CO-SPONSORED BY THE FOOD AND DRUG ADMINISTRATION CENTER FOR DRUG EVALUATION AND RESEARCH (FDA/CDER), THE CRITICAL PATH INSTITUTE (C-PATH), AND THE PHARMACEUTICAL RESEARCH AND MANUFACTURERS OF AMERICA (PHRMA)

12TH HEPATOTOXICITY CONFERENCE 14-15 March 2012

WHY DILI IDIOSYNCRASY? THE IMMUNE SYSTEM AND BEYOND: WHY DO ONLY A FEW PEOPLE SHOW SERIOUS LIVER DYSFUNCTION FROM DRUGS THAT NEARLY EVERYBODY CAN TOLERATE OR ADAPT TO?

WEDNESDAY
MARCH 14, 2012

The conference opened in the Solidarity Hall of the Lane Kirkland Center, National Labor College, 10000 New Hampshire Avenue, Silver Spring, MD, at 8:15 a.m., John R. Senior, M.D., chairman.
A G E N D A

Introductions and Brief Opening Statements

Session I: How and why do people respond differently to the same drug?

Session IA Are there dose-related and idiosyncratic hepatotoxic drugs
What are the differences between pre- and post-marketing data?
What is idiosyncrasy anyway? Is it dose-related?
Comments
Discussion

Session 1B What may explain the different ways people respond
It's the genome
Not the inherited genome but epigenetic variations
It's the immune system
Discussion

Session II: Lessons to learn from

Session 2A Examples to make us think
GWAS in DILI: promises and pitfalls
Lumiracoxib - can it be reborn by HLA typing?
iPS cells and patient-specific hepatocyte cultures
A fresh look an isoniazid hepatotoxicity
Discussion

Session 2B Focus on the patients
Is it autoimmune hepatitis or DILI?
Why and how are biologics different than drugs?
Are children not just little adults in how they respond?
Plasticity of the transcriptome in autism
Discussion

Adjourn
8:15  Introductions & Opening Statements

DR. SENIOR:  Good morning.  Let me ask Paul Watkins and Carolyn Compton please to come up to the table. Lana, do you have any instructions, ground rules for the meeting, that you want to tell us? Lana Pauls has been the wonderful organizer of this meeting; we can't do without her. Lana?

MS. PAULS:  Good morning everybody. I guess I was introduced. I'm Lana Pauls, and I have been working on this meeting with John for the last 11 years. So welcome to our 12th Annual Hepatotoxicity Conference. I feel like most of you are friends, because I've seen you for many years.

Logistically, the women's rooms are over there; the men's rooms are over there. The pin drives that you received when you came in have the slide presentations that were provided to me as of last Friday. Some of them, including my own, have changed, and the ones that you will see today are the ones that will be eventually posted on the website. We shall be posting these slides on websites both at C-PATH and at AASLD.

One other point if you're doing wifi in here, is the pass code, all caps NLC11. If you have any questions throughout the conference, contact either me or Heather Saniel in the back. Heather, wave your hand.

DR. SENIOR:  While Carolyn Compton is loading her slides, I want to say very briefly that we're sorry that Stephen Spielberg can't be with us today. He had a family emergency, and I offered to fill in for him. So I'll say a few words about him, and then ask Paul Watkins to say something about AASLD. We'll leave the rest of the time for opening comments to Carolyn Compton, who is the new chief executive officer of the Critical Path Institute in Tucson, Arizona.

Dr. Steve Spielberg is relatively new to the FDA, but he's well-known all
over the country, all over the world. He got his undergraduate degree at Princeton, did his medical degree and residency at Chicago University. He's a pediatrician, a clinical pediatrician with a lot of clinical experience. He's been an academic at Hopkins, at Toronto, 15 years in academia. He's also been in industry. He's been in government. He had a full range of experiences when he came to the FDA. Within the past year, he was named as the sort of super-boss, overseeing other offices for drugs, biologics and tobacco products, and reporting directly to the Commissioner. So we're sorry Steve can't be with us. Paul knows him well, and in fact, introduced me to Steve. So now I'll turn this over to Paul Watkins to say a few words about our other sponsor, AASLD.

**DR. WATKINS:** Well thanks John. It's a great treat every year when John and Lana put together this meeting. In spite of major competing meetings of the toxicologists and pharmacologists, it looks like we have a great turnout.

The AASLD, the American Association for the Study of Liver Diseases, continues to be very supportive of this meeting and very supportive of drug-induced liver injury in general, as has the American Gastroenterology Association, which at their annual meeting in the spring of last year, had a state of the art talk on drug-induced liver injury.

In addition, internationally at the Asia-Pacific Society for the Study of Liver Diseases, about a month ago in Taiwan had a state of the art talk on drug-induced liver injuries. Actually the U.S. DILIN network was the topic, followed by a session on drug-induced liver injury.

As I mentioned in the meeting before, there's now a special interest group for drug-induced liver injury within the ASLD. You need to be a member of that organization to be part of it. One of the activities that's sponsored by the special interest group in the AASLD is this single topic conference that's shown on the screens now, which will be held at Emory in
June. It will have two parts: 1) mitochondrial toxicity, which John Lemasters and Neil Kaplowitz have put together, an outstanding program on, that will work right into 2) a clinical session on acetaminophen toxicity, but also has some mechanistic components. So it's a nice one-two punch. If you're interested, I put up the website there for it. You can just Google AASLD Special Topic Conference and get right to it. Without further ado, I'll step down. I know another speaker's coming.

**DR. SENIOR:** Thank you. I want to say also that AASLD has been the host for our programs on the Internet for the past four years. The federal agencies, including FDA, NIH, CDC, have all been hampered by the implementation of so-called 508-rules, that are supposed to make material available to disadvantaged people, such as people who have poor vision, blind people. In so doing, they have made the information available to nobody, because what they require is that slides shown by speakers be described in minute detail and text provided. Now the logic escapes me --- they can't see the slides but they can read the text. Duh. But that's a federal rule, the 508-rules. Outrageously stupid, but there we are.

AASLD has been kind enough to put up our meetings: what everybody showed, what everybody said for the last five years, a very important service from AASLD.

Now our opening speaker of special substance is Carolyn Compton. Carolyn recently succeeded, on the 1st of February, to the position of the chief executive officer of the Critical Path Institute in Tucson, Arizona, with satellite offices here in Maryland near Washington. Carolyn is an extraordinary person. She is a Harvard-trained, Mass-General-trained pathologist. She's recently been at NIH for a number of years, and has relinquished that to come to the Critical Path Institute. We're very excited, because Carolyn has succeeded Dr. Ray
Woosley, known to many of you, and a friend for many years. They very carefully did a national search and found Carolyn Compton, and now we're going to hear what she proposes to do with the Institute.

**DR. COMPTON:** Opening Comments from Dr. Carolyn Compton, Critical Path Institute, Tucson AZ

(Applause.)

**DR. SENIOR:** Thank you. There's much to think about in what you said, Carolyn, and I think we're going to be drawing on your visions and concepts to work together in the future. Now we're going to start the first session. I'll ask Lana Pauls if she'll come up, to give us some science, not just logistics. In the meantime, while Lana's approaching, I want to introduce two visitors from China, Dr. Yimen Mao and Dr. Chin Ning. Will you rise please? Where are you? Okay. They just came in from Shanghai and Wuhan. Now you may not know about Wuhan. It's one of the smaller cities in China, only ten million people. Anyway, they are here under the auspices of Dr. Tim Shi, who's the head of the organization called GlobalMD. So we're delighted to have them as distinguished visitors.

After Lana and I have had a chance to say some words, we're going to get into the meat of the program, which is discussion, and I want the moderators to be ready to come up and say a few words. They are Mark Avigan, Chris Hunt, Michael Merz, Arie Regev, Leonard Seeff and Paul Watkins. Lana, take it away.

**MS. PAULS:** What are the differences between pre- and postmarketing data?

(Applause.)
DR. SENIOR: What is idiosyncrasy anyway? Is it dose-related?

(Applause)

I want to invite Arie Regev to come up first. Arie is right here, and then Len Seeff, Paul Watkins and Mark Avigan. You know who you are. Come on up.

DR. REGEV: Well, thank you John, and thank you Lana, and thank you for inviting me. I thank all of you for being here, despite the fact that there's an Society of Toxicology meeting going on in San Francisco right now. So this is a great privilege to be here. It is warmer here. It's going to be 80 today.

So just a few words of support and agreement with statements that we heard. We're going to hear many times that one of the major issues with stepping forward with the development of biomarkers and understanding of biotoxicity is collaboration. I think this is a very good place to start doing it. Using databases that are privileged and used by pharmaceutical companies, I think, are extremely important. The use of those databases together could be extremely powerful, and there are a few other points that we will discuss. I think that the main secret is collaboration. Discussions between clinicians and toxicologists and basic scientists, I think, are critical in this field. I'm going to mention this in my talk, where we see so much misunderstanding when separate groups are looking at issues related to hepatotoxicity, and so much waste of work and brainpower and excellent brainpower and money, when we do those things separately.

A lot of basic science that is wasted on questions that are not even relevant to the patient, as was mentioned here earlier. I think these are the things that we need to talk about. There are excellent talks today that will -- today and tomorrow, that will address these issues. So we look forward to it. Thank you.
DR. SENIOR: Thanks, Arie. If the other moderators would prefer to speak from a floor microphone, they may: you can see a couple of them scattered around. So let's go with Paul Watkins now. What we're trying to do is to get some feedback from moderators who have been really in this program for a long time. They know the rules, and they know the meat of this meeting is the discussion from the floor, and that's what we want to get, not just a series of lectures. Paul, do you want to speak from the floor with a microphone or come up here?

DR. WATKINS: Okay. A couple of comments. First of all, should real-world patients be involved in clinical trials? Sure, that would be great. But there's a practical limit on what a company can afford to do now in the current environment, and we all know the current model of drug development is in serious trouble, at least for chronic diseases. I'm no expert in this area, but the idea needs to be considered of staged approvals and maybe going into patient populations, where there's some opportunity for reimbursement of the cost through third-party payers. But I won't say anything more on that.

Is idiosyncratic hepatotoxicity dose-related? Well, as Jack Uetrecht has been pointing out for probably two decades now, you know, Avogadro's number is an awful lot of molecules, and if you take only one molecule, I don't care how susceptible you are, you're not going to get the toxicity. But the other way to look at it is how about somebody who doesn't have the susceptibility, and you'll hear a lot about HLA and adaptive immunity, and there's some good evidence, you know, with some drugs, that if you don't have that susceptible HLA allele, you're not susceptible to the toxicity. If you took that person and gave them 100 times the dose, would they develop toxicity? I don't know. I think the answer may well be no. I know of examples of drugs that cause
idiosyncratic toxicity, where people have taken an intentional overdose to kill themselves, misguided, and had no evidence of liver toxicity whatsoever.

My own thought is that it's probably a mistake to say these things are all on a continuum. I think there may be a fundamental difference, particularly in these delayed, severe, sudden liver meltdowns, that it's true for those susceptible people. You can go down to lower doses, and they wouldn't get it. But I don't think that non-susceptible people would necessarily get it, no matter how much you gave to them.

The next thing is just a comment that, you know, it's often said that when they talk about the problem with drug development today, still the major problem is efficacy, that drugs fail in clinical trials because they turn out not to work at the end of Phase III. You can point to statistics that will support that argument, but in fact I think it's misleading, because if you could go to a higher dose, maybe you would have seen toxicity, or you would have seen efficacy. You couldn't go to a higher dose because you didn't have the safety margins in animals during your Phase I trial. So I think it's misleading when people say that it's lack of efficacy that's the major problem in drug development. I think clearly the bottleneck is safety.

The next issue is in post-marketing clinical trials, you know, one of the stories that came out with lumiracoxib was here's a drug, the COX-2 inhibitor that was improved. You'll hear a lot more about this in the later session. But all of the sudden there were severe acute liver failures, liver transplants, and the company then went back and looked at their clinical trial data. They saw mild, often transient, treat-through ALT elevations. They found an HLA association, and lo and behold, the people with the severe toxicity post-marketing had that risk allele.

