay. One quick question to Sangeeta and then I'll give it to you. In your -- in the -- in the model, when you injure hepatocytes, do you see regeneration in the context of that system, that bioengineering system?

DR. BHATIA: Yes. We are just starting to do the RD staining to that and there are some cases where we are starting to see some, but we haven't done anything systematic yet.

PARTICIPANT: I have three questions for three speakers, starting with Dr. Bhatia. So have you done dose response effects? So you showed troglitazone at 100 micromole is doing bad things to the cells. So that's actually a high concentration. It doesn't really stimulate circulating concentrations. But if you were to, let's say, compare the dose response effect of that drug versus other family members of similar products that are known to be hepatotoxic, but they are much lower risk, would you get a shift in the dose response effect?

So you know, dose response -- that's a dose-response question. And then actually let me just ask my questions first. Dr. Paules, a question about RNA and serum in blood is
very exciting. The question is how-- given that intend -- reclassability of our message from hepatocytes, the question then is how variable is the measurement based on stability of mRNA blood? Is there a technical problem in the harvest and the quantitation across different transcripts? Because this will become -- if it is reproducible -- in fact, it really could become a substitution of good liver biopsies, so this could be a very important known tool if it is reproducible.

And then for Arie, the question is why is the transplant referrals and will you be missing the referrals that have grown around the tempos, if we have time to get to the surgery. So we'll start with Dr. Bhatia.

DR. BHATIA: Okay. So I'll -- so my question was about dose responses. So we have done dose responses for some things and mostly to see if we can see patterns of toxicity curves that would be consistent with the literature. So like a glutathione depletion shoulder and acetaminophen versus like dose-dependent response and cadmium. So we have those, but we haven't done systematic -- quite the glitazone systematically yet.

DR. PAULES: Okay. My question was about RNA stability in play. And the way we do our -- and so RNA stability is always an issue. The lipid cells have a decline, so stability is a concern. We collect whole blood and we put it in what's called a PAXgene tube, which stabilizes RNA immediately. We are actually looking at the contribution of method RNA from circulating peripheral blood with say primarily the lymphocytes. There can be a lot of artifacts introduced into these analyses depending on how you collect it. A lot of people have tried to do purifications and you can actually introduce artifacts in gene expression of from the purification protocol. And so we are not looking at circulating RNA. There are individuals who are looking at that, Rusty Thomas is the handler, essentially, doing that as well. But we believe the predominant signal we're seeing is from the vertical lube section.

DR. REGEV: And to address your question about the subpopulation. We are looking at the sub -- this is a subpopulation. And I was actually lying when I said -- I was talking about the extreme over the extreme. There is a more extreme group and this is the ones that don't make it to liver transplant, because they are too sick. And people who work in transplant centers know we have patients that die minutes after they go through the door. And those are the ones that we have no information about, unfortunately. So this actually looks at a very narrow subpopulation of those that did not improve on their own, but were strong enough to make it to the liver transplant, which is the difference. And that's all we have, unfortunately.
DR. SEEFF: Okay. On the other hand, I think it's well-known that African-Americans are less likely to get this preferred treatment, watch for that. So I guess part of the question is, is there a bias in who gets a transplant? Are they more -- you know, are those who are African-Americans more -- do they have more severe disease at transplantation, recognizing, of course, that the decision is based also on other factors?

DR. REGEV: You mean, that they will have less liver -- less like to --

DR. SEEFF: Well, that's why --

DR. REGEV: -- get the liver transplant?

DR. SEEFF: Well, they get a liver transplant, but with greater severity of the disease at the time of transplant.

DR. REGEV: Right. But then again, I'm not sure how this will affect the distribution of acetaminophen versus non-acetaminophen in this case.

DR. SEEFF: Yes. I understand that. Okay. Back there. We will come to you, Doctor.

PARTICIPANT: A question for Michael. You used 100 times the Cmax concentration neuro-cultures.

DR. ALEO: Yes.

PARTICIPANT: Do you think that can lead to an artifact explanation? Is that high concentration? And this follow-up part have you used the culture condition by Sangeeta to get your vile adduct canaliculi and the tight junction?

DR. ALEO: So the question is using 100 times Cmax. I think there is a possibility of driving a false positive by using such a high concentration, but with the validation said that we used, it didn't seem to have that effect. So the data is as it is. But I think there is a challenge in terms of understanding.

