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- lunch break -
Hello, everybody. I have some brief opening comments, that I’m calling Sweet and Sour Keynotes.

01. **Sweet and Sour Keynotes** – John Senior

# 1. We have been running these DILI conferences since April 1999, and we are now having the 17th. We held them every two years in the beginning, but since 2003, we've had them every year. I think we've had some successes. The first group we spoke to was the FDA reviewers. I called Hy Zimmerman in the spring of 1998 and said, “Hy, I think we have a problem at the FDA. They are approving drugs that kill people from liver failure.” He said, “Yes I agree, that is a problem.” I asked “Would you speak if I could organize a meeting of the FDA reviewers?” He replied, “I'd be glad to.”

# 2. That was in the summer of 1998. I roposed the program to our educational specialists. And they laughed at me and said, “That's idiculous. Nobody would come? That's so obscure. Drug-induced liver injury? Who would ever be interested in that? I said, “Well I don't know but let's see what the reviewers think.” Well, 250 of them signed up
immediately. And I went back to them and said well, maybe we have to rethink this. They said, “we have no room here at the FDA large enough to hold 250 people.” So we got a larger room at the University of Maryland Conference Center at Shady Grove --- not this one at College Park, but Shady Grove. It held 300 people. We cut off registration at 300. But we had 25 more walk-ins who sat on the steps or stood in back. And then we had to run it again for 75 more in the fall, Bob Temple and I. So we reached 400 reviewers. Well, that's nice. So what?

# 3. Well, they got the message that new drugs can kill people in liver failure. And they began to require companies submitting applications for new drug approvals to provide some data on liver safety, telling them: “If you want to get your drug approved, that's what you have to do.” So they began to educate the industry, and without knowing why, it was having an effect.

The industry folks said, "Well, you had that meeting. Why weren't we invited? We would like to come." So in 2001 at Chantilly, we invited industry and have done it since. So we now have a three-way audience: we have industry people,
regulatory people, and academic consultants to both. And we've been running it that way ever since, annually.

# 4. What happened was that approvals of new drugs that killed people from liver failure stopped. We haven't had one since 1997. With the help of the reviewers and industry, the problem of drug approvals stopped, if they caused serious liver toxicity, That doesn't mean that the system is perfect or the problem solved. But it certainly is better than approving drugs that kill people. It's better to avoid the problem than try to fix it after it's occurred. It's rather hard for FDA to get a drug off a market once they've approved it.

Hy Zimmerman came to that April meeting. By then he couldn't speak any more, but his dear friend Jim Lewis spoke for him and showed his slides. And Hy came up on the stage and sat next to me. He would scribble notes and I would speak them. When Bob Temple said I’m calling this “Hy's Law”, Hy said, "No" [shook his head]. He didn't want an eponym.

# 5. But Hy’s Law was catchy and Hy couldn't argue back. Bob Temple said he had been thinking about it for over 20 years since the appearance of Hy's first book in 1978. He
had been looking for cases. He said that what Hy observed and wrote about was true; It worked. If the case was drug-induced, hepatocellular enough to cause jaundice, it was potentially serious and might kill people. Well, that was an innovation. Temple pronounced it, and wrote it into the 2009 guidance to the industry on drug-induced liver injury.

# 6. Ted Guo and I worked together in 2002-3. to develop a graphic program we called eDISH (for evaluation of Drug-Induced Serious Hepatotoxicity). It was based on the Hy's Law concept, a two-step process. First, graphic depiction of all the subjects in a study, looking in each at the highest value of ALT and total bilirubin at any time. That was only the first step, not intended for diagnosis, but a screening, incidence, and selection step. The second step was to graph, in an XY plot, for selected individuals the time course of 4 variables: 3 serum enzyme activities and bilirubin concentration --all in the same plot, time on the abscissa and the amounts on the ordinate. Because there was such a difference in scales, we used log_{10} values to bring them into range and plotted the highest value of bilirubin and ALT for each in the 1st plot. The 2nd step looked at subjects who had high chemistries individually in
detail, and also looked at a medical history or narrative to help make a diagnosis of probable cause.

# 7. But too many people still think Hy’s Law is defined only by ALT and bilirubin values. So in that, we have failed, but are continuing, at this meeting to try to clear it up. A coalition of major companies has now been put together (IQ DILI Initiative). Maybe there’s a chance to persuade not only industry people but others -- I'm talking about patients- that's a lot more people. They’re the ones who have to be educated and persuaded. They are now being besieged by advertising --- listen to the evening news. Half of the time is advertising. How can we counter this? How can we get to people in general?

We discover DILI by finding ALT rises and subsequent bilirubin elevations, when we should then begin to make a diagnosis. It is not enough just to find hepatocellular jaundice; It isn't a Hy's Law case unless drug-induced. Physicians have responsibility to treat patients, but can't treat them correctly without knowing what to treat. So they have to make a diagnosis in order to start the right treatment. It's very simple, but it isn't being done.
DR. SENIOR: Now we have a special presentation from Dr. Yimin Mao from China. He comes as leader of a contingent of 10 other Chinese doctors who are interested in DILI. Dr. Mao, you have the podium. Neil is here.

02. Recognition of Neil Kaplowitz’ book

DR. MAO: Okay, thank you. Morning, ladies and gentlemen. I'm glad to be here, and I really appreciate John Senior giving me this opportunity. Actually, this is my seventh time coming here to attend this meeting, and I have learned a lot from it. The Chinese DILI network was established ten years ago, and during the recent years I think we got great support from the U.S. DILI Network, and this makes us a great benefit. Thank you, Paul.

The book, the 3rd edition of "Drug-Induced Liver Disease" was published in 2013, by Professor Neil Kaplowitz as the chief editor. I think this book is really useful tool for us to learn more about DILI, and so we spent a lot of time to translate it into Chinese to try to introduce this book to more Chinese doctors. I think this is not only a book but also a symbol -- a sign of American-Chinese joint exploration in the DILI field. And I also believe in the future that Chinese DILI network and the U.S. DILIN will
for sure will work together and learn from each other, carry out closer cooperation.

So at this moment, I would like to invite Neil Kaplowitz, Paul Watkins, and John Senior to come forward. I will present them with this Chinese version of the book as a gift. Thank you.

DR. KAPLOWITZ: I just want to say that it's really wonderful to see Laurie DeLeve and my textbook translated into Chinese. This book, if you look at the dedication, was inspired by Hy Zimmerman. We've been talking a lot about Dr. Zimmerman this morning. We realized right off the bat that we could never do what he did, to single-handedly write that textbook. Even though Laurie Deleve and I contributed several chapters each, many of you in this audience also were key contributors to that book. And you're beginning to make me think that maybe we should do another edition. Thank you.

(Applause.)

DR. SENIOR: I'm going to turn the meeting over now to the moderators for Session I, Arie Regev and Naga Chalasani.

SESSION IA.

Best Practices in Clinical Drug Development

DR. CHALASANI: So good morning. Let's get started. The lead-off speaker is Dr. Szabo, who is, I think
Dr. Maddrey just addressed her as Madam President. She's from the University of Massachusetts, served in 2016 as president of the AASLD, and is an authority on alcoholic liver disease as I think most of you know. So we're delighted to have Dr. Szabo talk about alcoholic liver disease as a drug-induced entity.

11. Alcoholic Hepatitis as a Drug-Induced Disorder - Gyongyi Szabo

# 1. Thank you, Naga and Arie. As the first speaker, it is my honor to invite you to join me in congratulating John Senior for his leadership in putting this program and the conference together. I think without his dedication and energy that went into bringing this conference together we wouldn't be here today. So thank you on behalf of all of us.