I think that's the paradigm for the future, which is going to be as serious cases are found, you may not have enough of those serious cases in the SAE
consortium or the DILIN network, which you'll hear about, to do any meaningful genetic studies whatsoever.

But if the company has kept DNA and can look for milder toxicities, and have sufficient numbers, even drug-treated match controls to make associations, then those few severe cases in the post-marketing arena will become very critical. I think that one of the mistakes that's being made in the Sentinel Initiative is the pronounced concern about patient privacy, and the inability to actually track back and find those patients who have these severe adverse events, to offer them participation in networks like the Severe Adverse Event consortium or DILIN.

So I think that's what I see as the future of really getting at mechanism susceptibility in the post-marketing arena, and then let's see. That's it. Those are my comments, and I hope they're provocative and lead to other people's comments.

DR. SENIOR: Thank you, Paul. Some good points. I hope we get more arguments. Where's Mark Avigan? Mark, are you here anywhere? Do you want to come here or do you want to go there? Okay. Mark always has a few words.

DR. AVIGAN: Well, you know, tomorrow we're going to have a session on biomarkers, and one of the points that I hope will be made, which is a provocative point, is that we're really interested in biomarkers that are predictors, rather than just prognosticators. That is, they would predict in advance an outcome, and that's very daunting as a challenge because the biosystem that we're dealing with is rather complicated, and different drugs we know, a priori, incite drug-induce liver injury through a variety of different mechanisms.
In addition, the biosystem is rigged much like I think of it as a commercial airplane. It has a lot of built-in safety features. A drug-induced liver injury really is, in some sense, a system failure. Usually, the system corrects itself. If you lose a certain part on the airplane, there's another redundant part, and that's how evolution tends to work. There's a fair amount of redundancy. So we're looking for outlier individuals who actually have gaps of one kind or another, so in fact, when they get challenged by a stressor, these antibiotics, they have a bad event and they actually ramp up their injury.

The question, of course, is from a biomarker perspective. Can we identify a core number of predictors that have a common thread across different drugs, and I would say the answer is still out with regards to this question. But certainly it's a very important one from the point of view of efficiency of drug development and patient management. So that's point number one.

Point number two is that, and I think Lana touched on this very nicely, this idea of enrollment of patients in trials. We tend to see in clinical trials very narrow patient groups, who are tested and shown to have an efficacy response to a pharmacologic therapy. But they may not represent the real world. One example of this is patients with pre-existing liver disease, and we're seeing now many development programs for viral hepatitis. The fad of the day now is type C hepatitis. There's a bunch of programs, development programs in process. So the question then is can you discriminate a liver event caused by a drug to treat a disease, from a pre-existing disease in the liver?

One kind of defensive approach, if you're a drug developer, is to keep away from patients who have, significant necroinflammatory disease, because it may be hard in some cases to discriminate patients with drug reactions from their underlying disease. So we need to have clarity about decision trees in diagnosis, and I think this is an area of an interesting challenge, where decision trees in terms of how we would actually work through the discrimination
functions of diagnosis, and clear stop rules. I think this is an important challenge.

**DR. UETRECHT:** I'll bet you in most cases, the drug would actually, if we had more information, would be exonerated. So it is in their interest to get that data.

**DR. SENIOR:** That's exactly true, but we can't determine that, because we don't get the information.

**DR. UETRECHT:** So it's your job to change the law, so that –

**DR. SENIOR:** Oh. You want Congress...

(Laughter.)

**DR. UETRECHT:** -- so that you can go back, with authority, and confidentially get better information on those patients. We have to find some way to do it. That's the way to do it.

**DR. SENIOR:** I have to tell you, the FDA does not dictate to Congress. It's the other way around. Thanks, Jack.

**MS. PAULS:** John wanted spirited discussions. The next person to speak is Linda Scarazzini, Director of the Division of Pharmacovigilance at the FDA.

**DR. SCARAZZINI:** Hi, thanks John. I completely appreciate your comments, although I disagree with you that the problem is the AERS database. The issue is the reports in AERS that are reported to us are incomplete. And to John's points, thank you for making all those points. Having been on the industry side
for 11 years before joining the FDA, I can tell you that we all need to do a
better job in terms of follow-up, and I disagree that it's not in the company's
best interest to go back and find out from the reporter and get as much
information you can possibly get, because 90 percent of the time the drug is
exonerated.

So why can't we get better information? We're never going to have a trial
that's going to answer the questions that you want to answer. You have to
have a better post-marketing system. Why can't we get better reports? Why
can't we get quality reports? I do believe there're some data and privacy issues
that we've had to deal with, especially in terms of going back and getting blood
samples. But I can tell you that, and when I was wearing my industry hat, we
did that as well. So it's a call to action. We need to do a better job in getting the
data. So I'm going to open it up. How can we do that?

DR. SENIOR: Let Neil Julie say a word, Leonard, but don't go away. Leonard,
Neil Julie's been standing for a minute. He deferred. All right, Leonard, go.

DR. SEEFF: I want to point out that I am the one who with John is doing
most of the reviews of potential cases of hepatotoxicity based on the AERS
database. I guess that something like maybe 15 percent at most have data that
permit me to be able to say this is definitely a case of drug-induced liver injury,
or is not a case of drug-induced liver injury. Mostly, there are missing data and
you cannot know for certain. But I agree with you, Dr. Uetrecht, that I think
that in most instances, one would find that this is not drug-induced liver injury.
There are lesser causes for abnormal enzymes. There're so many other courses
and you can exonerate the drug. I don't know how to improve upon this, but it
is terribly frustrating, very difficult. It makes it more time-consuming, because
we have to go back and say can you please send us? Or was the patient tested
for Hepatitis C? Did the patient have other drugs or whatever it was? When was this or that done? We get reports that say "drug toxicity" and no more than that, and there's no way of being able to identify it. So I think this is a terribly important area, and people really need to try to work much harder in getting the data that's going to be sent into the FDA.

DR. NEIL JULIE: First, as far as the AERS database, it would really help a lot if there was a longitudinal data population, and perhaps the option for the FDA to go back to the primary treater and ask some of those questions, which I think at this point is precluded, so you can get more complete histories. Because I don't think the AERS database is worthless; I just think that it's insufficient in terms of the information that it provides.

My other point is in terms of DILI modeling. One more cost effective way that I think could be approached is maybe we should shift to a more susceptible mouse phenotype, if we want to try and look for particular types of metabolic hepatotoxicity. For example, if we look at mice and troglitazone, they have a diabetes and a natural phenotype. You can then distinguish what's truly idiosyncratic perhaps, from what most dependent-susceptible host. Would that be possible? Has that been possible?

DR. SENIOR: I don't know. Arie?

DR. REGEV: Again, to respond on things that were said before, and I didn't know that, until I moved to industry, after many years of clinical practice. Most companies have hundreds of individuals, medical people that sit on the phone on a daily basis and try to communicate to physicians on cases that were reported. I didn't know that, but some of those phone calls sometimes happen 30 times, 30 consecutive phone calls to clinical practitioners regarding a
particular case, and there's no instruction in a single case, and there's no
instruction in any of the companies, I'm sure. I know about mine, the one that
I'm in. But there's no instruction in any of the company to limit the data that
is obtained. On the contrary, we know that the more data we have, the less
chance that it will be the drug. Usually, that's the pattern. But still there's a
huge difficulty of getting information, because of several reasons. But the
barrier usually is the amount of information that practitioners are willing to
give, and that is the issue that needs to be solved.

**DR. SENIOR:** Over here on the right.

**DR. FRESTON:** Jim Freston, University of Connecticut. A couple of comments
about the AERS database. It grieves me considerably to think that Leonard
Seeff is wasting his time dealing with those raw reports. Just a gratuitous
suggestion, and that is that they should be triaged at the very lowest level, and
if three or four key elements of information aren't there, they ought to be put in
a file, possibly never to be looked at again. Then the ones that meet that
threshold for evaluation should be looked at. But the use of AERS database on
a big scale is very promising, and we've learned a lot from it, the use of
disproportionality analysis, for example, using those large databases is starting
to be quite productive. But the main point I'd like to make is that it seems to
me, having worked at, consulted at pretty much every level in drug
development, I'm quite satisfied that we're at the flat part of the dose response
curve, with respect to designing Phase I, II and III studies. We can make
minor tweaks, yes. But the amount of effort and money to go into it, to get
improvements, is going to be a small margin.

On the other hand, after the drug is approved, I think there are
wonderful opportunities and incentives for the company, not just to learn about
more safety issues, or to identify them, but to enhance the efficacy of their products by adjusting the dose and studying new populations. Some of the populations weren't included in the Phase II and II studies, for very good reasons. You don't want to add too much noise there, and you want to be sure you demonstrate efficacy to get approval. Well, once you do and you have the pump primed, so you have money to do additional studies, you can bring those special populations, some of whom were excluded, into focused studies. You can adjust the dose, sometimes upwards, to enhance your opportunities of showing efficacy.

The way we've looked at drug development is almost nine-tenths up to the NDA and approval, and then people shift their attention within companies to other drugs and bring them along. I think that's the wrong way to look at it. There's much to be mined post-marketing. Just give you another quick example, using population PK studies, something that 20 years ago we thought was impossible. Well, now when we find a drug that's out there, that might have an interaction with yet a new drug that's been introduced, you don't have to bring those patients back in and do a big elaborate PK study in-house. You can do it with population PK. Again, this is an example of taking advantage of the opportunities after drug development, or after approval.

**DR. SENIOR:** Thank you, Jim. Jim's a distinguished consultant to many companies, as well as a superb physician, and past president of the AGA. Linda?

**DR. SCARAZZINI:** I just wanted to make a comment, bringing it back to what Carolyn had said about technology, and I think we have to remember that we are developing electronic health records and other things, where we need to put the term "adverse event reporting" at the time at the point of care. Because
when you take things out of the flow for a physician in their daily work, it's unlikely that they want to go back and pull a chart, do follow up. It diminishes return on investment; it diminishes when you're getting follow-up from physicians. If you don't have them -- having been a busy physician myself -- if you're not catching me at the time when I'm seeing the patient, when I'm stopping the drug, it's unlikely that I'm going to go back and pull the chart and continue. That's why you have hundreds of people making hundreds of phone calls and getting no response. Two weeks ago, the FDA spoke at the Health Information Management Systems Support conference, where we were encouraging creative solutions to build into electronic health records at the point of care, the ability to make it easy to report, to upload at that moment all the lab results and anything else that you can report at that moment, so that we have more information, so that Leonard is not sitting there and looking at incomplete case reports.

And I think that the other important thing is educating physicians of the importance of reporting at that moment, because we have a broad outreach program right now to work with young physicians, residents, et cetera, about the importance of adverse incident reporting, of putting it at the moment, at the point of care, and making it easy is another way that we're going to get this information.

**DR. SENIOR:** Don't go away, Linda. I wanted to respond to that. And who really speaks for the practicing physician? The practicing doctors of this country are already very stressed. Their incomes are being limited, and they really don't have time to prepare detailed reports of adverse effects. So they simply don't report them, or they delegate it to an assistant, who doesn't really understand the problem so well. Now what are you going to do about helping the practicing doctor to do the reports that you say is necessary?
DR. SCARAZZINI: Agreed. Make it as easy as possible -- and using technology. Having a drop-down menu, so that all of the other fields are completely filled in for that adverse event report, and you just describe the event or upload information as easily as possible. We're also developing a mobile application so you can do it on the go. We have to make it as easy as possible. Everybody will agree with me that now paper submissions, faxing it, having to go back to the chart and pulling records over and over, limits your ability to report as completely as you would hopefully want to. So we have to use technology to make it as easy as possible, and not take it outside of your workflow. I think they're two really important things, and I'd be -- I'd love to hear other people's comments on it.

DR. SENIOR: Jack.