When we did the validation process, we only did it at 100 times the Cmax for the reasons I indicated earlier in the talk. We are going back trying to understand if we could generate dose response relationships for each compound in that regard to see whether or not we can understand that relationship better.

And the second question was relating to use of the micro-engineering devices which are RTC. Our Cambridge site actually has had a collaboration with MIT in the use of those bioengineering devices as well.
DR. RUSYN: Michael to follow-up on this question, thank you, some of the publications from, especially, Peter O'Brien have reported well over 90 percent, that's simplicity, for some of his high content screening that he did. And I understand there was a larger data set that he screened together with Pfizer, but he probably had done a subset of that in archives of Toxicology a couple of years back.

DR. ALEO: Yes.

DR. RUSYN: So when you look at those publications, you know, look at your presentation, there is a bit of a disconnect on how good the sensitivity and specificities are if you take the subsets of those compounds. So I wonder if you have a comment on that?

And also, you mentioned that you are willing to share the data with other pharmaceutical companies. Are you willing to share that with academics for potential modeling in some other approaches that can, you know, maybe look a little deeper into chemical structures and other things?

DR. ALEO: So to get back to your -- well, I'll answer your second question first, since it's in my mind. And so we are going to participate in an ECVAM process. There are active missions that are actually involved with that. We were planning on sharing that DILI classification list with them for the publicly known compounds, but, at this point, there is not a step or a process in place to share proprietary structures or compounds in that regard.

And your first question, if you can remind me was what, Peter O'Brien's question regarding the sensitivity? So Peter used some different formats in terms of the endpoints that he was using. Also, he had a calcium that was part of that process. He had a smaller data set. He used HepG2 as well.

I can't comment too much more on the disparity between those sets, other than that the different formats and the endpoints that were used. For right now, what I presented is the validation for the four parameters that we are currently using, but we realize there is a great opportunity to expand both the endpoints as well as the potential for the format for increasing that sensitivity.

DR. SEEFF: Let me just stop at Dr. Saukkonen and then I'll come back to you.

DR. SAUKKONEN: First, a comment just to follow-up on that isoniazid and transplant issue. About 10 times as many people appear with isoniazid alone in treatment of latent TB infection. And pyrazinamide is only used for two months. So -- and Rifampin rarely itself
causes hepatotoxicity.

I have two critical care questions. As a critical care physician, I'm always interested in how to identify patients who are at greater risk for organ injury and how to preserve organ perhaps that are injured. The transplant data suggested that women and maybe some other demographic groups are much more likely to have severe injury, maybe not at greater incidence overall, so should there be a greater emphasis devoted to certain demographic groups in monitoring or in providing some type of, you now, antioxidant?

And second, you know, do you see any value in using these in vitro systems for screening antioxidants or what is used potentially in animal analysis such as carbon monoxide for suspended animation?

DR. REGEV: Thank you. Well, these are excellent questions. And right now, I worked the previous 10 years before joining the industry, we had no special meetings addressing the high risk in women. There is no different type of test or treatment.

There are general models that we use to try to assess severity of liver dysfunction and those are used all over the world. And they are considered pretty accurate, one of which is called the male -- the model for induced liver disease.

DR. SAUKKONEN: Yes.

DR. REGEV: And that is a combination of a few lab results, one of which is creatinine, so the increase in creatinine is a very significant factor. Although, most of these patients are already listed as they arrive in what we call studies 1, which is the highest possible.

And what they are waiting for is just an organ that will get there on time. So if they are lucky and they're in the right center or they have the available organ, they're going to get transplant and nobody will wait for any assessment of any additional kind. So I'm not aware of any additional assessments for antioxidants or any other special assessment that we do for the transplant.

DR. SEEFF: Okay. We have two questions here, then John back there and then we'll come back to Neil.

Could you use the microphone, please? We can't hear you.

DR. BHATIA: Yes, the question is about infections with plasmodium falciparum and whether it will be full maturation through all the liver stage and all the way through the merozoites stage, which is the blood infection stage.
I don’t know the answer yet. We just did -- we started with attenuated strain with a vaccine collaborator and we just started doing the wildtype experiments, which are much more precious reagents.

DR. SEEFF: You went off the question or are you just -- oh, I see. I'm sorry. You plan to -- John was next and then we will come to you, Neil.

