FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
OFFICE OF SURVEILLANCE & EPIDEMIOLOGY;
CRITICAL PATH INSTITUTE;
AMMERICAN ASSICIATION FOR STUDY OF THE LIVER

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DRUG-INDUCED LIVER INJURY CONFERENCE XVI: HOW SHOULD LIVER INJURY AND DYSFUNCTION CAUSED BY DRUGS BE MEASURED, EVALUATED, AND ACTED UPON IN CLINICAL TRIALS?

23 and 24 March 2016

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The Conference met 23-24 March 2016 in the University of Maryland Marriott Conference Center, Chesapeake Ballroom, 3501 University Boulevard East, Hyattsville, Maryland.

John Senior, Lana Pauls, Paul Watkins, Mark Avigan, John-Michael Sauer, and Arie Regev, (organizers), presiding.

ORGANIZERS
JOHN SENIOR
LANA PAULS
PAUL WATKINS
MARK AVIGAN
JOHN-MICHAEL SAUER
ARIE REGEV

Wednesday, 23 March 2016

Welcome and Ground Rules – LANA PAULS

SESSION I: HEPATOCYTE ADAPTATION AND POSSIBLE RECHALLENGE
Moderators: ARIE REGEV and JOHN SENIOR,

1.1 CHRISTINE HUNT
1.2 VID STANULOVIC
1.3 JULIE PAPAY
1.4 JOHN SENIOR
1D.1 Discussion
Break
1.5 AYAKO SUZUKI
1.6 NEIL KAPLOWITZ
1.7 LILY DARA
1.8 MALA CHAKRABORTY
1D.2 Discussion

Special Award to Lana Pauls for Outstanding Service
Lunch break

SESSION II: TREATING ADVANCED LIVER DISEASE WITH DRUGS
Moderators: MARK AVIGAN and DEBBIE BIRNKRANT,

2.1 JOHN VIERLING
2.2 PATRICK KAMATH
2.3 GREG EVERSON
2.4 MICHAEL FRIED
2D.1 Discussion
Break
2.5 ARUN SANYAL
2.6 BOB FONTANA
2.7 POONAM MISHRA
2.8 RUBY MEHTA
2.9 MARK AVIGAN
2D.2 Discussion

Reception and dinner break

P-R-O-C-E-E-D-I-N-G-S --- 23 March

8:01 a.m.

MS. PAULS: Okay. Good morning, everybody, and welcome to the 16th Annual Hepatotoxicity Conference. For those of you who don't know me, my name is Lana Pauls and I'm with the Office of Surveillance and Epidemiology at the FDA and I've been here since Day 1 with John. So, this is our 16th conference and it's been an absolute, wonderful experience for me.

A couple of things that you may not know about me, if you haven't been here before, is that I make the trains run on time. So, all the speakers will be on time, the breaks will be on time, lunch will be on time, as will the reception, and I'm pretty stringent about that. I have that reputation. I'm not sure if that's good or bad.

John had asked me to go over a couple of ground rules today, because in the past we've had a little issue with regard to when questions are asked by whom in the discussion periods. This conference is being recorded by a court reporter, Toby Walter, over there in the corner. He's going

to capture every word that is said, both during the presentations of the slides and during the discussion periods.

There're going to be two 30-minute periods for open discussion in each of the four sessions. So, there will be a total of four hours of open discussion over the two days. This is not an accident. We did this on purpose, because in addition to the presentations, these discussions and dialog are perhaps the most important part of these meetings, because we get a lot of subtleties that aren't necessarily presented.

So, before you ask a question, and I'm sure that one of the speakers and/or moderators if I am not up here, will remind you before you ask a question, please make sure you say your name very, very clearly, as well as the company that you represent, because Toby over there doesn't know all of you, even though this is his fifth or sixth conference with us. And it's really hard for him if he hears just a garbled name, to put the name with what is said.

What John does after the meeting, again for

those of you who have not been here before, he goes over the transcript word by word and matches it with the slides. And then the annotated slides and text of comments made during ensuing discussions are posted on the website. That's why it's so important to get these names. With that being said, I'm going to go ahead and introduce the not introducible Dr. John Senior.

DR. SENIOR: Thanks, Lana. (Applause.)

We're going to start out with an issue that has been rather controversial; that is rechallenge of patients who have already shown some indication of liver injury from a drug.

Now, it started out with considering approved drugs that were already out in the market, out in practice, but we have taken this to the previous step, which is to go into the drug investigation process before the drug is approved, and there's a big difference.

Before the drug is approved, the company or the company sponsor must report everything that happens. After the drug is

approved, it gets into practice. We have no control over medical practice, nor should we, but we don't get very full reporting of adverse effects after drugs are approved for practice. So, we're going to discuss some of the issues of rechallenge and whether it can be done safely or whether it cannot. The first speaker is Chris Hunt from Duke, and she's going to tell us that rechallenge is too dangerous.

1.1#1 CH: DR. HUNT: Thank you, John and Lana, for the invitation to talk today. I look forward to a lively debate. Before talking on rechallenge, I just wanted to review some of the issues that we're all familiar with, with the initial drug-induced liver injury.

1.1#2 CH: As shown by the U.S. Drug-Induced Liver Injury Registry, a prospective series, roughly one in 10 patients with drug-induced liver injury progress to death or liver transplant. And roughly 1 in 6 go on to chronic liver injury. If you look at -- and that's across all drugs in the U.S. And if you look at individual agents, we know that fialuridine actually resulted in roughly half of patients in the Phase 2A study succumbing to

death or transplant. So, just the initial drug-induced liver injury can be quite dramatic. Additionally, up to 1 in 20 patients with drug-induced liver injury have concomitant Stevens-Johnson Syndrome or Toxic Epidermal Necrolysis with substantive mortality. And lastly, after an episode of drug-induced liver injury, patients are at uncommon risk for drug-induced autoimmune hepatitis when receiving a related, or even an unrelated drug.

1.1#3 CH: After an event of drug-induced liver injury, it's important to see the liver chemistry is resolved when the drug is stopped. And this is called positive dechallenge, because you want to look at that resolved event and then examine positive rechallenge. As the drug is restarted, you can see a doubling of the ALT for hepatocellular injury, or a doubling of the alk phos and bilirubin for cholestatic and mixed injury.

1.1#4 CH: Drug rechallenge, unfortunately, is associated with even greater dangers than drug-induced liver injury with up to 50 percent mortality with the particularly notorious

halothane, an uncommonly used anesthetic now in U.S., with mortality particularly seen in the first month of rechallenge. In the prospective drug-induced liver injury drug registry in Spain, roughly one in eight patients rechallenged with the drug went on to death or transplant. And typically this is with hepato -- drugs resulting in hepatocellular injury. And Julie Papay has published a series of 88 rechallenges and found a two percent mortality rate in a large retrospective series, and found the fatalities were in patients with severe hepatocellular injury and jaundice.

1.1#5 CH: In addition to mortality, rechallenge is also associated with frequent jaundice, hypersensitivity and hospitalization. And as shown in both prospective and retrospective series, most rechallenge events occurred more rapidly in the Spanish series in less than half the time -- rechallenge injury was seen in less than half the time of the original drug-induced liver injury. And in Julie's series, liver injury occurred within a week of rechallenge for nearly half of drugs. In the greater scheme of drug use, most rechallenge

events are unintentional. Either the providers or the patients were not aware that they had drug-induced livery injury and, therefore, the drug was readministered. So, therefore, drug rechallenge is preventable and it's really important that the initial drug-induced liver injury is shared with the patient communicated as a significant risk.

1.1#6 CH: There was a very nice paper by Einar Bjornsson and Jay Hoofnagle looking at -- looking at all the drugs associated with drug-induced liver injury in the LiverTox NIH database and dividing drugs by classes -- by groupings with the red bar depicting drugs with greater than 50 drug-induced liver injury cases reported and a decreasing number down to the yellow bar with drugs where only one to three reports of drug-induced liver injury were seen. And interestingly in drugs associated with fatalities, you can see that fatality -- drugs associated with at least one fatality, you can see that most of these drugs also were associated with positive rechallenge. And fatalities parallel with positive rechallenge in this large series of

hundreds of drugs causing drug-induced liver injury.

1.1#7 CH: Let's examine the risk factors associated with a positive drug rechallenge. We know that hepatocellular injury is associated with the highest morbidity and mortality. And we've seen that in the rechallenge prospective and retrospective series. So, we're looking for drugs that cause reactive metabolites to form and, additionally, impaired adaptation or regeneration.

1.1#8 CH: Additionally, we want to examine immunoallergic drug-induced liver injury. We know that increasing numbers of drugs, even though they're a minority, have an HLA association with liver injury and have additional hypersensitivity with fever, rash, eosinophilia. We should also examine drugs resulting in mitochondria impairment. We know that some lipophilic fat-soluble drugs that can be cationic can actually concentrate a mitochondria. Additionally, we know some drugs can interfere with mitochondrial DNA. And we know that roughly four in ten drugs

withdrawn from the market were associated with the black box warning due to liver injury or associated with mitochondrial impairment pre-clinically. As reflected, now, we can see a new paradigm that's used to quantify levels of hepatotoxicity. The increased reactive oxygen species/decreasing ATP ratio is indirectly giving us information on mitochondrial impairment. Additionally, bile salt export pump inhibition has been put forth as a significant hepatotoxicity issue affecting most drugs withdrawn from the market or associated with a black box warning. However, also associated with nearly half of drugs not associated with drug-induced liver injury.

1.1#9 CH: So, let's see how these factors play out and which are most important. As an example, I'll use the old anesthetic halothane, because it's notorious for drug-induced liver injury resulting in 50 percent mortality in patients receiving the drug within a month after an event of hepatocellular injury with jaundice.

1.1#10 CH: It's associated with hypersensitivity. The halothane is oxidized through a

trifluoroacetyl halide which forms protein adducts, and also free radicals could form. Additionally, it causes mitochondrial impairment with inhibition of complex I and II, and inhibition of fatty acid and pyruvate oxidation.

1.1#11 CH: We also know from epidemiologic studies, and I'll share two case series, that the highest risk of injury is within the first month of anesthesia. And there's an increased susceptibility in females and in obese patients. So, this cartoon just sort of highlights the individual aspects of halothane rechallenge injury.

1.1#12 CH: Halothane gets into the hepatocyte, is oxidized to the trifluoroacetyl halide, which forms protein adducts sparking immunoallergic injury. Free radicals are formed contributing to cellular injury and inflammatory cascades, and mitochondria are impaired resulting in loss of energy. Mitochondria provide 90 percent of the cellular ATP. So, therefore, impairment has grave consequences for the hepatocyte. Mitochondria can regenerate. However, with the impairment, it

requires one to several weeks for mitochondria to regenerate and this is actually important to recognize.

1.1#13 CH: This graph depicts the percent fatalities over time in patients receiving halothane rechallenge after having an event of hepatocellular injury and jaundice. And as you can see, particularly within the first month of halothane rechallenge, fatalities peak with nearly half of patients receiving halothane rechallenge resulting in death. And this actually aligns with mitochondrial regeneration. It takes one to several weeks for mitochondria to regenerate after being knocked out by halothane.

1.1#14 CH: A more contemporary example of a drug associated with positive rechallenge is lapatinib, a tyrosine kinase inhibitor used in breast cancer. And similarly, lapatinib is metabolized by cytochrome P450 3A, forms an electrophilic metabolite which forms protein adducts. Some patients are particularly susceptible, because they have an HLA marker associated with injury.

1.1#15 CH: And additionally, lapatinib inhibits

the bile salt export pump resulting in bile acid efflux blockage. This results in bile acids accumulating in the cell. Then bile acids are mitochondrial toxins, it can impair mitochondria and cause cell death.

1.1#16 CH: So, we're seeing themes that there's actually interplay of patients, drugs and perhaps environment. We know patients with the appropriate HLA markers in genetics, immune response, inflammatory state and/or microbiome are interacting with the drug.

1.1#17 CH: And there are some drugs that appear to have a greater risk with mitochondrial liability, reactive metabolite formation or bile salt export pump inhibition. And it's less clear the role of other drugs or infectious agents, but undoubtedly interplay for positive rechallenge. This is a complex table, but you don't even have to read it. It's a heat map designed to profile -- the drugs on the left, the left column, are drugs associated with at least ten rechallenge events and the characteristics profiled in a heat map fashion with red meaning severe, orange meaning marked, yellow

meaning mild to moderate, and green meaning no issue, with white meaning no data -- at least no data I found.

1.1#18 CH: As you can see, these drugs associated with positive rechallenge in descending order as seen in the left positive rechallenge column -- actually, anyway, people can see below the red, decreasing positive rechallenge, you can see that some themes arise. It appears that mitochondrial impairment, immune injury and reactive metabolites are common. And bile salt export pump inhibition, not so clear. So, layering onto this table I've actually put in the yellow text below, drugs with non-serious drug-induced liver injury. And in the white text below that, drugs not associated with drug-induced liver injury. And we've compared these same characteristics. And as you can see, there's clearly a marked difference. And it appears that the positive rechallenge drugs differ from other drugs not associated with positive rechallenge even if they're associated with drug-induced liver injury with a preponderanc of mitochondrial dysfunction, immunoallergic injury

and reactive metabolites among those associated with positive rechallenge.

1.1#19 CH: So, it appears the risk of positive rechallenge is increased with mitochondrial injury, reactive metabolites and immunoallergic injury. And to conclude, we -- the overwhelming data suggests we should avoid drug rechallenge due to two to 51 percent morbidity and mortality. However, we need to collect data.

1.1#20 CH: This is really key particularly for drugs -- critical meds that are given cyclically like collagen medicines. We should be looking at large healthcare databases, as Ayako Suzuki is doing, and prospective DILI registries, as many of the IDS members are doing.

1.1#21 CH: And consider drug rechallenge only if there is a compelling benefit that exceeds the risk after careful assessment assuring a clear patient understanding of the benefits and risks and informed consent, and that there's not a therapeutic alternative for the patient and close clinical follow-up.

1.1#22 CH: I just want to acknowledge those who have

driven hepatotoxicity science forward and all those who have contributed their data to rechallenge.

1.1#23 CH: And I just would thank you.

(Applause.)

DR. SENIOR: Very nice, Chris. Beautifully delivered and right on time.

Arie:

DR. REGEV: Okay. So, to our second speaker of the day, and this will be Vid Stanulovic who is a safety physician and a consultant to pharmaceutical companies with the Semmelweis University in Hungary. And he will actually make the case of maybe it is not that dangerous.

1.2#1 VS: DR. STANULOVIC: Thank you for the introduction. And I would first of all like to thank Dr. John Senior for inviting me and for the whole team for organizing this event.

1**.2#2 VS:** I'm very happy to be here and I will be presenting the pro argument, the pro rechallenge, but it's kind of difficult to say I am in favor of rechallenge when I really have to agree with what

Chris Hunt -- (Laughter.) -- has been saying. So, the data show it. I mean, what else can I say about it? So, definitely, I mean, rechallenge can be dangerous. So, what I would actually like to do is to build upon this and see, well, it is dangerous, but, I mean, what can we do about it in certain situations of clinical need?

What I would like to argue that in certain situations the risk can be minimized, or in certain situations it may be just that it's due to, well, other factors, compounding factors. Therefore, there may be no risk at all, and how to provide guidance on what we can consider as justifiable rechallenge scenarios.

1.**2#3 VS:** Now, why not rechallenge? Maybe I should primarily start with the scientific basis rather than medicolegal. Well, scientific basis, we're assuming that it's some unknown susceptibility to an adverse drug reaction. Generally idiosyncratic exists in this patient, which we are not rechallenging, but that this same idiosyncrasy will not occur in the vast majority of other patients, which we are continuing to treat. So,

there should be some kind of a hypothesis, some kind of evidence that this is going to happen in this patient only and a very limited number of other patients. Let's really limit it to a hypothesis. I mean, we of course all know that really finding evidence is difficult. And of course the medicolegal commercial base is looking at it from the sponsor's perspective.

1.**2#4 VS:** I mean, really you don't want to stop your development because you can rechallenge this one patient and this patient died. I mean, this is something that would really halt the entire developmental project, again, when it comes to investigators, when it comes to prescribing physicians also in the post-marketing phase. I mean, this is something that would be a sensitive issue. It is definitely far easier to say, all right, let's see what else we can do about it. Let us really not rechallenge. So, there are good basis, solid basis for not rechallenging, but let us see why and in which situations can we consider rechallenge. If a patient is enrolled in a clinical trial, we're assuming that the patient needs

treatment. And initially it has been assessed that the benefit does outweigh the potential risks. Of course there are these situations of rare and neglected diseases where there is no alternative treatment with really limited treatment options if we're talking about some targeted therapy for rare cancers or other congenital, metabolic, genetic diseases, or let's reconsider rechallenge in case of failure or intolerance to second-line therapies. And really what we should be keeping in mind, that, again, I have to agree with Chris Hunt saying that we need to really have compelling evidence that there should be some knowledge base to expect that the benefit/risk balance may be favorable in this individual patient and we're talking really about the individual patient. We're talking about N=1 clinical trial here with taking a subset of a clinical trial population of a hundred patients or a thousand patients or whatever are the number of patients. I mean, if we are rechallenging this one patient who has experienced an adverse drug reaction, this isn't an equal clinical trial, which can be extended to N=1+1+1 if we are really

going ahead with further rechallenges.

1.**2#5 VS:** So, in case you decide not to rechallenge, what happens? And this is really what got me thinking about this whole idea. The threshold for premature discontinuation of patients in clinical trials is generally low. You go for the option of let us rather discontinue this patient than risk. And what happens, you have a high drug discontinuation rate due to adverse drug reactions than what I was in a situation to do with and subsequently argue, well, this is because of this factor, other factor, et cetera. And this is something that really is quite justifiable in a number of cases, but really we're talking here about a legal case where you are innocent until proven guilty. You are guilty until proven innocent when it comes to an adverse drug reaction. If discontinuing, you are talking, well, they say there is a high likelihood that the drug calls to the reaction, this is a possibility of idiosyncrasy potentially leading to fulminant, unpredictable onset, evolution of this case and you are also assuming that this is likely not dose-related,

because if it were dose-related, then you can say, well, you know, let's go for a low dose, et cetera, and you're saying there is nothing that can be done about it, there is no risk-minimization measure that can be put in place.

So, alternative approach. Let's go for retrospective evaluation and that's something that really is reasonable. But when you discontinue, what is your threshold for discontinuing? Will it be, well, the FDA DILI Guidance provides some thresholds. It's eight times above ULN or prolonged duration of five times ULN, but where do you put your threshold for your individual drug and your individual situation, your individual clinical trial or your patient? And what is it -- what is this threshold where you say I'm discontinuing now and I'm calling this an adverse drug reaction or when are we saying, well, you know, it's only two times above the ULN, it's not an adverse drug reaction, it's just a pure variation. So, here, really, we are talking about a combination of the approach of rechallenge and this retrospective evaluation. So, and this is what I

have kind of with a group of my colleagues, co-authors, and we have proposed this kind of an algorithm. What is it that you should consider if you want to rechallenge a patient?

1.**2#6 VS:** First of all, benefit assessment. Make 6sure that the patient really needs this and assess the benefit and risk of any other available treatment to treat this condition. Assess the risk and evaluate the initial reaction and pathogenesis, estimate the risk upon rechallenge, what is it that you can do in terms of predictive tests. Well, of course pharmacogenetic testing, et cetera. We all know this is all limited, but what can we do about it? Risk mitigation, what can we do to minimize the risk? And finally, or let's say last, not least, information and consent to the patient.

1.**2#7 VS:** And this is -- this is the publication in Drug Safety. And the general idea being -- the leading idea is that each adverse drug reaction is unique and that each drug -- adverse drug reaction combination requires a unique approach in evaluating benefit and risk, risk minimization and

considerations of ethics of rechallenge in this particular situation.

1.**2#8 VS:** So, evaluating this algorithm or protocol, or call it as you wish really in some detail presenting the risk, often the adverse drug reaction happens with a drug that has valid alternatives. Hypertension, we have plenty of options out there. You probably wouldn't want to rechallenge. Anticipated benefit may be marginal compared to possible risk. Again, are we talking here really about a serious indication, serious clinical condition, or are we talking about treatment for hair loss? And administration of another drug or alternative treatment may be without discomfort and should not have a high probability to induce adverse drug reactions.

And in all of the above cases, there clearly is no room for rechallenge, but then you're asking yourself what is the benefit of the second-line therapy. Well, certainly second-line therapy will be second line. If it were as effective and as safe as first-line, it would be first-line, it wouldn't be second-line. And then

again, failure of second-line and then fall back to first-line. So, consider the benefits.

1**.2#9 VS:** Risk, suspected mechanism. Wherever you can make a hypothesis about it, biomarkers, predictors, pharmacogenetic factors, antibodies. And especially when it comes to liver injury we are talking about a range of potential immune mechanisms. Likelihood of causal relationship, of course that's what we are considering. And what we all know is the variability in LFTs, which can be found in the general healthy population, which can be due to a whole range of benign reasons. And also what I would like to highlight is the risk of the rechallenge, and the reaction which may occur upon rechallenge is not the same as the risk of the initial reaction. It may be higher in cases that we are talking about sensitization, immune sensitization or already induced damage; it may be lower if we are -- if we have managed to set up an algorithm which minimizes the risk; or there may be no risk. Maybe this is just something that happened previously, this LFT elevation or these clinical symptoms that were actually not due to

hepatotoxicity.

1.**2#10 VS:** Risk mitigation. Well, again, in hepatotoxicity, and this is really why the approach to rechallenge in case of DILI has been so cautious, is that there is very little possibility for prophylaxis. Of course we are talking about acetylcysteine for paracetamol toxicity, but there are not so many prophylactic therapies in terms of risk mitigation. What are we going to propose as monitoring of LFTs or whatever other parameters that we're monitoring here, whatever parameters, indicators of -- early indicators of the reaction? And in terms of treatment, probably may not be known what may be administered. But when setting up a rechallenge algorithm, then this therapy, this is something that should be considered and made available. Make sure that the rechallenge is performed under controlled conditions. For example, inpatient hospitalization. Make sure that the personnel and facilities for the treatment for reacting as early as possible are available. And if the reaction is dose-related, start with a low dose. So, here again I was talking about, well, N=1

clinical trial and here we are talking about any +1 phase 1 dose-escalation trial in certain cases.

1.**2#11 VS:** And rechallenge consent. This is something that would be different -- if we're talking about clinical trial patients, this is something that would be clearly different to the initial clinical trial consent. Because in terms of the initial clinical trial consent we are evaluating the benefit/risk not knowing that the adverse reaction has occurred or may recur. And if the patient has been treated for a certain while, we may already have some indicators of benefit. If the patient may have been on treatment, we may have some sort of benefit there. And so, this is something that would be an addendum to a clinical trial consent.

1.**2#12 VS:** Now, in terms of examples of acceptable and successful rechallenge, I'm now talking about a DILI here. Drug desensitization, for example, and this is something that is quite commonly performed for, for example, penicillin, cephalosporins that desensitization is a commonly used procedure generally limited to specialized

centers. Again, I am -- one of the examples which I found is this trial in successful reinstitution, a rechallenge of agalsidase beta therapy Fabry disease, which is a rare disease of requiring enzyme substitution therapy. And what this trial was all about is that based on this initial trial, patients were prematurely discontinued if antibodies to the enzyme replacement therapy occurred, but what they did is then at the end of the trial they said, but what do we do with these patients who require enzyme replacement?

1.**2#13 VS:** So, they essentially set up a trial in which they enrolled only the patient which had been previously discontinued. So, this was an old rechallenge trial, some of the examples. And this is something that Julie Papay will be talking about, and this is actually what initiated the whole discussion, this 2009 publication. And my reaction and my interpretation reading this publication was actually quite different. What Julie found in her series is there were 648 positive rechallenge cases and 441 negative rechallenge cases. So, my thinking was, and the conclusion was,

well, rechallenge is too dangerous, but the conclusion which I reached reading this is, well, if we have so many negative rechallenges reported, how many negative rechallenges went unreported? I mean, if it's negative, who is going to bother reporting it? You will very simply say it's not an adverse reaction, it's something else. So, the true number of negative rechallenges is probably substantially higher. And what is the number of patients who are actually never rechallenged? Probably again we're talking about a substantial number since the approach to rechallenge is so negative. And so, I'm looking at these, well, 88 cases of possible probability including, well, two fatal cases. Well, what is the number of patients who didn't receive the therapy or in which there was no adverse drug reaction compared to these cases of DILI and thinking, well, how many patients were unjustly deprived of their first-line treatment because of a suspicion of an adverse drug reaction.

1.**2#14 VS:** And this is the large halothane study. And this is a study where I dug this out. And this

is a study which enrolled or a retrospective study of several hundreds of thousands of administration of general anesthesia.

1.**2#15 VS:** And what they are saying is -- this is the publication from '69 saying "It is clearly unwarranted to suggest that halothane anesthesia should not be repeated, for it is under the difficult circumstances of emergency reoperation that a wide choice of anesthetic agents may most urgently -- may be most urgently needed." [As read] And what they have actually found is that none of these patients who have been -- who have died due to massive hepatic necrosis, none of those actually had a reaction when they were first administered the drug. Therefore, that this occurred really with readministration without an adverse drug reaction the first time when this happened --

1.**2#16 VS:** And this is also what has been repeated in the European study. So, way forward, conclusions, the true dangers, idiosyncratic reaction should be isolated and rechallenge contraindicated, but blanket contraindication to

rechallenge may result in more harm than potentially harmful rechallenge.

1**.2#17 VS:** And unless cautious rechallenge is evaluated in clinical trials based on based available evidence, it may be attempted in the real world under suboptimal conditions by treating physicians who are not really fully aware of the conditions of -- optimal conditions of rechallenge. The medicolegal aspect should not take priority, and that just saying "no" to rechallenge is essentially leaving the burden on individual treating physicians.

1**.2#18 VS:** And then based on clinical trial experience, we should ideally be providing guidance in prescribing information. And of course last, not least, no scientific interest can ever take precedence over the benefit justifying the risk in the individual patient requiring treatment.

1**.2#19 VS:** Thank you very much.

(Applause.)

DR. SENIOR: Thank you, Dr. Stanulovic.

We're now going to hear the third talk from Julie Papay who worked very closely with Chris Hunt as colleagues at GSK.

Chris is now back at Duke, and Julie has moved on to another company that I don't know much about, but the thing that's so intriguing about Julie is she's begun to get some data on rechallenge from drugs under development.

So, let's hear what Julie has to say.

1.3#1 JP: DR. PAPAY: Thank you very much, John Senior. I'd like to thank John and Lana for the opportunity to come and talk about this very important topic. I would also like to extend a special thank you to my co-speakers for their very thoughtful presentation.

1.3#2 JP: I'm Julie Papay. I'm with UCB BioSciences, for the record. And I'd like to just say as a housekeeping item that the information I'm about to share today and the opinions are those of my own and not necessarily those of my employer.

1.3#3 JP: So, as Chris had alluded to earlier, it's very important to agree first upon the definition of "rechallenge." And Chris covered

this quite nicely, but just to recap very briefly, first there has to be a true initial drug-induced liver injury event and other causes have to be ruled out. There are a couple of causality assessment scoring systems like RUCAM; there are also expert opinions like the Drug-Induced Liver Injury Network, that can help clinicians define and determine drug-induced liver injury. Then, the drug has to be stopped, the subject has to recover from the initial injury, a decision weighing risk/benefit has to be made to determine whether it's appropriate to rechallenge. If it is, the suspect drug is readministered and either there's a recurrence of the event, which is deemed positive rechallenge, or there isn't, and that's a negative rechallenge.

1.3#4 JP: It's also important to consider the pros and cons of rechallenge as we look at clinical trial data. So, some of the advantages to readministering a drug is that it might enable a life-saving drug where other therapeutic options just aren't available. It may also confirm a true drug-induced liver injury diagnosis, and negative rechallenge

may tell us about adaptation, for example. Drugs like isoniazid, statins, tacrine and others may suggest a -- imply adaptation. Finally, one of the biggest pros, I think, of clinical trial data is that it can inform which subjects are more safely and appropriately rechallenged. However, you have to weigh this very carefully against the potential hazards, and those can include increased morbidity and mortality. As Chris had alluded to, fatalities have been seen with rechallenge. The time to onset of injury can be more rapid with rechallenge. The FDA generally advises avoidance of such rechallenge except under certain circumstances. Many of these rechallenges are infrequent particularly in the real world scenario after a drug is marketed and in use in clinical practice. And there really might be -- truly might be a high risk without rigorous clinical monitoring. With enhanced preclinical testing, toxicology studies and incorporation of routine monitoring and stopping criteria in drug development, cases of true hepatic rechallenge are infrequent.

1.3#5 JP: As a result, at the time of NDA review and approval, information about rechallenge is lacking and leaves many questions unanswered. Questions like who's the appropriate patient population to rechallenge, what dose should they be rechallenged at, for how long, how closely should you monitor that patient? So, the information is sparse. However, hepatic rechallenge in the setting of drug development and clinical trials actually offers the most optimistic data on drug rechallenge precisely because of the improved preclinical screening and testing that yields safer therapeutics and rigorous safety monitoring and stopping criteria that are in place that ensure -- help to ensure patient safety.

1.3#6 JP: So, although the data are limited, there are some recent examples that I'd like to share with you today.

1.3#7 JP: The first two are from new drug approvals. The first example is a drug use to treat hepatitis C in which three out of four patients experienced a positive rechallenge. The

second example is a cholesterol-lowering drug in which two subjects had positive rechallenge. And this information is available in the public domain, it's available on the FDA website in the medical reviews.

1.3#8 JP: The first example is an interesting example. This is actually approved as a single agent in the United States, daclatasvir, that goes by the trade name Daklinza. However, as part of the submission package to the FDA, three combination trials were included with the combination daclatasvir/asunaprevir. And the medical reviewer noted a 75 percent positive rechallenge in three out of four patients. What was also noted was that mean time to recurrence happened much sooner upon readministration of drug and the ALT elevations were actually much higher upon readministration, greater than ten times upper limit of normal with rechallenge in three subjects of which one also had concurrent rash. Fortunately, there were no cases of concurrent increases in bilirubin or eosinophilia. It was also noted that in two of the patients there was recovery

following the injury. However, in the other two it -- the information wasn't reported.

1.3#9 JP: What's also interesting about this drug is that this combination is actually approved in Japan and has been approved since 2014. And last year there were three case reports in the literature suggesting immunoallergic injury with the combination product most likely due to the asunaprevir portion of the combination. The immunoallergic injury was characterized by fever, increased lgE and eosinophilia.

So, briefly going through these cases, the first case is a 57-year-old male who experienced hypersensitivity reaction. His fever resolved after the drug was stopped, but his liver injury continued.

The treating physician did not feel that rechallenge was appropriate, because they were concerned about drug resistance. This patient had previously received other hepatitis C therapy and failed.

The second case is a 57-year-old female who developed fever, eosinophilia and increased lgE.

She rapidly developed hepatic failure and luckily recovered without hepatic transplantation. She was not rechallenged.

And the final case is a 66-year-old male who experienced DRESS Syndrome. After discontinuing the drug, the labs and clinical manifestations gradually improved.

So, important information about this combination product that was alluded to in the submission document and now we're seeing case reports come out in particularly Japanese patients suggesting an immunoallergic injury.

1.3#10 JP: The second example is a monoclonal antibody that is administered every two weeks for the treatment of high cholesterol. In the medical review, two positive rechallenges were noted.

The first case is a 42-year-old obese female who had ALT increases of mild intensity. The actual numbers were not provided. Her treatment was interrupted, and her ALT returned to baseline. Upon rechallenge, she had an ALT peak of 3.6 times upper limit of normal. This led to permanent discontinuation from the trial.

The second case is a 36-year-old male who had fatty liver disease that was confirmed on ultrasound. He had a peak ALT of 5.4 times upper limit of normal. Viral serologies were all negative. Treatment was interrupted and within four weeks his ALTs returned to normal. Upon reintroduction, he had five additional episodes of increases in ALT. And interestingly enough in this individual, treatment was continued and he actually completed the study.

What you should know about this program in general is that there were no Hy's Law events noted in the submission documents. And that the rate of ALT above three percent was very similar in the treated patients' overall population versus placebo patients. So, could this imply adaptation?

1.3#11 JP: Now, I'd like to share with you three examples that are available in the published literature. And as Chris had alluded to earlier, it is so important for manufacturers and investigators to share their findings whether they're positive or negative, because many times

this information goes unreported if it's not published or if it's not available in an NDA submission.

The first example is a compound that was in development for HIV in which there was one hallmark case of positive rechallenge. And I'll go into more detail in just a minute. The other two examples are from oncology indications in which there was a positive rechallenge rate up to 55 percent.

1.3#12 JP: So, the first example is aplaviroc, which was a CCR5 antagonist in clinical development to treat HIV. They had a hallmark case of positive rechallenge in a 37-year-old male. His first ALT elevation was 5.7 times upper limit of normal that occurred at week 12. He had normal bilirubin. He did recover from the initial injury and viral serologies were negative. Upon rechallenge, his ALT was much higher at nine times upper limit of normal and occurred much quicker, within two weeks. This individual permanently discontinued the study and his ALT normalized.

Within this program overall, there were two other Hy's Law cases noted. One was in a

treatment-naive patient, and the other was in a treatment-experienced patient. And overall throughout the whole program, six percent of patients had ALT elevations between two and a half and five times upper limit of normal. Given the totality of data, this program was terminated. And I would like to just say a very special thank you to those who actually took the time to publish these results and make them publicly available.

1.3#13 JP: The second example is pazopanib. This is an oncology drug that's FDA approved for renal cell carcinoma and soft tissue sarcoma. In this series, 103 patients were rechallenged. This included Phase II/III studies in a little over 2,000 patients. And, basically, patients had to have a relatively normal transaminases and liver function coming into the study and rechallenge was considered for isolated ALT above eight times upper limit of normal with the caveat that they were rechallenged only if they had clinical benefit from the product and that all of the following criteria were met. Once the drug was stopped, their transaminases had to come down, ALT had to come down

to less than or equal to 2.5 times upper limit of normal. Their bilirubin had to be less than one and a half times upper limit of normal, and direct bilirubin had to be less than 35 percent. They couldn't have any signs or symptoms of hypersensitivity. And importantly, the subject had to reconsent to having drug readministered. Readministration was also done at a reduced dose with close monitoring.

1.3#14 JP: So, what they found in the series was a 38 percent positive rechallenge, and that might beg the question, was there adaptation, because over 60 percent of the other folks had negative rechallenge. It's a good question. Perhaps the reduced dose resulted in a higher percentage of negative rechallenge. However, it is important to note that 38 percent have positive rechallenge.

1.3#15 JP: The reoccurrence occurred much more rapidly with a median of nine days. And upon rechallenge, it's interesting that most of the ALT elevations were between five and eight times upper limit of normal and there were no exceedingly high elevations of ALT greater than 20. There were also

no cases of liver failure after rechallenge. The authors of this paper conclude that rechallenge should only be undertaken when you have close monitoring of liver chemistries. And subsequently what was found was that there were HLA associations with injury suggesting immunoallergic injury. Excuse me for just one second. (Pause.) Seasonal allergies. Apologies.