DR. BLOOM: Jack Bloom. I just wanted to make a suggestion that might be carried into tonight's discussion. For all the reasons we heard this morning and others, we're going to continue in the immediate future to have uncertain signals, ambiguity as far as whether the data we're collecting or certainly not collecting, is really actionable as far as risk management and risk assessment. That in the context also of the risk-benefit equation, makes for obviously a real challenge, and whether or not this can be standardized or codified, remains a question in terms of the regulatory science. But right now it appears to be discretionary across the agency and with other international regulatory agencies. One of the key questions is whether or not one can really standardize something like this, based on those particular challenges.

DR. SENIOR: Bob Roth.
**DR. ROTH:** Bob Roth from Michigan State. As a toxicologist, I feel like I have to defend the discipline, I guess, from what John said about those of us who are in experimental toxicology, assuming that all animals behave the same. Of course, they don't. We recognize that. But he's right in the sense that although we recognize biologic variability in responses, we do statistical analyses and oftentimes when we have an outlier, we do a statistical test and we throw that outlier out. So I think you're right, John. There's a lot to be learned if we were not to throw those animals out. A problem is that we often don't know what to do with them. The other thing I might say is that you gave an example within experiment variability. But those of us who have been in experimental biology know that when you try to repeat an experiment sometimes, the dose that causes a particular effect shifts. So even in animals that you get from the same supplier and the same housing conditions that are genetically identical, you do get different responses. I think that as it relates to drug-induced liver injury and idiosyncratic injury, if we were to understand better why we get differences in responses, either within or among experiments, there might be some clues there about why people respond differently as well.

**DR. SENIOR:** Thanks. Bob is one of what I would call enlightened toxicologists, who's beginning to question some of the cherished standard beliefs that we've been following for 60 years. Chris Hunt?

**DR. HUNT:** Thanks, John. Chris Hunt, GSK. I just want to say I agree with virtually all of the points. Leonard's, Arie's, Linda's. I think we need better ways to get information. I completely agree with capitalizing. We know electronic health records are now being pushed. There're actually additional financial incentives for these to be broadly used. We have some folks from Kaiser and elsewhere looking at creative ways to use electronic health records. I
think we need to incentivize. I work in drug safety, and I completely agree we need better information. It seems like we really want to incentivize physicians to report, and perhaps you could creatively add it as a Category B for continuing medical education, or get a drug safety certificate or some kind of incentive, and –

**DR. SCARAZZINI:** -- a billing code.

**DR. SENIOR:** We can't hear you, Linda.

**DR. HUNT:** A billing code? I agree. She was saying a billing code, and some kind of concrete incentive, I mean, so people can start brainstorming. I think if you incentivize physicians, this is something that people really would like to see happen, and I agree, it has to be maximally efficient. So I think there're ways to do that creatively. Thanks.

**MS. PAULS:** I'd like to remind everybody that if you're speaking from the floor, state your name. This is absolutely vital because the court reporter is trying to capture everything, and what is said will go up on the web. So I appreciate the spirited conversation, but please come to the microphones to do that.

**DR. SHI:** This is Tim Shi from GlobalMD. Thank you, John, for allowing me to join this meeting. Well, let me make comments on data information, also for the people trying to record data for toxicity post-marketing. If for instance each department could create a data analyzer to focus on the post-marketed drug, it would release the stress from the physician being involved in data collection, data reporting. Now is not the time to discuss the issue of the e-record. Every hospital, every department should have e-record. But how you really can
utilize this data is the issue. How to utilize the data to analyze each drug, particularly in the patient treatment, is also the issue. So from the S-FDA in China, they all have issue to say the post-market issue, the data, how to get together. So there's a possibility in the department for the patient treatment. You get the data analyzer to get data to come together, to say this is how liver toxicity, this is data for the kidney toxicity.

So therefore, you can really analyze data, and to get together for the reflect to the market and reflect to the manufacturer, to see this data, this result, how to reflect this drug. Thank you.

**DR. SENIOR:** That was Tim Shi, as I said, head of GlobalMD, and really spearheading a major initiative to study DILI in China. Paul?

**DR. WATKINS:** Yes, I was going to respond to Dr. Julie's question. Are there other people who want to keep on the post-marketing surveillance issue? Dr. Julie brought up and the question of post marketing surveillance. I guess it would make sense if your product's going to diabetics, and there's a good diabetic animal model that would make sense. But I wanted to make people aware that there's a lot of work going on now in mouse genetics. So you can buy from Jackson Labs what's called the Mouse Diversity Panel, which is 35 different strains that are genetically very different, that have a lot of the genetics worked out. And if you can show a differential toxicity across the strains, you can actually have a good chance of finding what the genetic susceptibility is in that strain.

Allison Harrill at our Institute's doing a lot of work in this area, in particular with Pfizer, that's trying to find other certain strains that would have picked up toxicities that were missed, and usual strains that can be incorporated into pre-clinical screening, because obviously you don't want to
have to 35 different strains for that. The Jackson Labs' next big project is what's called the collaborative outcross, where they're taking eight strains of mice that have been genetically completely defined, their whole DNA sequence actually obtained, and then allowing them to breed to get sort of a random, human-like population.

After all, this isn't just an issue for idiosyncracy, that animals aren't a good model. Humans aren't a good model, the vast majority of humans. So to create a heterozygous sort of human population, and my understanding is those studies are yielding some interesting results. Then at the University of North Carolina, there have been a series of publications now on the next generation of inbred mice called the collaborative cross, and they plan by this summer to have 50 strains that they'll know the complete DNA sequence and have -- they're all homozygous at every locus. If you find an animal that is a good model, you can have an unlimited supply of that animal to do mechanistic studies.

**DR. SENIOR:** Neil.

**DR. JULIE:** Neil Julie again. I'm in effect a hepatologist, so as far as the idea about having EHRs include some kind of an easily launchable template for reporting, that could even be similar in some ways to MedWatch, that's a great idea. I think as far as coding for it, that's a nice idea, but we have a disincentive for doctors to report. We don't want to create a reverse incentive for them to over-report, which a code might do. But it would be great if various agencies like pharmaceutical industry, like the FDA could, you know, find some way for the administration to, you know, pony up a couple of dollars to do something like that and create something like that, because I think with EHR there's a great potential to come up with a template, find a way to strip off all the
identifying data so it's confidential, and then to import all the data that you'll need to get much better quality adverse event reporting.

DR. SENIOR: Okay. Jack.

DR. UETRECHT: Jack Uetrecht again. Let me be specific about what I think would be logical. And that is an active system, totally independent of the company, independent of the physician, where there are a couple of dozen people in hospitals around the country. A physician doesn't have to report anything. These people go around sniffing out trouble, and then they have the authority to go into the medical records and document, carefully, exactly what happened, and possibly get samples, although that would be -- I think you could deal with the confidentiality of the information that's already there without any trouble, because it would be the -- separated from identifiers. And compared to clinical trials and other things, it would cost trivial amounts of money. I think it's logical, but I don't think it's going to happen, unfortunately.

DR. VANCUTSEM: Hi. Paul Vancutsem, Biogen Idec. I'm a toxicologist, and I wanted to make a point about animals. I've seen a few compounds and not only in liver toxicity, but in -- for which we had some animals outliers, and we didn't really know what to do with the findings. Then we moved forward into clinics, and we ended up in the clinics saving a few human outliers that we didn't know what to do with, and we continued developing. But I think that the goal of the tox studies is kind of to enabling the clinical studies anyway. So and it's both of them are pretty messy systems, and I don't know if we want to fully try the system that much. I hear my clinical colleagues saying that they want to model this system. I don't think that we want to go to too clean a system in toxicology. In my mind, one of the solutions is to keep more of the
information that we are generating in these outlying animals, so that we are able, after we start seeing something in the clinic, to go back to them and explore the two systems in parallel, to know whether we can find a bit more information that would lead us in the right direction. And typically in tox, we don't see any real good system for that. When the study is done, nobody wants to reopen it. So maybe that's a new project needs to be a bit more open.

DR. SENIOR: Okay. Mark, you get the last word, because we're going to have a coffee break. The last word, Mark.

DR. AVIGAN: Okay. So I just wanted to comment a bit about what Jim Freston was talking about, with regard to disproportionality and assessment of spontaneous reports. There really are two levels. We looked at this a number of years ago in the AERS database. This proportionality of just crude numbers of reports, for certain drugs historically, actually, for those drugs that are real problems at the top of the hit parade, actually is not bad for a liver failure, for the most serious adverse event, in PT terms. That's the first cut, and so the idea of cumulative disproportionality over time with reference utilization, is a very useful initial measure, a so-called signal detector. But of course it then challenges the causality analysis and unit cases, and for that you need a fair amount of differential diagnostic information to exclude other causes. And there is some good news in this. I don't think we're totally stuck. The NIH actually has advertised that in the next few months, they will have an interactive website, so-called liver tox, which will be a structured report, which will take into account all the questions that will help the analyst, the FDA analyst also, decide whether this is a drug-related event.

So the first question -- can it ever happen with this drug? -- will potentially be answered. Not so much the frequency question, but can it
happen. The other idea is that to have expert networks of people who see these cases, who have some academic or finance motivation to report them in an organized way to the FDA and to other agencies. I'm looking forward to a reform over time in how, in the spontaneous report database, of having subsets of reports from more expert groups, that will allow us to look with more clarity at the unit causality question. So those are different things, and I think we shouldn't sort of mix them up. But reform is on its way.

**DR. SENIOR:** We're going to have a break for coffee. We want everybody back by 10:29, to hear an exciting presentation by Matt Nelson. Lana, do you have any instructions?

**MS. PAULS:** No.

Coffee break, 10:01 - 10:29

**Session IB: What may explain the different ways people respond?**

**MS. PAULS:** Wow, I've never been able to quiet down a room this quickly in my life. This is wonderful. We are going get started with the second half of Session 1, and I would like to insert a couple of announcements:

First of all, I'm not sure how many of you are aware of the fact that we do have an evening session, which is optional tonight, which is to talk about potential revision to the guidance, from 7:00 to 9:00 in this room.

In addition, for those of you who are staying here, dinner is not provided, because we have quite a heavy appetizer at the reception from 5:00 to 6:00. So if you want to grab dinner, you're going to have to pay for it on your own at the cafeteria, or go out to a different venue. In addition to that, for the 7:00 to 9:00 session this evening, we have xeroxed a couple of questions to stimulate
thinking, and they are out at the reception table. Oh, and lastly, March 20th through the 21st, 2013. This is our tentative date for next year, and I want you to know it misses Montgomery County school break, Howard County school break, the ASCPT meeting, the SOT meeting, Passover and Easter. So if I'm missing anything else that I have to skip, you need to let me know.

So, that being said, I'd like to ask Len Seeff and Paul Watkins to come up to the table, as they will be moderating the remainder of Session I. Thank you.

**DR. WATKINS:** Okay. Now we're getting into a very interesting session. It's so important to my own interests. I'm going to sit, probably right there, so I can see the slides clearly, and the first speaker, with the title "It's the Genome," is Matt Nelson. Matt Nelson's with Glaxo Smith-Kline, but importantly he's also co-chair of the Scientific Advisory Committee for the Severe Adverse Events Consortium, which I'm sure most of you know is an industry-sponsored consortium, devoted solely to finding genetic determinants for rare adverse drug reactions. Appropriately, the first one they went after was drug-induced liver injury. He's an extraordinarily clear thinker, and I think you'll all enjoy his presentation. Matt.

**DR. NELSON:** It's the genome

(Applause.)

**DR. SEEFF:** The next speaker is Dr. Igor Koturbash, who's a post-doctoral research fellow in the Division of Biochemical Toxicology, National Center for Toxicologic Research. His interest is in epigenetic mechanisms of carcinogenesis, and the mechanism's susceptibility to various toxicants and carcinogens. He's going to tell us that it's not the gene; it's rather epigenetic variations responsible.
DR. KOTURBASH: Not the inherited genome, but epigenetic variations-

(Applause.)

DR. WATKINS: We'll save questions for the discussion period. Our next speaker is Gyongyi Szabo, who's Professor of Medicine at the University of Massachusetts, who's going to talk about "It's the Immune System." I think it's straightforward. I guess the pointer doesn't work.

DR. SZABO: It's the immune system

(Applause.)