(Applause.)

John gave me the challenging task to talk about alcoholic hepatitis as a drug-induced disorder.

# 2. When in doubt, go to the internet. I find that Sobernation.com is pretty well established saying that alcohol, obviously, is one of the most commonly used drug of abuse, but it's very clear that it is a very dangerous drug because alcohol and alcohol-related death actually contributes to about 100,000...
deaths per year. That is about five times more than other type of drug-induced deaths in general. And, of course, in terms of the reasons, this website goes into various kind of explanations that are beyond the scope of this meeting. Certainly liver-related complications and hepatitis are the major causes of death, and with alcohol-induced cirrhosis are the major contributors to the mortality of alcohol.

# 3. So if you look at the clinical progression of alcoholic liver disease, I think all of us very well know that alcohol very rapidly induces steatosis in the liver, but if somebody stops drinking then the liver goes back to being normal. We have all seen individuals who show up with alcohol-induced cirrhosis after a relatively quiet course. Some develop hepatocellular cancer on the basis of this chronic cirrhosis. But I think what really distinguishes alcoholic liver disease from NASH and many other types of liver disease is really the acute phases of alcoholic hepatitis that occur and cause very severe clinical pictures. In some patients, these episodes of alcoholic hepatitis happen multiple times if alcohol continues to be used and often lead to a more progressive disease
# 4. What we also know is that alcoholic hepatitis is a very deadly disease. Depending on the MELD score and particularly fn MELD greater than 20, 90-day mortality rapidly increases. Whenever we talk about alcoholic hepatitis as a deadly disease, we think about it in comparison to many cancers. Look at 90-day mortality, then notable is the argument that alcoholic liver disease is more deadly than even stage IV metastatic cancer in most cases. That calls attention to the need for new treatment in alcoholic hepatitis that I'm not going to talk about today, but certainly underscores its importance.

# 5. What I'm going to talk about is a little bit of insight into the patho-mechanisms of alcoholic liver disease. I realize that this is a very basic concept compared to the rest of the talks that you're going to hear today. What we understand about alcoholic hepatitis and alcoholic liver disease at this point is that there are multiple components that contribute to the really severe form of disease development. One is hepatocyte damage induced by alcohol-induced reactive oxygen species and mitochondrial damage. But this also happens in the setting where alcohol in the gut causes a lot of changes, including impaired gut permeability and translocation of various
microbial components that, together with the danger signals that come from damaged hepatocytes, turn on a perpetual inflammatory response by activation of innate immune cells and recruitment of cells, resulting in a major cytokine storm and clinical manifestations of acute alcoholic hepatitis, much overlapping the biological effect of the cytokines.

# 6. What we know about alcohol in terms of the drug effect is that, during alcohol metabolism, multiple enzymes are involved and the very rapid alcohol metabolism to acetaldehyde by alcohol dehydrogenase. It's very toxic but short-lived because alcohol dehydrogenase further metabolizes it to acetate. The problem is that these enzymes get exhausted very rapidly, after a larger amount of alcohol use, particularly in case of binge drinking or in chronic alcoholics, when the mitochondrial major oxygen species system kicks in. Byproducts of this result in reactive products and particularly cytokine. The cytochrome P450 2E1 plays a major role in this, bringing us to the overlaps that alcohol metabolism can exert on drug-induced drug metabolism and drug-induced liver injury.

# 7. One of the classic models is the acetaminophen-induced liver injury where cytochrome P450 plays a major role and through the detoxification of NAPQI exhausts the
glutathione system, leading again to a major reactive oxygen species-induced hepatocyte death.

# 8. Multiple previous studies demonstrated that CYP2E1-induced reactive oxygen species particularly affect mitochondria, leading to toxicity, apoptosis, and/or necrosis of hepatocytes. At the same time, there is also activation of immune and inflammatory pathways that contribute to and perpetuate all this damage.

# 9. What happens is that hepatocytes6 are damaged and their various components spill out into the circulation. One of those includes micro RNAs that are short, only go into no-active sequence and non-coding RNAs, and these have multiple functions. And I'm not going to go into details because in previous meetings here you heard about micro RNAs and you will hear more about them in the future. But the interesting part of this is that the micro RNAs have multiple functions. They could be explored as biomarkers. They certainly have the very good ability to target multiple, target genes and also to potentially contribute to cell-to-cell communication.

#10. Micro RNA-122 is obviously not a stranger to you because this is the micro RNA that's most abundant in hepatocytes and contributes to various processes, including regulation of cholesterol biosynthesis; it also plays a role in hepatitis C viral replication.
#11. So, interestingly, if you look at serum micro RNA-122 levels, then it appears that in alcoholic liver disease in a mouse model and also in an acetaminophen-induced liver injury model, increases in serum micro RNA correlate with changes in ALT levels. And there are some potential indicators that micro RNA-122 could be an early plasma marker of APAP-induced liver injury because some of the changes can happen much more rapidly than increases in ALT that we find, at least in this animal model.

#12. This brought our attention to investigate micro RNA-122, not only in the circulation but also in the liver in alcoholic liver disease. What we found is that in a mouse model of alcoholic liver disease and in patients with alcoholic liver disease as well, in spite of the increase in the circulating micro RNA-122, if you look in the liver, we find a significant reduction in micro RNA-122 levels in chronic alcohol exposure.

#13. So that led us to evaluate the mechanistic role of micro RNA-122. My M.D./Ph.D. student, Abhishek Satischandran, did beautiful studies using a recombinant AAV8-mediated micro RNA-122 inhibitor, a tough decoy, that he used to reduce miR-122 levels either with or without alcohol feeding. He found that if he just reduced miR-122, even in the absence of alcohol, he found
increased serum ALT levels and that were even higher if he gave alcohol to the mice that had the micro RNA-122 down-regulation, leading to the question that micro RNA-122 might have a biological effect. And then he went on and evaluated some of the alcohol metabolizing enzymes and found that CYP2E1 levels were increased by alcohol. That's in the middle kind of gray bar in the Panel A that shows that alcohol compared to the scrambled vector recipe in mice increases CYP2E1, and this would be something that you expect.

#14. Some of the liver injury, particularly in acetaminophen-induced liver toxicity, is dependent on hepatocyte death where caspase-3 activation is a key factor.

#15. In previous studies, we found that in activation of caspase-3, there is this ER-mediated signaling that we discovered in alcoholic liver disease dependent on phosphorylation of the interferon regulatory factor 3, IRF3.

#16. So normally the IRF3 activation is occurring downstream of various TL receptors, particularly in TLR4, TLR3, viral-induced pathways that result in Type-I interferon induction.

#17. We found in previous studies that IRF3 also has a role in activation of caspase-3.
#18. The way this was detected led us to the observation that IRF3 phosphorylation actually is very directly involved in hepatocyte death in alcoholic liver disease, #19. So in summary, these results suggested that if induced acute liver failure results in phosphorylated IRF3, liver injury and inflammasome activation, STING attenuates serum ALT increase inflammasome activation and caspase-3 in the liver after an APAP overdose and STING deficiency can protect, at least in mice, from APAP-induced liver necrosis and mortality.

#20. Together, our results suggest that there are at least two new molecules that seem to be at the intersection between alcohol and drug-induced liver injury; that includes micro RNA-122 and the STING and IRF3-mediated pathways that collectively can link hepatocyte death and inflammation in acute alcohol- and drug-induced liver injury.