1.3#16 JP: This is an example that Chris had also shared with lapatinib in which there was a 55 percent positive rechallenge. This was observed in a lapatinib monotherapy study in breast cancer in almost 1200 patients. Overall in the program, two percent developed ALT elevations greater than five, five times upper limit of normal. And, again, there was a 55 percent positive rechallenge observed after reinitiation. HLA markers were observed, which suggest an immunoallergic injury. And most of the patients who had ALT elevations above five also had these markers. These markers are expressed in up to a quarter of women with breast cancer and less more frequently in Japanese, interestingly enough.

1.3#17 JP: The immunoallergic injury that was seen with lapatinib is actually -- the information is actually provided in the U.S. prescribing information along with warnings about hepatotoxicity and potential Hy's Law. And I think this is a very positive move forward to alert prescribers and patients and caregivers about severe and potentially serious hepatotoxicity.

1.3#18 JP: So, this data suggests that high positive rechallenge rates have been observed in recent clinical trials, and all of this despite rigorous monitoring with many people looking out for the benefit of the patient, which include the investigator, the sponsor and regulators.

1.3#19 JP: So, what happens in the real world when you don't have this rigorous oversight? It's likely that there's a higher morbidity and mortality in an uncontrolled environment. And we know that even fatal positive rechallenges could be unknown by the treating physician.

1.3#20 JP: This is a series that was previously shared and that my co-speakers alluded to. This was a rechallenge analysis that was conducted

looking at a sponsor's drug safety database. And what was observed was that up to three-quarters of the positive rechallenges included serious adverse events. Most of these events exhibited symptomatic jaundice or hepatitis. Usually the injury happened much quicker. And importantly, there were pretty severe cases. And Hy's Law was noted in up to 14 percent of the cases and there were actually two deaths. What should be noted is that most of these cases were spontaneously reported, 83 percent. However, we did have a handful of clinical trial data.

1.3#21 JP: We concluded that real world drug rechallenge can be prevented and should be prevented by first recognizing the initial liver injury and communicating that to the patient so that they can effectively communicate that to all of their caregivers. And equally as important is recording this injury in the electronic medical record. All of this to prevent possible rechallenge.

1.3#22 JP: In summary, positive rechallenge has been observed in up to 75 percent of patients in

recent clinical trials and many had an immunoallergic injury hallmark sign. Rechallenge requires truly a systematic benefit/risk assessment with key questions such as is the patient benefitting from the drug? Are there other therapeutic alternatives available for this patient? Has the patient truly been dechallenged and had a recovery from the injury? What else do we know about the drug, the molecule or the class of drugs in terms of HLA markers, hypersensitivity. And has the patient been alerted and made aware of this serious injury and do they -- are they aware of the benefit/risks and have they reconsented?

1.3#23 JP: In conclusion, rechallenge should generally be avoided due to potential for serious injury and fatalities and should be considered for critical medications only when you have a standardized rechallenge benefit/risk checklist.

1.3#24 JP: Again, I'd like to acknowledge the organizers of the meeting, John Senior and Lana, for inviting me to speak about this very important topic and to engage in dialog with my co-speakers. And I'd also like to thank colleagues who

collaborated on the rechallenge analysis. Thank you.

(Applause.)

DR. REGEV: So, the fourth talk is going back to the notion that maybe rechallenge is possible under certain circumstances. And this will be John Senior, who will discuss in a different version the isoniazid story to illustrate that point.

John:

1.4#1 JS: DR. SENIOR: I won't talk just about isoniazid, but I think we have to learn from the past, because we can't really see very clearly into the future. We sort of back into the future, with hope. I cannot speak for the FDA. I have been working there for 21 years, but I'm not authorized to be their spokesperson. So, what I say is my own cockamamie ideas and opinions.

1.4#2 JS: We are concerned about drug safety. Drug safety has been my principal concern for at least 15 of the last 20 years at the FDA. We have developed programs to try to prevent approval of new drugs

that were dangerous to more than some people. And we'll talk about that tomorrow.

1.4#3 JS: It's ironic that I, from the FDA, would be talking about do we rechallenge, when we know that in the discussions you've just heard, we know it can be dangerous, it can be fatal, but it's not always. In fact, it's very rarely fatal, but we don't know that. Chris in her closing slide, said, we need data. And Julie has given us at least a little data, but we have a precious, small amount of true knowledge. I agree with all the speakers that I think rechallenge is too dangerous to be carried out *in medical practice.* I don't think practicing physicians are equipped or able to deal with this difficult issue. I think the only possible way that rechallenge can be done safely is that it's done before approval for drugs under investigation, under legal requirement to know what's going on, to report all the data. We simply don't get the data and I don't think we can draw any conclusions without it.

1.4#4 JS: We know that rechallenge can occur, but we don't know the incidence, really. We have some

glimmering from what Julie has told us, but very little data. It may be that only one in 9 or one in 99 patients really shows a real rechallenge problem that is a significant, not just a little tweaky bump on transaminases. That doesn't hurt anybody. They don't even know they have it. So, we need to do -- we need to do more. There have been thousands of papers written on benefits and risk, but we really don't know how to compare those. So, we do know some things. We've learned a lot in the past, oh, 15, 16 years we've been discussing these issues. We have not approved new drugs that kill people from liver failure in the last 18 years, but we don't really know how to compare quantitatively benefits and risks. We know that people differ. They're not the same. There is no standard patient. Everybody gets the same dose. People differ in their reaction to the same dose of the same drug. We also know the drugs are different. Some are more dangerous than others.

1.4#5 JS: Now, let's just talk a little about isoniazid. Isoniazid is a simple compound. It's been around for 70 years. It was being used before

it was reviewed for safety by the FDA. It's been used since the '40s and it's used because it's valuable. It prevents tuberculosis in those people who are exposed to it who don't have active disease yet, but isoniazid alone can do it.

1.4#6 JS: It's a simple compound. An acid and a base. Isonicotinic acid and hydrazine. Hydrazine is a toxic chemical, but combined with isoniazid it's not, with isonicotinic acid.

1.4#7 JS: So, isonicotinic acid is a simple isomer of a vitamin, nicotinic acid, vitamin B3, niacin. It's a relative of nicotine. It's in tobacco and it was very useful and valuable. It helped prevent a serious disease and it's still in use.

1.4#8 JS: Somebody has estimated that a third of the world population is exposed to tuberculosis. A lot of the world is poor and ignorant and that's where TB is and isoniazid is still widely used. So, but we knew it could be dangerous. We knew it could kill people.

1.4#9 JS: And a study in Baltimore of some, what was it, 60,000 patients showed eight deaths, seven of which were in one city, Baltimore, and everybody

got terrified. And it took 20 years before they figured out how this could be avoided. And it was Charles Nolan who did it. Very patiently 20 years later, he finally figured out how to avoid isoniazid hepatotoxicity.

1.4#10 JS: It was simple. Ask the patient. Don't monitor transaminases every month for years. People get very weary of sticking their arm out and getting stuck and having results reported that are negative. And negative results year after year, month after month, get very boring and they stop doing it. So, instead of that, just ask the patient: how do you feel? And teach the patient to report immediately if they are a little fatigued, they've lost their appetite, whatever. And it worked like a charm. And he did a big study and, look at that, no deaths at all. No monitoring. No deaths. Just ask the patient. The patient, if instructed, knows it's in their own interest to report, because it's their life. They want to report. They want to get the benefit of isoniazid, but they don't want the risk. And so, ask the patient. They can tell you every day, not once a month, not once a quarter,

every day they can tell you how they feel. And if they report it to their doctor, he can check the transaminases and the rest of it and make a decision very quickly. So, that was an important study.

1.4#11 JS: Okay. Now, there was another important earlier study that was conducted as a result of an NIH protocol. And it was not at NIH, it was done somewhere else in Virginia at a local hospital where they had a great many patients who were sort of warehoused, mentally ill patients who were warehoused, but they were exposed to tuberculosis, active tuberculosis.

1.4#12 JS: So, they wanted to study a number of them to see if they would show any transaminase and bilirubin elevations. And so, they monitored those patients monthly and guess what they found. Three Hy's Law cases. Here's one. Look at that. Up goes transaminase in red, and up goes the bilirubin. No symptoms. It was not even known, because the way they did the study was to collect the blood for a year and then analyze it all a year later. That was a probably true Hy's Law case.

1.4#13 JS: And there was another Hy's Law case.

1.4#14 JS: And another Hy's Law case. All three cases were Hy's Law cases, none of which were detectable by -- none of which progressed to death or anything else. They all just got better.

1.4#15 JS: So, INH is still with us and tuberculosis is not going away. In fact, drug-resistant tuberculosis is much with us. So, we learned a lot from tuberculosis, from isoniazid.

1.4#16 JS: We learned that it can be used safely. And the way to use it safely is, ask the patient. Pretty simple. It doesn't cost anything, and saves a lot of wasted normal transaminase tests.

1.4#17 JS: So, we go back to the FDA Premarketing Liver Safety Guidance, which says, generally, it should be avoided, not done, but maybe we need to rethink the Guidance. FDA, nobody regulates the practice of medicine, nor should they. The practice of medicine has to be done according to the judgment and experience of the treating physicians. And the treating physicians are all kinds and all sorts. They have labeling to guide them, but they don't all really read the labeling that carefully. I was in practice for 25 years in

teaching hospitals at Harvard and Penn and I really didn't read the labeling all that carefully. I wanted to look it up to see what the average dose was, but I didn't even believe that, because I might have a little 88-pound woman and I'm not going to give her the same dose as a 250-pound linebacker.

1.4#18 JS: There's no such thing as an average patient, they're all different. And I knew that, so I took the labeling with a grain of salt. But still it's useful information. It's a good starting point. So, I'm not going to get into this. (Laughter.) I don't agree with everything that has been legislated to control the FDA, but we'll talk about that some other day. For now, what I'd like to do is really turn this discussion over to the audience to discuss all the issues that these very interesting presentations have made.

1.4#19 JS: My opinion is that rechallenge can be done safely without killing patients. I think the threshold for calling it a positive rechallenge is too low. Just a slight bump in the transaminases, asymptomatic, not progressive, it's too low. We know that the liver is a remarkable organ. You can

cut out two-thirds of it surgically, or you can kill two-thirds of it with chemicals, and the liver grows again very quickly, in a couple of weeks, a few weeks, back to its original size. A remarkable organ: Hepatocytes can regenerate. Not only can they regenerate, they can change, and they can become tolerant to a chemical that initially caused injury. We have to learn that wecan only learn that if we look. If we don't look, we can't see.

1.4#20 JS: Now, what do we have in the literature? We have horror stories about positive rechallenge, but who's going to report a negative rechallenge? If you give a drug again and nothing happens, are you going to report it? Are you going to publish it? There's nothing to publish. So, we don't know. We will never know. The only way we can possibly know is that it has to be done under conditions where the results *must be reported*, which is in the IND phase of new drug development. So, that is the best time to learn. That is the time to learn about what should be in the labeling. That is the time to teach physicians and patients what they should do in their own best interest.

Thank you.

(Applause.)

DR. REGEV: So, we have exactly 30 minutes for discussion and questions. Please, whoever has a question or a point or comment to make, state your name and where you're from. This is all being recorded.

I would like to start with a question I'm curious about, about the definition that we've seen here, at least from two speakers, regarding what a positive rechallenge is. And it seems like there is an agreement that, first, the drug is discontinued, symptoms should disappear, levels of ALT and AST need to go back to normal, but then there seems to be an agreement, at least between two of the speakers, that, when you start the drug again, a doubling of ALT constitutes a positive rechallenge. And I'm thinking, what if the ALT was 21 and now it's 42? Is it a positive rechallenge? Is there an agreement about this? So, at some point, I would love to hear if there are other opinions regarding that.

Jack:

DR. SENIOR: This is Jack Uetrecht. If we know them, we'll tell their name. If not, say your name, because we don't know everybody.

DR. UETRECHT: Jack Uetrecht, University of Toronto.

Two points: one is, I remember, and some people in the audience may remember better than I, ximelagatran, where patients were rechallenged and nothing happened. But what did we really learn? Because it didn't mean that the drug didn't cause liver injury, and the drug eventually failed anyhow. So, did that really help us? Minor point.

Second point, it was implied again that idiosyncratic reactions are dose-independent. Simply not true. A usual dose of a drug is about 10^{20} molecules. Because the mechanism of the idiosyncratic reaction is different than the mechanism of the therapeutic effect, there's no reason why the dose-response curve should be in the same range. Sometimes it is, just by accident. In many cases, it's shifted to the left. So, a

lower dose will still cause an idiosyncratic reaction.

Going back to isoniazid, you can usually start with a lower dose and desensitize patients. I'll bet, if you started at even a lower dose, you could desensitize everyone. And we haven't done a very good job of systematically rechallenging with a very low dose. I'm talking about a milligram, maybe even sub-milligram dose, when it's important for the patient. I'll bet you we could desensitize virtually everyone if it was important for the therapy of the patient. And we haven't done a very good job of studying desensitization. It's done with isoniazid. It's done certainly with penicillin and other things. You start with one 10,000th the normal dose, but that's still like 10^{16} molecules.

DR. SENIOR: Jack, what we learned from ximelagatran was that there are genetic markers that distinguish susceptibility. That was a very important lesson. What we found is that the population in Scandinavia that had a high frequency of the HLA marker had a high frequency of the

adverse effects, but the Asian population did not. So, learning that there are differences in patients, and here it was a genetic difference, was a very important point.

DR. UETRECHT: But did desensitization help as much?

DR. SENIOR: We didn't learn about ---

DR. KAPLOWITZ: And I would like to echo that --- resonate with it, in the sense that I really don't find the argument that rechallenge is going to be extremely informative to be really that valid. And, you know, if there's a diagnostic issue in an individual person out in the world, I suppose you could make that argument, as dangerous as it might be. But in the clinical trial setting I don't think it's really essential to prove that one patient has an adverse effect due to a specific drug when you have a population study. So, you know, when you rechallenge, it's either going to be, as Jack implied, either going to be negative or positive. And in the vast majority of instances, experience has been that it's probably going to be negative. And what does that really prove, you

know? So, there's adaptation or something else has happened.

I think what's interesting, really, is in the context of situations where an individual is having a really important clinical benefit, for example, from a cancer chemotherapy drug, like lapatinib or pazopanib or the new kinase, all the various kinase inhibitors that are out there. And the question is, how can we mitigate the risk of rechallenge in those type of situations, which are almost inevitably going to be accounted for by an adaptive immune-mediated mechanism? So, our oncology colleagues just typically treat rechallenge in these settings with steroids. So, you know, and claim there's anecdotal evidence that if you rechallenge with the chemotherapy, a kinase inhibitor, for example, combined with steroids, you can somewhat mitigate the risk.

I wonder, you know, I would be interested to see if Jack has any ideas about how to do the opposite of what he's doing, which is creating models to dampen the adaptive -- the immune tolerance effect. Can we promote immune --- is

there a mechanism for promoting immune tolerance that would allow us to rechallenge? Perhaps the small dose approach is one, but I think that's really the critical issue. And I really don't find the argument that we need data on rechallenge in clinical trials to be very compelling, unless you are going to develop an approach to mitigate a positive rechallenge.

DR. SENIOR: Neil, as usual, is looking ahead. He's talking about tomorrow's discussion on immunology, but he's absolutely right.

DR. REGEV: There is a niche, though, that --- is somebody trying to speak or --- anyway, there is a niche, Neil, in clinical trials where the question of rechallenge becomes an important question, and it's not something that has been discussed here. Very often -- and people who do clinical trials here know about it very well -- investigators would discontinue drugs much earlier than the company has intended for them to be discontinued.

So, many times, at three times upper limit of normal, --- an investigator who happens

to be a psychiatrist or an endocrinologist becomes nervous and discontinues the drug. And after some discussion, there is a decision by the company that it's probably warranted to reinitiate this drug and continue treatment because discontinuation was not warranted. At that point, there is some mystic fear of rechallenge. If we now stop the drug and restart it again, something terrible is going to happen. And I don't think that our discussion here really covers that very well, because under those circumstances I don't think there should be a big risk of restarting a drug that was three times upper limit of normal with asymptomatic, and then two, three days later we are restarting it again when it went back to normal.

Did you cover that, Chris, and maybe John? Did you cover that under the risky group?

DR. SENIOR: I don't really know how this can be done most safely. Now, Vid has talked about an algorithm. Now, he originally was intending the algorithm to be used by practitioners who are going to investigate liver toxicity, minor toxicity in an approved drug. But I think that

algorithm might be applicable also to consideration of new drugs under development. And in one of Chris's early papers, she actually had kind of a flow-sheet for what degree of elevation of enzymes and what bilirubin and so forth could be used to guide the process. Now, Chris, maybe you want to comment on that, because it was in, I think, your 2007 paper, as I recall it.

DR. HUNT: Yes. Excellent question, Arie. Is this on? Should be, okay. I don't think that we actually have good data to inform your question, and I fully second the comments made by Jack and Neil that we really need to understand, for critical medicines particularly, how we can safely rechallenge. Because there are certainly many times, as you pointed out, where it would be helpful to better understand rechallenge more broadly. But are other people aware of data? Because this is a very knowledgeable group.

DR. ROSENBERG: Can I make a comment that addressed a trend we were building on before this? So, I am Amy Rosenberg, FDA. And what seems to me to be critically missing in these rechallenge

ideas is a mechanism. And, as an immunologist, the mechanism speaks to me to be an immunologic one. It happens faster, it happens with lower dose. That speaks to a memory T cell response. And so, efforts to mitigate, as was being mentioned before, to mitigate by effecting the immune system, immunomodulation, are really, it seems to me, the way to go. And steroids are, you know, a blunt instrument, and the development of newer therapies that might induce regulatory T cells, which may critically shut off these kinds of reactions, and we know are very important in liver injury, that effort is really critical. So, identifying the mechanism, it's likely to be immunologic, and then seriously addressing how to mitigate for patients for whom this drug is essential.

DR. REGEV: Thank you. Paul Watkins, UNC.

DR. WATKINS: I think he was ahead of me there.

DR. REGEV: Oh, he was?

DR. CHEN: This is Minjun Chen from the FDA NCTR. And I like the discussion. Basically,

it's very interesting. As we can see, some drugs we can challenge, some drugs we cannot, because some drugs are very, very dangerous. Basically if you go a little further, asking this drug is more dangerous, what kind of patient is more, you know, in danger from the challenge?

I really like that Christine gave the color, you know, this kind of project can help us identify which kind of drug can be more danger for rechallenging. So, basically I can say if we can get more data coming in, it maybe can help you identify based on the drug property, we can identify what kind of drug can be rechallenged, what kind of drug cannot be rechallenged. If we can even further, maybe go connect it with patient data, so, what kind of patient, we can rechallenge or not. I think you should put that in the considerations.

DR. HUNT: I just want to thank Minjun Chen from FDA NCTR. You're doing a great job analyzing drugs and bringing -- advancing our knowledge base. And you are the person who developed the increase ROS, decrease in ETP as one

other paradigm to evaluate, and I applaud your work.

DR. CHEN: Yeah, I think the -- we maybe can consider more drug properties, you know. Basically we know that different drugs, you know, make different effect. It basically can help us identify what kind of drug may be more risky, you know. I think the --- and we have some more drugs accumulate data, for example, for some drug can be rechallenged, some drug cannot. Basically, I think we can find something, you know, can help us, you know, do this kind of job.

DR. REGEV: Paul.

DR. WATKINS: Yeah, Paul Watkins, UNC, Institute for Drug Safety Sciences. And if it's clear that somebody is jaundiced or has hypersensitivity, it would be a bad idea to rechallenge them. But one issue that hasn't been specifically addressed, I don't think, in the great discussion, is the fact that rechallenge probably occurs all the time outside of a physician's oversight. In other words, people lose their insurance, they don't think the drug is doing them

any good, they stop it, they restart it. Certainly, antibiotic courses tend to be repeated.

And so, I think an argument can be made that the company has a responsibility to show that ALT elevations that are clearly associated with their drug, if they are, what happens when that individual is rechallenged. So, I'd like to pose that to the panel.

DR. SENIOR: Arie, do you think that's so? Do you think sponsors have a responsibility to do that?

DR. REGEV: I think they do. And as I said, I think this happens much more commonly than the numbers that have been indicated here. This is a point, I think, that John brought up. We do have many instances where a drug is being interrupted during clinical trials. Now, the combination of being interrupted and having some ALT elevation is not that rare. And I think that, in many of those cases, there is what we would consider rechallenge, but it's not documented as rechallenge. It just goes unnoticed. So, similar to what John has said, I think there are many more

cases, and they're not well-documented and I think it should be. Many more cases of negative rechallenges that just go unnoticed and cause no bad outcome.

And still I think Chris Hunt's comment is correct. We do not have this data. So, it's very hard to make a final comment.

DR. STANULOVIC: Yeah, and if I can add to that, the classical definition of "rechallenge" is adverse drug reaction occurs, stop the drug, reaction abates, patient recovers, and then you restart, well, intentionally or unintentionally. But really what can happen for drugs that are, for example, administered weekly, monthly, or in cycles is that, well, each new administration essentially is a rechallenge, even for if it's once daily dosing, I mean, you do see potentially some kind of recover.

DR. SENIOR: That's right.

DR. REGEV: So, I have a very specific question. And this is for Will Maddrey. This is regarding at least -- it was presented at least in one of the slides. I think it was one of our

speakers. And this was about statins being classified in the high-risk hepatotoxic drugs. And I've seen this happening a few times. And I know you have a lot of experience regarding statins and hepatotoxicity and would love to hear your opinion.

DR. MADDREY: Will Maddrey, University of Texas Southwestern. You know, statins have had a remarkable career. Early on, as John will certainly recall and a few others in the audience, there was great concern about statin safety. And much of that was allayed when it turned out that some of the material that had originally come from Japan had been tainted in some ways. And then in the statin trials, it was rather clear that aminotransferase elevations were common, but didn't come to much. Over the years, statins have gone all the way over to the other side of being considered remarkably safe. In recent couple of years, with one or more of the statins there has been a concern that there are some latent, even autoimmune-type reactions from statins. I think this needs to be put into parallel.

With the number of drugs that we look at, you read a list of the concomitant medications a patient's on in a clinical trial, and we've rather much brushed over the statins, either I have, because I've seen so little of statin hepatotoxicity that was for certain. But maybe now, and I think Crestor is the drug that has come more to the fore, that there is a statin relation to even an autoimmune process. I haven't seen it yet, but I know several in this room have. I still think statins are rather safe to use. However, it is one of those things that every now and then needs to be reconsidered. Personally, I think statins, in general, particularly the classical statins, are quite safe.

DR. REGEV: Thank you.

DR. TILLMANN: Hans Tillmann, East Carolina University. A comment -- a question and suggestion I would have. So, the comment would be that lower dose to start with might not be an option for antivirals and antimicrobiotics. And potentially even cancer drugs might not be a good idea, because you can evade your response

eventually. So, even though it's a great idea to do it, in some therapeutic options it might not be.

Then the question would be, is the HLA signal usually stronger in the rechallenge cases than it is in the whole database? Do we have data on that? Because it would help to mitigate the risk if there is an HLA type and you have not a risk factor, then you're probably at a low risk.

DR. REGEV: I do not have an answer for this. Is there anybody in the audience that would have a comment regarding this question?

DR. TILLMANN: And that, perhaps, is a good transition to the suggestion that I would make, because, so, I think you can do rechallenge within clinical trials, but it doesn't necessarily reflect what you would need to know in the real world setting. So, therefore, I would think one should look into a mechanism where perhaps the FDA and NIH get together to create a consortium for data-based generation on rechallenge, perhaps with a concept that if someone has a patient who needs a rechallenge, that that case gets reviewed by expert in the relation to DILI to ascertain how

likely is it initial event was DILI, and then also how risky it looks to reexpose, and then get the data on what happens during the reexposure.

DR. REGEV: Thank you. We'll take a comment from the other side. Are you waiting in line there?

DR. SOERGEL: Yes, but it's not referring to the same question, sorry. This is Marianne Soergel from Actelion. I'm a drug safety physician. One element that I felt could be explored more is: are all clinical presentations of DILI similar regarding the risk for rechallenging? There are so many different and I +wonder whether we have data to differentiate whether the rechallenge of a Hy's Law should be handled like the rechallenge of an isolated asymptomatic transaminase elevation. And I would be interested from the speakers to learn whether t+here is anything that differentiates in the literature that has been looked at.

DR. HUNT: Yeah, there's no question that halothane -- the two case series I presented, halothane, those patients had hepatocellular

injury and jaundice on their first presentation. And then, within a month, you know, half of them -- those particularly who received it again within a month, you know, had fatality in nearly half. There's no question, as Arie pointed out, that there is lots of patients with modest ALT elevations and we simply don't have enough information that's published to say what you can say specifically about each patient, but we, as Minjun raised, I think we have a lot of information -- and Julie raised -- there's a lot of preclinical information on drugs. Not all drugs are created equal. And as Jack and Neil pointed out, there are also inter-individual differences if somebody has HLA markers. There's clearly a lot of risk differentiation, and as Hans pointed out, that we can do with the available information even in clinical trials to say which drugs, particularly critical medicines, would you consider rechallenging in a safe environment. I don't know if that answers your question. What are other people's thoughts?

DR. STANULOVIC: Yeah, I think that

what has been touched upon with that clinical presentation, as you phrase it, is of critical importance. And if I may be free to say, maybe the hepatocellular injury, direct toxic hepatocellular injury, is probably the simplest one to deal with in the sense that you're dealing with, well, let's say, numerical ALT elevations. Whereas when we're dealing with immune mechanisms, there are various potential mechanisms involved which should be considered there, therefore, a clinical presentation would be definitely of critical importance.

DR. REGEV: I think we'll let this side speak, and then go back to your side.

DR. BONKOVSKY: Herb Bonkovsky from Wake Forest. Just a comment about what Will was talking about. I think we agree that statin hepatotoxicity is extraordinarily uncommon when you consider the millions of people taking it, but it does occur and we've had some positive rechallenges, usually with autoimmune features. So, it is a real thing, but it's very rare. I just wonder, are we now, from this discussion, coming

to the general conclusion that, in future, the FDA is going to strongly encourage, maybe even require, sponsors and pharmaceutical companies for new drugs to include rechallenge when there are adverse drug reactions? I mean, that sounds as though what we're talking about, assuming that it's not, you know, severe hypersensitivity with very high fevers and so on. Is that what's going to come out of this?

DR. SENIOR: I don't think so, Herb. I think the FDA is not --- and their guidance is not a dictation of what people must do. It's simply a recommendation. It's not telling people what to do. I think the whole issue of rechallenge by companies is really up to them, because it's in their own interest to find out. So, I'm not sure the FDA --- and I cannot speak for their policy making, I don't make policy at the FDA -- I think the whole issue of rechallenge is fundamentally in the hands of the developers who are developing a new drug under IND. Now, Neil says don't worry about that because it's a rare event. But I don't think drugs should to be developed just for the average

patient, for the masses. We know there's a big difference between safety and efficacy. Clinical trials are the best information that is available to anybody to know about a drug. It's from clinical trials that much is learned, but clinical trials are organized principally to get approval of the drug for use by the people for prescription. So, the protocols generally select people who are likely to respond and they deselect people who are likely to have trouble. They don't want trouble. So, the safety problems, which are rare to begin with, are even rarer in the populations selected for clinical trials.

So, we result in the best information we have being incomplete, incomplete knowledge about safety problems. If only one in a thousand people respond with liver failure from a drug, how big a trial do you need? We can't study 200,000 or 500,000 patients. So, it's a problem, and we don't get good reporting after the drug is approved, because physicians who are prescribing don't report adverse events. It's not in their interest to do so; they're not paid for it; it takes a lot

of their time and they might get sued. So, they don't want to report them. They may report if they want to publish the paper, but they ordinarily don't report. Ninety-five percent of the adverse events that are reported to the FDA come from pharmacists, from patients, from families. They're reported to the company and the company simply passes them through without getting any more information. So it is almost impossible to determine whether the drug caused the problem or whether it was just a disease effect. So we're stuck with it.

DR. REGEV: We have one last question on this side, and then we'll have a break.

DR. ROSENBERG: Amy Rosenberg, FDA. Actually, I'd like to make a point about the alirocumab as being involved in liver injury. That only is supposed to be administered in the setting of maximum tolerated dose of statins. And was it looked at in terms of the administration of the prolent with respect to the increase in dose in statins to reach that maximum tolerated dose? Because I have a hard time understanding how, you

know, a monoclonal antibody directed to PCSK9 is going to give that same kind of liver injury that was spoken of. So, that's one point.

And also, the other question I had concerned mitochondrial injury that everyone mentioned. Do liver cells with mitochondrial injury resort to glycolytic metabolism? And is there an overall long-term risk of hepatocellular carcinoma in patients? Because we know tumor cells, for instance, really depend on glycolytic metabolism. So, thank you.

DR. PAPAY: Amy, thank you for your comment. I guess I have to speak to this really good question. Thank you for your comment, Amy. Admittedly, it was very difficult to find examples in recent NDAs, and the monoclonal antibody was a stretch to include it in the presentation. So, thank you for your thoughtful comments.

DR. HUNT: And in terms of mitochondrial injury, mitochondria can regenerate. And your point about cancer, I think one of the things is mitochondria are the key cellular energy sources, but they can regenerate.

So, even if injured, assuming the cell survives, they regenerate within weeks. So, unless you had --- your point could speak to if there's a mitochondrial toxin that's given long-term, you know, with chronic liver injury, potentially that could have other repercussions, but I have no information.

DR. REGEV: One last question.

DR. HAQUE: Hi. Thank you. My name is Asif Haque, I work for Astra Zeneca. I'm a global safety physician. I'd like to address that discussion we had about statins. I think it's a very relevant comment. Statins have had a very long and very widely used profile. The actual incidence of liver events is actually going down, reportedly. I think it's because it's been so well studied, managed and addressed. I just want to highlight the discussion about immunology, and besides the hypersensitivity reactions, is there any other specific data that we find? There's a mention of Crestor in terms of immunological response for liver injury. I just want to check if there's some additional information, or perhaps

you can provide some guidance. Because as a rule, statins we know have potentially an anti-inflammatory effect and they can have a really beneficial effect, as been discussed many times.

DR. REGEV: I don't know if Dr. Maddrey has a --- no comment. I agree with completely -- and this is a poorly documented observation, but I think the denominator with statins is so large by now.

DR. HAQUE: That's right.

DR. REGEV: Hundreds and hundreds of millions are being exposed. The fact that we talk about a case here and a case there, out of that denominator, just proves to me how safe this drug is, this group is.

DR. HAQUE: And with the current advent of all the protease inhibitors and all that, there's also a lot of potential for drug interaction, which could lead to increased AUCs. And that's also something that needs to be now explored and, you know, kept in mind. Because, in its own entity, statin will not cause it, but a potential interaction with a protease inhibitor or

some others could potentially cause AUC to go above that level. So, I think it's an evolving pattern, especially with the new approvals of all the hep C inhibitors and so forth. I think that's something in the future we can discuss.

DR. HUNT: Just to give a shout-out to a paper by a Kaiser colleague, the first author was Charles, they actually looked at something like 20,000 patients receiving statins in Kaiser and then looked at those patients who had ALT greater than tenfold. Of those, roughly ten or so got the statin, either the same or a different statin, again and there were positive rechallenges. And there's not additional information about whether this is autoimmune injury, but just that some of them did have significant rechallenge injury.

DR. REGEV: But this is a very important part. I didn't read that paper, but when we say "rechallenge," did they have anything in addition to just ALT elevation? Did they have jaundice, liver failure, anything else, or just ALT elevation?

DR. HUNT: They reported ALT

elevation, and I know that one of the --

DR. REGEV: Because that is a quality of the drug. We know that they do it.

DR. HUNT: Right. At least on rechallenge, I believe at least one of the patients had both ALT and bilirubin elevations and was hospitalized. Some of the patients on rechallenge were hospitalized, but I don't remember all the details anymore.

DR. REGEV: Thank you. That's important. Well, we have a break now exactly until 10:10. So, 20 minutes for coffee break.

(Whereupon, the above-entitled matter went off the record at 9:52 a.m. and resumed at 10:13 a.m.)

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DR. REGEV: Okay. So, the next session is going to be devoted to hepatic regeneration and adaptation, which is a continuation from our previous topic. We will start with a talk by Ayako Suzuki, who is at the Division of Gastroenterology, University of Arkansas Medical Sciences. And she

will talk about interactions between drug properties and host factors. Ayako:

1.5#1 AS: DR. SUZUKI: Good morning, everybody. I'm going to talk about the interaction between drug property and host factor in human hepatotoxicity.

1.5#2 AS: Drug-induced liver injury is a multifactorial disorder. Certain drug properties such as high lipophilicity and high daily dose are associated with severe clinical hepatotoxicity. However, not all patients who take drugs with such properties develop liver injury. Drug-induced ALT elevation develops with wide range of incidence, from less than 0.001 percent to 20 percent. And most of the cases, ALT elevation resolved even when medication is continued. In some case, it turns into non-progressive chronic liver enzyme elevation. And in some rare cases, serious life-threatening liver injury occurs. So, clearly, drug and host are two key players in determining DILI risks. However, how those two key players interplay and determine DILI risks, phenotypes, and outcomes has not been fully

understood.

1.5#3 AS: We recently proposed a concept of drug-host interplay in human hepatotoxicity. Various drug properties and host attributes may interplay at multiple levels and determine individual susceptibility to specific drug, phenotype, and outcome.

1.5#4 AS: I'd like to remind you that drugs have multifaceted properties. Physiochemical, pharmacological and toxicological properties. And also individual drugs have targeted or therapeutic biophysiological effect. And also at the same time, some drugs have various off-target biophysiological effects which may influence host response to drug insult and eventually impact individual susceptibility and phenotype. We also know that some drugs directly interact with immune system and induce specific immunoreactions.

1.5#5 AS: The hosts also have various factors which may influence relevant mechanisms to DILI. Not only the genetic variant, race/ethnicity, but also age, gender, sex hormones, co-medication, co-morbidity and gut flora may interact some of the

relevant mechanisms related to the susceptibility to drug-induced liver injury.

1.5#6 AS: So, we conceptualize that drug properties and host factors interplay at the multiple level in determining individual susceptibility to specific drug insult. And by modulating host response to injury insult, those two key players may determine clinical phenotype and outcome as well.

1.5#7 AS: In my talk, I'm going to focus on adaptive mechanism. But before I do so, I'd like to define "adaptation" such as "diverse host responses to minimize toxic cellular insults, inflammation, and tissue injury which lead to the resolution of cellular stress, cellular dysfunction, inflammation and tissue damage." And a compromised adaptation may result in clinically significant DILI occurrence and may lead to serious clinical outcomes.

1.5#8 AS: Here is today's outline. I'm going to share conceptual discussion over interaction at each level. And also I will briefly discuss how we should investigate and include our

understanding on drug-host interaction in the future.

1.5#9 AS: I'm going to start with cellular stress response, focusing on covalent binding protein damage and protein repair and degradation.