DR. WATKINS: The panel's next, so if we could have the other speakers up here, Doctors Koturbash and Nelson -- and now for the best part. I guess one question for Dr. Szabo, the interest with some HLA associations, as I had mentioned before, is when you go back to the clinical trials, actually more mild than even transient elevations in ALT that you can treat through, turn out to have an HLA association, suggesting that even the mildest liver injuries in fact have an adaptive immune component to them, rather than some sort of intrinsic toxicity that secondarily initiates a -- I mean it's still unclear. This is sort of an evolving story. But in any event, there are ALT associations. They have a clear HLA association, presumably in adaptive immunity. Yet they go away when you continue to treat. So can you give us some insight into T regs and any thoughts on how this could, would make sense to an immunologist? Jack Uetrecht may have some thoughts on this too.

DR. SZABO: Right. I think, you know, the timing and the extent of responses and the quality of the responses are going to be the one that's probably going to
give the end result. In terms of the immune response, and the timing is such that the immunity responses kick in right away, very early on, and that probably happens even before the ALT increases come up. Indeed, immunity response is kind of the first response. It's very, very rare that there wouldn't be some adaptive immune response. The question is that just because we don't see the adaptive immune response, that doesn't mean that it doesn't exist.

So in case of this kind of delayed damage that you're talking about, often what happens is that T cells that are activated, as part of this adaptive immunity, actually can be recruited to the liver, and that another component I didn't get to talk about is the liver is sort of the graveyard for activated T cells. During that process, what happens is that those activated T cells don't really kind of go into that grave so quietly, because bystander liver damage can happen. In fact, many of the kind of components that we see with increased ALT often could be just a result of this bystander damage. That could be the tail end of the immune response that's already quieting down, yet the damage still occurs. So I think that could be the mechanism for this -- I'm giving speculation obviously, for explaining this transient change that happens without future consequences.

DR. WATKINS: Okay, good. Question. Please say your name and where you're from.

DR. McKINNEY: Leslie McKinney, FDA. I have a question about epigenetics. It's a general question, because -- and it has more to do with how you get from changes in DNA methylation and histone structure to a specific cell response. Because everything that I've heard about epigenetics so far looks like it's more of a -- it's sort of like changing the redux state of the cell. You change the global, you know, DNA methylation state of the cell. But I don't see how, or I
don't know of any examples where that's been tied to specific changes in specific
gene expression, that kicks off some pathogenic process in the cell that's going
to lead to liver injury. Could you -- I know you gave us some examples of some
drugs, but if you could enlarge on those, because the ones you picked were
mostly carcinogens, and what we're interested here is like a more short-term
kind of organ damage. I'm not seeing the connection between epigenetic changes
and the more acute changes that go on in the tissues.

**DR. KOTURBASH:** Thank you for your question. That's a very good question,
because when I just got the invitation for this conference and I went to
PubMed, and then just typed DILI and epigenetics, I got zero hits. So there is
really -- like I said, there is really lack of information regarding particular
epigenetic changes that are associated exactly with drug-induced liver injury, or
with drugs in general. There are several reports in 2012 regarding epigenetics
and some of these are really a very, very new studies. What I wanted to show
today that we probably need to pay a little bit more attention to possible
epigenetic alterations that can occur in the cells, because as I showed, for
example, DNA methylation and histone modifications can regulate gene
expression. It has been shown, for example, that the number of cytochromes are
epigenetically mediated. Even cytochrome 2E1, which is one of the main
cytochromes, is silenced in the fetus, and it has been shown that one of the
mechanisms, because it is really severely hypermethylated the promoter.
Within the breast, the promoter is getting demethylated. So there of course
there is a broad field to explore the potential of epigenetic alterations in
response to various chemicals, including pharmaceuticals.

**DR. SZABO:** May I just add something?
DR. WATKINS: Sure.

DR. SZABO: In my mind, and I'm not an expert in epigenetics, but based on studies, for example, that were done, for example, in alcoholic liver disease, it has been shown that the exposure to binge drinking, for example, can cause certain changes in methylation that essentially is an epigenetic change that then over time can change the response to a subsequent challenge. That, in my mind, is the power and the importance of the epigenetic changes, because it might be something that happened earlier on, that now will determine the response of a new insult.


DR. UETRECHT: Yes. There is actually some evidence with drugs. There's very good evidence that the autoimmune syndrome associated with hydralazine and procainamide involves inhibition of DNA methylation. And although not everybody that takes these drugs develop clinical autoimmunity, if you give procainamide long enough, the incidence of positive ANA is almost 100 percent. Hydralazine a bit less. Hydralazine can also cause liver injury. In these cases, it's leading to activation of the adaptive immune system. There's one paper linking isoniazid hepatotoxicity. With inhibition of histone acetylation, not quite as strong evidence. There's another paper linking pyrazinamide hepatotoxicity in an animal model with inhibition of DNA methylation. Unfortunately, we weren't able to reproduce it. We didn't get the toxicity. Because we didn't get the toxicity, we didn't look at whether it in fact inhibited DNA methylation. So I think there is evidence with drugs, that cause idiosyncratic toxicity, including liver toxicity. But again, the final mechanism is the adaptive immune system. It just helps turn that on.
DR. WATKINS: Frank Sistare.

DR. SISTARE: Yes, a question for Matt Nelson. Matt, you had a really nice slide up there. I think it was allele frequency versus odds ratios, and it had like a typographical map that had, I guess it was like 100 patients or 1,000 patients, 10,000 patients, that kind of thing. My question is: can we use that data? Can we leverage that data to help guide us, in terms of using that historical perspective to draw linkage in the various compounds that are associated with those symbols and colors? Can you look at what was seen in Phase 2 trials, for example, in terms of ALT signals. I think what you were showing was like DILI, like really bad events. Can you look back and look at the frequency of ALT events, in small numbers of patients, to somehow guide us on how to draw conclusions on what we should be doing in Phase 2, in terms of doses and what kind of signals we should be seeing? What's the likelihood that we're going to see this later?

DR. NELSON: Well, I think that was a point that Paul made this morning in his opening comments, about once we identified genetic risk factors for serious liver injury, can we go back and recognize their role in perhaps less serious forms of liver damage? That's certainly true. I'm not sure how the patterns that I showed in that particular graph would inform that, because we're essentially depending on having found an association, we believe, to back and investigate that in perhaps more subtle cases of liver injury.

DR. WATKINS: John Vierling.

DR. VIERLING: Well first of all, I enjoyed all these talks. I think they're very
provocative. I have two questions, one about epigenetics and one about the HLA associations.

With respect to epigenetics, we have increasing evidence that for males, an ALT less than 30 and for females less than 19, is the picture of hepatic health, and that levels above that contributes to risks of all-cause mortality, which has been presumed to reflect systemic issues of inflammation, and that this ALT is such a signal. So where we have so much evidence of those increases above that norm ALT, see reactive peptide associations with all-cause cardiovascular mortality, my question is: what contributes to a change from a normal liver to an epigenetically altered liver, if there's a systemic inflammation as a background in adult humans?

DR. KOTURBASH: So your question is whether inflammation can contribute to epigenetic alterations in the liver?

DR. VIERLING: Chronic low level inflammation, which appears to be the rule rather than the exception in industrialized societies, and certainly in the U.S.

DR. KOTURBASH: Well, I would say probably yes, because this is a compromised cell, and in compromised cell, any alteration can occur. Honestly to say, to my knowledge, I don't have -- I don't really know whether such studies will exist showing, for example, direct correlation between cell inflammation in certain tissue and epigenetic alterations in it. But I would expect that chronic inflammation would result in part DNA methylation. Especially we have seen, for example, that different chemicals, introduced chronically or sub-chronically, that lead, for example, to hepatotoxicity, in parallel also lead to impaired DNA methylation and alterations and histone modifications. But I don't really have direct evidence to say yes, sure.
DR. VIERLING: That's not been studied in the animal models, where you give some type of stimulus for an external chronic inflammation, cutaneous, muscular, arthritis, and then look at the liver before and after? It might be a fruitful area to investigate.

My second question is the fascination about the Class 1 and Class 2 HLA associations, and since they've been very allele-specific, modeling should be in one's mind, as to which peptides may be bound within those HLA alleles as an activation signal and recognition by the T cell receptor repertoire, which of course is part of our idiosyncratic differences as human beings. My real question is: what do we know, based on these HLA associations, of what the antigens should be, that are allegedly activating the adaptive immune response?

DR. NELSON: I don't know anything about that. I don't know if you're aware of it. I know there are some that have investigated, but I don't know what's been found.

DR. VIERLING: Have they been modeled, to look at what peptides would actually fit in the actual antigen-binding grooves for these Class 2 and Class 1s. That again could potentially be fruitful, and narrow some understanding of whether we're dealing with a drug, a metabolite, an altered host protein. There must be an answer there with this fascinating association.

DR. NELSON: I seem to recall that Simon Mallal has looked into that, following the finding that B5701 was also associated with flucloxacillin, in addition to the abacavir hypersensitivity. But I have not seen any results from that.
DR. WATKINS: Either way, okay. Next. But name and institution please?

DR. OLDACH: Sure. Hi, David Oldach, O-L-D-A-C-H, from Cempra Pharmaceuticals in Chapel Hill. I have another question about inflammation. These talks were all great. Thank you very much. I think it would go to Dr. Szabo. We've been really interested in the work by Kevin Tracey, in sort of teasing out what he calls the inflammatory reflex, which looks at enervation of innate immunity. Specifically, the classic experiment was a vagotomy, an actual vagotomy animals that were exposed to LPS had increased mortality and increased liver injury. So we're wondering about whether certain drugs might actually reproduce some of these effects, by interfering with the inflammatory reflex, interfering with the dampening of immunity that occurs after an insult. There are specific receptor interactions that we think can do this, and we're wondering if you've given that body of work by Kevin Tracey and others, consideration in your thinking about idiosyncratic DILI?

DR. SZABO: Thanks for bringing that up, but that's a fascinating area of immunology, and I think they're just kind of still kind of coming to the mainstream. There're certainly multiple components of that. So the anti-inflammatory cytokine induction, that kind of part of that response that particularly interlocking-10 induced. The interlocking-10 induction and the anti-inflammatory effects of interlocking-10 is one of kind of the immediate risk for this response. Another interesting component actually is the link to the microRNAs, in terms of microRNA 132, that plays a major role in the regulation of some of these neuromediatory and neuroinflammatory circuits, that in my mind could be another sort of target to look at in relation to the hypothesis that you just showed and brought up.
DR. WATKINS: Okay, thank you. Next.

DR. DINSMORE: Steve Dinsmore, from Merck and Company. A question for Dr. Nelson. It's pretty clear that there are a number of autoimmune diseases, rheumatoid arthritis, ankylosing spondylitis. That the C-2 component will complement and so on. These genes are located on the short end of Chromosome 6, as is the marker for DILI, idiosyncratic DILI. Are there associations between diseases and this HLA B5701 marker?

DR. NELSON: That I don't recall. I don't know if there have been. None that I recall. There may be.

DR. WATKINS: Well, except to the extent I think some of the HLA alleles have been associated with susceptibility to other autoimmune diseases.

DR. NELSON: Yes, but not the 5701. I don't recall B5701.

DR. WATKINS: Well, is your question: are we ready to start using these markers to actually screen patients and personalize medicine, decide who gets it and who doesn't? We're going to hear an interesting presentation about lumiracoxib that I think's going to address this issue. It's the first attempt to try to do that. I know Lana wants to stop, but I think we actually have a couple of minutes. So the last two questions. Mark.

DR. AVIGAN: Well, I was just going to ask about the observation that there are not a lot of patients who have been described, who have had more than one drug-induced liver injury reaction to different drugs at different times. Which
then raises -- and if you look at the literature, you ask is there are certain
susceptible people, because they have, let's say, a high tonic rate of innate
immune hyperstimulation, because of another disease going on. You'd expect
them to be repeat offenders, but you don't really find that. So I wonder whether
you might comment on the question of susceptibility to these extraordinarily
rare reactions on the front end of the drug itself, versus the back end, which is
the ramping up of the reactions that is already at the non drug-specific phase.
Why are we not seeing more repeat offenders, individuals who have more than
one reaction to more than one product if they have an underlying pre-existing
hyperstimulated effect? You may just comment on that, whether that's what
really is rate-limiting with regards to serious drug-induced liver injury reactions.