#21. And I want to thank NIAAA for funding and these three individuals particularly: Shashi Bala who did the initial experiments in the circulating micro RNA-122, Arvin who did all the STING and IRF3 study, and Abhishek on the micro RNA-122 study. Thank you.

(Applause)
speaker. The next talk will be by Dr. Naga Chalasani. Naga is a David Crabb Professor of Medicine and Cellular & Integrative Physiology and Director of the Division of Gastroenterology and Hepatology of Indiana University School of Medicine. He's an internationally-recognized leader in the field of drug-induced liver injury, and he will talk about detection and management of DILI in NASH/NAFLD patients in drug development. Naga.

DR. CHALASANI: Thank you, Arie. Also, I want to thank you and Dr. Senior for the invitation to speak here and also to moderate the session. Congratulations, Neil, for a wonderful Chinese translation. I was looking for my name in that book.

DR. REGEV: He already read that book. Very good.

12. Detection and Management of DILI in NASH/NAFLD Subjects – Naga Chalasani

DR. CHALASANI:

# 1. As the NAFLD field has heated up, it has come up quite frequently that DILI is more common in patients with NAFLD. How do you monitor for it, given that there are baseline fluctuations in liver chemistries?

# 2. So why is this significant? I think most of you know NAFLD population prevalence is quite high. If you look with an MRI, it can be as high as 30 to 35 percent,
and there are more than 150 clinical trials, various phases, phase 1, 2, and some 3, more than 150 trials registered at clinicaltrials.gov. Elevated baseline aminotransferases are common. Sometimes you may even see increased alkaline phosphatase in the absence of an increase in ALT and NAFLD, and fluctuations are common, a little noisy and also adds to complexities. In a sizable proportion of NAFLD patients, as well as NASH, even advanced fibrosis can exist with totally normal aminotransferases. We are talking on the order of 30 to 35 ALT being not uncommon in somebody with established NASH. So the field is interesting and also complex.

# 3. I touched on the fact that ALT and AST tend to fluctuate, but they tend to stay largely under 200 IU/liter, rarely 250. Once it goes beyond, I think patients either may be on statins or maybe have low-grade autoimmune liver disease, but not more than 250 typically.

Autoantibodies are common. A third may have ANA and anti-smooth muscle antibody. Really I don't think that has any clinical significance. It could be a epiphenomenon. Also, increased ferritin is not uncommon in this patient population. Up to 500 and 600 ferritin levels are not uncommon.

# 4. Just as NAFLD doesn't increase the risk of all-
cause DILI, I have not seen any evidence that the NAFLD increases all-cause DILI across all drugs. Maybe if you have underlying fatty liver disease you may be at a higher risk for methotrexate hepatotoxicity. Some of the data are contaminated with hepatitis C patients. I’m not quite sure how rigorous those data are. There is some talk that tamoxifen may be more toxic if somebody has underlying NAFLD. Once again, it’s a possibility but not rigorously tested.

# 5. Maybe we have 10 - 15 well-done clinical trials in NAFLD,. In some other early trials there were occasional DILI cases. Most seem like compound-specific rather than underlying disease. I don't think we have seen any cases of DILI in Piven's or Flynn's trials. In trials with rosiglitazone, there was one case with DILI, Ben Tetry's (phonetic) study, and I think it was thought to be a prednisolone-related DILI. In a small trial from Australia with high-dose resveratrol, there was an increase in ALT. I don't think we're seeing DILI signals across NASH trials. It has been said before that DILI patients with underlying liver disease may have worse outcomes, first by Dr. Zimmerman in his textbook. There are now data to show that it may be the case, from Drug-Induced Liver Injury Network data funded by the NIDDK. In a paper that was published in 2015, about 10% of the
patients enrolled had underlying liver disease. DILI in patients with underlying liver disease had significantly worse outcomes, about 5% mortality in patients without underlying liver disease, but 16% in patients with pre-existing liver disease. We noted a higher frequency of azithromycin DILI, maybe just a coincidence, or over-represented in patients with underlying liver disease.

6. In earlier data about altered pharmacokinetics with obesity, there is a fair bit of literature in 70s and 80s looking at the drug disposition, but not to the same number of investigations in effect of fatty liver on drug disposition. When Steve Hall was at Indiana University, we showed that with more fat accumulation in the liver there is lower CYP3A activity.

7. In NAFLD, clearly there is an upregulation of hepatic 2E1, in one of the papers that we published. Jeff Farrell has done nice work in this area, as well. Increased 2E1 could be a bad actor predisposing to at least certain compounds. There is one paper on children with fatty liver, looking at acetaminophen at a standard dose, and I think there was a higher production of NAPQI. Whether that translates into hepatotoxicity I don't think there are any data. Although there is a lot of talk about altered pharmacokinetics as a risk for drug hepatotoxicity, when you carefully look in the
literature, very rare well-documented cases. You can see higher drug levels when drug dosing is doubled, for example 6-mercaptopurine, you see DILI but not necessarily a convincing relationship between altered pharmacokinetics, or drug interactions for that matter, and instances of well-documented DILI.

# 8. Baseline ALT fluctuations occur in NAFLD. You cannot really take upper limit of normal in monitoring for DILI. You may need multiples of baseline. Establishing baseline in NAFLD can be tricky, and agencies now are asking multiple readings during screening phase to establish a baseline. That's sort of becoming standard in NASH trials.

# 9. This is a commentary that Arie and I wrote for Gastroenterology, published maybe six months ago, on how to identify DILI in patients with chronic liver disease in early-phase clinical trials and how to manage them. This was a starting point, and some of you have challenged some of what we said. But I think, generally, there is a consensus on fatty liver or other underlying liver disease. If there is a baseline, it’s high, There is a general acceptance that you should look for multiples of baseline rather than upper limit of normal. There isn't a lot of controversy.

So here we stratified. For example, if somebody
has ALT 5x upper limit of normal, then we recommended repeat liver tests in 2 to 5 days and follow for symptoms. For those with ALT 300, as long as there isn't an increase in bilirubin and there are no symptoms, continue treatment and just follow the serial liver biochemistries. If the ALT goes above 8x upper limit of normal in those with normal baseline or if it touches 500, even if the bilirubin is normal and if there are no symptoms, we recommended interrupting the study drug, a work-up for competing etiologies, and only restart the investigational drug product if other etiologies are identified.

#10. An area that requires discussion here is whether to un-blind and continue the subject in the trial requires further discussion. It may be that you take the patient off the study and then you realize he was never exposed to an experimental compound. Regardless of what happens to AST or ALT, if the bilirubin doubles or in those with Guillain-Barre, if the direct fraction doubles, I think that indicates more severe liver injury because we have to interrupt and you have to be very cautious about re-challenging subjects. Liver symptoms feature heavily, but very rarely have I seen a case of DILI just from symptoms. When you suspect DILI just based on symptoms, it turns out to be something else, gallbladder disease or
mitochondrial toxicity. You may see a signal with INR or prothrombin time. For monitoring and assessment of DILI in patients with decompensated liver disease, it may be hard to come up with a general guidance that applies to all compounds and a very tricky patient population.

#11. So I'd like to highlight this paper. It was published last year by Dr. Hoofnagle and Dr. Bjornsson who are also in the audience. Really interesting, grading hepatotoxic potential of compounds that are available. I encourage you to look at this paper. Where I'm heading with this is: Should we be recommending that patients with chronic liver disease in clinical trials avoid some hepatotoxic compounds. It seems like a good practice to me.