1.5#10 AS: Some drugs are extensively metabolized in the liver. And these properties are known to be associated with human hepatotoxicity. Such properties likely interact with host factors influencing drug metabolizing enzyme. We know that females in general are associated with higher drug metabolizing enzymes, enzyme activities, and also a theoretical concomitant use of inducers or inhibitors change the activity of drug metabolizing enzyme and influence individual susceptibility to specific drug toxicity.

Reactive metabolites also cause protein damage in the cell and eventually lead to cellular dysfunction and cell death. In such setting, host ability of protein repair, protein degradation, or recycle may impact cellular survival and impact the patient's susceptibility.

1.5#11 AS: We recently performed a data mining

analysis using WHO database to assess drug-host interaction in liver event reporting. We classified over 300 of the drugs with known hepatotoxicity into four groups: no gender difference in liver event reporting; female dominant; female dominant, but only under age of 50; and male dominant. And then we characterized those drugs using the drug property information in LKTB database at NCTR. The analysis is still preliminary, but the drug property of significant hepatic metabolism associated with the higher prevalence among drugs with female dominant liver event reporting would suggest the interaction between this drug property and gender.

1.5#12 AS: Thioredoxin reductase is involved in protein repair in the cell and has been known for its protective effects in experimental drug-induced liver injury. A recent small genetic association study that shows that a single -- the haplotype of the SNPs associated with the occurrence. Unfortunately, they did not classify the drug based on the drug properties.

1.5#13 AS: This study is from the group of Dr.

Harrill. They've been using a mouse diversity panel to investigate the diversity of susceptibility. In this study, they exposed their mouse diversity panel to one drug with known hepatotoxicity and generated a drug-induced liver injury. They observed elevated serum ALT, hepatocellular hypertrophy, single cell necrosis, and phospholipidosis, but the severity of the injury differed significantly differed among the strains.

They divided into resistant strains and susceptible strains and they performed hepatotic gene expression analysis to identify which pathway significantly expressed between the group. What they found out was genes involved in protein ubiquitination pathway, drug transport, phospholipid metabolism, and lysosomal function are differentially expressed between those two group, suggesting that a protein repair/protein degradation mechanism may be involved in determining individual drug-induced liver injury risk.

1.5#14 AS: Mitochondrial toxicity, oxidative

stress, and ER stress are all interrelated and are important drug toxicological properties. Those are the drug properties may likely interact with host factors altering cellular stress response, such as increased cellular oxidants, depleted antioxidants, accumulation of fat, impaired cellular protein repair/degradation, and mitochondrial dysfunction.

One of the examples for this concept is the valproic acid hepatotoxicity in subjects with mitochondrial DNA polymerase mutation. But I think we could more broadly apply this concept. For example, nonalcoholic fatty-liver disease. That is the most prevalent chronic liver disease in most industrious countries which is associated with increased cellular oxidants, depleted antioxidants, fat accumulation in the hepatocyte, and mitochondrial dysfunction and increased ER stress. So, the clinically relevant question is, those patient with NAFLD may have increased susceptibility to the medication with those drug properties. We don't know yet.

1.5#15 AS: So, this is a model in Parkinson's

disease I borrowed because it's nicely described. Disease susceptibility based on the interrelationship between oxidative modification, mitochondrial dysfunction, and lysosomal dysfunction. A preexisting impairment, the lysosomal dysfunction, may augment drug toxicity caused by oxidative stress or mitochondrial dysfunction by accumulating damaged protein or damaged mitochondria in a cell. It has been known that glucocerebrosidase deficiency, as in Gaucher, has been associated with Parkinson's disease and cancer, but also recent experimental studies showed that the glucocerebrosidase deficiency actually augments acetaminophen-induced hepatotoxicity. Suggests that probably the patient with lysosomal dysfunction may have higher susceptibility to the medication with the drug property causing protein damage.

1.5#16 AS: There are several medications commonly used in clinical practice known to ameliorate ER stress. So, those medications could protect from hepatotoxicity caused by the medication. I quickly checked our previous data mining analysis using WHO

database. It turned out that those medications usually associated with significantly decreased reporting frequency of liver event, the four, acetaminophen, isoniazid, and valproic acid, but not amoxicillin/clavulanic acid.

1.5#17 AS: Now, I'm talking about inflammation and immune response. Regardless of initial toxicological insult, once hepatocyte is damaged, they release the damp molecule and induce cellular inflammation and immune reaction.

1.5#18 AS: So, the host factors influencing inflammation and immune response, such as genetic variant, sex hormone, altered microbiome, may significantly impact individual susceptibility to developing clinically significant drug-induced liver injury. Some medications induce immune-mediated drug-induced liver injury. Those medications likely interact at host factors influencing immune response as well.

1.5#19 AS: Tissue injury and repair. The different cell death pathway, the necrosis, apoptosis, they induce different intensity of inflammation and tissue damage.

1.5#20 AS: And drug-causing necrosis is usually associated with more severe liver injury, but host factors also change the cell death's pathway, such as gender and the cellular energy supply. And also many host factors, as listed here, are known to change the capacity capability of tissue repair, which all might influence individual susceptibility to DILI. Cholestatic injury is probably associated with different set of host factors, but there wasn't enough data to conceptualize the specific drug-host interplay in the setting.

1.5#21 AS: So, I'm going to talk a little bit about the future investigation, how we can improve our understanding in drug-host interaction.

1.5#22 AS: Probably we might need to introduce more biological variants, especially key biological variants -- age, sex, sex hormone -- to assess specific drug-host interaction.

1.5#23 AS: NIH announced back in 2014 that the old NIH-funded study should consider at least including female and males. I think it's a great idea, but also I can see a lot of challenges.

1.5#24 AS: In a clinical investigation, we should more introduce the drug property in our analysis. I've been collaborating with Dr. Chen and Dr. Tong at the NCTR, who developed wonderful knowledge base of drug property for future analysis. Probably we needed to have a more complete data set and also integrate clinical phenotypes in order to enhance our clinical analysis. And, also, we needed to have new investigational tools to cope with the complexity of this disease condition.

1.5#25 AS: This is the project I am currently working on. Using National VA Data Warehouse we are generating parent database, including more than a hundred causal agent and comprehensive clinical data to supply the specific customized table for future data mining, which will communicate with LKTB and NCTR, and findings will be shared with existing multidisciplinary collaborations to further facilitate the future research.

1.5#26 AS: This is the last slide. Heterogeneity in risks, phenotypes, and outcomes of DILI may be explained by multilayered interplay of drug

property and host attributes in adaptive mechanisms.

Future investigation incorporating drug properties, biological variants, and their potential interaction in study design will aid in better understanding of the pathology and facilitate future personalized drug safety.

1.5#27 AS: Thank you for your attention. These are my collaborators.

1.5#28 AS: (Applause.)

DR. SENOR: Thank you, Dr. Suzuki. We now have as the next speaker Dr. Neil Kaplowitz. Dr. Kaplowitz has spoken many times in this forum. He is world renown for his prowess as an investigator, but he's also still a very active clinician and takes service regularly at his hospital in Los Angeles. Neil:

1.6#1 NK: DR. KAPLOWITZ: Thank you very much, John. It's really a pleasure to be here, and I'd like to thank Dr. Suzuki for an excellent presentation. And she pretty much gave every -- her talk pretty much covered everything I was going

to cover, but nevertheless I'll slog you with it again, with a slightly different perspective.

So, I'm going to talk specifically about adaptation to cellular stress, but particularly hepatocellular stress, and propose the question -- or pose the question of whether this has a role in the severity and susceptibility to DILI, including idiosyncratic DILI.

1.6#2 NK: So, I have no conflicts related to this specific presentation.

1.6#3 NK: So, I'd like to start by reminding you, since this is a government-sponsored symposium, that former government employees are well known for telling us that there are known knowns, there are known unknowns and there are unknown unknowns.

So, in the DILI field, I'm not exactly sure where we are in this spectrum, but probably all three. And we know what we know for sure, and we know what we don't know, but who knows what else there is out there? And at this point in the field of the evolution of DILI, our understanding of DILI, although we've come an enormous way in the past 15 years and it's just amazing, we still have

huge barriers and unanswered questions.

1.6#4 NK: So, just a slight variation on Dr. Suzuki's definition of "hepatocellular stress," because it really applies, you know, it applies to everything that we see in medicine, that the pathophysiology of all diseases involve what one might view as a sort of yin and yang of stress and the response to stress, adaptive responses and maladaptive responses. And the ultimate battle between those determines whether individuals develop disease, or adapt to disease, or don't develop disease at all. So, liver disease-promoting triggers, such as drugs, viruses, alcohol, fatty acids, often mediated by organelle and oxidative stress, activate signal transduction pathways and transcription factors which promote gene expression programs or post-translational modifications which mitigate or promote injury, the final outcome being cell survival, death, or an inflammatory response. So, the question then is idiosyncratic DILI. We currently believe based on all this, you know, explosion of information about the adaptive immune

system, that IDILI mainly is mediated by the adaptive immune responses. And most patients with -- but the interesting thing is that most patients with susceptible HLA polymorphisms do not develop IDILI when exposed to the specific drug, or, at most, develop only mild injury, to which they adapt.

1.6#5 NK: Why is this so? Why is it that, despite in some cases striking susceptibility host factors, most people do not develop a problem? And of course you're going to hear a lot at this meeting about immune tolerance, and there's no question that immune tolerance seems to play a very important role in protecting the liver against an immune attack. But in this particular talk, I'd like to propose a hypothesis and speculate with you about hepatocellular adaptation to stress as playing an important role in protecting individuals from an immune response. Most drugs associated with IDILI cause organelle or biochemical stress in model systems. Many of you are from pharmaceutical industry and it amazes me how well-developed especially big companies are in

providing very detailed pre-clinical systems:, a cell-based, a gene expression, isolated mitochondria, membrane transport models, and so on to identify risk. And when you look at some of the literature, you see that using batteries of so-called DILI drugs and safe drugs, that these various model systems, even including isolated mitochondria, are pretty predictive. They're not perfect, but they're not too bad. And so a lot of reliance has been placed on them. So, the question is: how does this predictive in-vitro toxicology explain our clinical experience, particularly with respect to the fact that most of what we're seeing is immune mediated?

1.6#6 NK*:* This brings me to this half filled-half empty argument. There's no question that what these model systems are showing us is that there are hazards that occur in response to chemicals that enter hepatocytes and stress hepatocytes. The question then is, are these hazards a really pathophysiologically relevant phenomenon in the sense that they're integral to the development of the immune response or immune DILI? Or are they

basically just surrogates with the fact that these drugs are metabolized in liver cells and that they stress liver cells normally.

1.6#7 NK: So, if you take the optimistic view that all these in vitro studies are relevant, which I tend to, there are two ways in which, you know, in which that's the case. One is that even nonlethal stress of hepatocytes can induce danger signals which can certainly interplay in facilitating an adaptive immune response. And there's also plenty of experimental evidence that these stress responses, organelle stress and chemical stress in liver cells, can sensitize liver cells to the lethal consequences of innate and immune-mediated processes. So, liver cells that have poorly functioning mitochondria and so on are more prone to be killed, more resistant.

So, this is the overarching model that I would hypothesize, which it's a pity I don't have a pointer, but I guess there isn't one. Government technology. This happens to me every time I speak here, one glitch or another. So, starting with drugs either converted to reactive metabolites or

parent compounds, drugs can undergo covalent binding (in the box at the bottom), but this occurs particularly in the endoplasmic reticulum leading to drugs or parent drugs being sequestered in mitochondria, lysosomes and impair their function. They can inhibit transporters and that can lead to bile acid retention. And they can induce reactive oxygen species, a key factor. These chemical phenomena and distribution phenomena of drugs and liver cells induce organelle stress, which can be lethal or nonlethal. The key organelles in this respect are the mitochondria and the endoplasmic reticulum, as have been pointed out. Lethal stress, of course, will release DAMPS, but even a nonlethal stress can. This will, along with covalent binding, promote innate and adaptive immunity. The liver then may be susceptible, but the liver responds to organelle stress by adaptive responses which mitigate the stress and may dampen the entire process. That's really what I want to focus on.

1.6#8 NK: So, I just listed here just a few examples and this is a huge field, but I just remind you many of these are transcriptionally regulated,

some are not. NRF2 is a transcription factor which is activated by oxidative stress and reactive chemicals, induces the expression of antioxidant genes. So, that's an adaptive response, a very important one. The unfolded protein response can occur in the ER or in mitochondria. What this is basically is a response to either protein overload or malfolded proteins. And this elicits a program of especially the production of chaperone proteins which can bind and prevent the toxicity of the malformed or unfolded proteins. There are a variety of mitochondrial responses, including, most importantly, mitophagy. So, when mitochondria are damaged, there's a signaling mechanism that's initiated which leads to their removal. That removes mitochondria that are generating reactive oxygen species. Mitochondrial biogenesis, as Dr. Hunt alluded to earlier, mitochondria, “regenerate,” but basically as mitochondria are being removed, new ones are being produced. And this is all regulated by transcriptional programs. And then there's the fascinating, very active area of research into mitochondrial fission and fusion

and their role, and I'll mention that in a minute. There are a variety of post-translational modifications that are responses to stress that I won't go into. And I'm sure microRNAs probably play a role, although, there's very little exploration of this in DILI.

1.6#9 NK: So, stress occurs and there's this whole sort of range of phenomena that occur in response to specific types of stress that lead to protection and dampening. So, one could imagine how this -- the appropriated adaptation or maladaptation could influence the outcome.

Bile acids have, you know, been a very important area of investigation and bile acid retention can, you know, which is potentially toxic, can induce a variety of stress responses involving transcriptional programs that I won't go into now, but you can peruse that on the -- on whatever the FDA puts on the website.

1.6#10 NK: Just to talk a little bit about ER stress, ER stress is almost uniformly seen with drugs that undergo covalent binding. And it's because the adduct formation that occurs in the --

with ER proteins induces a malfolding response and maybe other glutathione depletion in the ER, which is -- glutathione being important for protein folding. So, there are many aspects to it, but also reactive oxygen species and bile acids can induce ER stress.

So, this leads to misfolded proteins and triggers the unfolded protein response, which is triggered really by the displacement of the chaperone Grp78 from the inside of the ER membrane where it's normally holding ER stress sensors in an inhibited form.

But when malfolded proteins accumulate, Grp78 is displaced and these ER senses, IRE1alpha, ATF6 and PERK self-activate and trigger a variety of responses mainly leading to increased chaperone through transcription factors and some post-translational modifications.

I won't bore you with the details, but if the adaptive response is insufficient or maladaptive, then apoptosis and inflammation occur related to a variety of different mechanisms that, again, I think are probably beyond our current scope.

1.6#11 NK: And mitochondrial stress can of course be triggered by inhibition of mitochondrial DNA synthesis by, for example, nucleosides. As I think Chris alluded to, cationic drug accumulation can impair mitochondrial function. Reactive metabolites can actually be sufficiently stable to enter the mitochondria and valproic acid and acetaminophen are pretty good examples of that. And signal transduction is a very important one. I'll come back to that.

Again, I don't want to go into this in great detail, but just to give you a sort of 40,000-foot view of this, there are mitochondrial adaptive responses analogous to ER stress adaptive responses.

1.6#12 NK: These include the mitochondrial unfolded protein response, which is transcriptional activation when mitochondria are impaired through a transcription factor which upregulates mitochondrial-specific chaperones and mitochondrial protein import machinery.

Mitophagy involves also mitochondrial stress or damage, induces stabilization of a

protein, which is a target for a ubiquitinating enzyme, and that leads to targeting damaged mitochondria to the lysosomes.

Mitochondrial biogenesis involves its own transcriptional program and then there's fission and fusion, which are very complex issues. And there are intrinsic proteins in the outer and inner membrane of mitochondria whose regulation and dysregulation modulate fission and fusion. And I could talk about any one of those five bullets -- I could talk about any one of those for an hour.

1.6#13 NK: Important to remember that the major source of reactive oxygen species themselves is mitochondria. And when mitochondria generates superoxide usually at either complex I or III, which occurs even physiologically, that is rapidly converted to hydrogen peroxide which then diffuses into the extra mitochondrial cell. And this activates signal transduction mechanisms, antioxidant defense, hypoxia responses, physiological -- this is -- so, mitochondrial reactive oxygen species are physiological messengers, but in excess they produce

pathological responses involving signal transduction, for example, activation of -- sustained activation of JNK, inflammation, cell death.

1.6#14 NK: JNK is a particularly interesting one. And I show you here on the upper panel a graph of the effect of phospho-JNK accompanied by ATP in isolated mitochondria on respiration involving State 3 respiration, which is the - after ADP addition, and maximum respiratory capacity after CCCP.

The point here is that an activated form of this kinase actually directly inhibits mitochondrial electron transport. And this occurs, as you've seen on the bottom, with either JNK1 and 2 activated, recombinant JNK1 and 2, and in a dose-related fashion.

So, the doses that are out towards between five and 50 are the kinds of levels we would see in acetaminophen toxicity after a few hours or other toxicity models whereas more towards the 0.5 to five would be the kinds of JNK levels we see in fatty liver disease or NASH.

1.6#15 NK: And this induces oxidative stress as well. JNK directly activates reactive oxygen species measured here by MitoSOX. And if you add calcium to this, it even amplifies this greater and that's sort of the mechanism.

1.6#16 NK: Then activated JNK through a variety of mechanisms binds through an outer membrane protein called Sab, which inhibits electron transport leading to production of reactive oxygen species which sustains JNK activation.

And in pathological circumstances, it's very bad to have sustained JNK activation. It's good when it's transient.

1.6#17 NK: I'm almost done, I think. So, we knocked out JNK in a liver-specific inducible knockout and it -- on the left you can see it completely -- virtually completely prevents acetaminophen toxicity knocking out Sab and it also prevents TNF-induced apoptosis shown below that.

1.6#18 NK: And we've worked out a mechanism for this in terms of intramitochondrial signaling, which I won't bore you with. And finally just to remind you, and you'll hear more about this in the

next talk, that these adaptive responses not only respond to cellular stress, but to cellular death-inducing pathways.

And we know that aside from like acetaminophen poisoning, which is mainly a necrotic liver injury, most liver injury with liver cell death or hepatitis of any type is an apoptotic type of disease. And so, mostly apoptosis. And apoptosis can be induced by death receptors or intrinsic stress within the cell through, you know, intracellular organelles.

1.6#19 NK: And the death receptor pathway in the case of some death receptors like the TNF receptor, is mitigated by activating NF-kappaB, which dampens the death pathway.

And there are a number of survival responses that mitigate apoptosis, and those are listed in the right lower side. They include the upregulation of caspase inhibitors by NF-kappaB and those are listed, the upregulation of anti-apoptosis Bcl members like Bcl-XL and Mcl1 in the liver, which fight the proapoptotic Bcl2 members at the outer membrane of the mitochondria

and, therefore, prevents cytochrome C release and apoptosis, and then there's, you know, antioxidant responses that are regulated by NF-kappaB and NRF2.

1.6#20 NK: So, conclusions. Okay. So, chemicals, like drugs, stress hepatocytes in a variety of ways. And I think I've tried to stress all the, you know, the myriad of fascinating things that happen. Many intricate adaptive responses dampen the adverse effects of stress and protect hepatocytes. Stress can affect the fitness of hepatocytes leading to increased susceptibility to the lethal consequences of immune attack or can generate sublethal danger signals. The balance of injurious and -- versus adaptive responses to drug-induced stress may be modulated by genetic and environmental factors. And analogous to the yin and yang nature of immunity, the injurious stress versus adaptive responses in hepatocytes may be an important contributor to the occurrence of IDILI, even if immune-mediated, hypothetically.

(Applause.)

DR. SENIOR: Thanks, Neil. We're

three minutes behind. So, we need wings to get to the point. Arie:

DR. REGEV: Okay. So, our next speaker will be Lily Dara. She's an instructor in clinical medicine at the Division of GI and Liver, USC Research Center for Liver Disease. And she will talk about the new data on adaptive processes.

1.7#1 LD: DR. DARA: Actually, the title of the talk changed.

DR. REGEV: It has?

DR. DARA: Yes, it has.

DR. REGEV: Okay.

It's Liver Cell Adaptation: Death Versus Survival, because we realize that tomorrow we have an entire session dedicated to adaptation. But I will touch upon adaptation a little bit, but more in the context of hepatocytes.

DR. REGEV: Okay.

1.7#2 LD: DR. DARA: So, hepatocytes in their microenvironment are in a steady state of homeostasis. Changes in this homeostasis due to a chemical, a toxin, a drug, an injury, a virus, can cause irreversible damage leading to cell

death. The elimination of these damaged and infected cells is critical to the normal development and homeostasis of the hepatocyte, the -- sorry, the entire organ, the liver and the multicellular organism. And as we talked about in the previous session, the liver can regenerate and replace these damaged hepatocytes and, thereby, restore homeostasis.

1.7#3 LD: Now, the capacity of the cell to adapt will dictate whether it dies or survives. The hepatocyte's ability for adaptation depends on the severity of the stress signal, as well as the cell's capacity to modulate gene expression and stress responses, as Neil pointed to in the previous talk. Failure to do so will result in hepatocyte death. So, it's really important for us to talk about the fact that the liver is an immune-privileged organ. As you all know, the liver is constantly exposed to new antigens and bacteria from the gut and their antigens. And if it were to react constantly to this stream of incoming antigens, it would be in a constant state of hepatitis. Tomorrow we'll have an entire session on immune tolerance. So, I will

very briefly talk about the tolerogenic liver microenvironment before I pivot to the issue of hepatocyte death.

1.7#4 LD: The main functional unit of the liver, is the hepatocyte. Hepatocytes express a variety of receptors on their cell surface. I'm only showing the TNF superfamily and the death receptors. Many other cell types make up the microenvironment; equally important are the nonparenchymal cells. Liver sinusoidal endothelial cells are very important in maintaining the state of tolerance in the liver. They are scavengers, and antigen-presenting cells as well, and they down-regulate antigen presentation by secreting prostaglandin E2, which is an immune dampening prostaglandin and has autocrine and paracrine effects, Kupffer cells are very important. They secrete cytokines such as IL-10 and TGF-beta, which dampen immune responses and induce regulatory T cells, inhibit leukocyte adhesion to the LSECs thereby also dampening responses, and also recruit macrophages from the periphery and convert them into dendritic cells,

which are mostly IL-10 positive, IL-12 negative, thereby promoting an immune-tolerant environment. Dendritic cells also differentiate regulatory T cells into T amplitude phenotypes, which are more promoting an immune-tolerant phenotype. Another important player is the stellate cell which can induce apoptosis of T cells and elimination of cytotoxic T cells and also induce regulatory T lymphocytes. Lastly, but very importantly, is LPS preconditioning and a phenomenon known as endotoxin tolerance. LPS in a lot of organs induces an inflammatory response in the liver. Because of the constant stream of low-dose exposure usually about 10 pg/mL to 1 mg/mL, LSECs and Kupffer cells are desensitized to this LPS. And LPS helps in inducing Kupffer cells to secrete IL-10, and promotes the lack of adhesion of the white blood cells to the LSECs, and also it desensitizes TLR receptors. All in all, this tolerogenic environment is where the hepatocytes are residing.

1.7#5 LD: Now, as I said, the hepatocytes are expressing death receptors. These are numerous. Their ligands are expressed by the immune system.

And it's important to point out, I think, as Neil mentioned, that apoptosis in the liver is a predominant model of cell death and it's executed by these death receptors. So, the interplay of the death receptors on the ligands on the immune system is really important. And since idiosyncratic DILI is adapted immune mediated, it's interesting to speculate that the resultant hepatotoxicity ultimately involves death receptors. And the mode of cell death is more than likely apoptosis.

1.7#6 LD: Now, why is this important? Cells can die many different ways. So, the signaling pathways activated by the cytokine TNF-alpha, are among the most extensively studied and well understood in mammalian biology. However, there're still a lot of unknowns. In a simplistic model, two signals emanate from this pathway. Either cell survival via NF-kappaB, or in the absence -- or in certain circumstances, cell death via caspase 8. *I wish I had a pointer.* So, as you can see at the top of the figure when TNF binds to its receptor, receptor-interacting protein kinase 1 or RIPKinase 1, is recruited to the TNF receptor. The adaptor

TRADD, as well as TRAF2, 5 and the cIAPs are also recruited. The cIAPs are erythropoietin ligases and so is LUBAC. So, what happens is that RIPK1 is then ubiquitinated and it forms a platform. This platform brings in proximity the IKK complex, as well as the TAB1 and the TAK complex, thereby activating the MAP Kinase cascade, as well as NF-kappaB. If RIPK1 is not ubiquitinated or if it's de-ubiquitinated by enzymes, as cylindromatosis, then it enters complex IIa or complex IIb. In complex IIa it promotes TRADD, FADD, Caspase 8-mediated apoptosis rise in complex IIb, or in the ripoptosome another complex forms, but ultimately the resultant outcome is caspase 8-mediated apoptosis.

1.7#7 LD: Now, NF-kappaB are a family of transcription factors that regulate survival. In adaptation at a cell level, especially in the context of hepatocytes, NF-kappaB is really important. The predominant form of NF-kappaB is a heterodimer comprising of p50, NF-kappB1 and p65 or ReLA. In a resting state, NF-kappaB dimers are held by inhibitory kappaB in an inactive state.

When released by IkappaB, they translocate to the nucleus where they bind the NF-kappaB elements and they upregulate survival pathways and then they turn on gene expression that dampen pro-apoptotic signals. It's really interesting because p65 knockout mice and IKKbeta-deficient mice die in utero of hepatocyte apoptosis. So, we know that if we take away NF-kappaB, you get hepatocyte apoptosis. It's very important for the adaptation of hepatocytes. These anti-apoptotic and pro-survival effectors of NF-kappaB are the cIAPs, cFLIP, Bcl-XL, among others.

1.7#8 LD: So, what's going on downstream of the TNF receptor, as we talked about, is activation of survival pathways through -- on the left-hand side you can see the ubiquitinated RIPK1 leading to NF-kappaB activation, or if RIPK1 ubiquitination is inhibited, apoptosis via complex IIa or IIb, or in cases where caspase 8 is inhibited in certain cell types, a RIPK1, RIPK3 activating MLKL and resulting in necroptosis. This can also occur with other death receptors such as FADD and TRAIL, as you can see. It's not unique to TNF. Actually,

it can occur with interferon.

1.7#9 LD: So, what is necroptosis? Necroptosis is a form of programmed cell death that is morphologically similar to necrosis. It's evolved as an alternative death pathway to apoptosis and it usually occurs in the presence of caspase inhibition. This was very recently discovered that the loss -- the reason why caspase 8 knockout mice die in utero or embryonic lethal, is the fact that when you take away caspase 8, you unleash necroptosis. Same thing for FADD. So, caspase 8 cleaves the RIPK1 and RIPK3 and keeps necroptosis in check. Necroptosis occurs in certain cell types, L929 mice, Mice fibroblase, MEF cells, Jurkat T cells, macrophages. And in some cell lines in addition to caspase inhibitors, you need to add IAP antagonists to unleash this death pathway. All cells that undergo necroptosis do express RIPK3 and MLKL.

1.7#10 LD: So, as you can see here in this cartoon, another way of depicting this is that if caspase 8 is inhibited in the red box, RIPK1 and RIPK3 oligomerize and they activate MLKL, which is then

phosphorylated and translocates to the cell membrane, punches holes in it and ultimately results in lysis of the cell. Necroptosis is a form of programmed cell death carried out by receptor-interacting protein kinase 1 and 3 and the pseudokinase-mixed lineage kinase domain-like. Now, as you can see here, RIPK1 and RIPK3 can bind together via that pink area called the rim domain or the homology interaction motif. And if RIPK1 is not ubiquitinated, then it -- RIPK3 aggregates on RIPK1 and oligomerizes autophosphorlyates leading to MLKL phosphorylation. Translocation to the cell membrane where it binds to the PIPs, or the phosphatidylinositol phosphatases, punches holes in them and then there you go. You have lysis of the cell membrane.

1.7#11 LD: So, the kinase activity of RIPK1 is thought to be necessary for this. And necrostatin, which I'm sure a lot of you have heard of, blocks the kinase activity of RIPK1. RIPK1 knockout mice are embryonic lethal, they don't survive, and it's actually very interesting how this happens. There're a lot of different functions

for RIPK1. It's not only functioning in necroptosis, it also functions in cell survival. RIPK3 and MLKL knockouts, however, are viable without any phenotype. Interestingly, RIPK3 inhibitors have been tried to block necroptosis, but instead of blocking necroptosis, they rather switch the mode of cell death to apoptosis. So, as you can see, this is more complicated than one may think in terms of therapeutic targets. It's just a warning that, beware.

1.7#12 LD: Does necroptosis ever in occur DILI? APAP DILI is a form of necrotic cell death. So, we knew that and we were interested in necroptosis, so we wanted to see if this is indeed the mode of cell death we're seeing. We know that APAP DILI is regulated, we know it involves signaling interfering with a lot of the MAP kinase cascade aggregates. This response interfering with MPT aggregates cell death from hepatocyte death from acetaminophen. Necrostatin, the RIPK1 kinase inhibitor, had been shown by multiple groups, including ours, that it can interfere with acetaminophen toxicity and prevent acetaminophen

toxicity. However, we knew that ultimately RIPK3 activation and MLKL membrane translocation is what defines necroptosis and RIPK3 knockout mice and MLKL knockout mice don't undergo necroptosis. So, we decided to look at this at -- by doing studies in genetic knockouts and knocking down RIPK1.

1.7#13 LD: What we first did is that we knocked down RIPK1 and we saw about a 60 percent protection, as you can see in the bars here, against acetaminophen at 300 milligrams per kilogram. We then did a high-dose survival experiment where we gave mice 500 milligrams per kilogram, which kills a lot of mice, as you can see, and the mice that were knocked down of RIPK1 survived. This was not due to NAPQI binding or GSH, which I haven't shown here. So, it wasn't a metabolic effect.

1.7#14 LD: So, we then looked at the RIPK3 knockouts and the MLKL knockout mice and we couldn't see any protection with RIPK3 knockout and MLKL knockout against acetaminophen. Now, this is important because this points out that then acetaminophen necrosis is not a form of necroptosis, because MLKL knockouts compared to

their strain match controls and RIPK3 knockouts compared to their strain match controls were not protected.

1.7#15 LD: However, although the RIPK3 knockouts were not protected, compared to the wild-type controls, look at the yellow bar and the white bar. If we knocked down RIPK1 in the RIPK3 knockouts, as you can see in the orange bar, we did see protection. So, there seems to be some effect of RIPK1 knockdown in protecting the mice.

1.7#16 LD: The next question was, is RIPK3 even expressed in the liver? In the original studies that came out from Genentech and from Vishva Dixit's lab where he described this RIPK3 protein at least using northern blots, there wasn't much mRNA in the liver. And most hepatologists and most people studying this have noticed that at least under beta conditions there isn't much protein in the liver. We did a lot of experiments trying to figure this out. Most of the antisera out there were nonspecific. They were polyclonol.

1.7#17 LD: As you can see, we got really messy bands. The second lane here are all RIPK3 knockouts

and they all picked up these antibodies. We did immunohistochemistry, and all we could see was using RIPK3 knockout animals also using a negative control with IgG was complete pickup of the necrotic area. So, this was practically useless. It's just to show you like how much money and time and effort we put on this.

1.7#18 LD: We used a lot of different antibodies and most of the antibodies didn't even recognize the positive control. And some of them even didn't recognize -- the positive control.

1.7#19 LD: So, anyway, RIPK3 protein, we finally were able to detect this using an RIPK3 monoclonal antibody developed at Genentech. We got an NTA, we finally got it and eventually were able to see a clean blot. As you can see here, there's very low level expression in the liver and there's no pickup on the RIPK3 knockout, but it's not induced after acetaminophen, which is what you would expect if you were seeing some sort of effect. And as I told you, the knockouts were not protected.

1.7#20 LD: The last two slides are coming up, and these are the interesting slides. So, there is no

RIPK3 protein in the hepatocytes. We've fractionated the hepatocytes. As you can see here, the Kupffer cells and the LSECs and the liver white blood cells do express RIPK3. But even though we loaded way more as you can see on the Ponceau of the hepatocytes, 50 micrograms compared to five to 10 micrograms of the nonparenchymal cells, we don't see any RIPK3 in the hepatocytes. And as you can see, the knockout liver is there to serve as a negative control.

1.7#21 LD: But interestingly although they didn't express RIPK3, the hepatocytes did express MLKL. And that's very fascinating. Although it is less than what you see in the Kupffer cells on the LSECs, it's very intriguing to think why is MLKL even there without any RIPK3, as I told you this last effector of necroptosis.

1.7#22 LD: So, conclusions. Nonparenchymal cells maintain a tolerogenic microenvironment in the liver. Hepatocytes triggered by death receptors can either die of apoptosis or adapt and survive by upregulating NF-kappaB. Acute DILI from APAP is a form of regulated necrosis involving

RIPK1 and JNK, I didn't get to show a lot of that data, which leads to MPT, but not necroptosis, because MLKL knockout and RIPK3 knockout are not protected. Mouse hepatocytes don't express RIPK3 and do not undergo necroptosis during acute DILI from acetaminophen.

1.7#23 LD: Unresolved issues are, is RIPK3 induced in hepatocytes in idiosyncratic DILI or other liver diseases, for example, NASH, alcohol? There's been suggestions that it can be induced. Are there conditions in which intrinsic stress activates RIPK3 and MLKL? And then in the absence of caspase inhibitor, does necroptosis even occur in vivo? Because as I told you, the default mode of cell death is apoptosis and necroptosis only occurs in certain cells when you inherit caspase inhibitors. And since nonparenchymal cells express RIPK3 and MLKL, is necroptosis important in certain situations, for example, in LSEC death, in NPC death or in IDILI? And what is the function of hepatocyte MLKL and how can it be activated if there's no RIPK3 to phosphorylate it?

1.7#24 LD: So, there're a lot of interesting

unresolved questions and that's the end of my talk. Thank you. (Applause.)

The last speaker this morning is Dr. Mala Chakraborty. Mala:

1.8#1 MC: DR. CHAKRABORTY: Hi. I would like to thank Dr. Senior, first, for giving me the opportunity to talk in this conference. And I'll be discussing about the role of adaptive immune response in DILI. Especially the role of myeloid-derived suppressor cells in the model.

1.8#2 MC: The problem in DILI is that it's nearly impossible to predict what new drug will cause hepatotoxicity and which individual will be susceptible to DILI. This is mainly due to the part of idiosyncratic nature of DILI and the lack of animal models for most of the drugs where mechanisms can be started and the susceptibility factors can be uncovered. However, our understanding of DILI is improving based on animal model of acetaminophen, halothane and some other drugs, and clinical findings are also helping us to understand the mechanism of DILI.

1.8#3 MC: Clinical evidence and studies show that DILI may be caused by allergic reactions against the liver mediated by adaptive immune responses. Common clinical features are fever, skin rash, hepatic eosinophilia. And the hepatic lesions always contains, mainly contains mononuclear cells, neutrophils and eosinophils and sometimes lymphocytes. Toxicity usually occurs after more than one exposure to the drug. And susceptible patients have humoral and T cell response against the protein adducts or unlabeled carrier proteins.