PARTICIPANT: With respect to the altered hepatocytes in your optimal conditions, I'm interested since the normal liver has the portal blood flow and it has a number of pathogens associated molecular patterns in the vicinity of the natural hepatocytes, whether any of those issues of endotoxin or other bacterial, some often comments, are important in terms of optimization of hepatocyte differentiation or maintenance of growth or whether they are irrelevant just tolerated by the normal mode with the liver?

DR. BHATIA: So I think -- I know the answer to what kind of house that you get is for what level of revolution you are looking for. So I think that for many of the examples I gave, we are talking about sort of the generic hepatocyte/phenotype and really not at that time/revolution of compartmentalized the upstream or downstream hepatocytes.

So I -- when I said that some of these micro-environmental stimuli will be important for, you know, wanting a cell that has a phenotype of, you know, the canalicular, but right now, we're sort of more focused generically on is it hepatocyte versus not.

So let me say, for example, that extracellular matrix and oxygen radiance and even 3-D typology can influence some of these things.

DR. SEEFF: Neil?

DR. KAPLOWITZ: A question for Dr. Paules. So I guess I hadn't connected on the idea that you were looking at gene expression in white blood cells. So the question then is what is the signal? This is occurring before there is any over liver injury, how do you rationalize and explain that it's the identical gene expression change in the cells let's say to the assays? Is it unique? Is this a unique issue of acetaminophen? Is acetaminophen metabolism in white blood cells an issue here? Is it possible that some -- you know, I guess, Sid Nelson had described these ipso conjugates of acetaminophen, lay vile, glutocyne and conjugates which are, you know, lay vile, but stay vile and can be released from hepatocytes and engage other cells.

So that's sort of the area that I'm curious about. And along those lines, if you look back at the mouse model, well, I don't remember exactly what was shown, but are these -- this
recapitulation of the gene expression changes in hepatocytes, I mean, liver and white blood cells, present in other toxicity models, other than acetaminophen?

DR. PAULES: Okay. So let me clarify something first. I did not claim that gene expression was identical and there were similarities in processes and pathways. And I think that it actually is fortuitous that we are looking at acetaminophen, because it probably is -- the white blood cells can metabolize the acetaminophen and are showing mitochondrial stress directly from the metabolism of the acetaminophen. It is likely also that they are responding to the liver. And whether that is from metabolites or from acclimation of the immune response, we don't know. But I do think that this is something that is fortuitous for acetaminophen and that will not be true for all compounds. So there may be some that would require an acclimation to a reacting metabolite that could not be activated in the blood.

DR. SEEFF: Are there any other questions?

PARTICIPANT: I have a question for Sangeeta. It is a very nice model what you showed, but from my experience with blood, which have more active white cells in the CDNER and phenotype Type I cells and Type II cells. Type II is about 3 percent.

As long as you have -- they are in contact with each other, you have contact with the patient. So not all the cells would turn out to be Type II cell. Once you have an insult would damage Type II cell, you need Type II cells to protect the position. In your model, you release the cells from contact, so do you check for certain interaction or if you have some with different shape or different way?

DR. BHATIA: I'm not sure exactly what you are getting at, except for that -- so there are cell types better in the liver that are missing in our model. So for example, the sinusoidal adelial cells are a main player. We have what we call tri-cultures now and we have had those back.

And interestingly, endothelial phenotype is also unstable in culture, but more stable than the endothelial cells are next to a healthy hepatocyte, so there is some cross-top there.

We are missing immune mediator signals completely. So you know, another idea is to add back cupra-cells from the same suit, so matched hepatocytes and the cupra-cells.

And with regard to growth in the uninjured case, we don't see any proliferation in the past slides, even though we know, of course, in vitro, that they can grow.

PARTICIPANT: Okay. One just small question also. Do you envision in the future, for example, take a biopsy wrong subject and then try to test to see or predict whether they would
have it or not?

DR. BHATIA: Yes, I think that was in the last slide, so the one kind of vision for personalized medicine would be to test patients hepatocytes. And the two ways I can think of would be can you grow biopsy or can you take a fibroblast biopsy, do -- make them induce brain-cloned stem cells and make those hepatocytes. So those are the two paths we're pursuing which are admittedly very far off and that's why we're not done.

DR. SEEFF: John Senior?

DR. SENIOR: John Senior again. To follow-up on Neil Kaplowitz' question, if this blood RNA is reflecting liver only in the case of acetaminophen, what happens when you look at all of these eight hepatotoxicants you mentioned earlier and all those azobenzenes and I think Moganov reported in the summary paper that the blood RNA reflected the liver RNA. Was that true for all those compounds?