I was really surprised to learn there is a study ongoing in France studying augmentin to treat alcoholic hepatitis. I'm like thinking it's the most common cause of DILI. And AASLD guidelines for treating spontaneous bacterial peritonitis, one of the choices to treat is amoxicillin-clavulanate acid, and this is the most common cause of DILI and yet it’s recommended. This also is open for discussion. Should we be cautious and list these compounds, Dr. Hoofnagles' the top hits, from hepatotoxic potential? Should we make a recommendation that these be avoided in clinical trials? There are no
data, but it seems sensible to me.

DR. CHALASANI: I think that may be my last slide. Thank you. Our next speaker is Dr. Raj Reddy, a well-known hepatologist now chief of hepatology at the University of Pennsylvania and also holds an endowed chair. He'll be talking about hepatitis B reactivation.


# 1. Thanks, Naga. Thanks Arie and then, of course, Dr. Senior for having me out here. I'm delighted to be here to talk about a topic that is interesting, and actually several in this audience, particularly Dr. Hoofnagle, has done more work than any on this area. But I'll present it on behalf of several investigators.

# 2. As an overview, hepatitis B reactivation is rather interesting. We've known about it for at least two decades, but lately it's received quite a bit of attention. It's a clinical syndrome that's characterized by an increase in viral DNA and transaminases, and some of these patients may be quite symptomatic and they may evolve onto liver failure. This can occur in both surface antigen positive and surface antigen negative but core antibody positive individuals. It can be seen in a
variety of situations, particularly in the context of immunosuppressive therapy and, more recently, there's been quite a bit of buzz of reactivation in the context of directly acting antiviral hepatitis C therapy. It can be seen in other immunocompromised situations, such as HIV infection, organ transplantation, et cetera.

It’s interesting that it may occur late, particularly with B-cell-depleting agents. It can occur up to 12 months after the drug has been stopped. And it is a very preventable situation.

3. In the literature, there’s unfortunately some heterogeneity in how you define hepatitis B reactivation. You can define it based on virologic features, or whether there’s an increase in one log of DNA, or de novo appearance of the virus. The Emerging Trends Conference has proposed a two log increase as a definition for reactivation. You can also define it as reappearance of surface antigen in someone who was negative prior to reactivation, and then biochemically, also. There's been a definition proposed where there's an increase of threelfold or more in ALT levels if baseline levels are normal, twofold or more increase over baseline levels if initially abnormal.

4. In this case sent to me by Dr. Perrillo, who has done quite a bit of work in this area, we can see that
the patient underwent breast cancer chemotherapy, had evolution of abnormal ALTs. Chemotherapy was discontinued. The patient had a significant biochemical and bilirubin flare. Hepatitis B therapy was initiated while the virus was controlled, and the patient went on to die. The ALT came down, but bilirubin continued to rise.

# 5. And I must say, sadly, this is not an uncommon situation. Every year I get calls from an oncologist at least two to three times that say a patient with breast cancer, an Asian woman treated for breast cancer had jaundice evolve, and the first thing they're talking about is drug hepatotoxicity. I said, no, this is not drug hepatotoxicity. Have you looked at hepatitis B? Sure enough, it's reactivation. It's not a phenomenon that is well recognized in the oncology world, but it's getting better.

# 6. So hepatitis B may reactivate in several situations, but what's important to recognize is that it can't reactivate in someone who just has antibody core. While intuitively we would think it also could react in someone who's got surface antigen positive infection that might be in an inactive state, but the most important point I want to make is that, if you look at row F, there's an isolated core antibody with negative surface antigen, and
these patients can reactivate.

# 7. So why do patients reactivate hepatitis B despite serologic evidence of viral clearance? These patients' viral DNA persists in the liver even after resolution of infection, because cccDNA resides in the nucleus of hepatocytes, and may become active. While replication is suppressed with an intact immune system, with immunosuppressive therapy it can activate.

# 8. So here you can look at it in a different way. One end, pre-chemotherapy. The patient is surface antigen positive. Viral DNA may or may not be present. ALT may be normal, and there are innate and adaptive mechanisms that control the virus. If during chemotherapy you suppress the immune system, there is viral replication. ALT may become abnormal. And when you stop chemotherapy or biologics, there is immune activation and liver cell necrosis, severe clinical manifestations, or death.

# 9. The probability of hepatitis B reactivation depends on several features: on the type of immunosuppression, the status of hepatitis B, and duration of immunosuppression.

# 10. So at one end, there's relatively low risk of reactivation because they're surface antigen negative, there's low level of replication, and these patients, if they're exposed to corticosteroids, there is very little
risk of reactivation. On the other hand, if you are someone who is replicative and then exposed to a drug such as a B-cell-depleting drug like rituximab, there's a high risk of reactivation.

#11. And then, of course, you have patients who are in between who have a moderate risk of reactivation. And, again, the risk depends on the type of immunosuppression.

#12. So there are several drugs that have been implicated in hepatitis B reactivation, and I'll address these drugs in the context of a reactivation.

#13. Now, there's an eloquent review written by Rohit Loomba and Jake Liang that I highly recommend that you read. It came out this year, where they outlined hepatitis B replication life cycle and hepatitis B is under control due to innate and adaptive immune mechanisms. There are several drugs that potentially can influence any of the pathways of hepatitis B replication and lead to reactivation. For instance, you have the JAK-STAT pathway and you can have kinase individuals influence on their B cells and T cells. You have B-cell-depleting drugs, such as the monoclonal anti-CD20 that can influence and cause reactivation.

#14. A couple of years ago, some of us looked at the data that's in the literature and stratified the risk of reactivation by the type of drug. Unfortunately, I must
say that the data are not robust, largely. And you can see the breadth of risk of reactivation, say with B-cell-depleting agents in surface antigen core antibody positive individuals that could be anywhere from 30 to 60%, and the confidence and estimate in terms of it being a situation for high-risk reactivation is A. But then you have anthracycline derivatives where the risk in surface antigen positive individuals is 15 to 30%. Corticosteroids also can induce a high risk for reactivation, but the confidence in estimate is B.

#15. Then you have moderate risk situations where the risk varies anywhere from 1 to 10%. You look at TNF-alpha individuals, other cytokine and integrin individuals, et cetera. The literature is rather sparse here, and the data are not robust, but the risk can vary anywhere from 1 to 10% with these agents.

#16. And then you have low-risk drugs, such as azathioprine, 6-mercaptopurine, methotrexate, intra-articular corticosteroid injections, et cetera, where risk is less than 1%.

#17. One of the earlier papers published goes back to 1991, by Anna Lok from Asia, where 100 patients with non-Hodgkin's lymphoma undergoing CHOP therapy were looked at and had evidence of hepatitis B reactivation. It was about 48%. A fifth of patients had jaundice and four
patients died. This was one of the earlier reports on reactivation of hepatitis B in hematologic malignancy.

#18. Now this is a more recent experience, and the reason I'm presenting it here is that we would think that antibodies to hepatitis B surface antigens would be protective against reactivation, but it's not the case.

#19. If you look at the hepatitis B reactivation group and at the various color codes that depict the types of anti-HBs, you can see that even those individuals in anti-HBs levels between 10 and 100 million IU/mL had reactivation. So in the context of rituximab, it would be wrong to think that anti-HBs would protect against reactivation.

#20. This is a meta-analysis and a review of FDA safety profiles done a few years ago. There were 27 case reports and 156 case series reports. And the important point about this is that in about 29% of patients there was reactivation more than six months after rituximab was stopped, and so that is the key point with rituximab. We can see reactivation even up to 12 months after rituximab has been stopped. And here even the anti-HBs positive patients had reactivation.