1.8#4 MC: HLA association also suggests the role of adaptive immune response in DILI. The first two studies, the publications showed large number of case-control association studies, including candidate gene, as well as genome-wide association studies. And the strongest association was found in HLA I and II and NAT2 also. The underlying mechanism of HLA association is most likely the involvement of T cells as antigens are presented in the context of HLA molecules.

The last publications have shown that B*57:01 allele is a susceptibility factor for

flucoxacillin-induced hepatotoxicity. They cloned the T cells from the liver injury patient and also they showed that nine T cells from the B*57 volunteer can be activated by the drug if they are presented in the context of dendritic cells.

1.8#5 MC: Though clinical evidence and HLA association suppose that adaptive immune response play a major role in DILI, we need to develop an animal model for the definitive experimental proof.

The drug we used in our animal model is halothane. Halothane is an inhalation anesthetic, was widely used, as Dr. Senior mentioned, until 1980s. And it was withdrawn from the U.S. market due to the liver toxicity, but still is being used in Middle East, Sub-Saharan Africa and third-world countries due to its low cost and effectiveness.

Halothane is metabolized in the liver by cytochrome P450 to intermediate trifluoroacetyl chloride, which covalently binds to mainly liver proteins and it can cause toxicity to the hepatocytes directly, or it can cause enough stress

to hepatocytes that secrete many proinflammatory cytokines, which activates the innate immune cells and subsequently the adaptive immune system. We reported that eosinophils in the initial injury of halothane-mediated liver injury, whereas IL-10 decreases the toxicity.

1.8#6 MC: This is our model. We injected female mice with halothane, 13 mM/kg, mixed with olive oil. And you can see after 12 hours of the halothane exposure, there's an increase in the ALT. And which reaches its peak at 24 hours. And the ALT level drops by 48 hours. We confirm the toxicity by histology. The top slide shows the vehicle-treated mice. There is no cytotoxicity, whereas halothane-treated mice show the massive centrilobular necrosis in the zone 3 area in the liver. And we assess the kinetics of the infiltrating leukocytes in the liver. And we could see the difference compared to the vehicle-treated mice at 18 hours after halothane exposure and which this is peaked at 24 hours.

1.8#7 MC: Folks have been trying to develop an animal model of DILI for a long time. In 1997,

Gandolfi, et al., published that they give three exposure of halothane in a guinea pig model, but they couldn't recognize any secondary immune response, which showed -- give a hint that tolerance might play a role in halothane-induced liver injury.

1.8#8 MC: In our model, also, we expose the mice to halothane. We couldn't see any different trifluoroacetyl protein-specific T cells or increased similarity.

1.8#9 MC: We could detect some antibody directed against trifluoroacetyl protein, but the trifluoroacetyl was very low. After one to 20 dilution, we couldn't detect any antibody directed against trifluoroacetyl protein. So, we and others hypothesize that the idiosyncratic nature of DILI is due at least in part to the immune tolerance in the liver. If we can break the immune tolerance in the liver, we may develop an animal model of DILI mediated by the adaptive immune response. And similarly, patients who develop DILI may be deficient in some liver tolerance.

1.8#10 MC: These are the steps we have chosen,

because they are representative of the human halothane hepatitis. First, we wanted to detect if we can detect the trifluoroacetyl protein in the mouse liver, as well as in the serum.

1.8#11 MC: Then A and B showed the liver TFA-protein adduct in the liver and then we could detect trifluoroacetyl protein in the mouse serum after 12 hours of the halothane exposure.

1.8#12 MC: When we tried to characterize those infiltrating cells in the liver by flow cytometry, we found out that most of the cells express the CD11b and Gr-1 on their surface.

And as the Gr-1 is the marker for two combined Ly-6G and Ly-6C, we found out that most of the cells are Ly-6G high and Ly-6C low. Only two percent of the cells are Ly-6G negative and Ly-6C positive. And the cytosine preparation of the CD11b Gr-1 high cells showed the morphology of mature neutrophil, as well as some immature cells which has been represented as immature cells with banded nucleus.

1.8#13 MC: And recent reports indicate that myeloid derived suppressor cells are one of the

main cell populations responsible for regulating immune responses in cancer, in infectious diseases and also in autoimmune diseases. MDSCs represent a heterogeneous population of immature myeloid cells that strongly suppress T cells and identified by the expression of CD11b and Gr-1 on their surface. And MDSCs are regulated by the transcription factors STAT1, 3, 5, 6 and NF-kappaB. The effector molecules which access the suppression of the T cells are IL-10, prostaglandin E2, TGFbeta, reactive oxygen species, nitric oxide and arginase. The other two subsets has been cited in the literature. One is the granulocytic kind, which express CD11b and Gr-1 high. And the monocytic kind actually CD11b positive Gr-1 low, but they are Ly-6G negative and mainly Ly-6C high.

1.8#14 MC: So, then we also in our animal model, we assess the kinetics of the CD11b Gr-1 cells. The CD11b Gr-1 cells reach its peak at 24 hours, but it drops by 72 hours. As in mouse, in the model neutrophils also express CD11b and Gr-1 on their surface. It's very difficult to differentiate between myeloid derived suppressor cells and

neutrophil other than their functional activity. So, one of the functional activity is the expression of reactive oxygen species. So, we measured reactive oxygen species in the CD11b Gr-1 cells by a fluorogenic dye, DCFD. You can see that though the total number of cells is higher at 24 hours, but the per cell basis reactive oxygen species is more at 72 hours, which indicated that after neutrophil leaves the liver, that those are the cells are present in the liver, they are mainly myeloid derived suppressor cells.

1.8#15 MC: To assess the functional activity, we measured the suppression assay of this myeloid derived suppressor cells by coating the plate with anti-CD3e and we used sorted CD4+CD25 negative cells or CD8+CD25 negative cells as a -- from the mice spleen as a target cells.

1.8#16 MC: And we used CD3 negative HLA-DR positive splenocytes as antigen-presenting cells and sorted CD11b+Gr-1 high cells, the MDSCs from the livers of halothane or vehicle-treated mice were used as effector cells and we cultured the cells for 72 hours. And we measured the T cell

proliferation by tritiated thymidine uptake. You can see when we didn't use any myeloid derived suppressor cells in the culture, the T cells were proliferated. And when we added the myeloid derived suppressor cells, the suppression of the T cell proliferation went down. And several factors have been implicated for the function of -- for the suppressive activity of myeloid derived suppressor cells.

1.8#17 MC: We also use different inhibitors like catalase for reactive oxygen species and other inhibitors, but only one inhibitor, the specific inhibitor for inducible nitric oxide synthase the myeloid derived suppressor cells mediated suppression of the T cell proliferation. We also measured the nitride in the culture supernatant. And when we didn't have any myeloid derived suppressor cells, we didn't see any nitrite. So, we concluded that these nitric oxides are coming from the myeloid derived suppressor cells. The similar results we found in the -- when we used CD8 positive T cells as an effector cells.

1.8#18 MC: After confirming that halothane

induces myeloid derived suppressor cells in the liver, we wanted to deplete these cells in our animal model. For that, we had to tritate a different concentration of the anti-Gr-1 antibody, because initial injury is important for the secondary immune response. From our recent study, we know that eosinophils also express Gr-1 in -- though they are low in concentration. But if we use high concentration of Gr-1, we deplete both the eosinophils and myeloid derived suppressor cells or neutrophils.

1.8#19 MC: From this antibody titration, we chose the concentration 20 microgram concentration per mouse. On day zero we injected 20 microgram of anti-Gr-1 antibody to deplete myeloid derived suppressor cells. Next day we give the halothane. After two weeks, we give another halothane exposure and we collected all the samples after nine days of the second dose exposure of the halothane.

1.8#20 MC: And what we found in the anti-Gr-1-treated mice, there is an increase ALT level and we confirm the data with the histology. As you see the isotype-treated mice, there are no

-- there is some microcalcification, but no cytopathic injury, whereas in anti-Gr-1-treated mice there are mild perivenular, lymphocytic infiltrations.

1.8#21 MC: After getting this toxicity nine days after second exposure of the halothane, we wanted to dissect the mechanism of this halothane-mediated liver toxicity.

1.8#22 MC: Next slide also shows that anti-Gr-1-treated mouse showed some small foci of plasma cells. Plasma cells is a classical indication of allergic hepatitis.

1.8#23 MC: And in the other slide you can see that apoptotic hepatocytes are surrounded by lymphocytes and macrophages, which also indicated that adaptive immune response might play a role in this model. And sometimes we had like high ALT level, we found that both necrotic and apoptotic hepatocytes, most of the necrotic hepatocytes were near the veins, whereas the apoptotic hepatocytes were in the -- in the damaged region. Those are indicated with arrows.

1.8#24 MC: And as we know that trifluoroacetyl

protein adduct is important in halothane-mediated liver injury, we first decided to know that anti-Gr-1 antibody has any effect on this -- on the metabolism. And we found that after nine days of the second exposure, there is no difference in the TFA-protein adduct in the two groups. And then we wanted to know whether this humoral antibody, humoral response against this TFA-protein adduct is playing a role in this model.

We found out that anti-Gr-1-treated mice, there is a significant increase of the total IgG after nine days of the second exposure and whether we couldn't detect any difference in the antibody after seven days.

1.8#25 MC: And then we analyzed the isotypes of this antibody and we found out that the main difference we found in the IgG1 and IgE. And then as IL-4 promotes the -- these two type antibody response, we measured IL-4 in the -- in those mouse serum. And we also found an increased IL-4 in halothane hepatitis expression, but we didn't -- they didn't -- there is no report of IgE, but there is a report of IgG, increased IgG4, which is also

type 2 response. So, next in our reports, we found that eosinophil plays a major role in the initial halothane injury. And halothane -- in halothane hepatitis disease, eosinophilia is always very common. We measured the total number of eosinophils in the liver after nine days and we confirmed immunohistochemistry staining of the major basic proteins in the liver. And as a chemo attractant of eosinophil, we measured the neurotoxin one and two, we found a difference in CD11b, but we didn't see any difference in neurotoxin two. So, next we wanted to see the depletion of hepatic MDSC prior to halothane treatment resulted in CD4 T cells or CD8 T cells in the liver. We found increased CD4 and CD8 T cells, the total number in the liver. And the CD4 cells were proliferated when we cultured them in the presence of trifluoroacetyl protein adduct. And we showed that these T cells are specific related to TFA. And we measured the interferon gamma as a measure of T cell proliferation.

1.8#26 MC: And next we depleted the CD4 cells and both CD8 cells in those mice treated after second

exposure of the halothane. Anti-CD4 depletion lowered the toxicity of those anti-Gr-1-treated mice. We saw a trend in the decrease in the ALT level, but that was not just statistically significant. Though we developed a model mediated by adaptive immune response, we didn't see any fulminant liver failure, which made us think that other tolerogenic molecules are playing compensatory mechanism in this model for CTLA4, pG1 or regulatory T cells. But in this serum when we measured IL-10 and TGF beta there is an increased IL-10 in the anti-Gr-1-treated mice. So, maybe this Kupffer cells are secreting this IL-10 where MDSCs are not present there.

1.8#27 MC: So, in summary, we can say that we have developed a model by depleting these myeloid derived suppressor cells where halothane produce the trifluoroacetyl protein adducts are produced and released in the blood and were able to induce both specific humoral and T cell response against the protein adducts. And this approach also led to a significant inflammatory liver injury that appeared to be mediated at least in part by adaptive

immune response.

1.8#28 MC: I would like to thank Dr. Pohl for giving me the opportunity to work in his lab, and our lab colleagues. And I would like to thank Dr. Kleiner for carefully looking at my slides and giving me his valuable opinion. Thanks.

(Applause.)

DR. SENIOR: Thank you, Dr. Chakraborty. We're now open for discussion. Questions. Here comes Mark Avigan to the microphone.

DR. REGEV: Maybe I'll ask as they're walking to the microphone, this is for Dr. Kaplowitz. So, again, this is a comment that you made before. And based on all the evidence regarding the role of the adaptive immune system, how much can we rely in the future on cell cultures and other pre-clinical and nonclinical devices to try to predict idiosyncratic hepatotoxicity?

DR. KAPLOWITZ: Well, that's a very difficult question to answer. I, you know, I have no skin in that game, so to speak, but I would say,

you know, my cup is half filled in the sense that I believe that it's telling us something about the potential for a drug to be immunogenic even though it -- the way it's doing it is by damaging hepatocytes or altering mitochondrial function.

So, at the very least, you know, it appears that most of the drugs that do not exert any effect on these model systems do not have a clinical liability. So, irrespective of whether it's mechanistically linked to adaptive immune response, it looks to me like for the most part it's giving us some indications and coupled with dose, you know, and perhaps lipophilicity and a few other measures, you know. And on the other hand, it's conceivable that, you know, Dr. Suzuki's approach will be just as good, I mean, in terms of assessing just pharmacology, you know. If a drug is metabolized in the liver, you know, it's one thing. If it's not metabolized in the liver, it's not likely to cause liver disease with the exception of biologics that are immune modulators.

DR. REGEV: Thank you.

DR. AVIGAN: So, I had a question over

here and it goes back to a question I had asked a number of years ago, which is still with these animal models that we heard about today and the idea of adaptation with redundancies built into the relatively complex biosystem to correct the perturbation and you have different pathways of getting rid of cells and also protecting them, as we just heard.

So, the question becomes if that's the basic problem where certain individuals have some defect in the machine which gets unleashed when the liver is challenged and can't correct because it's missing a key bolt in the house, so the house collapses, why don't we see more examples of human individuals who are repeat victims to different drugs because they have a defect in their ability to adapt? In other words, you would expect -- you would expect individuals who show idiosyncratic DILI to, let's say, Drug A to also have susceptibility to events with other drugs because their defect is a downstream defect to write homeostasis.

DR. KAPLOWITZ: Well ---

DR. AVIGAN: I'd be interested in hearing your points on that.

DR. KAPLOWITZ: Yeah, I, you know, just to start the conversation, you know, perhaps a lot of it has to do with the specific --- the HLA restriction. So, you know we don't know how many drugs, you know, are promiscuous in terms of their HLA presentations. I think that's a big factor. And also, you know, the status of, you know, adaptive responses depends on whether the planets are aligned in the right way, you know. Sometimes you could --- you could envision all types of variations, you know, in terms of diet, concomitant drugs, concomitant illnesses and so on. It's very complex.

DR. AVIGAN: Yeah, I would just point out that there's data ---

DR. KAPLOWITZ: Microbiome.

DR. AVIGAN: There is data on this.

DR. KAPLOWITZ: Oh.

DR. AVIGAN: And what, I mean, in the sense that if you look for individuals that are well characterized as a causal case of DILI, very --

there are actually very few instances in the literature that that individual had an index case with another drug.

DR. KAPLOWITZ: Right.

DR. AVIGAN: And that's a very important, I think, observation about ---

DR. KAPLOWITZ: Yeah.

DR. AVIGAN: -- what we're talking about.

DR. KAPLOWITZ: We published something like that a few years back with the Spanish registry, and I forget the number. There were a group of patients who had multiple episodes of DILI with different drugs and almost all of them had a clinical phenotype that looked like autoimmune hepatitis.

PARTICIPANT: Mark, I'll try to answer your question tomorrow. And, Dr. Chakraborty, I want to thank you for the presentation. Lance couldn't --- Lance Pohl couldn't be here because he's had a stroke, and he convinced me 30 some years ago that these things were immune mediated. I didn't want to believe it, because I'm not an

immunologist and I didn't want to get involved, but Lance certainly had a major impact on my career. So, I want to thank you. Neil, we talked about this. I'm not at all nearly as impressed with these in vitro assays as you. When I look at them, the drugs that are associated with the highest risk, isoniazid, propylthiouracil, felbamate, nevirapine, et cetera, most of them are negative. And when you put busulfan and other drugs in there and say, well, that's a positive hit, well, you know, it's a rather cytotoxic agent. So, I'm not at all impressed that these things are very good. I think flipping a coin would be just about as good and ---

DR. KAPLOWITZ: Can I respond?

PARTICIPANT: -- it's a very attractive hypothesis that something like mitochondrial injury would be involved, but recently somebody presented some work on mitochondrial injury and they said Phenformin was withdrawn from the market because it caused liver injury. No, it was lactic acidosis. It does block complex I of the mitochondria. It causes

lactic acidosis, but I don't know of any cases of liver injury. And metformin also blocks complex I in mitochondria, never causes liver injury, nor, to my knowledge, does it increase the risk of other idio --- you know, there might be an interaction.

And you might think acetaminophen co-administration, because it injures mitochondria. Both clinically, I don't know of any evidence of that. And we did a study in our immune model giving along with the amodiaquine at early time points acetaminophen and it didn't have any effect on the liver injury caused by amodiaquine. So, it's an attractive hypothesis, but I think we have to go back to the clinical data. What do we observe clinically? I mean, valproic acid, absolutely. No question.

DR. KAPLOWITZ: Sure. You know, I'm not -- I'm not going to defend that position vigorously, but I think you're being entirely too pessimistic. (Laughter.) And, you know, I'd be interested --- I think we have an audience full of people who use systems like that, probably, who might, you know, want to chime in and ---

DR. REGEV: And probably wasting a lot of unnecessary dollars.

DR. KAPLOWITZ: Well, you know, it --- and, you know, it seems to --- there you are. It seems to me that the drugs that you cited as being negative are not negative in everybody's hands and it depends on the model system. And so I'm, you know -- I'm not completely negative about this. I mean, I totally agree that ultimately, you know, what happens in human beings is very important. But if you're a pharmaceutical company and you're trying to do, you know, you have --- you're trying to work your way through a myriad of chemicals to do predictive, you know, toxicology and so on, that, you know, there is some value in identifying compounds that are more likely to injure cells or alter functional --- well, somehow we're not seeing the same thing.

DR. REGEV: The jury is still out.

DR. SENIOR: Okay.

DR. REGEV: I'm not sure who was --- I think you were first.

PARTICIPANT: Okay. One thing which

may address that, and I thank the panel for a nice overview, but I think ---

DR. SENIOR: Could you state your name and company?

PARTICIPANT: Oh, I'm sorry. Fresenius Kabi in Germany. What I found missing is the factor of diet, which may explain a lot about the individual variability, because the level of antioxidants and other factors make a hell of a difference and certain diseases have a deficiency of certain nutrients. So, maybe that's why certain diseases are more susceptible and why certain persons are more susceptible. It's a multifactorial. Everything must come together, and then you get this disease. And what's --- and diet we can influence, we can supplement. For example, we can put DSH, DHA, EPA or fish oils to have more antioxidant capability, or supplement vitamin D or certain amino acids.

My question to you is, what is your experience here and what should we include in the diet, or should we, if we have a susceptible agent, supplement and thereby reduce the incidence and the

severity of DILI.

DR. SENIOR: It's a Suzuki question. (Laughter.)

DR. SUZUKI: I will try. So, I'd be interested in a multiple factors influencing the susceptibility. This is very preliminarily, but we did data mining analysis using four, the different causal drugs and what kind of supplement and concomitant medication actually influence the reporting frequency of the liver event. What I found out was that folic acid supplementation widely decreased the liver event reporting regardless of medication. When I talked with the basic researcher and the system biology seems like a folic acid significant role in regulating the information. At this point, I think we don't have good evidence what kind of nutrient or diet actually influence the individual, the susceptibility to drug-induced liver injury, but I agree with you certainly we need to put more effort to better understand those and how environmental factors influencing disease susceptibility.

PARTICIPANT: If anybody has more ideas, we have some data on DHA and EPA. They do influence autoimmune response and the antioxidant capabilities. It's a fascinating field, I agree with you, and we don't know everything yet.

DR. SENIOR: John:

DR. VIERLING: Vierling, Baylor College of Medicine Houston. I'm interested in the speakers' thoughts on the possibility of translating this newer information about the injurious consequences in pathways and simulation within the hepatocyte, or the innate and inflammatory process within the whole organ to be interrogated in vivo. And specifically, I'm interested in whether or not any of these things lend themselves to detection with mass spectroscopy of the liver. And secondly, whether any allow the interrogation to be based on the microvesicles released from the liver particularly during injury.

DR. KAPLOWITZ: Yes. (Laughter.)

DR. VIERLING: Are you going to tell me how to do it at the break?

DR. KAPLOWITZ: I think those are really good ideas and I think, you know, clearly there's a tremendous amount of interest, you know, currently in microvesicles and exosomes and so on and the information they carry and intercellular communication as well, which undoubtedly is just an emerging area and I think there's a lot to be learned, but clearly, you know, important. In terms of mass spectrometry, that, you know, certainly MR spectroscopy of the liver has been, you know, it's been very successful in the heart, you know, in terms of ischemia and so on, ATP. It can be used to measure redox status. So, there are potential uses. It hasn't been widely used in the liver, but, you know, but it, you know, certainly for investigative purposes might be of interest.

DR. SENIOR: I wanted to follow up on that. John, you talk about mass spectroscopy. Are you talking about somehow measuring liver blood flow? What what are you driving at?

DR. VIERLING: Actually what I'm driving at is what's happening within the hepatocyte or the hepatocytes undergoing apoptosis

or necroptosis for reasons of these stimulated pathways. They clearly create a different biochemical milieu in different gene expression, and I'm just wondering if that couldn't be interrogated by mass spectroscopy looking for changes within a spectrum of predetermined and prespecified molecules of interest.

DR. KAPLOWITZ: Yeah, I mean, along those lines, I mean, I've given some, you know, I've thought about this over the years because, you know, if you're going to posit that mitochondria are an important target, then it should be possible to, you know, in some sequential way to identify mitochondrial liability. And I believe, you know, this has been done like with nucleosides in muscle in particular; is that -- isn't that right? Yes? So, you know, you could, you know, for example, if you put people on, you know, some HIV medicine and follow their muscle ATP levels over time, you can -- you could predict, you know, when adverse effects are beginning to occur.

DR. REGEV: I think the other side goes first.

DR. BELFER: Inna Belfer, CDER, FDA, from the Division of Analgesics. For the past 15 years I was studying pain genetics and I would like to share with you something that may help us all to understand better the genetics of IDILI or DILI. So, from Neil's presentation, you mentioned that most people that have these susceptibility, HLA mutations, they do not develop IDILI or develop as milder. So, the question is why. One of the possible explanations may be sex-specific gene affects. And this is a new field. That's why I am bringing it up. So, apparently there are three scenarios for gene affects. Some --- for the interactions between the genes and the sex.

Some are sex-specific, meaning that in --- the effect may be absorbed only in one sex. Sex biased that for the different degree, the genes affect the trait or the disease. And sex-antagonistic affects, and this is most important. So, from pain genetics, so several genes that work antagonistically. So, they predispose one sex, but protect another one. So, if you do the analysis of your HLA mutation data and just adjust

for the sex, like most of the studies do, then unintentionally you can -- you just mask the true effect. So, if this particular mutation works oppositely in men and women, you will never see this affect in your clinical trial as a genome. So, the practical issue, you should ---- you should treat the genetic data, the genetic cessation data as we treat the safety data. So, it should be sex-specific. And then you can see better the interactions, the pathways, and you can use for genetic mutations not only as biomarkers, but also use them in the predicting models. And so, back to John's pulling from the average patient to the real patient, you can see it in the context of patient-related other traits because we know that many other traits like diet, like stress and others, they modify the genetic effect. So, I just don't want you to underestimate the true genetic susceptibility factors that may be affecting DILI the same way as they affect other complex phenotypes. Thank you.

+ + + + +

11:55

Special Award for Lana Pauls

DR. SENIOR: Thank you. I hate to interrupt this very stimulating discussion. This is really good, but we're running out of time and we have an important announcement to make. I want to call Paul Watkins, Lana Pauls, Debbie Birnkrant, Mark Avigan and Sara Senior to come join Arie and me on the stage here. Paul has an announcement to make.

DR. WATKINS: Yes, we're going to take a few minutes out to recognize the tremendous contribution of an individual. And normally you would think I was talking about John Senior --- (Laughter.) -- however, three years ago we gave up on doing that annually when we realized he'd go on forever. And actually it's Lana Pauls who has made a surprise announcement. She's leaving the FDA to become the director of Global Regulatory Sciences for Quintiles.

That obviously sounds appropriately important and influential, but I think this is going to be an example like the song, you don't know what you've got until it's gone, because Lana and John have really been the team that have built this

great community of people interested in DILI that have really pioneered the regulatory science of drug-induced liver injury.

The logistics of doing this meeting, of giving everybody all the slides --- almost all the slides. I knew she'd wince at that, you know, on a wristband, then recording everything, including the discussions, handing that all out to the many international speakers that they have here for them to go through and audit them, putting it all on the internet so people who didn't even come to the meeting are able to get the full benefit of the meeting, is really extraordinary.

So, we're going to miss you greatly. And she's already promised she'll come back. And we don't know how we'll go forward, but somehow we'll manage. Thanks. (Applause.)

DR. BIRNKRANT: So, Lana, we have some more words to praise you, as well as a few other things. Let me start with this plaque that we have. It's an Outstanding Leadership Award. So, your colleagues want to recognize you for your contributions in organizing this DILI meeting over

the last 15 years. On behalf of FDA, AASLD and the Critical Path Institute, we'd like to recognize you for your leadership, your dedicated efforts and your inspiration in planning and implementing this important meeting for the last 15 years. Thank you very much. (Applause.)

DR. REGEV: Just taking the opportunity on behalf of many, many industry members that enjoy this meeting for many years, your name has become the synonym for this meeting. They may need to change the name of the meeting after you leave, but thank you so much for so many years of dedication and excellent execution of this meeting. I don't know --- actually, I have no idea who is going to take your place, but I don't envy her. So, good luck and thank you very much.

(Applause.)

DR. AVIGAN: I just want to add a government perspective, because I've been an observer of Lana since, I guess, 2003, and of course this meeting has grown in its notoriety. I mean, actually, its prestige and it's clearly to a large extent the result of Lana's excellent work. And

the word "DILI" has become a regular part of our four-letter word lexicon. I won't use other words in that lexicon, but to a large extent Lana can take a lot of credit for that along with John. So, congratulations and really good luck.

MS. PAULS: Thank you very much.

(Applause.)

DR. SENIOR: Sara and I have become very fond of Lana. And we want to give Lana 15 red roses for 15 years of DILI conferences from 2001 to 2016. Lana has welcomed an audience 4 times. She's been a moderator 5 times. She's been an invited speaker 6 times. How are we going to top that? --- Lana, we love you.

MS. PAULS: Thank you. (Applause.) You know I'm not sure what to say other than "thank you," because most of you who know me very well know that I don't like to be in the limelight. But I have been now for the last four minutes. So, that being said, thank you very much. I will miss you all, and I hope to be back here on a regular basis as a participant with Quintiles. Thank you.

(Applause.)

(Whereupon, the above-entitled matter went off the record at 12:01 p.m. and resumed at 12:57 p.m.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

(12:57 p.m.)

DR. AVIGAN: So I think we'll get started for the afternoon session. We're never shy to deal with very challenging problems and issues around DILI. And the afternoon session is dedicated to a problem that's becoming much more prominent on our radar --- and that is chronic liver disease and recognizing drug-induced liver injury in the context of various kinds of liver diseases.

We have a very stellar cast. Our first speaker is Dr. Vierling who is chief of Hepatology at Baylor College of Medicine, and his talk is on: “Can study protocols protect patients with liver disease from serious DILI?” John?

2.1#1 JV: DR. VIERLING: Well, I want to thank the organizing committee for the opportunity to address this topic.

2.1#2 JV: My disclosures are illustrated on this

slide.

2.1#3 JV: As background, we're all aware that identification of drugs or biologics with potential to cause serious life-threatening DILI requires causality assessments and comprehensive testing for alternative etiologies in each suspected case. Since idiosyncratic DILI is rare, the prediction of risk of serious DILI post-marketing relies on identification cases of interest meeting Hy's law as criteria during Phase II or III clinical trials. Subjects with pre-existing chronic liver diseases or CLDs, with or without cirrhosis, are challenging because current FDA guidance is based largely on criteria for studies that exclude subjects with abnormal liver tests. CLDs do not increase overall susceptibility for DILI, but multiple exceptions suggest that additional examples will be identified in future clinical trials. DILI in subjects with CLD is worrisome, because such subjects, with reduced hepatic function reserve, are less likely to recover and more likely to die. And finally, the probability of subjects with CLDs

enrolling in clinical trials is increasing, especially in therapeutic trials targeting components of the metabolic syndrome which is a risk for NAFLD patients, antimicrobials and specific CLDs, complications of cirrhosis, and hepatocelluar carcinoma.

2.1#4 JV: (*slide not shown)*

2.1#5 JV: Now to address my question, I will discuss four key issues,beginning with the question: What is the probability of enrolling patients with chronic liver diseases, CLDs, including cirrhotics, in drug development trials?

2.1#6 JV: The etiologic spectrum of CLDs associated with progression of cirrhosis is large and in developed countries, the cumulative prevalence of CLDs is increasing, largely due to NAFLD.

2.1#7 JV: Thus the probability of enrolling Americans with CLDs, with or without cirrhosis, in clinical drug trials is substantial. As shown on the left, random enrollment in general drug development trials could result in inclusion of up to 35 percent with a CLD and five to seven percent

with Gilbert's syndrome. As shown on the right, the proportion of enrolled subjects with CLDs would be increased markedly in studies of drugs for obesity, diabetes, hyperlipidemia, hypertension, or gout; conditions in which NAFLD in highly prevalent as the hepatic component of the metabolic syndrome.

2.1#8 JV: What about trials for patients who have CLDs? On the left, by definition, all patients enrolled for therapeutic drug trials have specific CLDs and enrollment often includes a pre-determined proportion of compensated or decompensated cirrhotics. As shown on the right, drug trials specially for cirrhosis and its complications of portal hypertension and hepatocellular carcinoma, HCC, are increasing. Those for complications of portal hypertension, by definition, enroll only decompensated patients who have the least hepatic reserve to tolerate DILI or other hepatic injuries.

2.1#9 JV: (slide not shown)

2.1#10 JV: Now, the global prevalence of cirrhosis is striking and frightening. This heat map

indicates the prevalence of between 100 and 200 cases per 100,000 in the U.S., and approximately 48 percent of the cases worldwide are due to alcohol.

2.1#11 JV: (slide not shown)

2.1#12 JV: (slide not shown)

2.1#13 JV: Well, the second question is: what are the risks of serious DILI in hepatic decompensations in subjects with CLDs?

The first issue is whether patients with CLDs have increasing susceptibility for DILI. Although many clinical trials have excluded subjects with abnormal liver tests, everyone is aware that many patients with CLDs have normal liver tests, including many with cirrhosis. My mentor and colleague, the late Hyman Zimmerman, studied the issue of susceptibility in patients with CLDs in the 1970s and concluded that CLDs did not confer an increased risk of DILI. However, subsequent studies have identified specific exceptions.

2.1#14 JV: For example, both disease-specific and non-specific susceptibility to DILI has increased in patients with CLDs reported in those receiving

highly active antiretroviral therapies for HCV, HIV co-infection, antituberculous therapy for HBV, as well as co-infected patients, and in subjects with specific UGT1A1 alleles.

2.1#15 JV: In contrast to initial data from NIDDK's DILIN, recent reports indicate that NAFLD patients have an increased risk of DILI with drugs for hypertension, platelet aggregation inhibition, and infectious diseases, as well as for over the counter NSAIDs, PPIs, and acetaminophen. Moreover, patients with a variety of CLDs appear prone to DILI caused by antimicrobials and the immunosuppressive drug tacrolimus. More examples are likely to be described as new drugs are studied in larger numbers of subjects with CLDs.

2.1#16 JV: Regardless of susceptibility, DILI in a subject with a chronic liver disease is definitely associated with increased risk of progression to a serious outcome. The DILIN reported that ten percent of 899 patients with adjudicated DILI have significantly higher mortality compared to those without CLDs. These DILIN data indicate a need to identify all subjects

with CLDs prior to enrollment in clinical trials.

2.1#17 JV: (*slide not shown)*

2.1#18 JV: Well, our next key question is: How do protocols currently protect subjects with CLDs, including cirrhotics, from serious DILI?

Now, despite whatever the stopping rule is, DILIN has provided a severity index score ranging from mild to fatal, that has criteria that relate to the same parameters we've already discussed. It is an important, I think, scale to keep in mind and is clinically useful. And those with an asterisk meet criteria for reporting as an SAE during clinical trials.

2.1#19 JV: Acute liver failure is the concern of a serious DILI reaction. And acute DILI can resolve due to adaptation, as we have heard, or resolve off drug. But it can progress to acute liver failure with a risk of cerebral edema and death or death due to liver related causes. However, increasingly with CLDs, we have the unique acute-on-chronic liver failure components that are seen in advanced fibrotics and cirrhotics. Now, all of these can be rescued by OLT when near fatal,

but multi-organ failure and sepsis often contraindicate that option and lead to death in both groups. Now as noted earlier, normal liver tests do not exclude the presence of CLDs and etiologic testing for CLDs should be performed in patients with normal liver tests. Abnormal liver tests generally indicate a chronic disease, but acute diseases must also be excluded. An assessment for cirrhosis is mandatory because of the risk of decompensation and the unique need for screening for gastroesophageal varices and HCC surveillance imaging.

2.1#20 JV: *(slide not shown)*

2.1#21 JV: Protocols currently employ non-invasive testing to include or exclude the presence of cirrhosis. Tests based on serum laboratory tests are shown on the left, and imaging methods are shown on the right. The most accurate method to detect cirrhosis appears to be magnetic resonance imaging, MRE. Now, overlapping 95 percent confidence intervals between fibrosis stages with these tests indicate that they are reliable only for the differentiation between stages F0 and F1

and F4, cirrhosis. In current drug study protocols, non-invasive tests for cirrhosis can result in misclassification of non-cirrhotics as cirrhotic as well as cirrhotics as non-cirrhotics. Neither error rate is known and should be studied.

2.1#22 JV: Hy's law's cases, as defined by the FDA in the NIDDK DILIN, are based on concurrent elevation of ALT three times the upper limit of normal and total bili two times the upper limit of normal. This remains an essential tool for identification of subjects at risk for serious DILI. Key concepts merit some reiteration. First, despite the name, Hy's law is not a law but instead an important clinical alert that should trigger comprehensive assessment and adjudication of the cause of concurrent elevations of both enzymes and bilirubin. Second, not all Hy's law's cases, even if due to DILI, progress to liver failure. John Senior provided examples of cases due to isoniazid demonstrating that point earlier today. However, the two-case rule has shown that the finding of two Hy's law's cases adjudicated to be due to DILI in a clinical trial is highly predictive of acute

liver failure.

2.1#23 JV: Now, identification of potential Hy's law's cases in tracking of individuals during the course of a clinical trial is greatly aided by analysis of both eDISH and the kinetics of increases in ALT and total bilirubin. At the top you see our traditional eDISH plot of the log of the peak ALT and total bilirubin expressed as multiples of the upper limit of normal values, indicating in the right upper quadrant that Drug X exclusively showed cases meeting Hy's law criteria compared to Drug C. The kinetics of abnormal liver tests provide additional information regarding progression, potential stability, or regression of abnormal values. Thus both eDISH and kinetics are complementary indications for the urgent need to assess for alternatives to DILI and adjudication of the need to discontinue drug or to continue close observation.