DR. NELSON: I can't comment on that.

DR. WATKINS: Do you have any comments? Well, the argument would be
either a series of necessary, but neither one's essential, or a series of essential but
not sufficient steps in becoming that 1 in 20,000, and one of those steps may be
leaking LPS from your gut or having a viral infection.

DR. AVIGAN: Right. Well, the one thing that speaks to this, of course, is
antibiotics being a very common category of drugs associated with drug-
induced liver injury, as they've been tallied by the DILIN Group and others.
One possibility around that is not only are they potentially immunogenic
through an adaptive immune mechanism, but also that many patients who get
antibiotics happen to have kind of a hyperstimulated --

DR. WATKINS: That's true. They have an inflammation. But of course there
also one of the few drugs given in multiple gram doses, and we talked about
that, as well as they're design being to kill things, bugs. But Bob, if you want to make a quick comment?

**DR. ROTH:** Yeah, I just wanted to comment on that. So I'm going to talk about our animal models tomorrow. But one of the things we found in them is that the nature of the inflammatory response is very important, in terms of whether or not you get a hepatotoxic response to some of these IDILI-associated drugs. For example, it not only has to be of a sufficient magnitude, but I believe it has to be an acute inflammatory response not a chronic one, where you probably have down-regulation of the whole inflammatory system. I think the nature of the inflammatory response might be very important, in terms of why few people get these reactions.

**DR. WATKINS:** And the last question.

**DR. WAKSMAN:** Havir Waksman from Amgen. It's very well known that at least with acetaminophen, which is a very fascinating model of hepatotoxicity, you have two groups regarding who are the ones who develop full hepatic failure and the ones that recover. It is well known, and American toxicologists, so it's well-known that late presenters are the ones who go through the path of potentially acute liver failure, compared to the early presenters, that they received the antidote, they get better, they go home.

**DR. WATKINS:** Yes, because of the antidote.

**DR. WAKSMAN:** Right. So I am a biased believer, and I'm biased completely, that the ones that they develop acute hepatic failure, which are the late presenters, is because they develop an extreme inflammatory cascade, and
compared to the other ones, to the early presenters, that maybe they do develop an inflammatory cascade, but it's not enough extreme to go to the path of liver failure. Now the question is for drugs that are in Phase I or Phase II, whether there's any cytokine biomarker which we may use, that precedes elevation of ALT? And again, I'm using ALT because that's what we use today. Whether, you know, there's some work from Debra Laskin from Rutgers, talking about TNF alpha, talking about IL-1. I did my own work with IL-18, blocking IL-18. I definitely show that ameliorates the injury. So the injury happens, and cytokines propagate that. So going back to my question, whether there's any biomarker we can use, even in pre-clinical models, not necessarily in humans, but the pre-clinical model we can say oh, this comes out first, and then you go ALT, INR, etcetera, etcetera.

DR. WATKINS: Right. So the question is: are there immune surrogate biomarkers that might help distinguish say benign ALT elevations, that don't mean anything from significant problems, or even in the absence of ALT elevations?

DR. WAKSMAN: And even if your work, what you publish regarding people with therapeutic doses of acetaminophen, that some people may develop a bump in LFTs, which was really an innovative approach, because what was believed that that doesn't happen, and you show that does happen, whether you, maybe in your work, maybe you check or found that that cytokines or inflammatory biomarkers, that they precede in those patients that they develop the LFT elevation, and they did well, because nobody develop.

DR. WATKINS: Right. I think the answer is there aren't any validated markers that will help you out. The question is are there putative markers right
now, that if you at all in industry had carefully saved and frozen sera that could
be retrospectively tested now? Jack, any IL-17? Is that what you're going to –

**DR. UETRECHT:** I think there probably are. Like I said, with clozapine, a
high percentage of patients have a bump in IL-6 very early.

**DR. WATKINS:** Can I stop you right there? How was that picked up? Who
had the samples to test that?

**DR. UETRECHT:** They did it prospectively. So somebody took a bunch of
patients, and we're going to be doing similar studies and looking at a whole
bunch of cytokines. We don't see it animals, unfortunately. So what you'd
really like to be able to do is do it in animals, so you never had to go to humans.
But I think there are markers there. Most of those patients will still develop
immune tolerance. They won't go on to get sick, but it still could be a
biomarker early in clinical trials. But until we're sure, I would hate -- I'm sure
there will be some false positives. So until we have more experience, I don't
want to say yes, we need to go out and do it, and kill compounds before we
know for sure what it means.

**DR. WATKINS:** Right. Well, I'm going to address this a little bit in the
Society of the Liver Safety Research Consortium tomorrow. But final panelists,
any comments, because Lana says we have to stop for lunch? Leonard, you've
been awfully quiet over there.

**DR. SEEFF:** I'm listening. I'm learning.

**DR. WATKINS:** He's listening and learning. Anything else? Well anyway, let's
give them a round of applause. Terrific session. That was good.

(applause)

**MS. PAULS:** Lunch is out on the mezzanine. Please be back at one o'clock.

(Whereupon, at 12:08 p.m., a luncheon recess was taken.)
DR. SENIOR: We're going to start the afternoon session, and there's been a change in the program. Unfortunately, Bob Fontana reports that there's been illness in the family. So the afternoon session will be moderated by Chris Hunt. So I'll ask Chris to come forward, and as she does so, I wanted to say a couple of words about this evening's program. We realize that we have not allowed a lot of time for supper. But we hope that as many of you as possible will come and join actively in the discussion. We've outlined a number of points that we think will excite your interest and perhaps your comments, and copies of that, if you didn't receive them already in the email, there's a number. I think they made 70 more copies, and they're out on the table outside. So pick them up and please, if you can, join this evening's discussion. We need your input. We need your commentary. We need your support or objections. We need to know what we're dealing with, as we go forward with this question of should the guidance from the FDA to industry for controlled clinical trials, be modified to cope with the new stuff that we see coming. So we call that to your attention and hope you will participate. Chris, I'm going to turn it over to you now.

DR. HUNT: Thanks very much, John, and thank you for an excellent program. The first speaker will be Dr. Paul Watkins, who is kindly covering for Bob Fontana, who had a family emergency. I just want to say welcome. Paul needs no introduction. As the chair of the NIH DILIN, and also the leader of the Hamner Drug Safety Group and professor at the University of North Carolina. He will be sharing with us "GWAS and DILI: Promises and Pitfalls." Thank you, Paul.
DR. WATKINS: Genome-wide association studies (GWAS) and DILIN  

(Applause.)

DR. HUNT: Thank you very much, Paul. Excellent talk. Now I'd like to introduce Dr. Lloyd Klickstein, who is the head of Translational Medicine at Novartis, welcome, who will be discussing with us "Lumiracoxib: Can it be Reborn by HLA Typing?" Thank you.

DR. KLICKSTEIN: Lumiracoxib: can it be reborn by HLA typing?  

(Applause.)

DR. HUNT: Thanks very much, Lloyd. That was great. Now I'd like to introduce Dr. Ed LeCluyse, who is a senior research investigator in the Institute of Chemical Safety Sciences at the Hamner Institute, and will be sharing with us "Pluripotential Cells and Patient-Specific Hepatocyte Cultures." Thank you.

DR. LECLUYSE: IPS cells and patient-specific hepatocyte cultures  

(Applause.)

DR. HUNT: Thank you for an excellent overview. Now, I'd like to introduce Dr. Jack Uetrecht from the University of Toronto, who's been a leader in immune injury and its association with hepatotoxicity for many years. Welcome.

DR. UETRECHT: A fresh look at isoniazid hepatotoxicity  

(Applause.)

DR. HUNT: Thank you very much, and if you could please stay up here, we'll go ahead and open up the floor for questions, and let me see. We have about 15
minutes, so if I could invite the speakers to join us, in opening up the floor for questions.

**DR. COLLINS:** Nate Collins, Cellgene. This idea then that these reactions may not be immune mediated, because upon rechallenge, you don't experience DILI again is explained nicely by this idea of tolerance. In fact, the tolerance, if it's occurring, ought to in fact actually be protective, such that it's going to be long-lasting. If you rechallenge these patients or animals, it should be much less likely to experience a second round of DILI. Do you have any data for that, for instance, in your mouse model?

**DR. UETRECHT:** I don't actually. I mean I think you eliminate the memory T cells. I don't know if you also retain the cells that would mediate the immune tolerance. You may start back at square one. I'm not sure. Certainly another animal model we have with penicillin, it's not immune syndrome, which also can occur in humans. If you stop the drug, let them recover, rechallenge, most of those animals will again develop autoimmunity. It doesn't occur any earlier, but I think sometimes these T regs may disappear as well. I mean, even with penicillin allergy, people usually, with time, lose their allergy. So I think it's about ten percent a year will lose their allergy to penicillin over time.

**DR. BLOOM:** Jack Bloom, very nice presentation, Jack. Question for Ed. Nicely juxtaposed talk with Jack's and Ed's. With arguably a genetically relevant target cell and important effector immune component, the macrophage, absent the effector component such as the Th17, consistent with all the hypotheses we've had for mechanisms of immune mediated hepatotoxicity, what would be the utility of having that relevant macrophage population? Also, I know that you guys have been thinking a lot at Hamner
about the mouse with both the human liver and immune component. Boy, to be able to create that liver component from these patients would be a pretty exciting prospect. I don't know what the feasibility of that would be; probably low. But that would also allow you to answer the idiosyncratic metabolism question, versus the idiosyncratic immune response to it if you had a human immune system and your patient's had a transplant.

**DR. LECLUYSE:** So at this point, I think it's meant to open the doors or present options for where to take these new technologies, and I think you're exactly right. This is just scratching the surface of where it can go, including humanized animal models. But yes, you know, we acknowledge that it's going to have some shortcomings, with even just a macrophage hepatocyte co-culture system, and I think our intention was, or at least we envisioned that, we hoped to capture some of the early events, but hopefully still relevant events that maybe are exhibited in this particular patient population, that in the absence of having cells from those patients, I mean, we're sort of hunting around in the dark. So that's where we're going at this point with it.

**DR. HUNT:** I forget who was next. Okay.

**DR. ELMOUELHI:** Mohamed Elmouelhi. Just couple of questions to add. In terms of the IPSC and the DILI Network, has there been any confidentiality kind of information or consenting, barriers or issues in terms of doing the studies you are doing?

**DR. LECLUYSE:** Well, I think probably Paul, who's dealing with it more from an IRB perspective, could probably address that. Thanks.
DR. WATKINS: It has been a challenge, you know, even within the DILIN Network, maintaining identity links to these individuals we're now getting a whole genome sequence on, you know, can raise some thorny issues. But we've worked closely with the IRBs and made the consents very explicit. In this case, with CDI, you know, these cells live on forever, and can be commercialized by CDI. They've agreed to make them available with written permission to other academic investigators. But all this needed to be worked through, and that's why we just started with four as a pilot study, to see where that would go.

The other issue, if you put human hepatocytes in a mouse, and, all the models I know, you have to ablate the immune system to do that. There are several groups, including Sean Su at the University of North Carolina, who we've collaborated with, who can actually put human immune systems and livers into mice, mainly to study viral hepatitis and treatments, et cetera. So I think that would be the next step, to actually create a little mouse hospital of all our DILIN patients, that have both the immune system and the livers of DILIN patients, where we have the complete DNA sequence.

DR. ELMOUELHI: And the other question, related also to the IPSC. Is there any potential for artifacts or changes when you do that, compared to the original cells? Do you sort of add any new characteristics?