#21. If we were to look at anti-TNF agents and HBV reactivation, this is a compilation of several studies. Patients who were treated were categorized into surface
antigen-positive and core antibody positive and surface antigen negative and core antibody-positive groups. These were patients treated with anti-TNF agents for inflammatory bowel disease and rheumatologic conditions. The important point here is that the reactivation in red was more seen often in those who were surface antigen-positive. There were relatively few who had isolated core antibody-positive in the context of anti-TNF who were reactivated. 

#22. Here are some data with abatacept and HBV reactivation showing an inhibitor of T-cell activation; Clinical studies have largely excluded patients who tested positive for hepatitis B at screening, yet there are reports of reactivation with this drug.

#23. There are several guidelines that have been written and are in the literature. There is some heterogeneity with regard to who should be screened prior to immunosuppressive therapy. Some guidelines suggest that all patients should be screened, including CDC, EASL, and APASL guidelines. Some of the guidelines recommend screening for patients based on HBV reactivation risk. Surface antigen and anti-core certainly are necessary to do, but some suggest doing hepatitis B viral DNA as well. Also some suggest that in isolated anti-HBc cases you also do a hepatitis B viral DNA.
#24. Now, there's a bit of heterogeneity in prophylaxis, as well. There's no argument that all surface antigen positive patients be prophylaxed in immunosuppressive therapy, especially high-risk immunosuppressive therapy. If someone is anti-core alone-positive, some suggest to prophylax them only in the context of detectable viral DNA, and then timing of initiation also is a bit different according to the guidelines. Some suggest at or before onset of immunosuppressive therapy. The ASCO guidelines suggest that patients may alternatively be monitored and treated with on-demand therapy if they have reactivation rather than starting on prophylaxis from the get-go.

#25. There's, again, heterogeneity in terms of duration and various guidelines, but the most important point I want to make is that, in certain situations in the use of B-cell-depleting agents, 12 months of prophylaxis is being recommended by both ASCO and AGA.

#26. There are enough data to support the use of prophylaxis. Here are randomized control trials of HBV prophylaxis where, clearly, intervention showed a decreased rate of hepatitis B reactivation with various drugs that have been used that are commonly used to treat hepatitis B. So I think the data are strong enough to support the use of prophylaxis.
#27. For the choice of anti-viral prophylaxis, the consensus is that we should use drugs with high genetic value for resistance, suggesting tenofovir or entecavir. Lamivudine, while it's been studied extensively, particularly when it was the only drug, is associated with resistance, so it's felt that we should use high-genetic value drugs. In some parts of the world where cost may be a factor, lamivudine plus adefovir may be an option.

#28. Now, I will briefly talk about a couple of the guidelines here. One was put together on behalf of the oncology and liver community where there was risk stratification made and certain prophylactic strategies have been recommended. For high-risk drugs, we obviously want prophylaxis.

#29. For moderate risk, it's either prophylaxis or not, on demand. And with low risk, low prophylaxis.

#30. Now, the AGA, based on the data out there, has come up with its own guideline. For high-risk people where the reactivation risk is more than 10% because of B-cell-depleting agent, their grade of recommendation is strong and there's moderate quality of evidence, and here we would recommend 12 months of prophylaxis. If the patient is exposed to anthracycline derivatives, 6 months is adequate.
#31. For moderate risk, your syllabus says prophylaxis is for 6 months. This was based on the hepatitis B serologic status and the type of drug they're exposed to. Unfortunately, it's a weak recommendation because the evidence is just not that robust in the literature. So there's really a need to generate good data in a number of situations.

#32. For low risk drugs such as azathioprine, 6-MP, and methotrexate, prophylaxis is not necessary.

#33. Lastly, I want to talk briefly about what's been going on in the hepatitis C field where there's been hepatitis B reactivation reported in the context of DAA therapy. Initially, there are case reports, two case reports, one surface antigen-positive and one core antibody-alone-positive patient, a patient who received DAA therapy. They were started on oral drugs. The red lines indicate drop in viral DNA, the blue line indicates increase -- I'm sorry. The red lines include, indicate drop in hepatitis C viral RNA and the blue line indicates increase in hepatitis B viral DNA.

So you can see on oral therapy, it came down but hepatitis B viral DNA went up. In one case, ALT and AST went up. And when hepatitis B therapy was started, hepatitis B viral DNA came down. Then FDA came out with a warning about the risk of hepatitis B reactivation in the
context of hepatitis C directly-acting anti-viral therapy.

#34. And this was based on about 29 cases that were submitted to the FDA; it has come out as a publication in Annals. Unfortunately, there's a good amount of data missing in these cases, mostly from Japan. Nineteen cases from the U.S. and 5 from other parts of the world. It usually occurred within 4 to 8 weeks of DAA therapy. There were 2 deaths and 1 transplant report.

And if we look at the pie chart, about 38% of the patients had very little to poor data. And then on the other side of the world, three patients antibody-to-core-alone-positive. What we understandably don't know is the denominator number of patients treated with directly-acting antiviral drugs. Globally, there're several hundred thousand people have already been treated for hepatitis C. So we really don't know how frequently this happens.

#35. So FDA recommends monitoring during DAA therapy for reactivation. This is obviously something that people have been debating as to how frequently we should monitor and how we should monitor these patients.

I'd like to present some data that my colleague, Marina Serper, generated from the VA cohort. She looked particularly at anti-HBc alone patients. She had 22,769
anti-HBc patients in the VA system who were treated with directly-acting anti-virals. And they excluded several because of missing data. There were about 19,000 anti-core-alone-positive, anti-core-positive patients, of which 222 also had surface antigen, but the vast majority were surface antigen negative, anti-core positive, 18,840 surface antigen, anti-core positive patients. And there were 6 cases of reactivation in the entire cohort, but I want to impress upon you that 5 of the 6 cases were surface antigen-positive. Only one was core antibody-alone-positive.

So the question is: are we making a mountain out of a molehill? Are we in a situation where we really should monitor these anti-core alone positive patients while on DAA therapy, or is this just not a common phenomenon in these patients and that they should really be treated like anyone else without anti-core positivity? #36. The AASLD has come up with guidance on this. They suggested that surface antigen-positive patients should be assessed for viral DNA prior to, during, or immediately after HCV DAA therapy. And if there is active infection, obviously you initiate hepatitis B therapy. For those who have low replicated or undetectable HBV DNA status, you monitor for HBV reactivation, but there are insufficient data to provide
recommendations for patients who are anti-core alone positive with or without surface antibody.

#37. So to conclude, reactivation of hepatitis B is a concern with immunosuppressive therapy. The risk is variable and dependent on the type of immunosuppressive therapy. All patients should be screened for surface antigen and anti-HBc prior to initiation of biologics and other immunosuppressive therapy.

Assess risk for reactivation based on HBV serology and plan immunosuppressive therapy. There are guidelines in place. Information is needed regarding HBV reactivation associated with DAA therapy for HCV.

#38. Thanks very much.

OPEN DISCUSSION - IA

DR. REGEV: Can we please call the speakers to the podium? We're a little bit late; we started a little bit late. We are going to begin the discussion now. If anybody has questions, the microphones are in the middle of the room.

I'm curious. I have one question to Raj regarding this new line of drugs for hepatitis B that are now attempting to actually kill the virus finally. We are now in a new mode of treatment, and one of the interesting issues coming up is the fact that some of
those drugs were associated with a very common flare during successful treatment. So not only HBV doesn't go up, but the patient seems to be fine and ALT goes up with AST, and the question is how do we differentiate these particular incidences from the drug-induced liver injury, as opposed to a good flare? Any insight on that?