2.1#24 JV: Independent of the variability of stopping rules, the DILIN severity -- excuse me, let me go back one. This slide illustrates the

stopping rule recommendations that have been provided regarding patients with normal ALT and AST at baseline or all patients regardless of their baseline values.

2.1#25 JV: And in black, one sees multiples of the upper limit of normals of AST and ALT, and in the orange you see the addition of those normals with the addition of elevations of bilirubin as well as prothrombin time as measures for function.

2.1#26 JV: This slide shows the results from our recent studies in which we had a randomized control, a placebo control trial, of patients treated with glycerol phenylbutyrate, an ammonia scavenger, for overt hepatic encephalopathy. And you will notice that many of our patients, at baseline or during study, met proposed indications for stopping rules.

What about eDISH? Well, you see at the bottom, in our patients who had MELD scores ranging from five to 26, and the majority of whom had Child-Turcotte-Pugh scores making them B or C Class patients, that the values plotted with only one patient in the upper right quadrant at baseline

having a Hy's law criteria. To the right you see every value for every patient plotted during the study. And one sees that 15 patients ultimately met Hy's law criteria, including 10 in the placebo group and 5 in the GPD group. The placebo is in the blue and GPD is in the orange. Careful adjudication of these cases and a serial analysis indicated that they were unlikely due to DILI. And they were continued in trial and monitored very carefully.

2.1#27 JV: Now, serious DILI in subjects with or without CLDs can be also graded solely on the basis of enzymes and bilirubin. This looks at the issue of the CTCAE from the NCI and categorizes Grades 1 through 4. And I show this slide because it, along with guidance from the FDA, is often mixed in the determination of stopping rules in current studies.

2.1#28 JV: Now, this shows the variability of stopping rule in some current chronic liver disease trials that I'm serving as PI for currently. And without giving a complimentary ophthalmologic test for everyone in the audience who can't read the

font, I want to call attention to those things in red because the gestalt is the important thing. It's the high variability of what the stopping rules are in current trials. They're all over the map, and as an investigator, it begs for some kind of clarification and guidance.

2.1#29 JV: *(slide not shown)*

2.1#30 JV: Now, despite whatever the stopping rule is, DILIN has provided a severity index score ranging from mild to fatal, that has criteria that relate to the same parameters we've already discussed. It is an important, I think, scale to keep in mind and is clinically useful.

And those with an asterisk meet criteria for reporting as an SAE during clinical trials.

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However, increasingly with CLDs, we have the unique acute-on-chronic liver failure components that are

seen in advanced fibrotics and cirrhotics. Now, all of these can be rescued when near fatal by OLT, but multi-organ failure and sepsis often contraindicates that option and leads to death in both groups.

2.1#32 JV: *(slide not shown)*

2.1#33 JV: *(slide not shown)*

2.1#34 JV: So we're now to the money question. What improvements and protocols can provide greater protection from serious DILI in patients with chronic liver diseases, including cirrhotics?

2.1#35 JV: Well, I've highlighted in yellow tests that might be able to help us in the future by providing additional information and enhancing our interpretation of abnormal liver tests, both at baseline and on study. And I'll highlight the fact that we want to be sure that our elevations are due to the enzymes in question, of hepatobiliary origins for alkaline phosphatase. We want to have certain SNPs tested to help interrogate the issues of the rises in bilirubin as well as the issue of cholestasis and, most importantly, quantitative liver function testing.

2.1#36 JV: Now the gold standard for determination of a compensated versus the risk of a decompensated patient is a fasting hepatic venous pressure gradient of greater than ten millimeters of mercury. This is not feasible, although it is being used in several current trials, and hence we need surrogates. And quantitative hepatic function tests may provide that surrogacy.

2.1#37 JV: Why is it important to know whether your patient is compensated? Well, it's because of the high rates of progression. In two years or less than two years, 25 percent will have progressed to a decompensated state and in five years, 50 percent. And many drug trials have durations of one to four years and, hence, this decompensation is a likely event that has to be understood and adjudicated as being due to the disease or due potentially to DILI.

2.1#38 JV: Now, in the compensated state, the decompensation begins usually with mild decompensation and progresses to more severe decompensation. Dr. Kamath will address the issues of MELD and CTP scoring in the next lecture.

So I will leave it at that.

2.1#39 JV: *(not shown)*

2.1#40 JV: *(not shown)*

2.1#41 JV: Now, three quantitative liver function tests which are non-disease specific are currently available and could be used in future studies. HEPATIQ is a FDA-approved software that analyzes data from a technitium-99 liver spleen scan and calculates profused hepatic mass. And it measures hepatic function and, most importantly, functional reserve.

HepQuant analyzes hepatic clearance of oral and/or IV administered cholate to calculate either a disease severity index or a STAT score, and these will be explained momentarily by Dr. Everson.

Finally, the Excalenz breath test measures exhaled CO13, CO2 generated by hepatic metabolism of ingested C-13 labeled methacetin. And this generates a PKPD curve as a functional profile that correlates with hepatic venous pressure gradient.

2.1#42 JV: Now, in response to concern about DILI and subjects with CLDs, with or without cirrhosis, future protocols should consider more explicit

guidance for serial assessment of the symptoms, the liver tests, the eDISH, and kinetic plotting, and strict adherence to stopping rules. Since the major concern about DILI is the risk of progression to liver failure in those with reduced hepatic reserve, direct measurement of hepatic functional reserve compared to baseline will likely become the key factor in determining whether a patient is discontinued or observed on therapy. Detection of any signal of interest for DILI should lead to the urgent reviews in the box. **2.1#43 JV:** And I'll highlight, again, that one wants to reassess in a serial manner after comprehensive testing for all etiologies other than DILI, MELD sodium and CPT, and repeat quantitative liver function tests.

2.1#44 JV: *(slide not shown)*

2.1#45 JV: Now, numerous investigative opportunities exist to refine our understanding. They are listed here and most of them are the subject of discussions at this conference. And I think it's from the information to be derived from ongoing studies that we may derive novel insights to refine our ability for risk assessment,

pathogenesis, diagnosis, prevention, and therapy, in this unique group.

2.1#46 JV: So in summary, enrollment of patients with CLDs, with or without cirrhosis, in trials of drug development is increasing due to the rising prevalence of CLDs and cirrhosis. This is going to be true in general drug development, obviously in disease-specific therapies, and in cirrhosis and it's complications. In general, CLDs do not increase susceptibility for DILI, but exceptions exist and they suggest that more are likely to be discovered. Subjects with CLDs and low hepatic functional reserves have increased risks of serious DILI and death. Currently study protocols provide protection for subjects with CLDs from serious DILI by alerting investigators for the need for urgent, comprehensive evaluation of cases meeting Hy's law or other criteria and/or exhibiting evidence of decompensated cirrhosis. Future study protocols should provide additional protection for subjects by direct quantitative testing of hepatic functional reserve at baseline, during any subsequent DILI event, the application

of biomarkers for DILI and its severity, testing for polymorphisms, for UGT1A1, OATP, and BSEP to aid interpretation of tests of abnormal bilirubin or cholestasis, and utilizing advanced imaging techniques with the goal of producing better evidence-based stopping rules for patients with CLDs. Thank you very much.

(Applause.)

2.1#47 JV: *(slide not shown or discussed)*

2.1#48 JV: *(slide not shown or discussed)*

DR. AVIGAN: Well, that was terrific. And we're already going to hear about these very difficult issues around what we measure and also how we recognize DILI in patients with chronic disease where the profile or presentation may be somewhat different.

So with that, we're going to continue to ask the question, how do we measure function? And Dr. Patrick Kamath from Mayo Clinic is a professor there. And his research on using MELD is well-known to many of us in the audience. He's published on this measure of liver function and the 6very important work that he's been doing. Dr.

Kamath?

2.2#1 PK: DR. KAMATH: Thank you very much, Mark and Debbie. And thanks to John and Lana for inviting me to talk on how best we can use MELD and Child-Pugh scores to assess the level, both at baseline and during treatment.

2.2#2 PK: In this talk, I will go over the basics of survival models; how we use the CTP MELD and MELD sodium scores, the MELD and Lille model combination, how we monitor during treatment for chronic hepatitis C and NASH, and some future directions.

2.2#3 PK: First, the basics of survival models.

2.2#4 PK: Prognostic models assess risk, and the difference between modern times and ancient times is that we are much better at assessing risk now. If you're a quarterback throwing a pass, if you're a Presidential candidate making an offensive statement about your opponent, or if you're a physician prescribing a drug, success requires mastery of risk.

Models are not restricted to medicine alone.

2.2#5 PK: So for instance, a penalty kick is really

where we assess risk the most. So what is Messi thinking? Should I kick left or should I kick right? What is the goalkeeper thinking? Should I dive left, or should I dive right? And whoever assesses risk better is going to come out on top here.

2.2#6 PK: And in fact, there's a model for assessing risk for penalties. So I'm sorry these slides seem to be cutting off, but 86 percent of kicks placed within the goal are successful. A hundred percent of goals kicked in the upper third are successful, but that's where you miss the most. Goalkeepers dive left or right 94 percent of the time. (Laughter.) But they dive correctly only 40 percent of the times, so you save 25 percent of the shots that you dive, you're going to save only 10 percent of penalties. But if you stayed in the center, you can save 33 percent of shots. And there are more goals scored towards the center than towards the side. And so this model would suggest that the goal keeper should just stand in the middle and raise his hands.

2.2#7 PK: So let's look at how good models are. How

do we assess accuracy of a model? In medicine, we use discrimination. Discrimination ranks patients at risk for mortality. It uses the c statistic as a measure, and all it determines is who will die first. So if the c statistic is 0.8, which is what's required for an excellent model, we mean eight out of ten times the patient with the highest score will die before the patient with the lowest score. And so it's useful for populations, for example, allocating organs for liver transplant.

Calibration, on the other hand, is when will the individual patient die. And it's useful for the individual patient to make treatment decisions, to consult families. And I think this is what's important for DILI too.

2.2#8 PK: Let's give you examples of discrimination and calibration. Discrimination is which team will win. So if you want an excellent model, you have to get -- eight out of ten times you have to predict who the winner is. All you need to predict is who's winning. That is discrimination.

2.2#9 PK: But calibration is even more difficult.

Calibration means what is the individual score of each team. Get it right eight out of ten times, you've got an excellent model. Who would have predicted Michigan would have lost, leave alone that score. So calibration is much more difficult.

2.2#10 PK: So once we know calibration and discrimination, the next concept I want to introduce is static versus dynamic models. A static model predicts, at a specific time point, the MELD and the CTP scores, specific Maddrey score. Willis is here, developed probably the best model, and he was way ahead of his time, I think the same case, superb statistics when they came up with this model. Way better than the CTP score.

Dynamic models, on the other hand, predict over a time course. You take scores at two different time points. And that is the Lille score used for alcoholic hepatitis where you look at the score after one week of steroid treatment.

I think for DILI you require both a static and a dynamic model. And we'll get into that a little later. And the question is what

about a change in delta score? How useful is that going to be?

2.2#11 PK: So now let's go to the specific scores, CTP, MELD and the MELD sodium scores. The CTP score has objective variables, bilirubin, albumin, and the prothrombin time.

2.2#12 PK: But it also has subjective variables which unfortunately decrease the accuracy of the model. Both encephalopathy and ascites are subjective

2.2#13 PK: So we say CTP score is looking at probability using a simple calculator. And its accuracy in discriminating mortality risk is about 75 to 80 percent. 70 percent or 0.7 is a useful model. Point 8 and above is an excellent model. Okay, it’s borderline.

2.2#14 PK: So what is the MELD?

2. 2#15 PK: The MELD is the model for end stage liver disease. It is a mathematical survival model which was created on patients undergoing TIPS which is for complications of cirrhosis. It estimates risk of mortality. You can use it over any period of time, but typically we use it over three months.

And we use three easily obtained laboratory values, bilirubin, creatinine and the INR for prothrombin time.

2.2#16 PK: This is the formula. What's important to know is the INR influences the model the most, next the creatinine, and least of all bilirubin. This is important again for drug-induced liver injury.

2.2#17 PK: So here are some sample scores. Bilirubin of 1, INR 1, creatinine of 1, gives you a MELD score of six. If the INR goes to 3, the MELD score goes to 19. The bilirubin goes to 3, the MELD score goes up only to 11. And if the creatinine goes up to 3, it goes to 17. So you see how INR is most important, next creatinine and least of all bilirubin. If all three go to 3, then the score is 33.

2.2#18 PK: So what do these numbers mean for the patient? A score of 22 means three month mortality prediction of ten percent, 29 is 30 percent, 38 is 80 percent. And essentially, any patient with a MELD score of 40 is not going to be alive in three months unless they receive a liver transplant.

2.2#19 PK: So what about the discrimination for the MELD score? And this has been done by my colleague, Ray Kim. Just focus on the ones on the extreme left. The MELD and the MELD sodium discrimination in patients waiting for liver transplant, 0.87, which means 87 out of 100 times the patient with the highest score will die before the patient with the lowest score.

2.2#20 PK: Calibration, on the other hand, is observed mortality was as predicted. On the X axis is the MELD score, on the Y axis probability of death. In green is observed mortality, what actually happened in the patient. In red was what is the predicted mortality with a MELD score, and in olive green, predicted with MELD sodium. See how accurate the predictions are at lower MELD scores. So until 34, excellent prediction, above 35 all models have so far, in the waiting list for liver transplant, have over-predicted mortality. And we believe that's partly behavior. Because over 35, you get a liver transplant. And so you do everything possible to keep the patient alive so they can get a transplant. And we believe that's

the reason they are over-predicting mortality.

2.2#21 PK: What were CTP scores? CTP score has only 11 points. So between five and 15 gives you 11 different points. MELD is six to 40, but really it's from zero to infinity. So MELD gives you at least 35 points. So there's finer calibration and finer discrimination. So CTP does have discrimination, does have calibration, but it's what we call coarse discrimination and coarse calibration. On the other hand, MELD score, just because it has many more points, it has finer calibration and finer discrimination. And so in this talk, I would propose that in the future we start using MELD score for the DILI studies.

2.2#22 PK: This is the MELD and Lille model combination, and this is a paper which came out in Gastroenterology last year. They combined all the data from alcoholic hepatitis studies in Asia, in Europe, and in the United States.

2.2#23 PK: This is the MELD and Lille model combination, and this is a paper which came out in Gastroenterology last year. They combined all the data from alcoholic hepatitis studies in Asia, in

Europe, and in the United States.

2.2#24 PK: They came up with a combination of a dynamic model, which was the Lille score, and a static model, which was the MELD score. And they thought this combination performed the best.

So for instance, look here. On the Y axis is the MELD score. And on the X axis is the Lille score. So that is taken a week after treatment. And the higher the number the worse the patient is doing. So 0.45 and less means the patient is doing better; more than 0.45 means the patient is doing worse. So if we look at that time point there, that patient has a MELD score of 21 to start off with. And a week later the Lille score was 0.45. The two month mortality in this patient is 15 percent. The six month mortality is 24 percent.

I think we should start using something like this, the change in score over a week to determine how the patient is doing in response to a drug.

2.2#25 PK: We looked at delta MELD on the waiting list for liver transplantation

2.2#26 PK: And this was published a few years ago.

2.2#27 PK: The arrows show you the different time

points at which MELD was calculated. And this arrow is the last MELD, and the event is death. So the MELD could have been calculated on the day of death, or it could have been any time before that.

2.2#28 PK: Notice here that, throughout all this, MELD stays equally accurate. But the delta MELD is most accurate on the day the patient dies. So essentially, if your MELD was ten, and on the day you died it was 40, sure, that's a big change. But we think that's just reflecting the fact that you're dying. It's not predicting. It's just far too late. And so we need a little better way of detecting who is going to die.

2.2#29 PK: Now, how about monitoring during treatment for chronic hepatitis C and NASH treatments?

2.2#30 PK: So what I would suggest for scoring systems for DILI, we do not have a validated gold standard for early determination of mortality risk in DILI by AST and ALT alone. That's often used to stop, but we don't know what the mortality risk is of raised AST and ALT. In general, I think, if the absolute three month mortality risk has gone

up five percent over control, I think that's a reasonable indication to stop a drug. If you don't have a mortality risk at baseline, then five percent is actually high. So that would be important, that risk. And what is your baseline mortality risk? That's going to vary quite a bit, based on what you have. For instance, if you have got alcoholic hepatitis, you've got a 30 to 40 percent risk of mortality. So risk above that is an indication to stop treatment.

2.2#31 PK: Now, this is a busy slide, but as far as I know this is the only study in hepatitis C which specifically focused on patients with cirrhosis. Notice in there that the median MELD score is ten. A median MELD score of ten means your risk of mortality at 90 days was 1.7 percent. So these are really not a sick population. And we don't have studies on patients who have been really sick.

2.2#32 PK: And as John alluded to before, drug-induced liver injuries superimposed on cirrhosis or chronic liver disease is a prototype for acute and chronic liver failure.

2.2#33 PK: And this is what happens. This is what

happens when we see patients. They get a florid inflammatory reaction. We don't know whether it's a drug. GI bleeding can do that. Alcoholic hepatitis can do that. Post operatively, you can have that. Viruses can cause that. Additionally, infections can cause a florid drug-induced, can cause a florid systemic inflammatory response.

2.2#34 PK: These patients get septic. They get second infections. They get fungal infections and, ultimately, multiple organ dysfunction syndrome. And irrespective of what caused it, it's a very predictable pathway. And as part of multiple organ dysfunction, you start getting intensely cholestatic, and the bilirubin goes up. So that makes liver tests even worse.

2.22#35 PK: These are data from Mayo. So it's only some of these had drug-induced acute and chronic liver failure. Compared MELD with the CTP score, MELD, better calibration, better discrimination than the CTP score. And the CTP score has only limited calibration.

2.2#36 PK: So to assess severity of acute and

chronic liver failure related to DILI, which is the really sick patients, you can assess it by the severity of liver disease. That's the MELD score. You can assess it by organ dysfunction. And there are several organ dysfunction scores.

There is what's called the CLIF-SOFA score which is now being used in Europe. But I think here is the problem. If you use organ failure scores, you are reflecting the dying process. For instance, as CLIF score says, if five organs are failing, you're more likely to die than if one organ is failing. I think that’s way too late for you to stop a drug. You have to pick it up way before organs stop working.

2.2#37 PK: And there are some studies of the MELD score in prognosis of DILI.

2.2#38 PK: So first studies from Asia, anti-tubercular treatment, mortality in 269 patients.

2.2#39 PK: You can see the area under the curve here. In green is for the MELD score. So the C statistic was 0.88 which means it was very good in predicting mortality.

2.2#40 PK: A study from Korea, multiple different drugs, with and without underlying chronic liver disease, the MELD score here was predictable mortality. Hy's rule was not predictable mortality in this group.

2.2#41 PK: And here it's even higher -- I believe there may be something wrong with the statistics.

2.2#42 PK: But the discrimination was 0.93. So it's extremely high. I think it's what we call over-fitting of a model.

2.2#43 PK: Moving to the United States, the drug-induced acute liver failure study with Adrian Reuben,

2.2#44 PK: --- MELD score and its components of bilirubin and INR were predictive of mortality.

#45**2.2#45 PK:** And again, from Europe, this is from Denmark, acetaminophen induced acute liver failure,

2.2#46 PK: --- again, MELD, 0.88.

2.2#47 PK: So to summarize the studies of MELD score and drug-induced liver injury, high discrimination has been over 0.85 as a predictor of mortality, in acetaminophen, antitubercular

treatment and other drugs. So again, I would suggest using MELD at baseline for DILI studies. And if they have underlying chronic liver disease, we should have serial MELD scores in these patients.

2.2#48 PK: The ACG guidelines for diagnosis and management of acute drug-induced liver injury recognizes a high MELD score is predictive of a poor outcome but does not specifically state that this is what should be done for monitoring of the patients.

2.2#49 PK: Future directions …

2.2#50 PK: …approach to diagnosis of DILI, and we're going to go over this again, diagnosis of DILI requires adjudication. In alcoholic hepatitis, which we are studying, I think that situation you can use ASG and ALT easily. Because in alcoholic hepatitis, ASG and ALT does not go over 250. So if it goes over 500, that's probably something else. So that's useful if you've starting a new drug. Chronic hepatitis C and NASH, again, if the ASG and ALT go up, that is likely DILI. But the issue is, is that enough for you to stop the drug?

Is that associated with mortality? We don't know. I'm most concerned here about mortality in these patients. On the other hand, even if the AST and ALT don't go up that high, but if the bilirubin goes up, now we know. This is going to be associated with mortality. And that should be a reason to stop the drug. And I think we need prospective data from treatment trials.

2.2#51 PK: In decompensated cirrhosis, where the bilirubin is already elevated, what do we do? For decompensated cirrhosis patients, we need to look at variceal bleeding and jaundice. And, I think, if at baseline your score was 6, and increasing MELD score of greater than 10 points, that is going to be associated with a five percent increased risk in mortality. And I think an absolute increase in risk of mortality of five percent, to me, is an indication to stop the drug. Bilirubin itself is not good enough for this. Creatinine of 1, bilirubin of 1, INF of 1 is a MELD of six. Bilirubin goes up to 3. Hy's rule is 2. Let's say bilirubin goes up to 3, MELD goes up to 11, increase in mortality 1.7 percent. If

bilirubin goes up to 12, then the risk of increased mortality is only 5 percent. So bilirubin really has to go up to increase mortality. On the other hand, if the bilirubin went up to 3, but the creatinine and the INR also went up a little, that score is 16. And that's a 10 percent mortality increase. So I think we should move away from using bilirubin alone to decide drug-induced liver injury. Because bilirubin elevations alone are associated only with small increases in mortality.

2.2#52 PK: So to summarize, MELD was a CTP for DILI. Use whatever you're comfortable with at baseline. Some people still want to use CTP. I think it's okay for coarse classification. However, there are subjective elements. And if you are a regulatory body, and you want to look at the data, you want to know what happened to the patient, that's very difficult if you don't know about ascites and encephalopathy. You're relying on the observer. In addition, the coarse discrimination and the lack of calibration makes CTP a poor tool to make management decisions in the individual patient. We think MELD is better for

fine calibration as objective. You can pull the data, look at it any time that you want to. And an objective system with accurate calibration is what you're required for early diagnosis of DILI.

2.2#53 PK: Let's get back to the penalty kick, okay. Messi kicked left. The goal keeper dived left and saved. So what is the conclusion? No model is perfect, and neither is Messi, okay. (Laughter.)

2.2#54 PK: So the take home message is today our prognostic models in DILI are a work in progress. I think MELD scores should be recommended for current and for the near future studies. But we should have models to predict DILI risk and mortality. We can add additional parameters. They may add to the accuracy, but there's a diminishing incremental gain. And every time we add on something, we have to balance between accuracy of prediction and the practical problems in implementation. Thank you.

(Applause.)

DR. AVIGAN: Well, that was terrific. And as we dig deeper, this is going to even get more

complicated. Because we are going to be talking and asking questions about patients with different levels of severity of liver disease before they start the drug, as well as with different diseases. And we want to then make predictions that will help us decide when to continue or when to discontinue drugs.

So with that, Dr. Gregory Everson is here from the University of Colorado. And he will talk to us about non-invasive testing for liver function.

2.3#1 GE: DR. EVERSON: All right. Thank you very much. My thanks to the organizers for allowing me to speak to you today. I'm from Colorado. Most of my work's been in hepatitis C. And one of the things in Colorado, when we get together as a hepatology team, we used to do a rafting. I was in the back of the raft with the oars person a few years ago. And we're all, as hepatologists would be, talking about this or that, hep C this, hep C that, hep C this. People are dying from hep C, blah, blah, blah. And he turns to me, touches me on the shoulder. He says, "Are you a doctor?" Of

course he's, like, 20 years old, well muscled, chiseled guy. I said yes. What kind of doctor are you? A liver doctor. And he says, "Oh, my God. You know, I had no idea that Pepsi was so bad for you. I drink it all the time."

(Laughter.)

2.3#2 GE: DR. EVERSON: So I hope I don't confuse you too much with this talk on liver function. Of course, nowadays Pepsi is the culprit, because we're all talking about NASH, and fatty liver, and so on, and so forth. So he had that premonition that maybe that was not so good for you. I reconfigured this question to answer three simple questions for you. Can we measure hepatic reserve? Because the question that was posed to me was you have a patient with chronic liver disease due to hepatitis C. Can you measure its natural progression, its natural course? Can you measure a treatment effect? Those were the two questions posed to me. And I thought, well, for a DILI conference, really, as John Vierling was pointing out, really what we want to measure here is how much reserve does that chronic liver disease patient

have. Can we identify patients who might be at risk for decompensation if they were to have a DILI event? And if they have DILI event, can we track progression or recovery from DILI? And the answer to all this is yes, yes, yes.

2.3#3 GE: I'd like to get away from this podium now, but -- so I have several disclosures here. I'm going to tell you about a test that we developed at the University of Colorado that we did move, we are trying to move towards FDA clearance. So I am the founder of this startup. So I'm not making any money right now but whatever. We're working on that.

2.3#4 GE: So the first question was can we measure hepatic reserve?

2.3#5 GE: Well, as has been pointed out by some of the speakers, in fact, we have some really great non-invasive methods. We have ways to estimate fibrosis. How many here do a liver biopsy today? You still do liver biopsies for staging?

PARTICIPANT: Not for staging.

DR. EVERSON: Not for staging, right. You don't stage liver disease any more. In hep C we

did it every two years, right. Bob Fontana, do you do staging liver biopsy? No one does it anymore. Because we have all these great, you know, non-invasive methods. Unfortunately though, estimating fibrosis by the non-invasive techniques doesn't really give you an estimate of the hepatic reserve. It tells you if they're cirrhotic, non-cirrhotic, so on and so forth. Now, let's see, merged are these other modalities of measuring function and physiology. Because these more likely would indicate how much functional reserve a patient with liver disease has, chronic liver disease has. And you've heard a little bit about Hepatiq which is a liver spleen scan where you can measure the perfused hepatic mass. And it's an estimate of function.

2.3#6 GE: You've heard about breath tests, the breath ID test and the breath test. It also can give you an idea of hepatic reserve. And then there's this dual cholate shunt.

2.3#7 GE: And I'm going to focus on the latter test, because I know that data better. And the other modalities are emerging with data. They'll

be presented to show that, in fact, they too can measure hepatic reserve. If we look at dual cholate, it's a test that has two components, an oral dose of cholate, which is shown at the bottom of the slide, and then an intravenous dose of cholate. And all the sampling is done in the peripheral vein. And what it takes advantage of is the normal physiology of cholate. You can see there're multiple transporters across the intestinal epithelial cell. And there're multiple transporters into the liver. So it has this ability to be absorbed going to the portal circulation. And then what's measured in the peripheral venous sampling is the spillover. So let's say a dose of D4 cholate's given orally. It's not extracted by the liver. About 20 percent normally appears in the peripheral venous compartment. The intravenous cholate tags the 100 percent bioavailability to the systemic compartment to allow you to calculate a shunt fraction, the percent of the oral dose that appears in the systemic compartment.

2.3#8 GE: Now, the elements of the functional

impairment measured by this technique is shown here. First of all, regardless of ideology, all chronic liver disease impairs the function of the liver and also disturbs not only the systemic circulation but also the portal circulation. And, of course, the fourth element is, with the chronic liver disease, they develop portal systemic shunting. And all components are actually picked up by this dual cholate method. On the left is the test administration. It's a low tech test administration requiring only an intravenous catheter. On the right are the clearance curves generated from the dual cholate shunt test. And from this we can calculate a disease severity index which is measured from these clearance curves and the shunt fraction.

2.3#9 GE: Now, that's kind of impractical to do in a large scale situation or to screen populations

2.3#10 GE: So we also went back and remodeled this whole data set to look at a single point test which is one blood draw 60 minutes after oral administration of D4 cholate. In a sense, this is a drink and draw test emphasizing the western motif

from where I have come from. Now, so drink and draw.

2.3#11 GE: Anyway, both the STAT test and the shunt DSI test can be modeled further to quantify the percent hepatic reserve. And just to show you different data sets that we've looked at with this model, those are the fibrosis stages from different groups of patients. And these are all hep C patients. And you can see that there's a nice, significant decline in reserve with worsening liver disease.

2.2#12 GE: So we think these two tests actually do measure hepatic reserve. And also I would emphasize other function testing also measures hepatic reserve to a greater or lesser extent.

2.3#13 GE: The second question that I pose to you is can we identify the at risk patient population? Who are the patients at risk with chronic liver disease, who otherwise are stable, may decompensate if they have a DILI reaction?

2.3#14 GE: Well, we think that looking at the HALT-C cohort allows us to segregate out the population who is at risk. Remember, HALT-C was

a prospective study where the median follow-up of the patients was 5.5 years after a baseline test. And these patients at baseline had bilirubin of 1, an INR of 1, and a creatinine of 1. MELD was 6. CTP was 5 or 6. So how do you pick out an at risk population from that? Well, this is where these kinds of tests, functional tests, may also have some advantage over standard laboratory tests or clinical models. What's shown here is tertiles of the shunt DSI test. And you can see in blue across the top that there's almost no risk of a complication in up to seven years of follow-up in the tertile that had a DSI score of less than 15. In the green, you see the risk for an outcome which is the patients who had a DSI score greater than about 20, or 19 or 20. And you can see the outcomes listed.

2.3#15 GE: Those are hard clinical outcomes. Those that had the CTP progression, two-thirds had subsequent clinical outcomes. The same holds true for the single point STAT test, same data set analyzed with the single point test.

2.3#16 GE: So what you have here is evidence that

both the shunt DSI and STAT are able to select an at risk group. Here are STAT greater than 1.1 or DSI greater than 19 which selects a group at risk for clinical outcomes.

2.3#17 GE: Now, what has that got to do with DILI? Well, if you take compensated cirrhotic patients or compensated fibrotic patients, and they do have a DILI reaction, even a mild DILI reaction, you might push them along on this green trajectory more quickly than would otherwise naturally occur. And one of the big questions, of course, is if you're going to use a functional assessment, is it really that much better than, say, a fibrotic assessment? So we also then looked at these tests in reference to fibrosis. Across the top shows the hazard ratio for cirrhosis versus non-cirrhosis by liver biopsy and the prediction of the patients who would have a subsequent clinical outcome over that, you know, five to seven years of follow-up. And you can see the hazard ratio for cirrhosis was four, highly significant, hazard ratio of four.

2.3#18 GE: When you add fibrosis to a standard clinical laboratory test or demographic

information that also predicts outcome, you see it remains significant. Here it was 0.3. When you add either STAT or DSI to the same model on top of all the other variables, on top of fibrosis, on top of laboratory tests, and demographic information, the functional assessment, either STAT or DSI, becomes not only the dominant determinant of outcome, but it basically eliminates the significance of the other variables in predicting clinical outcome. So a functional assessment, at least by these criteria of analysis, suggests maybe more relevant than fibrosis assessments, standard laboratory or demographic information in these stable patients, the stable chronic liver disease population. So we would conclude that shunt and STAT identify the at-risk patient.

2.3#19 GE: Now, can we track progression or recovery from a DILI event? We've actually done serial testing now in seven cohorts, three from the SOLAR 1 study which was using direct acting antivirals in patients with severe decompensated cirrhosis or liver transplant patients with either fibrosis or cirrhosis. And we found that, in fact,

we can measure changes in the DSI and the STAT that occur in response to clearance of hepatitis C. Also in the HALT-C trial, we measured not only improvement in the DSI in patients achieving sustained virologic response but also progression in those who did not get treated or those who were non-responders. And then the seventh cohort was primary source in cholangitis patients, just to show that this is not just a test limited to hep C. And PSC patients, we've demonstrated some slight progression in a one-year follow-up, although that did not achieve statistical significance.

2.3#20 GE: So what about in a single patient? Is something like this really practical, or can you use this to follow a single patient? What's shown here on the graph is a patient who, in the SOLAR 1 trial who, like, on day seven of treatment in the SOLAR 1 trial developed a spontaneous bacterial peritonitis and, as a result, had acute chronic liver failure. The MELD score at the bottom on the dotted line went from about 16 up to about 22. The DSI started at about 29 or so. It went up to over

40. With treatment, the peritonitis cleared. The patient went on to sustained virologic response. The MELD score improved, actually dropped below their baseline MELD. But interestingly, the DSI remained very high. Thirty-one is the threshold for decompensation with that test. And despite the improvement in MELD and the stabilization in SVR, this patient had ongoing ascites, varices requiring treatment, and encephalopathy. So the DSI is reflecting disease severity, perhaps better than MELD in this situation.

2.3#21 GE: The other thing that -- here's another patient from SOLAR 1 who had 12 weeks of therapy and achieved an SVR and had low MELD score, 10 or 11, the whole time, and had a DSI that improved a little bit with SVR but remained in this high range for risk and missed his last test in the study, because he had a gastrointestinal hemorrhage, probably varices, at an outside hospital.

2.3#22 GE: So again, showing that the at-risk for complication might be better defined by a functional test than a clinical model. The other

thing that we think is relevant, and this is probably true of the other function tests available, that you can develop a function map for chronic liver disease.

2.3#23 GE: And here's an example, if you will, of a patient with PSC who had a flare, and I don't mean a hepatitis flare, during their course of follow-up. On the right side you'll see three dots in the upper right hand corner. The green is what the range is for healthy controls. The yellow is for mild hepatic impairment with early stage disease. Orange is early decompensation, and red is you better get to transplantation.

2.3#24 GE: This was a patient who was stable, had two baseline tests which were highly reproducible, went on to a flare where he showed decompensation. That's the red dot to the left. And then that was supposedly his termination for the trial. But he got treated with prednisone, did well, went home to Fruita, Colorado. He called me up about three or four months later and said, Dr. Everson, how's my liver doing? And I said, well, your standard laboratory tests were doing well, the MELD had

returned to normal. And he said, no, no. I want to know what my function is. I said, well, you're out of the study. I can't get you back in the study, you know. Well, can't you at least do my test again? So I wrote an exception for him for the protocol. We did his DSI a third time, or a fourth time. He's the only one in the whole study who got the fourth test. And he actually had shown improvement in his DSI, back to baseline. So despite his flare and the acute-on-chronic decompensation, with treatment clearance of the autoimmune hepatitis went back to his baseline functional level. And now he's four years later or three years later. And he hasn't had a decompensating event since. So we think these tests can track progression or recovery from DILI.

2.3#25 GE: How do they fit into this model? On the Y axis, you'll see bilirubin. And, of course, this is John's paper, just a couple of years ago. But I think there is a potential that functional tests, whether it be this test or other functional tests, can quantify the underlying hepatic reserve. They can identify an at risk group. And they can track

progression or recovery from DILI.

2.3#26 GE: And I'd just like to acknowledge all the people who worked on this particular function test with me over the years. Thank you very much.

(Applause.)

DR. AVIGAN: So we've heard about different approaches to getting a snapshot or a dynamic measure of changing function. And there will be many questions around where there may be pitfalls in each of these kinds of measures that will come up later in the discussion.

But now we're going to go towards specific diseases where FDA is seeing a lot of these diseases with patients with chronic liver disease and asking around DILI.

And Dr. Michael Fried is going to talk to us about hepatitis C. And his talk is going to be acute hepatotoxicity in HCV-infected cirrhotic patients treated with DAAs. Dr. Fried?