DR. WATKINS: Sure. In fact, speaking along the lines of epigenetics, if you will, some residual memory that comes along with those cells if you reprogram them. I think those are some of the issues that have still yet to be resolved, that we're looking into. Where it's going to be relevant or not in the case of looking at drug-induced liver injury types of issues, we don't know. I think, again, this -- it allows us to ask questions that we didn't use to be able to ask, and I think it gives us the opportunity, through these panomic approaches, to
look for maybe signatures or pathways that are unique to this subpopulation of individuals that have at least exhibited that kind of susceptibility. But yes, we do acknowledge that one of the issues is that there is some residual -- again, it's molecular memory that comes along, or epigenetic memory from the original cell type. So as you could imagine, even depending on what somatic cell that you originally took from the patient to derive your particular cell type, it's going to sort of come with a little bit of baggage too. So we're still exploring that.

**DR. HUNT:** Yes. Were you next?

**DR. WHERRY:** Yes. Janice Wherry from Bristol Myers Squibb. This question is for Lloyd, regarding the study where you looked at DNA in the patients who actually had the liver events. Did you have a control group of patients who did not have the liver events, but were also treated in terms of looking at specificity of the alleles that you found?

**DR. KLINKSTEIN:** Yes. The question was: what was the control group? And the answer is we did a 4 to 1 selection of controls to cases, and we got them all from the target population, or from a corresponding study with matched exposures in doses. So we did it as best as we could.

**DR. HUNT:** Tom, were you next? I'm not quite sure who was next. Okay.

**DR. VIERLING:** John Vierling, Houston. Ed, I was wondering if you could share your thoughts in the development of your dynamic systems? How are you going to try to recapitulate the zonal differences of hepatocytes, based on both metabolism and oxygen, and the phenomenon that drug metabolism does
appear to be somewhat zonal. Therefore, the environment is not just the cellularity, but it's also the microcirculatory environment. And with respect to bile salts, which you've shown nicely are being pumped out into those canalicular structures. Since they're acting as, you know, potent stimulators; in the case of hepatocytes, FXR with the chemo, that would be subject to re-uptake by the hepatocyte. One might want to wash that away. I'm wondering if you've got flow as part of your dynamic system, to change the nature for the study of those cells?

**DR. LECLUYSE:** Yes. So you've touched on a lot of important, particular but related topics. So in regards to the zonality of the system, we weren't intending to mimic obviously all the micro-environments in a single device. At least we feel that's a bit complicated at this point, in terms of where the technology is. But it gives you the option to mimic individual zones in individual devices. I didn't specify that, mostly because of time. But each of those devices do incorporate flow. So one of the things we can do is actually control flow dynamics, including flow characteristics, oxygen, tensions, things like that, to intentionally mimic local environments of the periportal region versus pericentral.

One of the things that I didn't mention also is just as it is in the liver, you'll have different phenotypes represented in the cell cultures too. Just as like an hepatocyte is not an hepatocyte is not an hepatocyte within the different micro-environments, right? One of the things that we haven't really touched on in this discussion is, you know, the nature of how that relates to some of these hepatotoxicities that are observed, that I think you're alluding to, even if indirectly. So I think this gives us the option to even define those characteristics under controlled conditions, and look specifically at how that might be a factor in all this. I'm not sure I got to your last point. Is it related to –
DR. VIERLING: No, I think you're addressing it. If you're having flow, it's a washout of the bile acids, which are basically going to act as hormones for nuclear receptors and potentially take what would be a normal excretion through the biliary system of those, and bathe potentially the cells. But if you're washing them away, I think that solves that problem.

DR. LECLUYSE: Yeah, and that's again an excellent point, and why at least, from my perspective, you'd want to use one of these more dynamic flow or bioreactor chambers, because the standard typical microtiter plate assays in a static culture environment don't recapitulate the dynamics whatsoever, right, of the liver in vivo. I think that affects both the types of toxicities that are going to be exhibited, including artificial types related to just building up of reactive metabolites, bile acids and byproducts, if you will. If you have healthy functional hepatocytes, I always like to say they will kill themselves by everything, by the nature of the beast, you know, under those conditions. So I think having these flow dynamic systems is going to improve the overall results and the accuracy of the results we get. So thanks.

DR. HUNT: Bob, and then Frank.

DR. ROTH: Bob Roth from Michigan State. I had a question for Ed and for Jack. First Jack, a provocative talk as always. Rather than be too provoked, I'll wait until my talk tomorrow, I guess. But I did have -- one comment about cyclosporine. You know, cyclosporine can also inhibit mitochondrially induced cell death. So I'm not sure how -- you know, one has to be a little careful in interpreting that data. I had two questions actually. That's just kind of -- one, you didn't show any histopath for the amodiaquine data, unless I missed it, and
I wondered what the histopath looked like in those livers.

**DR. UETRECHT:** It's very mild. There is an increase -- I mean you don't see hepatic necrosis. Again, the ALT, the highest was like 100. We do see an infiltration of cells. So there's especially M-2 macrophages. There's lymphocytes, there's Th17 cells. But you don't see hepatic necrosis. This is a very mild injury.

Back to the cyclosporine. I absolutely agree, although cyclosporine inhibits 3A4, and the bioactivation of amodiaquine is 2C9, I think, and we won't stop here. I mean we're going to do rag mice; we're going to try to inhibit by knocking out specific lymphocytes, anti-IL-17. Even though I think Th17 cells are involved, I suspect the final damage is due to cytotoxic T cells, not Th17 cells. I think they just produce the environment for the cytotoxic T cells to do damage. So still a lot of work to do, no question. But all the evidence is pointing in one direction.

**DR. ROTH:** Have you tried in the middle or near the end of the mild toxicity that you do get, to give LPS or something else that might induce a danger signal to convert that mild response over to one that's more robust?

**DR. UETRECHT:** We didn't towards the end. We did towards the beginning, and we get an earlier onset of the injury, but it still recovers.

**DR. VIERLING:** My question to Ed is how did you go about choosing the patients that provided your cells? Did you do that based on their HLA genotype?

**DR. LECLUYSE:** That's a great question and actually, Paul can you answer
that one, based on the four patients that were chosen from the network, to be --
that we're going to -- yes.

**DR. WATKINS:** Well, the exact four that have yet to be chosen, one has been
chosen to this date, and we picked the four drugs where we had done at least
exome sequencing on initially. Since it's been brought up by the Genetic
Subcommittee of the DILIN Network, maybe we should look at an augmentin
patient as well, so that we have one perfect INH patient who's agreed to do it so
far.

**DR. HUNT:** Frank.

**DR. SISTARE:** Frank Sistare, Merck. My question is for Jack. I want to make
sure I understand the similarities and differences in your models, from people to
rats to mice. So if I understand correctly, isoniazid in a human and in a mouse,
you can form the neoantigen. So you get good evidence of covalent binding.
You get lymphocyte invasion in the liver. No?

**DR. UETRECHT:** No. That was with amodiaquine. As I said, we can get –

**DR. SISTARE:** You don't see that in mice. You don't see the lymphocyte.

**DR. UETRECHT:** With isoniazid, we can get liver injury, but it doesn't look
like what happens in humans. It's not a model. With amodiaquine, we see a
model that does look like mild injury that resolves with what I propose as
immune tolerance. But we had not been successful in doing that with isoniazid
in animals.
There are a bunch of animals which have impaired immune tolerance which
we're going to try, and we've tried things like buthionine sulfoxamine, which inhibits glutathione synthesis. It was protective. I don't know why. You know, that electrophile reacts selectively with glutathione, and yet when we decrease glutathione levels, it didn't make it worse; it prevented the reaction. No matter what the mechanism is, what is that? I don't know. We'll find out.

DR. SISTARE: All right. So then let me go back. So with humans with isoniazid, you can also get this positive lymphocyte transformation test much later. You can go in there, you can take lymphocytes out, and you can add this HSA covalent INH and you can get a positive lymphocyte. With amodiaquine in the mouse model that you have, can you do something similar? Can you get a positive lymphocyte?

DR. UETRECHT: We tried it with a drug, a negative test, which is not surprising because the injury was mild. We can make them react to a metabolite and within a few weeks, we will have tried it with amodiaquine modified protein.

DR. SISTARE: Okay, and the last question, what about non-human primates? Is there any model like this in a non-human primate, where you can mimic anything that looks like this immune mediated DILI in a human?

DR. UETRECHT: There's so many things that we can do. Going into primates is not something --

DR. SISTARE: So there's nothing published. There's nothing out there that's ever been demonstrated to your knowledge?

DR. UETRECHT: No. I don't know that primates are that much a better
model than mice or rats. I think for each drug it's different, and I'm just not
prepared to go into primates. I mean these studies, so we can do up to 11
markers at one time to phenotype cells. That's getting expensive enough. If I
have to go into primates, I don't have the budget for it.

**DR. SISTARE:** Yeah. The only advantage would be is we do have a model like
that, the ability to go into translational biomarker discovery becomes really, I
think, enabled in that regard, because you know, to go from the primate, from a
non-human primate to a human primate. So we can reproduce a model like this.
I think that translational step becomes easier.

**DR. HUNT:** Paul, did you have a quick comment, and then we have to wrap up
the session.

**DR. WATKINS:** Is David Kleiner still here? All right. Too bad, because he
would be able to tell us whether microvesicular steatosis is seen in human INH
toxicity. I think he told me it is, but we can ask him tomorrow if he shows up.

**DR. HUNT:** Great. Well thank you all. Really appreciate it. A great
discussion, thanks. We're going to take a 30 minute break, and please be back
at three o'clock.

**MS. PAULS:** Okay. For those of you who are in the room and those of you who
are venturing into the room, for this afternoon and tomorrow morning, we will
have a sign-up sheet at the registration table. We try and get people together,
to make it much more cost effective for you all to go to the three airports in the
area. If in fact you need to go to the Metro or go to one of the train stations, the
train station, please let us know that as well. So that what they will do is ask
you for your name and your approximate time of departure. Please recognize
that in this area traffic can be a bear on a Thursday afternoon. So give yourself
a good hour and a half to two hours to get where you want to go. Chris?
(Whereupon, a short recess was taken.)

Session 2B

**DR. HUNT:** Thank you, Lana. And welcome to the second part of our Session
2. I'd like to welcome Dr. Keith Lindor, who's the Executive Vice Provost of
Health Outcomes at Arizona State University, welcome, who will be discussing
with us, is it autoimmune hepatitis or DILI? Clearly, a thorny issue. Welcome,
thank you.

**DR. LINDOR:** Is is autoimmune hepatitis or DILI?

(Applause.)

**DR. HUNT:** Thank you for an excellent talk. Now I'd like to introduce Dr.
Ross Pierce, who is currently the Acting Deputy Office Director, Center for
Biologics Evaluation and Research in the Division of Hematology, who will be
talking to us about why and how are biologics different than drugs. Welcome,
thank you.

**DR. PIERCE:** Why and how are biologics different than drugs?

(Applause.)

**DR. HUNT:** Thank you for a very nice overview. Now I'd like to introduce Dr.
Heide Stirnadel, who is from GSK Worldwide Epidemiology at GSK, who will
be sharing her data on pediatric liver chemistries, and also looking across
compounds at how drugs affect children versus adults; her talk is entitled "Are
Children: Not Just Little Adults and How They Respond." Welcome Heide.

**DR. STIRNADEL:** Are children not just little adults in how they respond?

(Applause.)

**DR. HUNT:** Thanks for a great job. Now I'd like to introduce Dr. Mark Alter, who is on the faculty at the Center for Neurobiology and Behavior at the University of Pennsylvania, and will be kindly sharing with us "The Plasticity of the Genome in Autism." Thank you, welcome.

**DR. ALTER:** Plasticity of the genome in autism

(Applause.)

**DR. HUNT:** If all the speakers could come back, please, and we'll open up the floor for questions. Also, if Lloyd Klickstein is available, we had a dangling question of his talk. Great, thanks. Maybe if we could start off with his questions, since we didn't get a chance to address it when he was last on the panel, because we ran out of time. Lloyd, do you want to provide the question about the ethnic differences? Great. And then we'll open up the floor for questions.

**DR. KLICKSTEIN:** Thank you. Christine asked me to come up and address very briefly the question of ethnicity, because I sort of left that hanging at the end. We see that as a huge challenge going ahead, as we think about how ethnicity is defined, and how we will adequately include people of varying ethnic backgrounds in sufficient numbers, to be able to make any conclusions regarding risks for DILI, if that exists for the particular drug. One of the more interesting things we found is that in our clinical studies, ethnicity is self-
assigned by the patients, so they reported. When we do the GWAS, we can do
an ethnicity analysis on the basis of the SNP profile, and it's often somewhat
different than the patient self-report. So which one do we believe? And we're
going to need some guidance on that as well from regulators.