DR. PAULES: All right. So actually, after I passed the microphone off, I remembered that you had a second component to your question. And I didn't answer it. And the eight compounds we saw, on that PCA that I showed you, that there was significant correlation with liver injury in the blood signal. And so those 30 genes showed a nice separation from normal and those compounds that were injuring the liver. And it was specifically to those individual animals that had liver injury. So there is a signal derived from the liver that -- well, there is acclimation of the immune response or secretion of cytokines or whatever in that data stress -- signal. So we were able to identify with those eight. But I would still want to clarify and say we don't claim that this is going to work for everything.

DR. SEEFF: Any other questions? Four more. Do we have any microphones? Come.

PARTICIPANT: I'm from FDA. I think if you have a new placenta, they seemed malformed. Drop me an issue that was based by my discernment at NIH that it is not associated with the acute liver failure. We saw it when we did data mining analyses, so there was data received that this is the sometimes report, we saw very clearly. And there is a need to prevent these adverse events with an organizing formation would be better and start looking up acetaminophen database and identify the signals from one database can be reproduced in another database. And we can start -- and we must start, there are treatment options, then we can start analyzing these issues much better. But we need informatic in place to be able to do it.
DR. SEEFF: Well, that was a statement, not a question.
PARTICIPANT: No, it was a statement.
DR. SEEFF: Anyone else? Another one here? We still have time.
DR. ULRICH: Roger Ulrich. So I'm just kind of mulling over this, the rather striking incidence of renal insufficiency or renal failure, what the CME have been. And so I'm wondering how, you know, causal that might be. So that's a question to Dr. Regev.

And then to Dr. Paules the question is is the signature that you see in white cells that are shared with the liver also shared with the kidney?

DR. REGEV: I'll start. Well, it seems to be causal. I mean, this is something that is not known. We have seen it for years. And it has never been documented in an orderly fashion. Will Lee just presented a pretty good documentation of the occurrence of that. And the interesting thing was that he mentioned also that it may occur even when liver injury is not as bad. So there is a direct effect here. So I think we are definitely dealing with something that is a double hit of some sort. And it exists and even without the significant liver injury. So yes, it's definitely a true phenomenon.

DR. ULRICH: Yes, I agree.

DR. PAULES: So, Roger, thanks a lot. I hate to say this it's embarrassing, but we have the samples and we haven't analyzed them. I mean, you know, it's -- we have the same budget constraints that everybody does. We have a lot of really nice kidneys frozen down to analyze, but we haven't.

DR. SEEFF: Well, we have time for at least two more or if you want -- oh, yes, one here and one there.

PARTICIPANT: Just to follow-up on what was your point in terms of the kidney injury. Has there been any kind of assessment of the additional markers for the kidney injury?

DR. REGEV: You mean acetaminophen induced-renal injury? Not as far as I know, but I, you know, would love to hear what anybody else knows about it.

PARTICIPANT: What markers are you referring to?

PARTICIPANT: The prediction, you know. You can have some prediction of whether the kidney injury would happen or not if you start seeing this kind of injury.

DR. REGEV: It's a good question. I don't know of any, but if anybody else wants to comment?
PARTICIPANT: You know, there are sis -- statin-C for example and other KIM and other kind of molecular work and progress to develop.

DR. REGEV: There is an open venue for you, so I think it's something that should be looked at, but I don't think anybody has any -- as far as I know, there is no good data on this.

DR. SEEFF: One other burning question, perhaps the last one now.

PARTICIPANT: Would the in vitro studies -- if the patient get a drug, usually they have some kind of information right on different disease. When you put cells in vitro, you put them in immediate. They are clean. They don't have any maybe increase of factors. There is no stress. So do you think about probably adding something to it to maybe mimic what is in humans that are having problems, maybe it is some chance worth taking. Probably taking some serum from this patient and add it to an in vitro study and maybe, you know, stimulate some gene that may see something there.

DR. BHATIA: Yes. So the answer to your question is we thought about the sort of flavors in these. So, for example, we thought about adding some of the inflammatory cytokines that are known to modify acute injury or in combination.

So I mean, I think really the sky is the limit in terms of tailoring them for a particular application.

DR. SEEFF: Well, this has been a very exciting session, and I think a round of applause for the speakers is in order.

(Applause.)

DR. SEEFF: And we will reconvene at 1:30 after lunch. Thank you.