2.4#1 MF: DR. FRIED: Well, thank you very much. I'm delighted to be here today to talk about the topic of hepatitis C and acute drug-induced liver

injury with direct acting antiviral agents.

2.4#2 MF: Here are my disclosures.

2.4#3 MF: I think everyone in this room, of course, is familiar with the incredible successes that we've had with combinations of drugs from a variety of classes of direct acting antiviral agents that have led to almost near universal cure for patients with hepatitis C. My clinic is one of the happiest places in the world these days, seriously. And the Phase III data has clearly demonstrated that these drugs are generally safe and well tolerated.

2.4#4 MF: If we focus specifically on patients with cirrhosis, which has been the largest group of patients treated in terms of Child Class A cirrhosis, there are a number of post hoc analyses that have been performed with a variety of different drug combinations. With ledipasvir and sofosbuvir as shown here, over 500 patients looked at in the post hoc analysis, Child-Pugh A cirrhotic patients, about six percent has serious adverse events associated with that regimen and three percent when ledipasvir and sofosbuvir was combined with ribavirin. There was only one death

which was not considered related to hepatitis C treatment.

2.4#5 MF: From, again, a post hoc pooled analysis of a number of studies that have looked specifically at patients with Child-Pugh A cirrhosis treated with the three drug regimen of paritaprevir, ombitasvir and dasabuvir, with or without ribavirin, you'll see over 1,000 patients here. And about two percent, a little over two percent had a discontinuation due to adverse events. And about one percent had an adverse event that was related to a Paddock decompensation that led to discontinuation. You'll see the ones that are listed here. The clinical outcomes, most of the time we had events resolved. But there was one death in the patient with Child-Pugh A that was associated with this retrospective analysis.

2.4#6 MF: Similarly, recent data from the most recently approved combination of a protease inhibitor and an NS5A inhibitor, elbasvir and grzopervir in Child-Pugh A cirrhosis, also demonstrates low rates of adverse events or discontinuations associated with this regimen.

Drug related AEs overall was about 40 to 70 percent in that population. But if you look at serious adverse events, there was it only three percent. Deaths only occurred in two patients, one in each of those regimens. And only one had a discontinuation that was judged to be due to an adverse event. And you'll see that really none, except one patient, had a discontinuation due to an abnormal ALT in those studies. So for Child-Pugh A cirrhosis, it seems that a number of studies have looked specifically at this but demonstrated these drugs to quite well. And we've seen very high rates of sustained biological response, very low rates of discontinuation, and low rates of serious adverse events.

2.4#7 MF: But when we start talking about patients with more advanced liver disease, namely those who have Child-Pugh B or C, those with high MELD, those with evidence of prior decompensation, understanding the impact of these drugs in those populations is particularly challenging. We know that it's difficult to measure, as we've heard from our other speakers, to measure these new onset

elevations of ALT or bilirubin against an already abnormal baseline. We recognize that these patients have high risks for intercurrent, acute-on-chronic liver disease, and some of them are shown here, sepsis, common spontaneous bacterial peritonitus, choledocholithiasis, coincidental or portal vein thrombosis, and some recent reports of, interestingly, reactivation of hepatitis B due to the impact of viral interference on resolution of hepatitis C infection. So even more things that we have to think about. And the spectrum of the natural history of cirrhosis, along with the progression of underlying disease, I think is perhaps the most challenging. As we've heard before, there are different degrees of compensation versus decompensation across the spectrum of cirrhotics. Yes, we can try to grade them as Child-Pugh A, B, or C, or MELD score. But even within those, we still.3 have fine gradations. And depending on where you enter the cascade of the natural history of hepatitis C and cirrhosis, you might be far from the edge of the cliff or just with one leg stepping over the cliff. And this becomes

one of our most challenging groups of patients to take care of.

2.4#8 MF: Now interestingly, until the era of direct acting antiviral agents, we really didn't worry too much about it. Because we just didn't have safe enough treatments to even think about treating patients who had very advanced liver disease. In the interferon-based era, we rarely, if ever, would treat patients who had any hint of hepatic decompensation. So this concept of falling off the cliff depends on where you are in that progression of hepatitis C and trying to differentiate that based on clinical parameters as well as some of the laboratory data that you've heard here. So we're starting to see studies now of direct acting antiviral agents that are specifically being done to evaluate the impact of treatment in patients with decompensated disease.

So in the SOLAR 1 and SOLAR 2 studies that looked at Child-Pugh B and C cirrhosis -- this data here is just from the pre-transplant population -- you'll see that, yes, we had very high rates of sustained virological response across the board

that, again, we would not previously have been able to even imagine in the interferon-based era. But we're also starting to see, compared to the Child-Pugh A data that I showed you, we see serious adverse events of 28, 29 percent, discontinuations due to adverse events approaching five to seven percent, which are much higher than we saw with the Child-Pugh A cirrhotics, and also an increase in deaths associated in those patients who had decompensated cirrhosis going into treatment.

2.4#9 MF: And again, it all comes down to this drug related versus relationships to their underlying liver disease or a combination of those things? Additional data looking at, in this case, daclatasvir and sofosbuvir with ribaviran, a smaller study that looked at 60 patients, about 75 to 80 percent of them were actually Child-Pugh B or C. Also showed increases of serious adverse events, discontinuation of all drugs due to an adverse events, there were about 20 percent. Although in this case, ribavirin seemed to be one of the biggest culprits for the reasons for discontinuation. And you'll see a few patients

had laboratory abnormalities, ALT or bilirubin elevations that resulted in treatment emergent adverse events and discontinuation of therapy.

2.4#10 MF: Recently, in October we saw the FDA warning come out from a review of the Adverse Event Reporting System that showed 26 worldwide cases attributed to hepatitis C treatment for patients who are receiving paritaprevir, ombitasvir, or dasabuvir with or without ribavirin. There is a temporal association with liver failure and hepatics dysfunction within one to four weeks of starting therapy. They were not associated with elevations of ALT. Ten patients had a hepatic failure and either died or required liver transplantation. And 16 patients had varying degrees of hepatic dysfunction. It was noted that some of these cases occurred among Child-Pugh B or C patients in whom this regimen was actually contraindicated. Now, when we look at these worldwide cases that are voluntarily reported, we recognize that the detailed clinical data may be lacking to adequately exclude other ideologies or whether this was a function of naturally or

progressive liver disease. The denominator of what's going on with these patients is not known, how many patients are actually treated with these regimes and ultimately attributed to these number of patients with liver failure.

2.4#11 MF: And then recently we've also seen additional case reports of other DAA regimens that have been associated with decompensation and liver failures. So clearly, as we treat more advanced patients with liver disease, we are seeing more impact in terms of negative outcomes in these populations. All this data in aggregate, and the pre-clinical studies, have led to recommendations from the prescribing information where certain regimens are relatively or absolutely contraindicated in patients with Child-Pugh B or C cirrhosis.

2.4#12 MF: And you'll see, again highlighted in red, one of the common threads here is that these three regimens contain protease inhibitor as part of the regimen. And likely the reason that, one of the reasons that that may be a role is shown here. That when you look at the impact of hepatic

impairment on the metabolism of these drugs, particularly protease inhibitors, you see that the protease inhibitor class has the biggest -- hepatic impairment has the largest impact on the protease inhibitor class and, hence, potentially could be one of the main mechanisms why patients have developed increased problems with protease inhibitor regimens.

2.4#13 MF: So we wanted to look at what's happening in usual clinical practice. And I'm a co-principal investigator with Dr. Dave Nelson at the University of Florida on the HCV target study. And I'm going to spend some time looking at the data that we generated in usual clinical practice in patients with advanced liver disease.

2.4#14 MF: The HCV target was created to understand the impact of these new hepatitis C therapies that were started in 2011, looking at what's actually being used in clinical practice at both academic and community centers.

2.4#15 MF: It's sponsored by multiple pharmaceutical companies. And over the years we've enrolled over 9,000 patients at 58 sites

around the world along with generated a lot of data for various presentations, including this one.

The HCV-TARGET Collaboration is a collaborative effort of academic experts, regulatory agencies, pharmaceutical sponsors, and patient advocacies along with our clinical sites.

2.4#16 MF: And the data from the entire electronic medical record is centrally abstracted, narratives, labs, phone calls, to develop information about demographics, comorbities, and adverse events, serious adverse events, and ultimately outcomes of those patients. We also include multi-level data monitoring to ensure completeness and accuracy of the data compared to the source. Here's just a snapshot of the sites that are currently involved with HCV-TARGET.

So just focusing on the last iterations of HCV-TARGET, 2.0, 3.0, we've enrolled over 5,700 patients. And you'll see that 44 percent of those patients were cirrhotic. You'll see the demographics that are shown here. And I'll just point out that, if you look at a history of decompensation which clinically we defined as

having evidence of prior variceal hemorrhage, the presence of a ascites, hepatic hydrothorax, hepatic encephalopathy, or concomitant medications that are specifically used to treat those indications, you'll see that about 20 percent of the patients overall have evidence of prior hepatic decompensation, so over 1,000 patients within this data set.

2.4#17 MF: Now, these are distributed across multiple treatment regimens. This is not a randomized trial. These are treatments that are being chosen by the physician, well, sometimes by the physician, more often the payers, unfortunately. But nevertheless, you'll see that what you see here is that the frequency of the incidents of cirrhosis, you'll see anywhere between 25 to 45 percent. And the frequency of prior hepatic decompensation is shown here somewhere between eight and 30 percent depending on what regimen you're looking at. So we recognize that this is not a randomized trial. And if we're evaluating the incidents of adverse events and serious adverse events in this population, we have

to carefully look at other factors that can impact those findings. Because the level of disease severity differs across the different treatment regimens.

2.4#18 MF: Looking at causes of death, and we won't go into all of those, but highlighted in orange we had a total of 37 patients that died. And you'll see that 21 of the 37 had potential liver related deaths, whether it was related to deaths not otherwise specified which, in the worst case scenario, would all be liver related, GI hemorrhage, hepatic encephalopathy, hepatic failure, et cetera.

2.4#19 MF: So we chose to look, for the purpose of this presentation, at three specific aspects, changes in bilirubin, changes in new hepatic decompensation, or patients with prior hepatic decompensation who then developed additional complications associated with their liver disease. So we see, of the 5,700-plus patients who started treatment, about 84 percent, 4,800, had an evaluable bilirubin change. Now, I will say that HCV-TARGET does not mandate when clinicians or

patients get laboratory data or if -- this is purely observing what's happening in the real world. So you'll see that not all patients gave an evaluable change in bilirubin.

2.4#20 MF: But of the 4,800 that did have an evaluable change, about 108 or two percent had a baseline increase of bilirubin greater than or equal to 3. And we chose that threshold recognizing that ribavirin would have an impact with the hymolysis in increasing indirect bilirubin. And I will say that, also, indirect bilirubin is not routinely measured in clinical practice except under certain circumstances.

2.4#21 MF: And what you see here is that, of 108 patients, the breakdown of which regimens were associated with a change in bilirubin, as shown here ranging from about 0.5 percent up to 6.2 percent in regimens with sofosbuvir, simeprevir, and ribovirin, well again, the vast majority of patients did not have a change in bilirubin. To look and see what the outcomes were of these patients with a change in bilirubin, we see that of the ones, the 108 that had started treatment, only 24 or 22

percent had discontinued treatment prematurely. And 21 of those were due to an adverse event. The remainder completed treatment. And you'll see that three-quarters of them actually achieved sustained virological response. The causes of death for the eight patients that are shown here, kind of a mix between cardiac arrest, death not specified, three with hepatic failure on simeprevir and sofosbuvir. If we look at the adverse events leading to discontinuation, again, highlighted in orange are those that potentially, as preferred terms, could be construed being related to the liver. You'll see ten out of 21 had liver related adverse events leading to discontinuation. And causes of death are also shown here. Now, we wanted to look at the impact of bilirubin elevations in addition to changes in ALT to try to get some impact with whether or not this was a true drug-induced liver injury according to Hy's law.

2.4#22 MF: We looked at the changes in bilirubin in those who had concurrent, greater than twofold increases of ALT during treatment, trying to be a

little less specific but perhaps a bit more sensitive in this population. And we see that, of the 108 patients, only 22 had a concurrent elevation, sorry, only 17 had a concurrent elevation of serum ALT during the course of treatment and follow-up. Ten of these 17 patients actually had liver transplants during treatment. Eight of these ten had previously been decompensated. And when you look at the kinetics of what actually happened to them during treatment, it was rare to see one that potentially would be associated with the drug treatment itself. Most of these look like natural progression, but they continue to be adjudicated. And of the patients that had liver transplants, you'll see that six actually went on to SVR, two relapsed, and two still have outcomes pending. Of the other patients who had concomitant elevation of ALT for a variety of reasons, there was one SVR and two deaths which are shown here, one due to cardiac arrest. And one, without a prior history of decompensation, died from an unknown cause but did have elevations in bilirubin and ALT starting about six weeks after

initiation of therapy.

2.4#23 MF: So we're able to do some multi-variable analyses to look at the impact of baseline treatment factors as well as the individual regimens on these outcomes. Baseline predictors of bilirubin change, you'll see, as might be expected, low albumin, higher total bilirubin of baseline, cirrhosis, and a history of decompensating events are all associated with a higher likelihood of having a change of bilirubin of an increase in three or more during the course of treatment. Looking at a number of factors associated with the drug treatments themselves, you'll see that specific regimen here was not associated with the outcome of interest. But in the top line, ribavirin use, as would be expected, and I was happy to see this as sort of an internal control, was associated with increased odds of bilirubin increasing over three milligrams per deciliter during treatment.

2.4#25 MF: When we looked within regimens, with or without ribavirin, you'll see sofosbuvir, simeprevir, with ribavirin versus sofosbuvir, simeprevir without ribavirin. You can see that it

confirmed that there was more of an association with ribavirin than the specific regimen itself, and that you see that here.

2.4#26 MF: If you look at regimens without ribavirin specifically, one regimen compared to all the other regimens without ribavirin, you'll see that ledispasvir, and paritaprevir, ombitasvir, dasabuvir, were not associated with the outcomes of interest. But when you look specifically at ribavirin containing regimens, interestingly daclatasvir containing had a slightly lower risk for increasing bilirubin while the PrOD regimen associated with ribavirin, compared to other regimens, had an increased risk associated with increased bilirubin.

2.4#27 MF: Now, looking at hepatic decompensation, we see that -- and again, this was defined by the measures that I mentioned, the presence of a new event, such as nuance at ascites, nuance at variceal hemorrhage, nuance at hepatic encephalopathy, only 36 patients had developed that during the course of this treatment. And you see it's distributed across the various regimens.

The patients without prior decompensation were a mixture of those who had non-cirrhotic as well as those cirrhotics. And the vast majority of those with the decompensating event were indeed cirrhotic. If we look at those who had a prior history of decompensation, that they had some evidence of those events prior to starting therapy, we had over 1,100. You'll see that 180, now 16 percent, developed a change in their clinical status consistent with the new event of decompensation, either an exacerbation of a prior event or a new type event. And you'll see here, again, the distribution ranging between, well, a very small number of patients, six who were treated with PoD, but anywhere from ten to 28 percent, or eight percent of the 28 percent with the new evidence of the hepatic decompensation.

2.4#28 MF: These are the AEs, again, as I showed you previously, liver related AEs in orange. Twelve out of 29 were associated with potentially liver related adverse events leading to discontinuation.

2.4#29 MF: And the causes of death, 11 out of 17,

potentially liver related mortality.

2.4#30 MF: So looking at the same kinds of analysis as we did for bilirubin with those in patients with no prior history of decompensation, so potentially the Child-Pugh A group as well as patients without cirrhosis, we see low albumin at baseline and cirrhosis having an impact, as you would expect.

2.4#31 MF: And when you look at the factors associated with some of the regimens, we did not see any specific association of a regimen with the outcome of interest in relationship to hepatic decompensation and those without a prior history of decompensation.

2.4#32 MF: Same thing when you looked at the same regimen with or without ribavirin. We could not discern an impact on the addition of ribavirin in terms of a history of prior -- of inducing an event of decompensation.

2.4#33 MF: And within the regimen, looking at those without ribavirin and those with ribavirin, actually compared to all the other regimins, we could see that there was no specific indication in those who did not have a prior definition of

decompensation.

2.4#34 MF: Now, looking at this more severe group, perhaps the most interesting group -- that's the group that had a history of prior decompensation prior to therapy, the group that, again, in the past you might never have thought about treating with interferon-based regimens but are now being treated quite extensively -- we see that, again, albumin at baseline, higher bilirubin at baseline, and cirrhosis at baseline were associated with the likelihood of having a new decompensating event. Now, it's interesting. We don't talk a lot about albumin. But whenever we have looked at outcomes, sustained virological response, toxicities, albumin has always fallen out as an important marker. And I think it's one of those things that often gets overlooked, is that synthetic dysfunction that's showing that patient is really at that cliff that we have to keep thinking about. And whether you look at it on a continuous basis or a cut off, in this case it was 3.5, you do see that albumin falls out as an important predictor.

2.4#35 MF: Looking at those, again, with a history

of prior decompensation, a few regimens, as shown here, showed a slight association with the outcome of interest. But again, this could be an impact of ribavirin, because you see here that ribavirin was strongly associated with increased odds of increasing a new decompensating event.

2.4#36 MF: With those who had, if you look at the regimen with or without ribavirin, in simeprevir, sofosbuvir, those treated with ribavirin had increased odds of a new decompensating event in that population. And there were some patients that were treated, very few, that we didn't have enough population to look at, for example, the PrOD regimen, with or without ribavirin.

2.4#37 MF: And similarly, if you look at within the regimen without ribavirin compared to other regimens, or without ribavirin compared to other regimens, you'll see that the specific regimen was not associated with the outcome of interest, in this case a new decompensating event among patients with a prior history of decompensation.

2.4#38 MF: So overall, it's gratifying to know that, even though we're treating very severe,

patients with very severe liver disease, the overall event rate for hyperbilirubinemia and hepatic decompensation was low, even among these patients with advanced cirrhosis. The features of advanced liver disease, including cirrhosis, baseline elevation of bilirubin, decreased albumin, and I want to stress that, prior decompensation, are associated with increased risk. Among patients with a change in bilirubin for all causes, less than 20 percent had a concomitant ALT increase. And these were usually associated with some other kind of clinical event, although hepatotoxicity could not be excluded in a minority of these patients. Despite changes in bilirubin and new hepatic decompensation, the treatment regimens themselves were infrequently discontinued. And patients often achieved sustained virological response. In the multi-variable analyses of hepatic decompensation across multiple regimens, ribavirin was modestly associated with that increased risk. And the specific DAA regimen did not seem to have the greatest impact in those populations.

So a few caveats and take home, this is, it continues to enroll, HCV-TARGET. It's an ongoing study, new data is being acquired as we speak. So what I'm presenting is only a snapshot at this time. **2.4#39 MF:** Differentiating hepatotoxicity from the natural course of disease in an observational, non-randomized study is a complex undertaking for which multiple factors must be explored. Clinicians clearly, I think we'll all agree, should monitor closely all patients at risk for decompensating events as shown here. Those with baseline evidence of advanced liver disease -- and I want to stress again that albumin story -- so that the appropriate investigations and interventions can be performed in a timely manner. As far as next steps, well, we talked about trying to get something going with eDISH prior to this meeting, but time certainly would not allow that. And we'd love to see how this data looks in that kind of analysis and using other analytical tools to explore the association of HCV therapy along with clinical decompensation. And I think it's important to recognize that these are very complex

patients. And often the data that we get from voluntary reporting is often incomplete and is very challenging to attribute to disease versus drug. So attempting to standardize the information that we see with voluntary reporting would be very important, again, with the HCV-TARGET model where extracting all the data from the full electronic medical record so we try to minimize that problem. But for voluntary reporting outside of those arenas, trying to standardize those, I think, would be really important. And even modifying the MedWatch 3500 form specifically for drug-induced liver injury among patients with baseline liver disease could be important.

2.4#40 MF: I'd like to thank the staff at the clinical and data coordinating centers of HCV-TARGET who have worked hard and also all the participating sites, many of whom are in this room as well. Thank you very much.

(Applause.)

2.D1 DR. AVIGAN: Well, that leads to our discussion period of questions to the speakers.

We have seen a lot of data, and this is a difficult area, because of heterogeneity of the diseases that are being treated. We'll hear more about the NASH end of the spectrum in the second half of this discussion.

The tempos of diseases are different in different patients. And the whole issue of whether we want a static or a dynamic measure of deterioration, when a patient already has baseline cirrhosis, gets a drug, and quickly gets worse in terms of liver function, to determine how to manage that patient, how to diagnose what the problem is, and then when to discontinue, is a lot of detail around the mechanism and also the rate at which worsening occurs.

So this is going to be very nuanced. And I think that the purpose of this session today is just to get people to think about a problem that will require a fair amount of work.

I want to start with, while people are coming to the microphone, just asking Dr. Kamath. He was talking about using the MELD with a 10 point change from baseline as a kind of benchmark for a

decision to stop, because you don't want to fall off that cliff or off the waterfalls if you're in a canoe.

But my question would be, but you used arbitrarily 6 as your starting point for MELD in your example. But I wonder whether, if you had a target population that was being treated with a drug, let's say to improve encephalopathy, for hepatic encephalopathy as an example, so your MELD scores would be not 6, but they would be roughly 20 or something, how much latitude would you feel before you would kind of pull the switch?

Because again, there's a question here of latitude and sliding scale. And in the second half we'll talk more about this question of how much can we generalize or how specific we have to be about the details of the individual patient. Dr. Kamath?

DR. KAMATH: Thank you, Mark. So the question really is: how fixed is this 10 points? So I took the ten-point for someone at baseline who has no risk of mortality.

So for instance, Michael's data, the

decompensation rates were really very low in that. So in that group of patients, an increase in mortality of even five percent to me is too much. And that would be an indication to stop.

On the other hand, alcoholic hepatitis, which currently we have studies going on, the baseline mortality in that group is 20 to 40 percent. In that group you'd certainly say that the five percent increase in mortality is probably acceptable.

Generally we look at studies. We say when is something effective? We say an absolute decrease in mortality of 15 percent, a relative decrease in mortality of 25 percent. So certainly it will change in the group of patients.

So if you don't have a risk of dying, if you increase the risk of dying, that's a problem. When you've got a huge risk of dying, then maybe you're willing to take a chance, because you know that without that treatment you're going to die anyway.

So it's going to vary quite a bit. And I have one slide to say it depends on what your

underlying disease is. Whether it's cholestatic, they are decompensated, or whether it's just chronic liver disease, it's going to vary quite a bit.

DR. AVIGAN: Well, thank you.

DR. VIERLING: Mark, could I make a comment --

DR. AVIGAN: Yes, please.

DR. VIERLING: -- regarding that? In our study that had MELDs from 5 to 25 for hepatic encephalopathy, one of the observations, which is true I think in every transplant center in the world, is that patients with MELDs of 20 can often have an increase in MELD which is transient and will reverse. One cannot use the MELD as a ratchet, that once elevated it will only continue to go up. And the principal issue then is usually the rise in creatinine because of an acute renal insufficiency. This is often the predictor. And when that happens, of course, you no longer renally excrete the conjugated fraction of bilirubin. So both bilirubin and creatinine rise simultaneously. So I think that we have to have a bit of a nuance

as to how one interprets a rise and whether it has features that would suggest reversibility. And that can be addressed from a clinical standpoint.

DR. AVIGAN: Arie?

DR. REGEV: So thank you. These were very impressive presentations. And I have a question for Michael Fried. The thing that fascinates me regarding those patients, all of us that treated patients with end stage liver disease, we know, as you described, that they have completely spontaneous, out of the blue, deterioration that you have no idea where it comes from. And in your case, some of those where it ended up being attributed to a drug, even with no preceding elevations of ALT and AST, I mean, take me through that. How is that causality assessment done and reaches the conclusion that rather than a sudden drop, which we see in the natural history of hundreds and hundreds of patients, this particular one is caused by the drug that you gave him for hepatitis C?

DR. FRIED: So I didn't want to leave the impression, I hope I didn't, that the patients

I showed through HCV-TARGET were all attributed to a drug. If I did that, wow, I did not mean to do that. But I think we're seeing in the literature that, you know, when you look at the temporal association, and this is why I said at least if you look at the warning for the paritaprevir, for example, those 26 worldwide cases, there's a temporal association to some of that decompensation and liver failure. So we have very detailed data on looking at the kinetics of what happened to those patients. As I think I mentioned, actually, that most of them did not actually appear to be directly related to the drug of the ones that I showed you. So I want to clarify that. But there were a few that you just, a couple that we're still in the process of adjudicating, that potentially without any other, they didn't have choledocholithiasis. And we had examples of acute choledocholithiasis in some patients. We had examples of, you know, sepsis and SVP that occurred in those patients. But there are some that did not have any of those clinical associations. And those, I think, we have to take a closer look

at. It's a tiny minority though. I think the numbers that I showed you are vanishingly small overall. And I want to make sure that the message comes across that I still feel that these drugs are quite safe. But we still need to be vigilant in certain populations. Because we've never treated this population before until the last two years, really. So the ones that I showed, it was clear that ribavirin had a major impact.

DR. AVIGAN: Yes. I'm going to repeat the question. So this is a very, this was actually one of the things really caught our eye at FDA when we started getting into this question. And there is a literature about these kinds of advanced cirrhotics who get these untoward reactions on HCV therapy. The question is why, when they get worsening liver function and clearly they go into liver failure in some cases, why don't they get a bump in their ALT? Their ALT has been relatively flat. It doesn't look like the Hy's law in that sense. On the other hand, they have deteriorating function. It's not just hemolysis. So the question is what is that and why don't they have

the ALT rise?

DR. FRIED: Yes. I don't have a good answer for that. But as you saw, ribavirin clearly had an impact in those patients. And you're much more likely to have a bigger ribavirin impact in the patients with cirrhosis than those who are non-cirrhotic, interestingly. I'll say in unusual clinical practice, I mentioned that direct bilirubin is not often measured. So we don't have that ability to determine that. But I can't say why specifically. We saw some -- and as it's been reported in the literature, liver failure associated with a normal ALT or minimally abnormal ALT.

DR. AVIGAN: Bob?

DR. FONTANA: Yes. Hi, Bob Fontana from Michigan. So along the lines of the phenotype, if you will, that could be different of DILI in a patient who's already pretty far along the curve, what about the alk phos levels? Do you have those pre-treatment at the time of bilirubin elevation? Is it more cholestatic?

DR. FRIED: Yes. We don't have that

routinely captured. We don't. We can go back and look at that, but we have not looked at those --

DR. FONTANA: It might be helpful as we go forward, particularly at least to capture that prospectively. And the other point is perhaps, as we move towards ribavirin free regimens, that might be a little cleaner model to see --

DR. FRIED: Right. And that's why we tried to look at, in the multivariable model --

DR. FONTANA: Right.

DR. FRIED: -- to look at ribavirin free versus with ribavirin to see what the, you know, subtraction impact was on that.

DR. SANYAL: With respect to what Mark was saying about it, why doesn't the ALT go up when the liver function is deteriorating? Well, we have to realize where ALT is coming from. And ALT does not measure any liver function at all. It only measures the leakage of intracellular enzymes into the plasma. They're not ordinarily there. They're intracellular enzymes, the transaminases. And so there's no connection really between the ALT, which is a measure of injury to the liver

itself, and leakage of enzymes into the circulation, and the function of the liver in a non-function test.

DR. AVIGAN: Dr. Hunt?

DR. HUNT: Hi, Chris Hunt, Duke University. Excellent talks, really enjoyed them all, and raised so many questions. And I'm wondering how -- and I don't know which speaker wants to address this question, but I just sort of have a three part question. I'll keep it really brief. One is we know that as cirrhosis progresses that we have changes in cytochrome P450 activity. And how do you filter that onto the other existing changes? Additionally, with treatment there's endogenous interference that can modulate CYP activity. How do you filter that? And lastly, I think Michael alluded to hep B reactivation. And what part of that -- we know a lot of people don't know their hepatitis B status, and perhaps their providers are unaware. How often does that actually also impact, you know, given these fall off the cliff phenomena that have been reported in the literature?

DR. VIERLING: Maybe I can tackle at least the first part. Time didn't permit to show a slide I'm fond of to call attention to the fact that, grossly, we certainly know that architecturally there are three forms of cirrhosis, the macronodular forms, as tend to bridge portal to portal, and portal to central, and have profound impact in the cirrhotic on the central zonal activity, in which is the highest concentrations for the CYP P450s, as well as the machinery to manufacture the prothrombin complex which we measure as PT-INR.

On the other end of the spectrum, you have biliary cirrhosis which is really just spikes of fibrous tissue that align and create sort of a jigsaw effect with regeneration. And it actually protects the central zone. And clinically we know that these people tend to go along forever, and then they fall off the cliff with primary biliary cirrhosis or other forms of biliary cirrhosis. In between, we've got the micronodular cirrhosis of alcohol which is extremely important. So I think the point I would make is that, in a cirrhotic, we

do not know about the microcirculation and the retention of lobular architecture and gradients that are important to the answer to your question. And we certainly do not know, in cirrhotics, how drugs are distributed, which cells take them up. And that is a piece of missing information, probably not entirely discernible from standard PKPD measurements. Because all we know is that with hepatic impairment there's more in the circulation and there's less overall uptake. But the question still remains, where is it being taken up? And where is it being metabolized? And further studies are needed.

DR. AVIGAN: Arun?

DR. SANYAL: Yes, great talk, guys. I have question for Patrick. So the phenotype of an average cirrhotic patient has changed pretty dramatically over the course of the last decade. So most of our patients that we see with cirrhosis have diabetes, long-standing diabetes, and many of them have chronic kidney disease. And so in the assessment of MELD and delta MELD, how does the presence or absence of chronic kidney disease

factor in? Or is that really irrelevant?

DR. KAMATH: So the question is: if your underlying kidney disease that's causing your elevation in creatinine, does that make a difference? When we originally developed the score, we excluded all patients in whom we thought had underlying kidney disease. We also excluded those who were septic, because the MELD goes up and then comes down. So the original cohort had no, as far as we could tell, had no underlying kidney disease. But later we looked at the UNOS waiting list, liver transplant, and it did not seem to make a difference. If your kidney is bad, whether it was from diabetes or from liver disease, your outcome is the same.

DR. WRIGHT: Terry Wright, Genentech. I have two questions if there's time, the first for Dr. Avigan. And that relates to cancer even in therapies. Patients with hep B and hep C have been largely excluded from these trials. So what do we know about the safety of giving these drugs to virally infected livers and whether we should be determining that pre-approval or in a controlled

setting? Because undoubtedly, the epidemiology of these viruses overlaps with the cancers that these patients get. So these drugs are going to be given post-approval.

DR. AVIGAN: Well, I don't know the exact answer to your question. I think it's an important question. What we do know is that we're getting more of these immunotherapies in the pipeline. Again, it's various T cell subsets. Although they're targeted to, in the case of cancer therapies, to sort of up-regulate the cells that are auto-reacted to kill the cancer cells, you also perturb the whole T cell network, because the receptors that you're targeting actually are shared by various subsets of T cells and other cells as well. So what you get in terms of viral, sort of maintaining viral homeostasis if you have a chronic virus, for example, whether you get reactivation, for example, because B virus is held in check by T cells. It's an important question and may be variable depending on the patient. So there may be actually a very specific, detailed answer that would require study to know the answer. It

is an important question though.

DR. WRIGHT: And my other question actually relates to the panel as a whole. As we think about developing drugs for hepatocellular carcinoma, we're developing drugs for patients who almost all have cirrhosis, even if you select compensated cirrhotics, Child A cirrhotics. And some of the drugs that are being given have the potential to have adverse effects on the liver. If you actually look at the Phase III trials of sunitinib versus sorafenib, the sunitinib was shut down because of adverse drug reactions which were higher in the sunitinib and the sorafenib. And that included variceal bleeding, variceal bleeding related deaths. And so my concern is that, as we're giving these multi-kinase inhibitors or VEGF inhibitors, we're actually changing portal pressures, changing hepatic regeneration, affecting collateral blood flow. So my question to the panel is how is the underlying cirrhosis in HCC patients going to influence the risk/benefit of giving the drugs when you're treating carcinoma?

DR. AVIGAN: Dr. Everson might have

some insights on this. And just to tag a question along with that, because hepatoma is disease where this comes into play, you may get a fair amount of shunting because of the disease AV formations without a lot of necessary intrinsic loss of hepatocyte function in some of these cases.

DR. EVERSON: Yes. There're two components there. One is that got us interested in this function testing in the first place was, in the HALT-C trial, we had patients with compensated cirrhosis and advanced fibrosis. And when you look at the functional impairment across that well compensated population, it ranged from near normal function all the way up to, hey, you better get almost a liver transplant, because you're decompensating. And in fact, those patients that were really at the top of the poor function heap, they ended up having a decompensating event within two years. So we think that, at least I think this is a good place for some type of functional assessment in addition to the cirrhosis or fibrosis assessment. I think there are complementary assessments where you get an idea of their fibrosis

stage but also have a functional element to further stratify. The methactin breath test, I just saw some recent data. I don't know if it's been published yet or not. But there is some data there that correlates with a cutoff for portal hypertension. And we have some data as well that correlates with the DSI and STAT with risk for varices and risk for portal hypertension. So I think these could be, this could be a good spot for use of function testing. That is, those patients who have very poor function might really be almost high risk for this type of therapy. The other thing that comes back is the shunt. Right now the radiologists, I think, do a shunt test, some of them, especially when they do the beads therapy, the TheraSpheres, I think. And so they, I think, knowing whether or not they have a shunt ahead of time that's quite substantial might actually allow you to go to a different therapy than, say, the beads that can shunt to the lungs and cause significant pulmonary injury. So I think this is one particular application that could be quite good for functional assessment, both shunt assessment

as well as function assessment.

DR. WRIGHT: John, you were nodding your head. Do you have any thoughts?

DR. VIERLING: No. I very much agree with what Greg is saying. And I think that the other issue would be exploring all possibilities of targeted therapy which, of course, is done with chemoembolization, whether with eluting beads or just standard single injection of chemotherapy to take advantage of the neovascularity in arterial profusion of the HCC.

DR. AVIGAN: We have, I think, just a few more minutes. If there are some outstanding questions there are -- I would actually want to ask Dr. Fried, just before we terminate this first part of it, about the clinical presentation of some of these patients who are decompensating. And I would just draw our attention to some of the recent literature that describes a kind of case phenotype. There was an editorial by Jay Hoofnagle recently in the Journal of Hepatology about these kinds of decompensations in patients treated with a variety of different kinds of antivirals against HCV. And

he notes in his editorial that it's not a specific drug that causes the problem but, in fact, different drugs that are direct-acting anti-viral agents against HCV. There's a subset of patients who have this response of rapid worsening. And in a paper that he was editorializing about it from Frankfurt, I think Wilker and colleagues, the phenotype was very rapid onset, worsening after initiation of treatment within a few weeks, and lactic acidosis. And I just wonder what your thoughts are about that particular phenomenon.