The biggest differences were Caucasians mischaracterizing themselves as
Hispanics and vice versa, but I suppose it would depend where in the world you
did it. We all know that the genetic diversity in Africa is greater than the rest of
the world put together, and I'm sure things would be even more stark there.
Thank you.

DR. HUNT: We can open up the floor for any regulatory comments or general
comments about the ethnic differences for a safety biomarker.

DR. TILLMANN: A simple explanation, perhaps, for the genetic
misclassification could be, I think there is a study out of England, that about
eight percent of children are born to dads which are misidentified. So that
accounts already for a big proportion.

DR. HUNT: Thank you. Mark.

DR. AVIGAN: Well, I think this is going to evolve very quickly actually. I
heard on NPR just last week that in the recent Census, intermarriage between
ethnic groups is rising, in the United States, at least, to extraordinarily high
levels. So within a generation or two, this idea of which pure ethnic group
do you come from will be more of an esoteric question that will reflect sort of
past rather than future. I think that eventually, what we're really interested in
obviously are genetic susceptibility concentration effects. From what I observe
at the FDA when this question comes up around labeling, for example, in the
case of carbamazepine and HLA B*1502 for SJS, which got on the label recently, the language is somewhat circumspect, the way the label is written, it's pitched to the most kind of pure and most -- the demographic group in which the marker has most bang for its buck, which in that case is Taiwanese, with a negative predictive value of not having the marker as 100 percent. The case in point for 1502 testing is given in very specific terms. I don't think that that reflects a specific guideline per se. It's just kind of what people feel makes the most sense. Clearly, testing for markers of susceptibility for hypersensitivity or for drug-induced liver injury will be driven demographically by the presence of the marker in a particular population, as well as the incidence of DILI in that population from that drug. And if they're very, very rare, then it wouldn't make any sense to routinely screen everybody. On the other hand, if there's very good negative predictive value and the incidence of these effects are high, then obviously screening makes sense. So I think that there's a question here of discretion, and the agency has been somewhat careful not to be too prescriptive with regards to instructions for testing, as far as I can see.

DR. HUNT: Great, thank you, and I guess if I can -- and that was Mark Avigan from the FDA. Apologies. I meant to remind people to share their name and affiliation when they ask questions, and over to Leonard please.

DR. SEEFF: Keith, obviously this is addressed to you. First of all, thank you very much for that summary of autoimmune hepatitis. I must say, I was hoping desperately that you were going to tell us how to distinguish drug-induced AIH from so-called idiosyncratic AIH, because this is one of the most difficult things we face all the time. From what I heard from you, there are maybe some subtle differences, but not enough in the individual patient to really tell the difference. I suppose one issue that would be helpful is clinically,
if the patient recovers without treatment, then you can say well, this is -- and they developed an acute illness, that this is probably -- and they received a drug, and even though they develop ANA or smooth muscle antibody, this is probably drug-induced. On the other hand, let me try something out, and I say this with trepidation because in this hall there are people who are the great experts in this area. Should there be a difference in actual fact between drug-induced autoimmune hepatitis and so-called idiosyncratic hepatitis?

After all, if I'm correct, we are not born with the disease. Something has to precipitate it. We are born perhaps with a genetic predilection, but something has to precipitate it. Could a drug be precipitating autoimmune hepatitis and therefore -- unless the stimulus, the antigenic stimulus that precipitated this is different and has a different likelihood of causing severe disease, should there be a difference? Because what we've tended to say is if they recover, then it's the drug. If they don't recover, it's autoimmune hepatitis. But couldn't this be the precipitant that, you know, that the idiosyncratic issue is?

So this is a problem, which that leads me finally, that paper that you refer to, you know, I'm embarrassed at the fact that you, of all people, I'm saying I haven't read that and I was hoping to set it aside. If they were comparing drug-induced autoimmune hepatitis with idiopathic hepatitis, how did they actually chose the cases? How did you -- at Mayo Clinic, how did you actually come up with the fact that this is drug-induced and this is not drug-induced?

DR. LINDOR: Well, Leonard, those are good points. I wish we had an easy answer too. I think the distinction that we try and draw between these two conditions is probably not as clear as we wish it would be. There are examples of certain drugs that have been associated with prolonged chronic hepatitis, looks
like autoimmune hepatitis. So we know that drugs can be amongst the precipitants that do that. I think sometimes we look at treatment response, and you alluded to that. If a patient's on a suspected drug, and we withdraw it, and their liver tests normalize, that was probably not autoimmune hepatitis. Many times we're on the other end of the spectrum, where we're faced with a patient with relatively severe disease, bilirubins of 10 or 20 or so, and we are concerned about whether that patient has time to wait and see what the effects of drug withdrawal are. Many of those patients will be treated, and treated with corticosteroids. A patient who has either drug-induced liver injury and has the drug withdrawn and is treated with steroids for an autoimmune hepatitis, will both likely respond. Most patients who have autoimmune hepatitis, who are treated with corticosteroids, with or without azathiopine, will require 18 months to 24 months of therapy. At the Mayo Clinic, we did liver biopsies to demonstrate histologic regression before tapering them off. Not everybody does that, and a number of people will relapse. I think sometimes in the cases that turn out, in retrospect, to be more likely to be drug-induced liver injury, those were cases in which the response after the drug withdrawal and initiation of steroids is very quick, and maybe normalization was reached in a liver test within a matter of weeks. The immunosuppression was withdrawn, with or without a follow-up biopsy, and the patient didn't have any evidence of relapse. Sometimes the response of treatment, the rapidity of the response and the course after drug withdrawal help us in retrospect better determine which of the two categories was most likely. But again, that's different than seeing a patient at the bedside initially, and trying to make the distinction.

So I think, because there isn't such clarity oftentimes between the two conditions, despite our wishes that there were, we oftentimes end up treating people for both, withdrawing drugs as we can, and initiating immunosuppressive therapy.
DR. SEEFF: In that regard, by the way, to add to my comments this morning about inflammation coming to us at the FDA, to try to make a distinction, often what happens is that these cases are not followed long enough to know whether the enzymes have returned to normal. I would again plead, that if there is a patient that develops what looks like autoimmune hepatitis, and the drug is being considered as a possibility, that the patient be followed to see whether they recover and to send that information to us, which would make it a lot easier then to make a decision one way or another. I have had a number of instances, in what looks like autoimmune hepatitis, and it stops when the values are still 300 or 400. Can't make sense out of it. So it's just a plea on my part.

DR. LINDOR: And to answer the second half of your question, we have the luxury of having, because this was a retrospectively gathered series, we could go back and either find patients who really had no evidence of any implicated drug, or had longer-term follow-up and response to therapy.

DR. HUNT: Great, thank you. Mohammed, please.

DR. EMOUELHI: Mohammed Emouelhi, Novartis. Comment to Heide. You should, in your conclusion that the children could be more susceptible to the liver injury. But look at your demographics. You showed that the liver disease group had a lower body weight or not healthy kind of weight. And being malnourished or other reason for nutritional perspective could result in depletion of the defensive mechanism, the glutathione, as well as the UDPGE, and that can predispose them to higher incidence and risk for drug toxicity. Is that something that has been considered in your study?
DR. STIRNADEL: So I think the low birth weight particularly comes from, there were a lot of trials which were done in Africa in malarial children. So yes, all the points made are correct. I think what surprised us that we still didn't find a lot of combined elevation in these children. Also, they were malnourished and they were very diseased, having severe malaria at the same time.

DR. HUNT: Thank you. Yes, please.

DR. BOURDI: I am Mohammad Bourdi, NIH. I have two questions. The first one about the DILI in kids. So my first question is: do you see high increase of susceptibility in girls like you see in adults, in younger patients? And the second question is about since we see all the time there's susceptibility in people that are 65 and older, do FDA have any guidelines for like pharmacy company to test their drugs in aging mice, instead of keeping like young and not old animals? The last question is for Dr. Pierce on biologics. Do you think that in the future, there will be more case of DILI in using biologics? Because now in asthma there's a lot of use -- of trying to use anti-cytokines, antibodies to block like I-4 or I-13. These are protective in the liver. So maybe do you think there's going to be, are we going to see more risk in the future? Even 14F alpha antibody, studies have shown that DNF alpha is important for liver regeneration. If you block -- I mean, reduce the liver regeneration in the liver, maybe you're going to increase susceptibility to drugs in coming cases. Thank you.

DR. HUNT: So I guess to Heide first.

DR. STIRNADEL: Okay. What's the question? Oh, the gender. I think, although particularly in the younger age groups, the liver injury frequencies
that we saw was a lot more similar in females and males, at the same time. As we go more to the adolescent states, there was a slight difference of higher incidence in males. So we're working, and at the moment we're looking actually at a progression according to age as well, and to see if different thresholds might be applicable across the different age groups. But so far the gender itself, when we looked at the preliminary graphs, did not see a big difference in any thresholds we would need to apply.

DR. PIERCE: Regarding the question as to whether we would be, you know, more likely to see more hepatotoxicity among biologics in the future, I mean, it certainly is a possibility, and as you point out, many of the neurobiologics are targeting various aspects of the immune system, and we heard about, you know, an impact on tissue repair systems. If you impair systems that are, you know, repairing an injury, then that may ultimately, you know, change the trajectory of the course of the injury, so that it progresses to the most severe or fulminant category. But, you know, that would just be speculation. So I hope not, but we are certainly continuing our vigilance in clinical trials and post-marketing in this regard.

DR. HUNT: And perhaps for the last, the middle part of your question, does anybody from FDA have any commentary about studying geriatric animals? No takers. Okay. Sorry, over to the far left. If you could please introduce yourself?

DR. KULLAK-UBLICK: Yes. Gerd Kullak-Ublick, Zurich and Basel, Switzerland. I have two questions, one for Dr. Lindor and one for Dr. Stirnadel. The autoimmune -- given the similarities in autoimmune hepatitis and DILI that you showed, what is your feeling on the use of steroids in DILI?
We have some examples. Budesonide is somewhat effective in flucloxacillin-induced DILI. So what are your feelings on the use of steroids in DILI? The question to Dr. Stirnadel, you showed the spontaneous reporting rates for DILI in children, which are obviously very dependent on the way pharmacovigilance networks are set up in different countries. And at any rate, they're only useful for generating signals, and not for calculating incidence. We have some very good adult databases, pharmacoepidemiological databases, such as GPRD in the UK. Should we be supporting a systematic evaluation of pediatric patient files, connecting pediatrics in private practice?

DR. STIRNADEL: I start first. No, I think very good suggestion. I think the idea was to use this adverse event databases, to kind of very quickly see if we can actually see differences in adults versus children, and we wanted to do the proof of concept to kind of show that we can actually do that. I mean, we do a lot of these safety studies in GPRD or in the big U.S. claims databases. But often it's very difficult to define hepatotoxicity, in particular when it's associated with, if it's drug-induced hepatotoxicity due to co-medications, co-morbidities or even a follow-up, and often there are some LFT measures done. So I mean hopefully in the future, with all the linkage, with UK Biobank, et cetera, that we can actually use these databases to identify the cases a lot better, and so we can do a lot better prognostic studies for incidence and actually association of risk. Thank you.

DR. LINDOR: I'll take the question about use of immunosuppressants for DILI. It's really one that depends upon how severe, the clinical severity of the case, and also the time course of the response to the withdrawal of drugs. So if the patient has mild disease and their liver tests become normal very quickly, of course not. If they have severe disease and there's a prolonged response and
they actually get worse, oftentimes clinically we're pushed to initiate a course of steroids. So I don't think it usually does any harm to the individual patients. It just -- it may save a number of patients from needing transplant or other adverse outcomes.