**DR. REDDY:** So you're talking about drugs that get to cccDNA; is that correct? So as to whether this is a flare of virus or drug. So I'd guess I'd have to depend on other B markers in trying to define if this is a flare of B or whether this is drug-induced. I'm not sure how else I could differentiate it. So the viral DNA is rather -- I would expect it to go down. But if it's not gone down, it's gone the other way, or surface antigen -- well, there's always surface antigen positive. One thing that has become available is surface antigen titer measurement and whether that might provide some insight is a question. But apart from looking at viral markers more carefully and, of course, looking at the time course of appearance of this phenomenon, I'm not sure how else one can differentiate. Obviously, that's going to be an interesting challenge to try and sort out.

**PARTICIPANT:** Yes. I have a question for Dr. Reddy. How do you understand the reactivation you get of hepatitis B with antibodies that target B cells, not antibody-
secreting plasma cells, not the long-lived plasma cells that continually secrete antibody but the B cell precursors of those cells? It suggests to me that there's an ongoing need for new B cells to convert to antibody-secreting plasma cells in the state of latency. It's a little surprising. I would have expected, for instance, that with rituximab treatment you would see these kinds of flares because that kills off the antibody-secreting plasma cells. So I'm curious to know what your take is on the specificity of that response.

DR. REDDY: I was going to have Gyongyi, who is an immunologist, help me out here because I'm not sure I can address it.

DR. SZABO: Hepatitis B is not my strength, but I find this entire sort of hep B reactivation particularly during hep C treatment very compelling and intriguing. If you just think about how the directly-acting anti-virals affect the hepatitis C infection, the idea is that you hope immune response and the anti-viral immune response to get rid of hep C virus. And then why is that now all of a sudden that is not having an effect on hep B? So if I had to speculate, I would say that probably is not related to the type of interferon that you need immune kind of responses, but, most likely, somehow the antigen-specific T-cell responses get sort of switched in a way
that the hepatitis C immunity works, but somehow for the hepatitis B that sort of is not going to be enough to take control of the hep BV infection. In terms of the B-cell precursors, I think it's very fascinating. I don't know if immunoglobulin switching is not happening fast enough to get the antibodies produced. I think that could be kind of a target there to look at.

DR. REGEV: Mark.

DR. AVIGAN: Yes, thank you. Just anecdotally, when I was director of a division that saw the rituximab signal early on, and I didn't believe it because the target of rituximab is B-cells and not T-cells, and the idea of cytotoxic effects of infected cells with T-cells was not appealing, but the rituximab effect was very dramatic. So I think that the point that you're raising is very interesting and bears some thought. I want to ask you, Gyongyi, a question about miR-122 effects and the potential interaction between these two pathways since you show that the effect of down-regulation of miR-122 has an effect to actually augment inflammation and damage with alcohol and perhaps other mechanisms, as well. So that's a very intriguing finding. I just was wondering whether you have an idea of what the target of the miR-122 is as an RNA molecule and whether it's interactive with staying in the RF3 pathways or some
common sort of mechanism as a downstream target?

**DR. SZABO:** I think those are fantastic questions. I don't think that I have an answer to that, actually. This is something that, you know, we are developing these, kind of identifying these questions. The miR-122 has so many targets, and I think most of the targets have been related to cholesterol metabolism. So at this point, I don't think that any of the inflammatory cytokine genes necessary are direct targets of miR-122. So in terms of the pro-inflammatory effect or what we see in animals after reducing miR-122, I don't think it's necessarily a miR-122 effect. It might be just related to the hepatocyte death that is being induced and as a secondary induction of inflammation. In terms of STING and miR-122, I don't know -- I don't think that is a direct target of miR-122.

**DR. AVIGAN:** And just on the effect of miR-122, is it a sequence-specific effect? If you alternate the sequence of the RNA to something slightly different, do you still get the effect? If you mutate or create a --

**DR. SZABO:** To my knowledge, nobody has done that.

**DR. REGEV:** Jay.

**DR. HOOFNAGLE:** Yes, Jay Hoofnagle from Bethesda. I'm glad we admit that alcoholic liver disease is drug-induced liver disease. Let me point out, though, that
alcohol is a terrible drug, and we have all these drug
development people here and I would encourage you to
develop a better drug because the problem is you can't
control the dose and you see the effects of it. And its
kinetics are all bad. Everybody is always withdrawing
and so forth. Anyway, that's my comment about alcohol.

DR. SZABO: I didn't say it was good.

DR. HOOFNAGLE: I'd like to make a point on other things.
I hate these upper limit of abnormal things, three times,
five times, eight times, ten times. Every group has a
different cutoff, and I, frankly, think that it's
meaningless. It's meaningless to talk about the upper
limit on normal, particularly when the baseline is high.
You say it has to be three times. Well, why is that?
What's the evidence of that? Why should it be three
times if it's slightly elevated? Because this is
separate. The injury that's being caused is separate
from what's there already. And it also is confusing to
the community. Even if the bilirubin upper, I don't even
know what twice the upper limit of normal bilirubin. I'm
a hepatologist, but I don't know what you're talking
about. I think it would be very good to go to the actual
levels and say this is a level that is serious or
whatever you want to call it and forget about the upper
limit. I mean, what is five times the upper limit of
normal? You have to think about it, don't you? But if you say 200, if you say 500, if you say 1,000, those are meaningful, and I think the community, if we want the community to pay more attention to that, will understand that better.

You know, the NCI, the AIDS Clinical Trial Group, and the FDA have completely different cut points for upper limit of normal, and this type of confusion could be stopped by going to the actual absolute values.

DR. CHALASANI: I think that's a good point, Jay. And I have also spoken support previously about going to an absolute, whether it's 200 or 250. Once again, it's going to start with consensus. One of the arguments that was previously made about an absolute cutoff was machine-to-machine variability. But today that doesn't seem like a big deal. Most of the instruments, you get a sample, cap surveys very good reproducibility. So I think you make a very valid point. I don't know where you start it, whether it is going to be the FDA that is going to make that recommendation or the academia, but I think it's overdue I think.

PARTICIPANT: Okay. I have a question for --

DR. REGEV: Can you state your name, please?

PARTICIPANT: Okay. You show the APAP model for in the clinicals and not to all for APAP patients who have got a
liver injury. So in your experiments, can you tell something about differences for APAP and liver injured model? How about the ratio of the liver injury in all APAP? Is there some difference for APAP and liver injury model in mice, rats, or human?

DR. SZABO: So the experiment that I showed you, and I'm sorry for not getting into the methods too much, it was a sub-lethal dose of acetaminophen given to mice. I don't have data on rats, and, obviously, we didn't do this experiment in humans as an experiment.

DR. REGEV: So just a reminder, before each question, can you please state your name and affiliation? This is for the transcribing person to be able to put it in. Yes, sorry.

DR. SHYAM: Hi. Oops, too close. Morning. My name is Rishab Shyam from PureTech. A quick question on the micro RNA story. I was wondering how generalizable it is to other diseases you looked at the case of acetaminophen. I'm just curious are there any thoughts of applying it to other cases where there's manifestation of liver injury? And I might have missed the dynamics of how the level changes in response to disease. How sensitive is that? I'm curious. Is it post-injury, pre-injury? Can you predict it? I might have missed it. And, lastly, how does that fit into the next rubric or
set of markers that you look for in addition to just the
ALT levels? How do you see that all playing together?