DR. FRIED: Yes. So we have not seen any specific cases of lactic acidosis. Although again, unless it's reported specifically within their medical record, we wouldn't capture that in the target. I think that, I know that the rapid drop in viremia has been also associated with the sudden change in the clinical status. And it's hard to explain why that would be the case, you know. Because if you look at hepatitis B, for example, where you have decompensated patients who actually get better in that situation, when you have improvement in their MELD score, improvement in

albumin, improvement in outcome where we're almost not transplanting any patients anymore, it's completely incongruous with what has been reported in hepatitis C. So it's still not clear where that comes from. And in fact, it's just the opposite of what we hoped to achieve. If we could, you know, all of us are hoping that we could still see the same impact on hepatitis C that we see with hepatitis B of a patient who's decompensated, who potentially could obviate the need for transplant. And while we're seeing some improvement in MELD score in some of those patients, the long term outcomes on whether or not they actually can obviate the need for transplant or just kind of linger in, you know, what we've called MELD purgatory, where they're just not well enough to get off the transplant list but sick enough to have a poor quality of life, is something that we need look at the long term follow-up.

DR. WRIGHT: Terry Wright. Again, I have a question for Dr. Avigan or maybe for the panel. With these immunotherapies with essentially the reversal of T cell exhaustion, we've talked a

little bit about the role of adaption for drugs that may otherwise not be that hepatotoxic. Is there the potential now for making patients at risk for drugs that might normally not be that hepatotoxic but actually more hepatotoxic, such as simvastatin and prochlorperazine, and in that setting should we, as commissioners, wait to study that? Or should we preemptively, proactively try and advise to take patients off any potential drug that is not essential for their care, for their cancer care?

DR. AVIGAN: I think that you're asking a very important question. This is going to become actually a question that people will want to hear more about as these drug development programs go through for approvals, et cetera. I can't really answer your detailed question. But I will point out that tomorrow we will have a session dedicated to this question of tolerance. And one of the issues that's swirling around and the reason why we're having that session is that there are now development programs to basically up-regulate T cells, or down-regulate them, where the treatment population is not just cancer, but now it's also

autoimmune diseases. So we're going to see more of this kind of question. And we're going to see liver injuries as an unintended consequence.

DR. BIRNKRANT: Just to follow-up on that somewhat clinically based question, I was wondering how the panel feels about when new therapies are started. And given the fact that these hepatitis C treatments are now pretty short compared to previous therapies, they're approximately 8 to 12 weeks, let's say, would you recommend stopping, let's say, a statin for that period of time to allow the patient to take the hepatitis C treatment just to try to avoid potential drug toxicity?

DR. EVERSON: Yes. It kind of depends on the regimen that we're using. But we certainly have done that in those situations where there's an anticipated DDI. And there's typically no problem with that. I mean, hyperlipidemia is a chronic problem. You can be off your drug for eight weeks and get cured of your hepatitis C without a problem.

DR. BIRNKRANT: Okay, thanks.

DR. EVERSON: I would like to comment on one thing that came up here, if I may. The interesting thing about the current era of getting, of the DAAs for hepatitis C, is that we now can take chronic inflammatory liver disease, get rid of the inciting agent and monitor recovery. And we've been doing this in small studies, you know, a few patients here, a few patients there. And it's very interesting, because you see there're three phases of healing that happen. Initially, when that virus is cleared, and it happens very quickly within four weeks, their R&Es are negative, the ALT drops, their inflammatory markers drop. And there's improvement of the microcirculation of the liver. And then there's hepatocellular functional improvement. And of late improvement is the fibrosis resolution. And in our experience with the testing we're doing, we do not see any significant change in consequences of portal fibrosis for up to 48 weeks of follow-up. And we're following those patients a lot longer. So I think, you know, if you look at yourselves out there in the audience, and you look at your skin, how many of you have a

scar on your body? At what age did you get the scar? How long have you had the scar? Now, the liver scar is different than the skin scar, I understand. But the point is scar resolution is a very different animal. And I think it's different in B than C. And I think that's why, in the B patients, they just don't have that same level of dense, fibrous, micronodular scarring. It's more of a, you know, you've seen it on biopsy. Maybe the pathologist could comment on that. It doesn't seem to be as dense a portal fibrosis as we see in C.

DR. DARA: All right. Quick question actually for Greg. I'm a hepotologist. When we talk about liver function, obviously we always think about Factor 7, albumin, the function of the hepatocyte synthetically. What I don't understand about your model, maybe you can explain it to me: is it a measure of portal hypertension, like an indirect way of measuring HVPG? Can it differentiate between a non-cirrhotic cause of portal hypertension like NRH and cirrhosis? Like, you know, why are you calling it synthetic function

and not just portal pressure?

DR. EVERSON: Well, it's a function test, because it's clearance of the molecules dependent on hepatocyte uptake. So it's a measure of hepatocyte uptake, of hepatocellular specific function. Cholate uptake is specific to the liver. When you think about these clearance techniques, whether it's metabolic clearance or of this type, it's basically the product of flow times extraction efficiency. With cholate it is a high extraction efficiency. So it's more of a flow dependent clearance. So it tracks the circulatory change better than, say, you know, more so than a metabolic test which is measuring an intracellular metabolism function. So the reason why we call it a liver function test is it's not only measuring the hepatocyte uptake and the function at that level, but it's also tagging the downstream physiologic impact of liver disease on both the total circulation, the portal circulation, and the shunt. So it's a composite global score.

DR. DARA: But if you have portal hypertension --

DR. EVERSON: It's well into portal --

DR. DARA: -- and you're shunting away from the liver, how can you measure the uptake?

DR. EVERSON: No, no. So maybe we can talk outside. But it correlates with HVPG. And HVPG is flat until you get portal hypertension. And then HBPG goes up. And then when you get collaterals it flattens out again.

DR. AVIGAN: Yes. I think what we'll do, you'll take this offline. Because I think there're a lot of follow-up questions, fairly so. But I think it's an important set of questions. You have a question, finally?

DR. TILLMAN: Hans Tillman, ECU. For the studies which are specifically on cirrhotic patients, do you see that biopsy might come back? Because we need to know whether, what kind of cirrhosis pattern people have. Because the adverse event might also depend on whether it's more focusing -- so on three, it's more vulnerable. And you might have a substance which might be, has a narrow window. But it can be safely applied if someone has still very good function in the Zone

3?

DR. EVERSON: I think biopsy is just going to be used very selectively. The staging liver biopsy, I think, is pretty much gone from practice. Now, will it be required in other studies? --- perhaps. And if you're interested in histology, of course biopsy is key, you know, if you need to know the inflammatory pattern. But other than that, I think staging biopsy is a thing of the past.

DR. AVIGAN: Yes. I think we're going to, at this point, take a break. And then we'll kind of come back and do Session P.

(Whereupon, the above-entitled matter went off the record at 2:52 p.m. and resumed at 3:13 p.m.)

DR. BIRNKRANT: Okay, we're going to get started. Welcome back to Session 2 Part 2. We'll be discussing the challenges of assessing and managing DILI in a setting of NASH and chronic hepatitis C liver disease.

We have a great group of speakers during this half of the session, so I would appreciate if

everyone could take their seats so we can get started. Could someone close the doors as well?

Thank you. Our first speaker is Dr. Sanyal. He's a Professor of Medicine at Virginia Commonwealth University in Richmond, Virginia. He has over 25 years experience as a hepatologist and has served as president of AASLD.

He is also a founding member of the Hepatology Board of the ABIM. His research focuses on two areas, cirrhosis and complications and alcoholic and non-alcoholic steatohepatitis. His presentation is entitled: “Can We Treat Liver Function to Assess Treatment Affects in Course of NASH?” Dr. Sanyal?

2.5#1 AS: DR. SANYAL: Thank you. So thanks to the organizers for asking me to speak here. My focus is, well DILI is a part of the talk but a lot of the talk is actually, as the title suggests, the use of tests to assess treatment effects.

2.5#2 AS: I have my conflicts up here, and none really for this specific talk.

2.5#3 AS: So probably a good place to start is to point out that this term of liver function test,

is actually not accurate as I think Dr. Senior pointed out. And what we commonly refer to as “LFTs” in, you know, clinical slang, these are really markers of liver injury. But they can be used both for assessment of efficacy as well as safety. And actual measurement of liver function per se involves different set of tests, and we heard a little bit about these. My talk is more going to be focused on markers of liver injury rather than the quantitative function assessment of liver function in the context of NASH, mainly because there's not that much data to talk about in the context of NASH yet.

2.5#4 AS: So when we talk about efficacy, we think about two things in the setting of NASH. One is a decrease of demonstrating that there is a decrease in disease activity. And number two, that there is a decrease in fibrosis because fibrosis is a very good biomarker of risk of mortality in patients with cirrhosis with NASH.

2.5#5 AS: So before we can talk about assessing treatment efficacy looking at liver enzymes, a good place to stop and think for a moment is where,

what's our starting point? What is the normal background level of liver enzyme in patients with NASH because it's only when you know where you're starting from that you can assess the changes that are going to happen once you commence therapy.

2.5#6 AS: So the mean AST and ALT in patients with fatty liver disease, and NASH is a component of that, actually not that high. And the majority of clinical trials you will find that the mean AST and ALT end up somewhere between 75 and 130, around 100. And so there are a lot of patients who actually have relatively low values of AST and ALT in the setting of this particular liver disease. And the AST ALT does not really correlate with the severity of the histological injury that you see in these patients. Now about 13 years ago we published this paper showing that the full spectrum of the staging of NASH can be seen in individuals who have persistently normal liver enzymes or ALT in this setting. Now you'll note that the threshold for our lab at that time was almost 70. And one of the things to also remember is there's a fair amount of inter-laboratory variability in the reporting

of ALT. Some of it is based on methodology, but also based on how these norms were originally identified 50, 60 years ago. And more recently in the last ten years now, there's been considerable attention paid to identifying what is a normal ALT.

2.5#7 AS: Now normal reflects a range of values which reflect a healthy state as opposed to an unhealthy state. So in defining the upper threshold for normal, you got to ask so normal for what. Normal for separating people from with chronic liver disease from people who do not have chronic liver disease? Normal with respect to predicting mortality? So you got to place it in the proper context.

So probably this landmark paper from Daniele Prati in the Annals of Internal Medicine where they looked at individuals and the spread of ALT and tried to identify which thresholds best identified those who had hepatitis C and viremia. And they ended up with this threshold of 19 and 30 for women and men which now widely people have started using as sort of the upper limit of normal for defining a healthy versus an unhealthy state. And certainly

there are data that even if you have slightly abnormal ALT using these new thresholds, your all cause mortality and liver related mortality might be impacted.

2.5#8 AS: So with that background, let's look at some of the results of the clinical trials and look at how AST ALT relate to improvement in disease activity.

2.5#9 AS: So here are two sets of data. On the left are the data from the PIVENS trial which is the adult trial. And these are data of patients getting vitamin E versus placebo. Vitamin E is in red. And on the right is again vitamin E versus placebo in the pediatric trial, the TONIC trial. And the reason I show this is to point out that if you look at the first few weeks, regardless of whether patients were in placebo or in the treatment arm, everybody's liver enzymes got better. And it's only after about eight to ten weeks that the two arms start separating out. So one of the dangers in assessment of efficacy in early phase trials by just looking at liver enzyme changes is the timing at which you made those

assessments. So if you cut the study off too early, you might miss an effect that when there actually is an effect because initially everybody's numbers get better. We can argue about why that is so, maybe everybody behaves better to start with because they're in a clinical trial. And the separation with drug effect only occurs after a period of time.

2.5#10 AS: So then there's been a re-analysis of ALT from the PIVENS data. And this is analysis done by Jay Hoofnagle. And looking at the ALT six months into the study, end of treatment at 96 weeks, so this was a two year trial, and then looking at the off treatment response at 120 weeks.

2.5#11 AS: And somewhat arbitrarily, the ALT response was defined as a drop below 40 IU which was at the time felt to be normal and a decrease of 30 percent or more. And this number really came from hepatitis C studies where if you had an SVR, typically it is associated with a 27, 28 percent drop in your ALT. So 30 was a nice round number to go with. So in the assessment of improvement of ALT, if you separate out the ALT responders this

way from the non-responders and you look at the histologic change in patients who receive treatment, that is the green bars here are the patients on vitamin E, the red is the placebo arm. And you look at the delta in the NAFLD activity score, you see a grey, in the responders almost 80 percent of patients have a decrease in disease activity while ALT non-responders, they also have improvement. But again, it doesn't correlate that well.

2.5#12 AS: So looking further, it turns out it's actually much more complex. And so if you look at the AST ALT over time in this individual patient and also track the weight, you can see that again, taking a page out of the hepatitis C playbook, you can see what was defined as an early sustained responder, early meaning at six months the AST ALT had dropped below our thresholds for ALT response and the patient remained a responder to end of study and even after treatment, continued to have normal liver or improved liver enzymes.

2.5#13 AS: This is a patient who actually had a ten kilogram weight loss over the course of the study

with vitamin E. And as you can see over here, there was not only at the lower right corner in the box you see not only there was a dramatic improvement in the activity score, but fibrosis improved as well. So this is of course not the most common outcome because most patients don't lose weight when they're on vitamin E. Here's a patient whose weight remained flat. And you can see that they did have an improvement in disease activity at end of treatment, but then once they came off therapy they relapsed.

2.5#14 AS: And here's a late responder who also relapsed, another person who really did not have a dramatic weight change.

2.5#15 AS: So there's this interaction between AST ALT change and the change in weight. So if you divide the subjects into three groups, those who had weight loss more than two kilos, those who had no loss defined as plus/minus two kilos, and then weight gain of more than two kilos. In the weight loss group you actually see that vitamin E and placebo are actually fairly comparable and they do quite well. On the other hand, if you don't lose

weight or if you actually gain weight, that's where the drug therapy appears to pull away from the placebo arm. So weight loss maximizes benefits but when weight loss does not occur, compliance with therapeutics is important to get the best shot at a treatment response.

2.5#16 AS: So this of course allows us some opportunities for refinement, and we should maybe look at earlier time points rather than six months and look at the trajectory of AST ALT change in that timeframe to see if we can better define if those can relate to histology. And we might use that along with changes in weight to develop more sophisticated algorithms if you will. And if we do find something like that, then we need to find out if these are treatment agnostic or really specific to specific types of treatment.

2.5#17 AS: Now very interestingly, this just came out, like, literally last week or two weeks ago. And this is a paper from Cuba. And Cuba's in the news nowadays so I figured it would be topical to talk about a paper from Cuba. So this was a study where they took 280 patients with biopsy proven

NASH and they gave them some dietary counseling and then let them loose and brought them back in a year later. And the only intervention in between were periodic phone calls from a dietician to the patient. That's it. And 28 percent of those patients actually showed significant histological improvement, and a pretty dramatic number of people actually lost weight. So that paper came out last year, maybe a year and a half ago showing the histologic improvement. This particular study took that same data set and essentially tried to develop a non-invasive prediction model of imprudent resolution of steatohepatitis. And what they again found is that if you lose weight, if your ALT normalizes, interestingly if you had a high disease activity score at baseline and if you did not have type II diabetes, then that sort of predicted the area under the curve that they came up with and they divided the cohort into a test and a validation group. They ended up with an AUC of about 0.93, 0.94. Very, very dramatic, certainly very provocative. And this needs to be replicated to see if this really holds up.

2.5#18 AS: I think the bottom line is that we're now beginning to see from the PIVENS trial, from the TONIC trial, from this study that if you lose weight and your liver enzymes go down, there's a pretty good correlation with improved histology or disease activity. On the other hand if you don't, then I think all bets are off. Things don't really correlate with histologic improvement.

2.5#19 AS: So moving on to improvement in fibrosis, so AST ALT alone really don't relate to changes in fibrosis over time. So this is a poster, it's only been presented as a poster. We're in the process of writing this up, so it's relatively new and as yet unpublished data. But essentially what we did here was we took the FLINT trial which is another trial done by NIDDK and NASH clinical research network, randomized control trial of obeticholic acid which is FXR agonist versus placebo. And what we computed here was changes in number of non-invasive panels. So we looked at three different things. One is the FIB4 which is based on age, AST ALT and the platelet count. The second is the AST to platelet ratio and

the third was the NAFLD activities and NAFLD fibrosis score that was developed by the late Paul Angulo. And we tried to see if changes in these scores occurred, number one and number two, how these changes related to improved changes in histology. So the data for obeticholic acid is shown on the left. And as you can see this is based, there are three sets of data for stage one, stage two, and stage three patients. And you can see over here that patients with stage three had the biggest drop. People with stage two disease had an intermediate drop at 24 weeks. Stage one had a lesser drop. And then the stage twos and threes at 48 weeks sort of, it all leveled out for the other two. For stage one patients it sort of leveled off somewhat higher up.

2.5#20 AS: Placebo patients on the other hand did not really show any significant changes in their FIB4 score over the duration of therapy which is a 72 week treatment trial. Now these are AST to platelet ratio data. And you see similarly that in the obeticholic acid, the AST to platelet ratios dropped, however the placebo arm data again did not

show any significant changes. Data are divided into stage one, two, and three.

2.5#21 AS: When we looked at the NAFLD fibrosis score, we however did not find that this was sensitive to change. So it was just the FIB4 and the AST to platelet ratio that was sensitive to change. So I think that this is being driven mainly by changes in AST and some interaction with platelets in this setting.

2.5#22 AS: So when we look at the percent change from baseline to six months and tried to look at those who had fibrosis improvement at 72 weeks versus those who did not have fibrosis improvement at 72 weeks when we did the follow up biopsy, what we found is that a ten percent improvement in the median FIB4 values at 24 weeks was associated with a greater than one stage improvement in fibrosis and a 50 percent drop in similar analysis with APRI showed that 50 percent drop in APRI was associated with a similar improvement in fibrosis.

2.5#23 AS: Unfortunately, when you do the AUROCs, they're still fairly modest. And part of it is that the positive predictive value is actually

quite low. The negative predictive values are okay, not fantastic by any means. So there's still room for improvement over here and room for refinement. But it does provide some proof of concept that it may be feasible one day to continue to refine non-invasive parameters and be able to even identify improvement in fibrosis over time.

2.5#24 AS: Then we move to toxicity assessment. So obviously this is critical and this is the focus of this meeting, and the NASH is no different than any other liver disease.

2.5#25 AS: There's a need for early detection of toxicity, severity assessment, stopping rules for individual patients, and for a trial as a whole. This is stuff that you guys know much better than I do, and this is, you know, a classic stuff that's been put out there as guidance.

2.5#26 AS: More recently, there's been this analysis from Europe where they looked at again the AST ALT ratios, the AST levels, and also increase in bilirubin to try to find the sweet spot that might identify that if a person came in with drug induced liver injury, could you actually identify

who would go into acute liver failure or need a transplant. And so this is to stratify patients who come in with drug induced liver injury in terms of their severity and triage them up accordingly.

2.5#27 AS: So what they found over here is that if you look towards the right, if you did a cut off and they came to the 17.3 times the upper limit of normal of the AST through some convoluted math, but ended up in that subset of patients of 238 out of 804, they found that those who also had hyperbilirubinemia and over here they again had a 6.6 times the upper limit of normal as a threshold, they found that this actually related with reasonable specificity, although I think the sensitivity there are still some issues. So this is again a work in progress in my view.

2.5#28 AS: More recently, we've been sort of working, and this is also work in progress, on using adaptive Bayesian models to identify individualized normal ALT values for a given person. And of course, this requires knowledge of baseline values. And so this is still modeling work that is in progress, but the idea here is that over

what you develop is a range of values that are within the range that the person's liver enzymes fluctuate based on what time of the day you draw the sample and the person's underlying liver health status. And the early data suggests that in the setting of NASH, the ranges expanded, that the values fluctuate which is no surprise to a greater degree, and it's larger than the individual ranges.

2.5#29 AS: But potentially this might provide a way when you start off with a chronic liver disease where the liver enzymes are fluctuating, if you have two or three baseline values, you might be able to model and develop the individualized normal range for that person. And if we're able to get to that point, then this may allow early detection of deviation from disease associated fluctuations in marker so you can say that this change is more than what you would expect simply on the basis of the person's baseline disease. Of course then the next question would be what does that mean. You know? Is such a deviation associated with a higher risk of significant liver injury or actually developing a clinically meaningful liver outcome. And so

obviously a huge amount of work needs to be done to further -- but I think this is an important potential avenue of work and if it pans out, should provide some help in this area.

2.5#30 AS: So I'll finish by talking about, you know, where I think one could go with looking at liver enzymes in addition to biomarkers and quantitative function, there's obviously a lot that can be done. But we can take a more granular look at early ALT changes once you initiate therapy to evaluate benefit treatment effects. And models of personal, normal ALT levels obtained during ALT study state may actually be very relevant in the future as a general way of taking care of just as providing healthcare that early when you become an adult, early on in your adulthood during your routine wellness visits, if you have a few of these ALTs hepatic panels drawn two or three times, it establishes your normal range so that when you first deviate you might be able to actually identify when somebody develops fatty liver disease or any other form of chronic liver disease. There's a lot of alcoholic liver disease out there

as well. And then of course we need to develop models to determine the best response when the ALT does trend out of those ranges.

2.5#31 AS: I would like to acknowledge, you know, all the people who made that data I've shown you possible, NIDDK for funding pretty much all of it, NASH CRN and particularly Jim Tonascia and his team at the Data Coordinating Center, Jay who did a lot of the ALT analysis, and David without whose reading the histology, none of those publications would have been possible. Some of the FIB4 analysis were done by Reshma and her group at Intercept and the ongoing work with the personal normal ALT values is being done in collaboration with Covance. Thank you.

DR. BIRNKRANT: Thank you very much for an excellent talk. It set the stage for the next set of presentations that we'll have. Our next speaker is Dr. Fontana who is a professor of medicine and medical director of liver transplantation at the University of Michigan. He's a principal investigator in the DILI network

where he also serves as co-chair of the DILIN steering committee. He is currently the chair of the ASLD hepatotoxicity special interest group. The title of his presentation is Diagnosis and Management of DILI in Patients with NASH. Dr. Fontana?

2.6#1 RF: DR. FONTANA: Thank you. When Arun and I were asked to give these talks, we weren't sure who should go first so I'm glad he did. I'll see if I can sort of complement some of the concepts that he brought up already.

2.6#2 RF: So I think fundamentally, really what we're trying to sort out is when someone's on a drug who has NASH, if you see a change in their liver enzymes, alk phos, bilirubin, you know, is it a flare of the disease naturally, or is it DILI. I think that's really what it sort of boils down to. So can we be smart enough to figure that out prospectively? And I might mention the potential value here of a liver biopsy on therapy which may prove to be useful. And then I'll share with you some data that we've obtained in the DILIN network of patients who had pre-existing liver disease who

developed DILI to see what that looked like and if we can differentiate that from sporadic DILI in the general population. And then just some brief comments about what to do and then really kind of where we're at currently.

2.6#3 RF: So we all know that DILI is infrequent but important, you know, from Einar Bjornsson's study, population based incidence is 10 to 20 per 100,000 patient years. But when you come to an individual drug it's probably 1 in 10,000 to 1 in a million in clinical practice. And it's really a clinical diagnosis of exclusion. So we have to make sure it's not alcohol, it's not gallstones, it's not acute hep C, et cetera. And then it gets more complex in that we know that DILI is less than one percent of acute liver injury and then it's a sort of a pleiotropic entity in that you can have a variable latency with a specific drug, you can have a variable histology, and you can have a variable severity. So it really makes it challenging to say this is definitely DILI. And in addition, in the US at least, polypharmacy is common and increasing. So this makes it even more

complex in clinical practice. And then the component of most scoring systems of dechallenge requires you to wait to see what happens which can be an issue in clinical trials. And so fundamentally, we all are struggling for an objective confirmatory test.

2.6#4 RF: So let's now apply these concepts to non-alcoholic fatty liver disease. You know, with most drugs that do cause drug hepatotoxicity, it's almost always within 12 months of starting the drug. There are few rare exceptions of, for example, nitrofurantoin and minocycline and things. So I think we can expect the same thing in fatty liver disease. It should occur if it's going to occur within the first 12 months. Hypersensitivity features are great if they're there. But I don't expect that they would be more common in fatty liver disease. It's currently less than ten percent of sporadic DILI cases. And then really fundamentally the question is is this again just the underlying liver disease or is this DILI. And we'll talk about the ALT pattern like we were talking about with Mike Fried. Maybe

it's different when you have more advanced disease than less advanced disease. That may be an important clinical observation. And of course in clinical trials, there's probably no prior reported cases to go back on. So really the clinical investigator and the sponsor have to have a high index of suspicion to diagnose DILI, particularly in a clinical trial.

2.6#5 RF: Now again, it's a pleiotropic entity. DILI is not one form of liver injury, it's multiple different forms. And we know this from prospective studies as well as retrospective case series.

2.6#6 RF: So histologically, it can look like a whole bunch of different liver lesions, including drug induced steatosis and steatohepatitis which now confounds the picture if you have preexisting steatosis or steatohepatitis. So when we say NAFLD, as you know the acronym is non-alcoholic fatty liver disease, and that's sort of the general entity of just fat in the liver, and as Arun alluded to, really the studies and the disease that we're most worried about is when you have steatohepatitis

with fat and inflammation, and then the fibrosis is really the driver of the natural history. And you know, just to go through a couple key points, it's non-alcoholic. So what does that mean? Well that's less than two to three drinks per day. But how hard do we go to find that out even in clinical trials to make sure that there isn't an alcohol component? You should make sure your patient doesn't take a drug that is associated with hepatic steatosis at baseline. That should be pretty easy from an exclusion criteria of the drugs that we know that cause hepatic steatosis.

2.6#7 RF: And I think at least in clinical trials, it seems to me there would likely be a baseline liver biopsy to be confident that the patient really does have biopsy proven NASH.

2.6#8 RF: For biopsy-proven NASH, there is this scoring system that the NASH CRN with Dr. Kleiner has developed. And you know, it's a combination of the NAS component of the score which is a combination of how much steatosis is in the high power field, how much lobular inflammation and how much hepatocyte ballooning. And then the fibrosis

staging here is different, it's zero to four as opposed to zero to six with most viral hepatitis. So when you look at patients who have NASH, and this is a paper from the NASH CRN, when you look at the individual components of the score, these patients all had NASH. They then said well there's patients who have definite NASH which they scored as greater than five points on that scale of zero to eight, or probable NASH which is three to four points, or just simple steatosis. And what you can see is there's some people who have evidence of steatohepatitis with no steatosis. So again, this is a complicated issue in terms of the individual components. And again, the amount of fibrosis which is likely a marker of the duration of the disease is also highly variable.

2.6#9 RF: So what happens in clinical practice? So there's lots of these series. This is one that was published last year. Liver biopsies in 222 patients with suspected NAFLD who were undergoing a biopsy. And the point of this is that 56 of these patients or 23 percent actually had a normal ALT at the time of the biopsy. But amongst this large

cohort, only about 24 percent actually met histologic criteria for NASH. So again, NASH is a small subgroup of fatty liver. And the point of this is that in the blue bar, you can see that there's a fair number of people with normal ALT who have biopsy proven NASH. And even advanced fibrosis you can have with normal ALT which again reiterates what Arun said, that the ALT is not a marker of the severity of liver disease. Now what about, you know, untreated patients? There's surprisingly not a lot of data on untreated NASH patients that I'm aware of. This is the NHANES study conducted in 1988 to 1994 which as you know they took a large group of Americans and then they had their diagnosis of fatty liver. But this was just looking at liver enzymes in the general population, and after you've excluded patients who have hepatitis B, hepatitis C and who drink a lot.

2.6#10 RF: And they just repeated a second blood test in the same patients an average of 17 days later. And you can see that in the general US population in early '90s, initially six percent of Americans had an elevated AST, six percent had an

abnormal ALT, 18 percent have an abnormal GGTP, and 12 percent have an abnormal alk phos. So I think that's a little higher than what we would all have projected. But more importantly is that if you just repeat the blood test with doing nothing 17 days later, you can see that actually as Arun said, there's a lot of fluctuation fairly quickly. So of those 115 with initially abnormal AST, only 74 were still abnormal on a second blood draw and so on. So there's some natural variability here without intervention over just 17 days.

2.6#11 RF: So what about making a diagnosis of DILI? There's in the DILIN network we're using this expert opinion based process which is the probability based upon prospective follow up with excluding all the other known causes of liver disease. And even in the DILIN network where we're really going out of our way to enroll only patients with bonafide DILI, when we follow them it turns out about 14, 15 percent actually had something else which wasn't apparent initially when we first enrolled them in the study.

2.6#12 RF: So there's also this RUCAM, and I'm not

going to go into great detail here but the RUCAM really has problems particularly if you have underlying liver disease to start with because you're supposed to exclude underlying liver disease when you use the RUCAM. So the RUCAM was not meant for patients with underlying liver disease. So it probably needed to be adapted in some way for those individuals.

2.6#13 RF: So what about DILI that happens? So this is from one of the papers we published last year. This is consecutive patients enrolled into the DILIN prospective study who all had high causality scores. So we didn't include the patients where it was something else. And we had about 900 total cases. And when we looked at it, we realized that we had about ten percent or 89 patients who had no pre-existing liver disease before they developed their DILI. So this is prospective, but it's purely observational. So the mean age of those patients was 52 compared to 48 in those without underlying liver disease. Really was no obvious clinical difference beyond the fact that there was more diabetes which is not

surprising because 36 of those cases were known HCV and the rest were presumed fatty liver/NASH with abnormal ALTs.

2.6#14 RF: Of interest is drugs that case DILI in patients with underlying liver disease versus not. It's very similar in the two groups. Antibiotics are the leading cause, followed by herbal and dietary supplements and cardiovascular agents. We're not seeing a predilection to one class of drugs or the other. Remember, these are patients with mild compensated disease, not patients with decompensated liver disease who then develop DILI into the DILIN protocol. But the proportion with were hepatocellular, cholestatic, and mixed was similar in the two groups. And the mean ALT was also similar as well as the mean bilirubin. So remember we're worried about susceptibility as well as potential outcome. So how did the patients do? Remember we do a detailed causality process. Largely because we knew these patients had underlying liver disease, their causality scores tended to be a bit lower in the patients with preexisting liver disease. When you look, 31

percent met Hy's Law criteria. But there was the same proportion in the non-chronic liver disease. Of interest, the rate of death was substantially higher. It was 16 percent versus 5 percent which is what we're all worried about that if you have preexisting liver disease, you may not do as well. So although they look the same when they first presented, this again is clinical practice, their outcomes were a little bit more concerning.

2.6#15 RF: So then how do you go back to differentiating NASH from DILI? So again, this is from the DILIN network with 249 suspected DILI cases where we had liver histology. And David Kleiner went through and very carefully described all the liver biopsies that we sent. And he came up with these six patterns histologically that helps a pathologist resolve that this could be a drug. But also in addition to saying what's happening in the DILIN database is also is there a role for histology that correlates with the likelihood of the overall score. And that is so if there's eosinophils in the liver biopsy that is associated with a higher causality score as well

as less ductular reaction, so those two histologic features may give you a clue that it may be more DILI than not. And then of course in terms of outcomes, not really surprising here. If you had zonal necrosis, ductular reaction, or underlying fibrosis you didn't do as well with DILI. And interestingly if you had eosinophils or granulomas, that's a good thing in that they did better.

2.6#16 RF: So we do have some histologic biomarkers for both diagnostic certainty as well as outcome. So let's get back then to the conundrum here of NASH clinical trials. So the good news, I think, will be that everyone will likely have a baseline liver biopsy when they get into the study. They'll be prospectively followed, we'll at least have monthly labs. You'll have a placebo arm as Arun showed to see what happens in the, you know, untreated control if you will, whether they're losing weight or not. So you'll have some comparator data at this point in time since there's nothing else approved right now. And then I think the FDA would appropriately require some PK studies

to make sure we know what the proper dose is for patients today with advanced fibrosis and so on. Now the drug that will be given, whatever the mechanism of action will be will be for 1 to 24 months. What will be the stopping rules? We'll discuss that here in the next few talks. You know, perhaps ALT greater than three times upper limit of normal, or their baseline as Arun alluded to. And then if they start to hit those thresholds, testing them more frequently, what will be the stop criteria. And then again, whether or not we should really be rigorous and maybe require a liver biopsy or suggest a liver biopsy in someone who you think may have DILI in a clinical trial to learn what it looks like in patients with NASH, particularly since you'll have their baseline liver biopsy.

2.6#17 RF: And I think, you know, as DILI investigator and adjudicator of these cases, it would be extremely helpful to get all these serologies in these cases if possible. And also because you have preexisting liver disease, I really think you need to have liver imaging in all these patients because if you have chronic liver

disease you're more prone to have gallstones and then you can get choledocholithiasis. Also, if you really have advanced fibrosis, you could have bad luck and have a spontaneous portal vein thrombosis which can lead to deterioration of liver function and worsening portal hypertension.

This is NAFLD studies, so they shouldn't be drinking, but let's just prove that and maybe get a urinary ethylglucuronide when this happens to make sure they're not drinking. List of meds, what if it's due to something else and not the study med. So these things should be standardized and should be achievable. But at the end of the day, we all know how complicated this is. So I think most studies will have probably an expert adjudication panel to try to vet these cases.

2.6#18 RF: And again, having those baseline values, perhaps a run-in for a month of what's that patient's baseline might be particularly useful. So really at the end of the day, you're going to try to boil things down into is it the NASH, is it DILI in NASH, or is it just DILI? And, you know, we have these different lab criteria. And as Arun

said, you know, we have some data on untreated NASH, but how much variability. Maybe there are a few patients ALT of 250 at the upper limit of normal's 30, that's 10 times almost upper limit of normal for that patient. So certainly having baseline labs would be helpful.

And again, if we have a biomarker for NASH that's distinct from DILI, that would be very helpful so that when the flare happens, is it more the NASH or is it the DILI. And is CK18 for example going to be helpful for NASH? Is HMGB1 going to be useful for DILI, we don't know. And then I think there will likely be a role for histology if we can get a liver biopsy, particularly in patients with suspected DILI.

2.6#19 RF: To summarize, I didn't have time to cover everything. But from review of the literature, there's really not a lot of convincing evidence from at least DILI registry studies and reports that preexisting liver disease like non-alcoholic fatty liver disease predisposes or increases the susceptibility to DILI in general. I think that's reassuring. Perhaps the only exception to that

would be methotrexate and that's a bit controversial actually. I think from the data that I showed you as well as what we heard from earlier today, if you do get DILI and you have preexisting liver disease, you can certainly have more morbidity and mortality. So there's a little bit more at stake here from a hepatic outcome. Lab criteria for DILI diagnosis probably are going to need to go up to 3 to 5 times upper limit of normal or better would be the baseline. And then how do you define the baseline? Is it two labs, is it three labs? What's the time interval? This all needs to be sorted out. And I think certainly an increase in total bilirubin will be important. I mentioned to you my suggested diagnostic algorithm. Remember, hepatologists should be taking care of these patients so you might have better compliance with getting the work-up done. Causality instruments need to be developed for patients with underlying liver disease. I feel that expert opinion is going to play a big role. This highlights again, as we've discussed in the past, we really need a DILI specific lab biomarker,

and I think we'll hear a little bit more about that tomorrow.

2.6#20 RF: And then we'll leave it up to the FDA to pull it all together and tell us what the stopping rules should be for NAFLD in clinical trials. Thank you.