**DR. HUNT:** Great, thanks. I'm not sure exactly who was next. Does anybody know? Arie, okay.

**DR. REGEV:** Two questions to Keith Lindor. One was regarding the differentiation between autoimmune and drug-induced. I was specifically wondering about a specific finding. There was mentioned fibrosis, and when there is a significant amount of fibrosis, I would think that this would be a pretty strong way to differentiate, and I would be happy if you could elaborate about that. The second question is: one of your slides mentions that diabetes is a risk factor for drug-induced liver injury, and I was wondering if you could elaborate about that as well.

**DR. LINDOR:** The reference that related to diabetes said that patients who have some evidence of mitochondrial dysfunction, and they reference diabetes as a cause, were more predisposed to drug-induced liver injury. You all are more of the experts on mechanisms, so I'd be interested in the discussion about that. The other question related to the presence of fibrosis, which was seen in the cholestatic form of drug-induced liver injury. I think that maybe it just means that in patients who have -- the hepatocellular ones are oftentimes more of an acute picture. I think the fibrotic, the cholestatic form may be a little bit more chronic-looking, if we think of some of the cholestatic drug injury patterns as maybe having more chronicity. So I wonder if that's not the piece that they were looking at.
DR. REGEV: So for hepatocellular presentation, fibrosis would be a pretty strong indicator of autoimmune. That's what I would expect, but I was wondering what you were thinking.

DR. LINDOR: I think that autoimmune hepatitis usually, particularly if it's been undetected or unrepresented, can go on to develop fibrosis and cirrhosis as we've shown. I think that it's -- the presence, in the hepatocellular picture, the presence of fibrosis, I think, would lean a person towards autoimmune hepatitis, whereas fibrosis in a cholestatic drug-induced liver injury may be not so strong a predictor of autoimmune hepatitis.

DR. HUNT: Great, thanks.

DR. TILLMANN: Hans Tillmann, Duke. Also a question concerning DILI versus AIHA. Did you look into immune globulin levels, specifically IGG-2 and the antibody titer, because that might help differentiate?

DR. LINDOR: No. Those weren't looked at. This was the data, the clinical data was retrospective. The prospective work was the look, the review of the liver biopsy. So we didn't have IGG-2 levels in this group.

DR. HARVEY ALTER: Excuse me. I'm Harvey Alter, the proud father of Mark Alter. But I'll address the question to something else. Actually Leonard's comment, and coming from a hematology background, there seem to be parallels.

If you look at red cell destruction from drugs, there's at least three known mechanisms. One is a direct membrane injury. But more commonly, it's a drug
attaching to the red cell membrane, inducing an antibody, which then attacks the cell, and even more commonly, it's a drug interacting with an antibody and the immune complex is what's damaging the cells. The latter two mechanisms are indistinguishable from autoimmune red cell destruction, the only difference being you know the drug in one case and you don't know the inciter in the other case. So it's somewhat semantic as to whether you call it autoimmune or drug-induced. The mechanisms are similar.

**DR. LINDOR:** I don't disagree with you. I think that that's oftentimes the case. Most of the patients I see end up having cholestatic liver disease, and the cholestatic drug injury are some of the ones that have the really prolonged course, and may go on to even requiring transplant. So, at least in that realm, it's common to see a prolonged cholestatic response that can be difficult to distinguish from some of the other chronic cholestatic diseases that we think of as having an autoimmune basis like PBC. In autoimmune hepatitis, I think the same thing happens. I think that sometimes we know that there are drugs. We don't seem them as often now. Nitrofurantoin would be maybe a classic example of prolonged use of a drug -- it's withdrawn, and the patient still has what looks like typical autoimmune hepatitis. I think in the past, the terminology for that condition has changed a lot. Some of the old terminology, chronic active liver disease or chronic hepatitis, may be more, actually more accurate. When we move to autoimmune hepatitis, I think then it really put more specificity on what the presumed mechanism of disease was. I think that sometimes we're seeing the similar pattern of injury, and the terminology we now use, autoimmune hepatitis, describes a cause, which may not be accurate.

**DR. HUNT:** All right, thank you. Who was first? John.
DR. VIERLING: Yes, Vierling, Houston. Dr. Pierce, I wanted to ask you about the issues of the monoclonal antibodies, which are active against TNF alpha. It would seem, from a hepatology perspective, that we started to see these reports on individual cases of autoimmune hepatitis, again with the same kinds of caveats we’ve been talking about, and that Keith Lindor has been emphasizing, rather late after the introduction of those agents, which were predominantly being used, and infliximab obviously is the first one out of the chute, in inflammatory bowel disease. Then later, it started to be used more widely by dermatologists and rheumatologists. Here, we see if you just sort of look at the years of the reports, it would be my perspective that we started to see these reports more recently than we did at the time of the introductions in the inflammatory bowel disease population. Is there something kinetically about that? You're more privy to the timing of when the reports became available, and how widespread they are. But it seems that there was a gap from the first introduction, and sort of a lack of report of these cases, and we're seeing more now. I'm just wondering if the populations being exposed have something to do with the toxicities being reported now as autoimmune-like.

DR. PIERCE: Actually, it's my colleagues in CDER who are more privy to the time course of those reports. But in 2003, there was an analysis of 134 cases, I believe, and more close look at 50 of them for drug-induced liver injury, or rather autoimmune hepatitis, with any of the TNF inhibitors. There were 43 of them that had confounding factors, so that 14 percent of them were felt to be more pure cases. So there's been discussion of whether the risk really varies according to the indication, and this is certainly a possibility for, you know, some of the compounds. But overall, my impression, as kind of an outsider, actually, to this field, is that there doesn't seem to be terribly strong evidence for that. But that it certainly is possible that early on, the clinical practice is
more restricted to a limited number of indications, and then as it expands and
as it's used in also more common conditions, you know, the reports start to
come in. Also, you have to think about whether what type of communication is
put out your physicians about the existence of these phenomena, in terms of
Dear Doctor letters or other communications, postings on FDA website. So it's
not uncommon for there to be somewhat of a lag, and then once the ball gets
rolling, reports beget other reports, because people start making the association.
Oh, this is something that can maybe occur, and well, we're seeing it in, you
know, rheumatoid arthritis patients. Well, maybe we haven't seen it yet in, you
know, chronic inflammatory bowel disease patients. So I've got one now. Well,
I'll go ahead and report that. I wish I could give you a more specific answer,
but that's all I'm privy to.

DR. AVIGAN: Well, I had two comments. One is to chime in on this question.
So we reported a couple of years ago -- Dr. Bezabeh, who was here before, was
the first author -- about a similar, almost like an outbreak. There was a flurry of
cases of acute liver injury, hepatocellular injury associated with exposure to
natalizumab, which is a monoclonal antibody which blocks alpha-4 integrin in
MS. It's used to treat patients with MS. And after just one or two doses, a
certain number of patients developed this thing, and it was reported. When we
looked at the cases, they had some footprints of autoimmunity. In other words,
patients were reported to have auto-antibodies, so smooth muscle, DNA,
double-stranded antibodies. But they didn't have all the criteria that you
would like at the bedside to call it idiopathic autoimmunity. Their titers
weren't all that great. But they had some features of autoimmunity. So the
question, when we were looking at these cases, beyond doing causality but with
regards to mechanism, it wasn't so clear to us whether the autoimmune effect
was epiphenomena, or whether it was a driving mechanism that was caused by
a change in immunity caused by the drug. We ended up writing a general
discussion, and we weren't sure whether the cause was excipient or immune
modulation, or even a virus that we couldn't identify in the liver. Just as a
pointer, I wouldn't be surprised in the future if we accrue more cases in
treatment plans that use monoclonal antibodies or immunosuppressive
therapies with biological agents. It may turn out to be more general
phenomena. So that's just a point you might want to consider. Discuss it if you
want to.

I wanted to just talk to Mark Alter's point about attractants. Tomorrow,
I'm going to be talking about biomarkers, and the problem, you know, you were
framing it from the perspective of the system going into certain sort of
homeostatic syncs that it tends to go to, based upon sort of preset epigenetic
sort of settings, and you were tying that to autoimmunity. So in DILI, there
also may be this idea of being able to forecast, based upon the initial conditions,
a very complicated series of steps towards a certain direction that the system
will take, and potentially be overwhelmed. Beyond epigenetics, this is a general
question, as a frame for heading the system towards a particular homeostatic
sync or attractant, what other features of the system will direct the outcome,
and very specifically, in weather forecasting, what's very important in terms of
making a prediction of where the system will go, to which attractant it will go,
is based on the initial conditions, the actual -- the general conditions. And that's
called the butterfly effect. I'm just curious what you, beyond the epigenetic
profile, what other features would come into play, to forecasting, you know,
sort of that concept?

DR. ALTER: So I think, I guess with respect to the butterfly effect, I think this
is very on the other end of the spectrum from that, because the butterfly effect
would suggest that small variations in initial conditions can lead to vastly
different outcomes, whereas this is saying that there's very defined outcomes, at least for specific cells, that can only kind of move between one state to another, or at least that's the idea. So as far as what's going to determine where things go, I think the ideas that I would think about are kind of two components. You have to destabilize the first program. So that would be one kind of general thing. You could think about things like opening chromatin might destabilize the program. You need a stimulus to kind of push things to get going, and then you need to restabilize at the other end. So I think things that destabilize. So things like HSB90, co-chaperone type proteins, things that make connections between, that help to create or help to form these self-organizing networks are going to be important for destabilizing and restabilizing. So I think stress responses are one thing you could think about. Other things would be I think epigenetics are going to be important, and then like things, just like general, thinking about it from a transcriptional, general transcription factors and modifications of those might also –

DR. AVIGAN: Right, and as you mentioned, the yin and yang of regeneration and cytoprotection, which the liver is very ready to do. So it's sort of just to predict downstream effects of when the liver will be overwhelmed or not, has to take -- to model it has to take that into account as well.

DR. HUNT: Great, thanks, and that was Mark Avigan from the FDA.

DR. VANCUTSEM: Hi. Paul Vancutsem, Biogen. I think that Dr. Avigan actually pulled from that question on the DNA. It's a question for Dr. Pierce mostly. With biologics, when there is liver injury after administration of biologics like -- the case of TNF alpha was mentioned, how much do we know whether it's a direct action of that biologic, or how much do we think it's
facilitating the space for, let's say, a small molecule or virus, to actually cause the liver injury, and does that really matter when we develop a drug?

**DR. PIERCE:** Could you elaborate a little more on the second mechanism involving the small molecule? I didn't quite follow that.

**DR. VANCUTSEM:** Basically, with the danger hypothesis, there's a lot of ideas that actually changing the immune milieu favors -- well, when the reactive intermediates are produced, favors recognition of these reactive intermediates and an immune reaction against -- well, it's something that becomes autoimmune. So if we give compounds, biologics that influence the immune milieu, are we basically opening the door to having reactive intermediates recognized that are produced by other molecules? And if it's the case, does that matter and how could we deal with that?

**DR. PIERCE:** Right. I think those are, you know, interesting ideas, and I've been looking to try to see how much work has been done, in terms of trying to get at the mechanism in the, you know, cases that have been seen with biologics to date, and I haven't seen very much. So I think most of what I've talked about and what you're suggesting to date may be in the area of possibilities and speculation. So I look forward to more concrete mechanistic work being done.

**DR. VANCUTSEM:** I hoped you had more of an answer than that, but thanks anyway.

**DR. PIERCE:** I hoped so too. But if you will send me your email, if I can get more information on that, I'd be happy to share that.
DR. VANCUTSEM: Great, that would be nice. And maybe Dr. Uetrecht, you're in immune toxicology. Do you have more to tell us about that hypothesis?

DR. UETRECHT: No.

DR. VANCUTSEM: Okay. I'll give you my email also. Thank you.

DR. HUNT: Are there any other questions, comments, thoughts? None? Okay, well, thanks, all. Thanks for a great general discussion.

MS. PAULS: We are wrapping up for the day. The reception is ready and waiting for you, and it will be available until 6:00 p.m. tonight. For those of you who are interested, please join us back in this room at 7:00 to talk about the FDA guidance. Thank you, and we convene tomorrow at 8:00 a.m.

(Whereupon, at 4:50 p.m., the meeting was recessed.)