DR. SZABO: Right. So in the animal model, and this was
a paper we published quite a few years ago in Hepatology.
We evaluated circulating RNA-122 levels after various
types of liver injuries in mice. So it was alcohol, I
showed the APAP. We also used a kind of immune-induced
injury with granular formation using TLR9 and TLR4, like
a stimulation or NASH. And in all of those cases, we
found increases in serum micro RNA-122. And if you look
in the human literature, there are increases in serum
micro RNA-122 in pretty much any kind of liver diseases.
So what that tells me is that miR-122 increase is not
specific necessarily for a particular types of liver
injury. It probably is just a marker of hepatocyte
injury. It may or may not -- actually, it seems like
there is some correlation with the extent of injury
because we see way higher levels within APAP as opposed
to other type where AST is lower. Dr. Lee actually had a
very nice paper on micro RNA-122 from a drug-induced
liver injury that showed that in drug-induced liver
injury you see an increase. But, again, I don't think
that there is any evidence that this would be specific
for any particular types of liver diseases.

DR. CHEN: This is Minjun Chen from FDA NCTR. I have a
question for Dr. Chalasani. I think you recommend we should avoid use of drug with hepatotoxicity potential if a patient has a pre-existing disease. I think we all agree with that. And you also gave a recommendation for equating hepatotoxic potential with chemical report, medication report with A, B, C, D, F, G. I think for FDA, for the chemical trial, we probably don't have this case report. So how we can assess, you know, for the hepatotoxic potential for the new drug, you know, in the chemical trial? Do you have a recommendation for that part?

DR. CHALASANI: I would actually ask Dr. Hoofnagle. So he's asking about the paper that you and Dr. Bjornsson had about the hepatotoxic potential. What do you suggest about the new compounds? And I think you briefly, at the DILI meeting, you had some thoughts about having some new categories. Okay. It's going to come up tomorrow. Okay.

DR. CZERWIEC: Hi. Frank Czerwiec from Otsuka, and my question is for Dr. Szabo. I love experiments of nature, even if we cause them, so your knockout information or details for STING and IRF3 were very interesting. I'm wondering if the converse might also be true, whether in different strains of rodents or, in fact, in different humans or different humans with different diseases you
can find an increased susceptibility to DILI that is evidenced by an upregulation of those pathways at baseline? Is that in existence or are those data available?

**DR. SZABO:** I think you are hitting a very important point. That certainly could be a next step of investigation. I don't think that the concept of this path is potentially having a role in, you know, drug-induced liver injury is something that anyone looked at. So I'm glad to be here and your ideas to do. But, you know, polymorphisms in humans exist for those genes, and that's certainly something that could be explored. But to my knowledge, there is no data on that.

**DR. CZERWIEC:** So GWAS and other things haven't specifically looked at it, but these may be potential targets for that type of evaluation for idiosyncratic susceptibility?

**DR. SZABO:** One can hypothesize that based on the data that I showed, yes.

**DR. CZERWIEC:** Thank you.

**MR. DASH:** Ajit Dash from Roche Genentech. My question is for Naga. This pertains to the altered metabolism in NAF and NASH and hypothetical risk it might pose for increased chance of DILI. You spoke about CYP3A4 and 2E1. I wanted to take on transporters and how they're
impacted by NAF or NASH. There are some animal studies and I think there's one paper on pediatric NASH. In your experience in clinical trials, did you see an impact of transporter alteration and any effects?

**DR. CHALASANI:** I've not seen clinical evidence of -- I'm aware of the transporter data from Tucson, so I think there seems to be some alteration in transporter expression at least in samples, pediatric liver samples, as well as pediatric kidneys for that matter, you know, some of the MDR. But I've just not seen any evidence that translates into meaningful alterations in pharmacokinetics or drug interactions.

**DR. LEE:** Yes, hi. Lee, Dallas. Dr. Szabo mentioned our paper, and I just wanted to go over one of the points and ask her a question back. In our series, when we looked at miR-122 in acetaminophen cases with the 5,000 ALTs, they had very high miR levels as if it's just leaking out of cells. But in the hep C patients with even just an ALT of 45, they would have levels nearly as high as the acetaminophen cases. So the question is what's happening there? Is miR-122 secreted in certain situations? Do you have any ideas as to how it's leaving the cells if the levels are similar to what we see in, you know, very high ALT settings?

**DR. SZABO:** So that's interesting. I must admit I don't
think I remembered that part of your paper with the hepatitis C infection.

**DR. LEE:** Yes, that's one of our control groups.

**DR. SZABO:** So in the serum, miR-122 is depending, I think, on the type of liver injury, actually. We find that it could be either sort of a free proto-infection probably bound to ALGO2 or some of the other carrier molecules, or it could be encased in or in the cargo of extracellular vesicles, and that may make a difference in the kind of -- I don't know, methodologically, how it was captured in terms of physiology, and then why would there be more or equal levels. I think it's very interesting. I mean, when it comes to extracellular vesicle contents, it's very clear that certain microns actually get sort of preferentially sorted to extracellular vesicles from cells. And that could be potentially a difference between hepatitis C and APAP, but I don't have any particular mechanisms for that. I don't think anyone has ever looked.

**DR. LEE:** Could it be that very high levels in acetaminophen is what turns off the acetaminophen injury?

**DR. SZABO:** So I don't know. I don't think that there is any experimental data for that. The only thing I can offer is that in alcoholic liver disease we find that the increased micro RNA-122 in the serum actually is in, when
it comes out from hepatocytes it is in extracellular vesicles and exosomes. And if we put those exosomes onto a naive macrophage, then that increases the susceptibility of those macrophages to respond to LPS and produce more inflammatory cytokines, suggesting miR-122 that normally is not in the macrophage but now it gets introduced through an exosome from a hepatocyte origin, that can change the phenotype of immune cells.

DR. REGEV: So, Will, I have a question about the data that you just mentioned. So you measured at least once, I assume, and the levels were very high. Did you then measure additional tests over time and it remained very high even in patients with ALT of 40?

DR. LEE: Yes. I can't remember how many serial ones. We actually did some through treatment, and it did go down to some extent once you got an SVR in the hep C treatment cases. But it didn't go down right away, interestingly enough. Even on treatment, it was still elevated as if it's almost an inflammatory marker.

DR. REGEV: So miR-122 remained elevated at similar levels to APAP levels throughout the --

DR. LEE: Yes. Maybe 80 percent, but it's certainly up in similar range, which seemed quite bizarre in a way.

DR. CHALASANI: Last question, please.

DR. AJAO: Adebola Ajao, FDA. I just wanted to make a
comment about the VA study that was looking at hepatitis B reactivation in patients taking DAAs. I believe that study was presented to us late last year, and I think one of the limitations for that study was that only a fraction of the patients were actually followed up. I think it was around 20 percent. So in order to really understand who's at risk for reactivation and to quantify the risk, we need large studies that are followed-up patients with good follow up in order to be able to really understand what is the magnitude of your risk and what is the risk factor for reactivation. I think that study was the first large study that looked at that question, but the limitation was that only a small proportion of patients were actually followed up.

**DR. REDDY:** Well, this was just presented at DDW this May. Maybe it's the same study, but my colleague, I think, started looking at it this year. So it's going to be hard to get data on a huge cohort of patients with good robust data. Again, you know, if you look at, globally, over a million people have been treated for the hepatitis C and treated in Asia where the prevalence of antibody to core is quite high. And who has hepatitis C? People who have been, and that is in the U.S. and used IV drugs, and there is a high prevalence of antibody to core in them. And, you know, suddenly now it's become a
concern. And I grant you that we need more data. But we also want to be careful in trying to generate some concern because the idea is to globally eradicate hepatitis C now. If you were to say antibody to core alone positive patients should not be monitored, you know, a fair number of resources concerned areas they don't have ALT, they don't have RNA access. They're just giving them 12 weeks of treatment and then hoping that they eradicate the virus. So that's where I have a bit of a challenge. And I think we really have to be careful in the core antibody alone patients. It's quite prevalent, and I personally am not concerned. Well, let's see if more data comes out we'll see in time.