DR. BIRNKRANT: Thank you very much. That was quite informative. Well our next two talks will focus on FDA challenges related to recognizing, assessing, and managing acute DILI in the setting of HCV liver disease and in NASH.

So our first speaker will be Dr. Mishra who's the deputy director for safety in the Division of Antiviral Products at the FDA. She has been involved in the review of direct acting antivirals for the treatment of chronic hepatitis C and pre-market and post-market review of safety data including review of DILI cases. Dr. Mishra?

2.7#1 PM: DR. MISHRA: Thank you, Debbie. So I would like to thank Dr. Senior and other organizers for giving me the opportunity to share my

experiences today.

2.7#2 PM: The views expressed in this presentation are those of mine and do not represent official policy of Food and Drug Administration.

2.7#3 PM: Today I'll share some examples of recent approaches and experiences in evaluating potential signal of drug induced liver injury in clinical trials evaluating direct-acting antiviral agents for chronic hepatitis C. I'll discuss both trials in patients with compensated liver disease as well as in patients with decompensated liver disease. Then I'll discuss one of our recent post-marketing experience with direct-acting antiviral agents.

2.7#4 PM: So the public health burden of chronic hepatitis is huge both globally and in the United States. Incidence of hep C infection in US is decreasing, but the chronic hepatitis C related complications are increasing. And this is in part due to the aging of infected population. And without effective interventions, more liver related complications were predicted in the next 10 to 20 years.

2.7#5 PM: Successful treatment for chronic

hepatitis C has resulted in attainment of sustained virologic response as we have seen. And multiple observational cohorts have shown strong correlation with improvements in clinical, important clinical, outcomes such as reduction in liver disease complications, including liver failure, decrease in development of hepatocellular carcinoma, and decreased liver related mortality as well as all-cause mortality.

2.7#6 PM: This slide shows you the evolution of treatment for chronic hepatitis C. So, starting back in 1991 with interferon monotherapy for 6 months, the SVR rates were less than 10 percent. Now in 2016 we have multiple interferon-free regimens or oral regimens and we have achieved SVR rates ranging from 97 to 99 percent. We even have treatment options for specific populations such as decompensated liver disease, patients with decompensated liver disease, post liver transplant patients as well as those with end stage kidney disease.

2.7#7 PM: So focusing on the topic of discussion today, there are challenges in the evaluation of

potential DILI cases in these trials evaluating treatments for chronic hepatitis C. The biggest challenge is: how should we interpret increases in serum transaminase as well as increases in bilirubin values in these trials? Which criteria should we use for identification of potential DILI cases in these patients who often have elevated baseline liver enzymes? And as we have heard during previous presentations today, we have to think about the patients with preexisting liver disease who may be more susceptible to drug-induced liver injury in terms of their compromised hepatic result and the serious outcomes in case they undergo drug induced liver injury. And then the final challenge is: how should we discern natural progression of disease from drug toxicity? And this has become even more challenging in the setting of advanced liver disease such as decompensated population.

2.7#8 PM: So I'm going to share today an example of DAVP's approach. And I want to note that this is one single example from one clinical development program. And of course there was variability in different clinical programs, so I'm not saying that

this is the best approach, but I'm sharing one of the approaches which was taken.

2.7#9 PM: So for daily assessment in these trials, there were three specified criteria for assessment which were agreed upon with the drug sponsor prior to the initiation of clinical trials as well as prior to the submission of marketing application. They were served by general principles which were utilized to develop the criteria for identifying potential cases in these trials, evaluating subjects both with compensated liver disease as well as without advanced liver disease.

And as part of some clinical development programs, potential DILI cases were independently evaluated by a panel of hepatology experts convened by the drug sponsor. And again, this varied from one clinical development program to the other.

2.7#10 PM: So here are some of the following general criteria which were used for assessment of serum transaminases. So we used a relative cutoff rather than using absolute cutoff for ALT or AST. What I mean is that we did not use an absolute cutoff value like ALT greater than 500 or 600 or 800.

Rather than we preferred using fold change from baseline value or from treatment nadir value. Change from individual's value on treatment was preferred for the reasons which have been already discussed today, because upper limit of normal in this patient population with elevated values is at baseline may not be appropriate. When we know that patients who are receiving direct-acting antiviral agents for treatment of chronic hepatitis C usually see a decline in their AST and ALT values very rapidly during the first few weeks of treatment. So a change from on treatment nadir value was considered a more sensitive marker of potential liver injury in these patients. And on treatment nadir value was their new baseline so as to say for the potential liver injury.

2.7#11 PM: Moving on to bilirubin monitoring for identification of potential DILI cases, total bilirubin values are often elevated at baseline in patients with decompensated liver disease. In addition, monitoring of total bilirubin value may not be helpful in patients who are receiving ribavirin which is a component of many DAA based

regimens, and it is known to cause indirect hyperbilirubinemia. In addition, some DAAs can cause increase in bilirubin values which is attributed to bilirubin transporter inhibition. So direct bilirubin is considered to be more reflective of liver function and injury. And we use baseline values as we know that bilirubin values do not rapidly decline when RNA declines on treatment.

2.7#12 PM: So first I will discuss some of the criteria which were used in trials evaluating patients with compensated liver disease. I have provided the reference at the bottom of the slide. And this all information is available on public domain. You can visit Drugs@FDA website and this is included in medical reviews which were done for this particular project. So the assessment of potential Hy's Law cases was used in these patients with compensated liver disease. We also looked at grade 3 and 4 liver enzyme elevations within phase III trials. We looked at bilirubin elevations within phase III trials. And there were pre-specified criteria which were required for

treatment discontinuation. So any of the subjects who had confirmed elevation of ALT or AST greater than 5 times baseline value or greater than 5 times on-treatment nadir value were required to stop the drug. Another criterion was confirmed elevation of ALT 3 times baseline value and total bilirubin greater than 2 times upper limit of normal. And then any patients who had confirmed elevation of ALT greater than 15 times upper limit of normal was to stop all study drugs.

2.7#13 PM: What we observed from these trials using these criteria, a comprehensive assessment was done. We found that no on-treatment cases of serious hepatotoxicity were observed within the phase II and phase III trials. There were no Hy's Law cases identified in these trials. There was no subject who met the protocol specified level related stopping rules during the on-treatment phase. And ALT or AST increases greater than 5 times upper limit of normal were infrequent. They were seen in less than 0.5 percent of cases and they were generally transient.

2.7#14 PM: So now moving on to the criteria which

we used in our trials evaluating patients with decompensated liver disease. As these patients had more advanced liver disease, we used more stringent criteria in this patient population. So any increase in ALT or AST greater than 2 times baseline value, we looked at any subjects who had increase in ALT or AST greater than 3 times baseline on-treatment not nadir value, or any increase in direct bilirubin greater than 1 milligram per deciliter from baseline value. Again, for the reasons I pointed out earlier, direct bilirubin was chosen instead of total bilirubin. And there were certain pre-specified lab criteria for our treatment discontinuations. And these were a little different from the ones which we used in compensated liver disease population because we thought that these patients will have elevated, more elevated baseline values. So we used AST or ALT greater than 10 times baseline value or nadir value, or ALT greater than 15 times upper limit of normal. And there were several additional clinical criteria which were used for a more comprehensive assessment of this patient

population.

2.7#15 PM: So to identify any possible cases of DILI, we looked at all treatment emergent deaths or liver transplantations, any treatment emergent serious adverse events of hepatic failure, and we also looked at hepatic AEs with preferred term of hepatic failure or acute hepatic failure or hepatotoxicity or any of these terms which, any of this events which led to treatment discontinuation during the trials. So based on our evaluation of these trials in decompensated population, we did not identify any safety signal of DILI. But you know, these regimens have been recently approved. So we will keep monitoring in the post marketing setting and we'll see how it goes.

2.7#16 PM: So I'm going to share a recent post marketing experience. Many of you might have seen this drug safety communication which came out in October of 2015 in which we warn of serious liver injury risk with one of the three drug regimen.

2.7#17 PM: And what we found in this particular case was that these serious outcomes were reported mostly in patients who had evidence of advanced

cirrhosis even before starting treatment. So some of these cases occurred in patients for whom these medicines were either contraindicated or were not recommended.

2.7#18 PM: Going back to the observations from pre-marketing evaluation for this particular drug, during clinical trials we had seen elevations of ALT greater than five times of upper limit of normal which occurred in approximately one percent of all subjects. These ALT elevations were typically asymptomatic. They occurred during the first four weeks of treatment and declined within two to eight weeks of onset with continued treatment. So these were basically attributed to adaptation. These transaminitis increases were attributed to the protease inhibitor in the regimen because transaminitis was observed at higher doses of the protease inhibitor in the regimen. There were grade three or greater ALT increases which were observed in 25 percent of females which were using concomitant estradiol-containing medications. There were no cases of ALT elevations that were associated with liver failure that was attributed

to this particular drug. And again, no cases appeared to meet criteria for Hy's Law.

2.7#19 PM: So moving on to clinical presentation in post marketing cases, what we saw was this presentation of liver injury in patients with advanced liver disease was very different. We know that paritaprevir exposures are increased significantly in patients with Child-Pugh B and C cirrhosis, AOCs reaching approximately 62 percent and 945 percent. ALT elevations did not appear to the predominant presentation in these post marketing cases with advanced liver disease. Elevated bilirubin values without ALT elevations was commonly noted with clinical signs and symptoms of decompensation these post marketing cases. And these reported hepatic events mostly occurred within the first four weeks of treatment initiation. So this observation led to labeling revisions.

2.7#20 PM: We included a section under warnings and precautions informing providers of the risk of hepatic decompensation and hepatic failure in patients with advanced liver disease. And there

were certain monitoring recommendations as well. So again, these reported cases typically occurred within one to four weeks of initiating therapy, and these were characterized by the acute onset of rising direct serum bilirubin levels without ALT elevations and were seen in association with clinical signs and symptoms of hepatic decompensation. So to conclude, DILI assessment in clinical trials evaluating patients with pre-existing liver disease such as chronic hepatitis C is challenging. We need pre-specified criteria and consistent approach to evaluate DILI in these trials. Data driven recommendations about clinical monitoring in clinical practice may reduce or elevate the risk of DILI in clinical practice. Post marketing surveillance is crucial, and data from real world observational cohorts may be helpful.

2.7#21 PM: I would like to thank Dr. Birnkrant and Dr. Murray as well as other team members from the Division of Antiviral Products who evaluated this data and helped with the slides. Thank you.

DR. BIRNKRANT: Thank you very much. I think Dr. Mishra made it look pretty easy, but it's a very difficult situation.

Our next speaker is Dr. Ruby Mehta. She's a pediatric gastroenterologist in the Division of Gastrointestinal and Inborn Errors of Metabolism Products at the FDA. Prior to joining the FDA she was an assistant professor of pediatric gastroenterology, nutrition, and hepatology at the University of Tennessee. And her talk will focus on the challenges related to assessing and managing DILI in the setting of NASH.

Thank you.

2.8#1 RM: DR. MEHTA: Thank you. Good afternoon. Thanks to Dr. Senior and Dr. Avigan for allowing me to present on this topic. I will focus this talk on knowledge gaps in assessing DILI in patients with NASH.

2.8#2 RM: The views and opinions expressed here are my own and do not represent official guidance from the FDA. I have nothing to disclose.

2.8#3 RM: At enrollment, patients with NASH may have elevated transaminases. In cirrhotic

patients with advanced liver disease, elevations in total bilirubin, prothrombin time, and INR are also relevant.

2.8#4 RM: During the trial, incremental increases in baseline elevations of liver biochemistries in NASH patients could indicate worsening of NASH, fluctuation in the natural course of NASH, drug induced liver injury, or other liver diseases.

2.8#5 RM: There are limited data available on natural history of variability and time course of progression of liver biochemistries in both pre-cirrhotic and cirrhotic NASH population. It is important to define biochemical criteria that will reliably detect both DILI in both these NASH populations.

2.8#6 RM: So how can we bridge the gap? An evidence based foundation is required for developing a framework for monitoring and responding to liver biochemistries in NASH trials. In the interim, we can characterize patient's baseline values and develop a plan to address foreseeable clinical circumstances.

2.8#7 RM: Pre-treatment baseline is a key frame of

reference for interpreting on study changes to establish baseline liver biochemistries for ensuring the stability of underlying liver disease. Are two screening laboratory values adequate? How far apart should these two values be collected? Is eight weeks acceptable? Are historical or pre-trial values acceptable, and if yes, of what duration? Should a mean or a median value be considered to establish the baseline value? How much variability for establishing the baseline value prior to start of the study treatment is acceptable? How should pre-treatment fluctuating liver biochemistries as evidenced by an elevation in the second laboratory value obtained be addressed? For example, if the first ALT value is 60 and the second is 90, is this patient eligible for trial?

2.8#8 RM: Should a third value or additional values be obtained to determine eligibility? When is the patient considered ineligible for trial enrollment? During the trial, in order to delineate the definition of new baseline liver biochemistries, then a patient's biochemistries

improve with therapy, consider the patient's documented baseline variability.

2.8#9 RM: Also, it is essential to consider the new nadir baseline variability for individual patients if the drug reduces the transaminases or the other liver biochemical tests. There are limitations in the assessment of these changes over time as the biochemical trends may change during the course of the treatment. Therefore, the incremental change in biochemistries that should reasonably trigger a DILI work up must be defined.

2.8#10 RM: FDA does not have any specific guidelines at this time for treatment interruption, however a few considerations are presented. If a pre-treatment baseline value is less than two times upper limit normal, then the treatment should be stopped if the ALT or AST increases to greater than five times baseline.

2.8#11 RM: Few other examples are also mentioned here. To be consistent with the DILI guidance, a thorough evaluation for a co-existing, non-drug related etiologies are required. If an alternative etiology is identified and the liver

biochemical tests have a trend to baseline, then consider restarting the therapy.

2.8#12 RM: A thoughtful and cautious approach is required to assess elevations and liver biochemistries for treatment interruption and discontinuation. A prospective plan for assessment of liver adaptation is needed.

2.8#13 RM: In a symptomatic patient, of course, regardless of the magnitude of these elevations, the treatment must be discontinued followed with investigations.

2.8#14 RM: A more conservative triggers for study interruption or discontinuation are required for patients with advanced stage liver disease. These patients have high risk of rapid decompensation or acute worsening of their clinical status due to low hepatic reserves or hemodynamic and immune status, and be on multiple medications mainly to drug-drug interaction and toxicity from concomitant medications.

2.8#15 RM: Changes in aminotransferases may not adequately assess hepatocellular damage when identifying DILI in patients with advanced stages

of liver disease. Therefore, customized criteria for monitoring, treatment interruption, and treatment discontinuation may be needed. There are limited available data on the natural history of biochemical vari

ability and time course of progression in decompensated liver disease, and the biochemical criteria that can reliably detect DILI in decompensated liver disease.

2.8#16 RM: Special considerations for the trials and decompensated liver disease patients, should other parameters be considered as DILI signals in patients with advanced stages of liver disease such as worsening of MELD scores or decompensation event? Should they be added in the evaluation algorithm?

2.8#17 RM: Thank you.

DR. BIRNKRANT: Thank you very much.

Our next speaker is Dr. Mark Avigan who actually needs no introduction. And he'll be discussing the development of a DILI guidance.

2.9#1 MA: DR.BIRKRANT: Our next speaker is Dr. Mark

Avigan who actually needs no introduction. He'll be discussing the development of a DILI guidance.

2.9#2 MA: Well, because of the late hour, I'm planning to give a ten minute version of a talk I planned, and most of it has already been said

And I do need an introduction. Sometimes I do, just for my own orientation.

DR. BIRNKRANT: Oh, okay.

2.9#3 MA: (not read)

2.9#4 MA: Most of these introductory slides just point out key domains and elements of the current FDA guidance where there are gaps that should be noted.

2.9#5 MA: Key overarching points about FDA guidances to take into account are made in this slide.

2.9#6 MA: Pertinent elements contained in the current DILI guidance, in particular, are shown here.

2.9#7 MA: Other pertinent elements contained within the current DILI guidance are shown here.

2.9#8 MA: Other elements in the current DILI guidance are shown here.

2.9#9 MA: Other elements in the current DILI guidance are shown here.

2.9#10 MA: Some of the key elements in the current guidance are shown here.

2.9#11 MA: Despite all the information that is provided, there are a number of unresolved issues in the current DILI guidance that I have listed here and in the next few slides..

2.9#12 MA: Another unresolved challenge in the current FDA guidance is summarized.

2.9#13 MA: Another unresolved challenge in the current FDA guidance is listed here.

2.9#14 MA: In addition, a huge unresolved challenge in the current DILI guidance has to do with patients with underlying chronic liver disease. But I want to just make a few points and then we can go on to the discussion of this issue.

2.9#15 MA: In focusing on relevant domains to build DILI guidance for patients with chronic liver disease there are critical requirements, methods and rules to consider.

2.9#16 MA: These requirements must address important challenges in DILI assessment in the

presence of chronic liver disease that we've heard about. And one of course is that there are distinct clinical, pathologic, and lab test signatures in different underlying liver diseases. So there's this Aristotelian question of do we lump or do we split. The second point as we've heard about from some of our speakers earlier in the session is that rises that occur when acute injury occurs on top of chronic liver disease, rises of ALT and the functional measures such as bilirubin and INR don't go hand in hand necessarily. And we actually can see a splay of different kinds of signatures depending on what the baseline characteristics are. And at one extreme, with mild ALT elevations, we could see further rises. But at the other extreme, we might -- as we heard with reference to these patients with severe cirrhosis and DAAs who had these extraordinary reactions -- they basically had very dramatic elevations of bilirubin but not much with regards to ALT changes. So there are different signatures to be mindful of as we study this problem further.

2.9#17 MA: There are other challenges as well, as

we think about this whole umbrella of different reactions. Sometimes, it can be difficult to distinguish cholestatic from hepatocellular DILI unless we have very kind of refined analyses. And we heard about ribavirin and its association with hemolysis, but we haven't completely ruled out a potential hepatotoxic interaction of this drug with certain other DAAs. Another point is that worsening liver tests may reflect loss of treatment effects such as resistance to hepatitis C drugs and not necessarily DILI, per se. And finally, we have to be very mindful of pharmacological mechanisms because many of the pharmaceuticals that we're using are cleared by the liver. There are many steps where either blood levels or tissue levels can be changed because of underlying abnormalities in steps of liver metabolism and clearance that then leads to the crossing of toxic drug thresholds.

2.9#18 MA: So I just want to basically again show you some real cases just to sort of bring this home. And the first case, and we heard about these signatures already but I just want to kind of tell

you some example cases. And you'll see that there are some gaps in the differential diagnosis. One case of a gentleman with HCV with class Child-Pugh A cirrhosis who pre-treatment had ALT levels of about two times the upper limit of normal and borderline elevated bilirubin which of course is in micromoles per liter, and started on a regimen of DAAs --- this is the quadruple treatment with ribavirin -- and nine days later developed basically severe liver injury with hepatic encephalopathy and jaundice. But the ALT surprisingly didn't really change at all. It was actually perhaps even lower than it was before. But the total bilirubin now was at four or five times what it was -- actually, what am I saying. It was more than that, it was almost ten times. So this was a very dramatic rise in bilirubin and then within a few weeks the patient had hepatorenal syndrome, hepatic encephalopathy. Again, the liver tests didn't show much change other than the fact that total bilirubin and INR continued to climb as did the creatinine. So the MELD score was worsening and the patient died.

2.9#19 MA: This is another case of a patient which it was published by Jessica Dyson at Newcastle last year, or this year with her colleagues. And this is an individual with HIV well controlled with treatment who had ACV who embarked on treatment. And we started out before treatment with a total bilirubin that was already abnormal. So this was Child-Pugh class B cirrhosis patient. And within two weeks, again in a short timeframe, developed worsening jaundice, higher bilirubin, and got kind of worked up. The bilirubin was conjugated in form. So that was done. At least they did a fractionation. The ACV levels, RNA levels did not budge, they were still suppressed. And a work up with some of the usual viral suspects, other viral suspects were negative. And in this case, a liver biopsy was performed and that goes to Bob Fontana's point because it showed not just cirrhosis but acute hepatitis with eosinophils and rosettes and there was an impression by the pathologist that this was consistent with a drug reaction.

2.9#20 MA: So the Aristotelian question as we think about guidances -- with all these different

kinds of presentations and profiles -- is whether we should split or lump these conditions into sort of an instruction list. Remember that guidances should highlight generalizable approaches that can be practically applied yet account for rule exceptions. And so this is a point for discussion.

2.9#21 MA: Some the questions to resolve in developing a DILI guidance for underlying liver disease are listed here an in the following slides.

2.9#22 MA: A question to resolve in developing a DILI guidance for underlying liver disease is listed here.

2.9#23 MA: An additional question to resolve in developing a DILI guidance for underlying liver disease is listed here.

2.9#24 MA: Some additional approaches to consider regarding requirements for liver testing are listed here.

2.9#25 MA: Finally, some other questions to resolve in developing a DILI guidance for underlying liver disease are listed here.

2.9#26 MA: I want to mention criteria that identify cases of interest with reference to

baseline characteristics of each individual study subject. We heard from some of the speakers that instead of looking at the upper limit of the reference range, look at the fold-rise above that individual's baseline. But the utility of this approach doesn't preclude the idea that we would also look at the upper limit of the normal reference range as well. And I'll show you why that should be the case. This question requires more research, of course. And then, for individuals who alrfeady have liver dysfunction at baseline, we would certainly want to look at measures of worsening liver function, which by itself might indicate that this is a patient of interest with possible DILI and who needs further scrutiny.

2.9#27 MA: So as I said, we would probably not want to exclude upper limit of reference range as a frame of reference in studying individuals. And here is an example of a study population that was plotted on eDISH. We'll call this Drug X. And you can see that using the upper limit of the reference range, there were cases with acute elevations of ALT shown by the triangles and blue squares,

indicators of individuals who received the study drug, whereas the green circles represent the individuals treated with placebo. And you can see most of the cases of potential interest are actually in the right lower quadrant where the boundaries are defined by two times the upper limit of normal. And some of these cases, when we got clinical information about them, seemed to be causally linked to the drug. But there was no huge fire -- in other words, we aren't seeing what we would call Hy's Law. Looking at Case A, who had a peak ALT level of over ten times the upper limit of normal on treatment, when we plotted the same data for the same treatment population -- with a different set of parameters or boundaries - in this case, fold increases over individual baseline levels - we get a very different impression of what the liver signal was and what the result was.

2.9#28 MA: What you see is - using again that major abscissa line between the right lower quadrant and upper quadrant being two times the baseline of each individual study subject - you can see that there was a rather dramatic perturbation

with the treatment for increases above baseline both of ALT as well as bilirubin levels. And Case A, although not passing the threshold of a Hy's Law case, as I showed in the previous slide, actually had a sevenfold rise of bilirubin above the pre-treatment level. So if we were just to look at this second graph alone, we might get the impression that we had a major liver injury of a patient in this clinical trial. When we looked at a broader set of standards, that wasn't the case. The only reason to show you this is that as we move forward and we learn more about studies with patients with chronic liver disease and we zero in on patients of interest and plot them, we will have to be very careful how we proceed with setting graphic standards for analysis. And I suspect that we'll further integrate more than one set of criteria to actually isolate and investigate patients and not just have one rigid point of view by looking exclusively at either the baseline levels of each individual, or alternatively at the upper limits of reference levels. Rather, both should probably be looked at.

2.9#29 MA: Questions to resolve in developing a DILI guidance for underlying liver disease are listed here and in a few of the following slides.

2.9#30 MA: So we've talked about scenarios and approaches to consider for close observation in terms of differential diagnosis. And we need to of course rule out certain other causes that come into play, and I don't want to spend more time on these because we've heard about this issue already.

2.9#31 MA: Another question to resolve ina DILI guidance for chronic liver disease is listed here.

2.9#32 MA: Another question to resolve ina DILI guidance for chronic liver disease is listed here.

2.9#33 MA: Another question to resolve in a DILI guidance for chronic liver disease is listed here.

2.9#34 MA: An additional question to resolve is listed here.

2.9#35 MA: And I'm just going to close at this point because I think we want to have some discussion here and make the following observations about this work in progress, as we consider the prospect of writing a new guidance for DILI in clinical trials.

First, preexisting liver diseases pose significant challenges in developing clinical trial guidelines to evaluate and manage DILI.

Second, because of differences in the type and severity of individual's underlying liver disease, quantization of changes of serum markers during study drug treatment standardized to pretreatment levels may not correlate with the degree of acute liver cell necrosis and reduction of liver function that can be attributed to drug toxicity. Therefore, nuanced approaches in a DILI guideline are required. And finally, the development and validation of reliable quantitative markers of acute drug induced liver cell death and reduced organ function in chronic liver disease is a priority need.

2.9#36 MA: So I think that sort of captures what has been said and we'll have to take in some points of view in your comments. Thank you.

DR. BIRNKRANT: Okay, so now we're looking for a discussion of the challenging topic of assessing DILI in the setting of chronic liver

disease.

DR. WRIGHT: Terry Wright, Genentech. I wanted to follow up on a comment from Dr. Mishra about the epidemiology of hepatitis C in the US.

And I think we should reflect that it may be changing with the opioid and heroin use which is now increasing in younger people and point to a New York State epidemiological report in January 2016 reporting rates of hepatitis C in up to 2012 in New York.

And half of the cases actually were individuals who were 50 years and younger. So I only, perhaps I'll ask the panel to consider really whether maybe our previous conceptions of the epidemiology, the studies done by CDC a number of years ago actually may need to be revisited in light of changing epidemiology.

DR. BIRNKRANT: Thank you for that question.

DR. MISHRA: Yes, I don't disagree with you. There have been recent reports of increasing incidents in certain sub-populations and we are fully aware of that data. And I totally agree with

your comments.

DR. BIRNKRANT: Go ahead.

DR. WANG: Hi. Tao Wang from the FDA. So I have a question. In addition, because now more evidence suggest the immune system play a role in this DII. So my question is is any possible using some immune parameters instead of, in addition to this like a traditional liver function, detection as a marker to assess the toxicity before a patient can take drugs?

DR. AVIGAN: Well, it's actually, it's an important question and not a new question. And so of course the detail of the question is what do you mean by immune because there have been over the years different kinds of tests of immunologic reactivity to drugs and index individuals with liver reactions or hypersensitivity reactions, whatever, one being the lymphocyte stimulation test which is still being done in some countries almost routinely. There of course has been waxing and waning enthusiasm about it with regards to its predictive value. And I don't know whether anybody else wants to comment. I know that whether

Bob wants to --

DR. FONTANA: Yes, I mean, we can comment on that that we've, in the DILIN network we looked at a cohort of consecutive patients who already had DILI so we didn't have their baseline values before they started the drug. And with the work that we did, we weren't able to come up with a lymphocyte proliferation assay that was clinically useful. So in our hands, we weren't able to achieve the results that have been reported in other countries.

DR. BIRNKRANT: Go ahead.

DR. MARQUEZ: Hi. Loretta Marquez, Janssen, pharmaceutical drug safety physician in oncology. And the question is I know the focus of today has been on hepatitis C and NASH patients. But oncology is another area of importance of preexisting disease, not only because of the liver metastases but most importantly, 85 percent of patients with pretreatment of chemotherapies are reported to have NASH or steatosis as a common chronic disease.

So what are the FDA thoughts about applicability

as the same concepts within this therapeutic area, this group of patients, and is there any ongoing studies right now in this particular population also in terms of DILI network?

DR. BIRNKRANT: So the question has to do with the applicability from one patient population to another? So oncology patients with underlying liver disease, can the data from that group be applied to hepatitis C or NASH in general?

DR. SANYAL: I thought the question was the other way around.

DR. BIRNKRANT: Oh.

DR. SANYAL: Did I get it? Okay. So I think there's a good starting point. But we also have to consider that some of the chemotherapeutic agents directly affect sinusoidal endothelial, et cetera. So then we have some other mechanisms of injury. So I think it still warrants independent generation of data and independent analysis, although the initial guiding rules from what we've learned from hep C where I guess the most amount of data for chronic liver disease exists could be a good starting point.

DR. FONTANA: Yes, I would agree that I think as we just heard from the last four talks, you know, the criteria, the presentation and outcome may be different in hep C versus fatty liver, and I have a feeling it's going to be very different in oncology patients. So the work up needs to be similar, but the definitions and the incidence, and also the tolerability if you will of hepatotoxicity may be a bit higher in oncology practice. So that also sort of makes things a little bit more complicated.

And perhaps also in the drug development, I can't comment on that but I don't know if anyone --

DR. SANYAL: Yes. One aspect also in talking about fatty liver disease and drug induced injury -- because we've just talked about the clinical aspects over here -- is that when you get fatty liver disease, you're changing the metabolic status of the liver, the fatty acid composition. You have alteration in isopentanoic and docosahexaenoic acid ratios which are very important for the biology of maintaining the health

of the hepatocyte. You have changes in hepatic eicosanoids. Interestingly, while systemic, lipoxygenases get activated. In the liver, actually the lipoxygenases, many of them get down regulated. How that alters the, you know, the micro environment, I think we haven't even started beginning to, you know, we have barely scratched the surface there. So I think we need to understand the biology of how all the alterations that we've learned about in NASH, when you throw a drug in there and particularly in the setting of polypharmacy which is the common reality of our patients. I think we need to learn a lot more.

DR. BIRNKRANT: What could be helpful also is we found it helpful in hepatitis C and that is to have an adjudication committee to help look at the cases with you.

DR. AVIGAN: I think that's right. And I would just add one other thought which is that different cancer drugs actually work differently from each other. So you know, the classic drugs you think of, cell cycle inhibitors that are pre-dose related and so that you would be

considered about sort of crossing over a dose effect. But there are other treatments in cancer now that are really in that "idiosyncratic" which again we heard from Jack these are still dose related. But there's a shift in the dose response to a different level. So there may be still some, so the answer I think is it depends on which kind of drugs we're talking about. But generally, the concepts would be the same.

DR. TILLMANN: Hans Tillmann, ECU. Would it be wise to define what upper limit of normal you want to use because I think we're moving to an upper limit of normal of 19 and 13, but your recent guidelines were written when we had upper limit of 60 or if you were in a veteran hospital you can have 75 ALT and you're still normal.

DR. AVIGAN: Well actually, this was not even normal. This was the upper limit of the reference range. And you can attack that because reference range are actually is all over the place because the, you know, I mean, we heard with NASH you can have patients who have 1.5 times the upper limit of real normal or even normal or two times.

So in a sense the question is that's sort of what the problem is in a way. But what I was trying to show you with the eDISH is that if you just go the other way and just go for full individual, there are other problems that start surfacing that you have to think about in your analysis which can mislead you. So I'm not sure that it's just a very simple, we may need a very flexible graphic program where the analyst can look at it in both ways.

DR. BIRNKRANT: Go ahead.

DR. KULLACK-UBLICK: Yes, Gerd Kullack-Ublick, University of Zurich, Switzerland. Just a question for Dr. Sanyal. You showed that obeticholic acid, which is an FXR agonist, can help to resolve fibrosis in NASH. Now the FXR agonists seem to improve steatosis as well in the NAFLD setting. So do you think, you know, given that some drugs, cancer chemotherapy, amiodarone, valproic acid cause DIS or DISH, do you think it would be worth trying to use FXR agonists to prevent steatosis in those regimens?

DR. SANYAL: I think that's a loaded question. Can I plead the 5th? I don't know. I

think you have to think about the mechanism of action. With the mechanism of action of fat accumulation, phospholipidosis with amiderone and the mechanism of action is actually quite different than the development of steatosis in garden variety fatty liver disease that we see. So I'm not sure how --

DR. KULLACK-UBLICK: It's mitochondrial toxicity which probably would also work with --

DR. SANYAL: Right. But I'm not 100 percent sure whether obeticholic acid will or will not improve something like amiodarone toxicity.

DR. HUNT: Hi. Chris Hunt, Duke. Just wanted to make one suggestion for consideration. We now have abundant data in NASH with a clinical research network and, you know, oncology, National Cancer Institute, numerous studies in hepatitis C, large studies that we can actually use for natural history to understand, you know, what is the natural history of liver chemistries in all of these areas and where are the deviations seen? And it seems like this would be

a ripe time for modeling efforts to look at how to best -- I mean, Arun was eluding to it and numerous people have talked to it. It just seems like this would be a great time to pursue that, to really understand when we see deviations and which ones are particularly notable because the data will really help it leap out to us. So thanks.

DR. SANYAL: Chris, you're right on point. And actually, Dr. Dimick and I, we've been talking about doing this for about a year. It's just we've got to stop going to meetings and actually do some work for a change to get it done. But you're right, I agree.

DR. BIRNKRANT: Any other comments or questions?

DR. RODRIGUEZ: Ignacio Rodriguez from Roche. Just to be provocative because I've been thinking a lot about this issue, should we use the normal limits or the reference ranges or the normal values. And you were saying that in covering something that you didn't know about it. So how will we handle if we have a patient with ALT at baseline of ten just for the sake of argument and

a bilirubin of 0.5 that moves amazingly to a bilirubin of 1, doubling, and ALT of 100, ten times. Is that a signal?

DR. AVIGAN: Well, that's what I was getting at. In other words, I think you have to pre-specify what has been called, and I think importantly called cases of interest. So what you're initially doing is you're looking at the response across the population of treatment effects on the liver, and then you're making a cut for cases that you really need to monitor carefully and do differential diagnosis on to work them up. And the way you make that cut requires some determinate, pre-specified determination. But exactly the boundaries for that cut require I think a little more research because I think the point is you wouldn't make the cut for some diseases just on the basis of full baseline of that individual because as you point out, you could get a perturbation, but it's a little smoke but not fire. So you would have to establish parameters for where you would be committed in that individual to pull them aside, carefully observe them, and work them

up. And it may be more than one parameter. But I don't want -- I think it's a nuanced answer, and I think you may end up with a guidance or guideline in your protocol which would have two ways of doing it and asking for two different criteria.

DR. SANYAL: So Mark, I've also been thinking that we've always sort of in very simplistic way thought of drug-induced liver injury into injury where the cell dies. But then you could have injury where the cell is just sick. And when it's sick what I mean is that, you know, a lot of its transporters stop working and you have this isolated hyperbilirubinemia, et cetera. But certainly for when the cell dies, what's the data on CK18, circulating CK18 vis-à-vis ALT as a way to evaluate drug toxicity.

DR. AVIGAN: Well, I think that's a great comment and we'll hear more about that tomorrow actually. That's a nice kind of segue for tomorrow. And I think we're at the end of this session time, right? So we'll at this point get Lana to come up and give us instructions about what's next.

MS. PAULS: Thank you very much. Thank you, everybody for staying all afternoon. For those of you who would like, there is a free wine and beer and hors d'oeuvre reception at the end of the hall when you go out.

And in addition, for those people who have never been here before, every registrant should have gotten a USB bracelet when they came in that has all the slide presentations plus the abstracts, bios, and pictures of all the speakers and moderators. So take a chance to look at that at your leisure, and we will convene again tomorrow at 8:00 a.m. Thank you.

(Whereupon, the meeting in the above-entitled matter was concluded at 4:47 p.m.)