DR. CHALASANI: All right. Thanks to all the speakers for a wonderful session. We'll take a 15-minute break and be back here by 10:05. I think there's coffee and maybe snacks outside.

(break, 9:51 to 10:10 a.m.)

DR. CHALASANI: Can we please get settled for the next session, please?

DR. REGEV: So, okay. The next two presentations, the two talks, will hopefully start a debate. And the topic of the debate will be something that we have been seeing in recent years, and have been
discussing more and more, and this is the phenomenon of chronic liver injury developing after acute idiosyncratic hepatocellular injury. It is very specific, those patients that come in with acute hepatocellular injury, and some of them do or do not turn into a prolonged, sometimes lifelong, chronic liver injury.

And the first presenter will be discussing the opinion that this is a prominent phenomenon that should be taken extremely seriously. The next presenter may say we may need to take it seriously, but may say it's not as big as we think it is. So the first speaker to support the notion that it is a prominent phenomenon will be Robert Fontana, who is sitting right here next to me. Bob is a Professor of Medicine and Medical Director of Liver Transplantation at the University of Michigan. He's a principal investigator of one of the six DILIN clinical sites, among many other things that he does with drug-induced liver injury. He will talk about chronic liver disease after acute hepatocellular DILI. Bob.

14. **Chronic Liver Disease After Acute Hepatocellular DILI**

- Bob Fontana

# 1. Thanks, Arie. I'll do my best to represent my views on this, and as you said, maybe we'll have a lively debate or discussion afterwards.
2. I'll first review briefly the natural history of DILI, and then focus in particular on data that we've generated about what chronic DILI looks like, who may be at risk, and what predictors might be. This might be an opportunity for an interventional study.

As John Senior mentioned this morning, before you start to model things, you first have to establish that you do have idiosyncratic drug-induced liver injury, which is a whole topic in and of itself, and I'm not going to go through causality methods otherwise than to say that you have to come to a diagnosis first.

3. This requires you to find certain features such as a temporal association between exposure to the drug and the liver injury onset. In most drugs, that's less than six months, although there are very important exceptions. Then you stop the agent or suspect product, and then you see improvement, but that doesn't always happen if you have fulminant hepatitis.

Then you look for the phenotype. What are the labs? What are the symptoms? What is the histology, and is this compatible with what has been reported? Is a pattern recognizable?
This gets convoluted by the fact that patients take multiple drugs. So how do you know it's drug A versus B. You simultaneously have to rule out competing causes, but as we've heard earlier today, underlying liver disease is more and more prevalent in our patient population. This gets confounded; alcohol is always in the background, and in an aging population, pancreatic or biliary disease.

So we continue to work on this from a clinical perspective without an objective confirmatory test. That being said, you can establish a diagnosis of DILI overall. # 4. And what do you do? It's important to recognize it so that you allow no further harm. You want to stop the suspect drug as quickly as you can. You know there's no specific treatment currently advisable beyond supportive therapy, fluids, rest, hospitalization.

A lot of us use urso sort of indiscriminately in patients who're itching. It’s probably a benign drug. We tend to use steroids in the sicker patients if they're not improving or if they happen to have immunoallergic features from presentation, not prospectively established.

It's important to recognize the importance of the first thing, which is to stop the drug. Retrospective data have shown that if the drug is continued, there is a higher risk of acute liver failure or that the DILI can progress.
There's a study from 1999 from the U.K. where they retrospectively looked at patients identified as having DILI, and followed them. In these highly selected cases, 39 % who had biopsy-proven DILI, had either an abnormal ALT or abnormal liver imaging at five years of follow-up, suggesting that there is a subgroup of people who may go onto progressive chronic liver disease, but the analysis was retrospective.

# 5. In DILIN, we've been prospectively studying patients throughout the U.S. for the past 13, 14 years at multiple sites, as many of you know.

# 6. Entry criteria were: anyone more than age of 2 who can be enrolled within 6 months of injury onse, defined as AST or ALT >5x ULN on at least two consecutive blood draws or alk phos greater than 2x, or total bilirubin greater than 2.5. We did allow patient's with known chronic HBV, HCV, and HIV into the study.

# 7. The study design was quite simple, observational. Someone taking medication, gets injury, meets entry criteria. We have to enroll them within six months, and then we follow everyone for another six months after their baseline visit.

When we designed the study, we thought that we might have patients who might end up with chronic disease, and if they did, we would then want to follow them out to two
years. I'm going to show you data on the two-year follow up. We've now extended it to four years. But don't have data yet on the four-yet follow-up.

# 8. Naga already referred to the paper here from the first 899 high causality cases, published a year ago. A single prescription drug acetaminophen, was responsible for 62% of the cases. Antibiotics cause 45% of the cases, with amoxicillin clavulanate being the leading cause of liver injury in adults in the United States, followed by INH and nitrofurantoin.

We have a fair number of herbal and dietary supplement cases as well as multiple products implicated simultaneously. And this is a cohort, so we have a broad distribution of ages, with 6% who are pediatric patients.

# 9. Mean age overall was 49, with slight female predominance. Demographics in the U.S. show a predominance of caucasians. BMI average was 27, but we tended to get fairly sick patients. The peak ALT was 1000, peak bilirubin was 13, and the proportion of hepatocellular to mixed and cholestatic of 50 percent in 25/25, is essentially what's been seen in other prospective registries as well.

So with all that in mind, we have the opportunity now to see what happens in a large group of patients who have drug-induced liver injury.
#10. This was a paper we wrote about three years ago, looking at high causality, a slightly different cut of data on 660 patients with high causality scores. We asked what happened to them within six months of injury onset. There were about 9.4% who had died or gotten a liver transplant within the first six months of injury onset, so 91% recovered.

We have prospective data establishing now those numbers with entry criteria. Amongst those who recovered, some of them went on to have chronic injury and others didn't. In our cohort of the ones who didn't die or get transplanted, about 19 percent had chronic liver injury by our definition, and the rest had self-limited or resolved liver injury. These are the clinical features that should be worried about in clinical practice.

#11. Here are data comparing those who died or got a liver transplant to those who didn't. There're some interesting differences. Asians, for unclear reasons, had a higher rate of death in transplant, as did diabetics, higher AST or ALT levels and bilirubin levels. Not surprising.

We tended to use steroids, I think, out of desperation more than anything else in the patients who didn't do well. And when you do multivariate analysis, we actually had a pretty good model with area under the ROC curve of 0.89. There was a multitude of factors. In Asian
races, low albumin or low platelets, high AST, high bilirubin, and underlying lung disease were all independent predictors of who was going to die or need a liver transplant.

#12. Now there's a newer paper that's just been, it's on the Hepatology website by Skip Hayashi, who's here, describing these deaths in a little bit more details. We obviously knew they had died or had been transplanted, but then we went back and looked at all the deaths in more detail.

In this analysis of 1,089 causality adjudicated cases, we found 107 fatalities. We looked not only at death within six months but also up to two years, because what if someone wasn't doing well and needed a transplant at 12 months after the DILI onset. Interestingly, it turned out that DILI was the primary cause of either the death of the transplant in 64% or a significant contributory factor to the outcome, but 22% died of other causes. Most of those cases had underlying malignancy or heart failure or other organ failures. When they died or needed a transplant, most of them showed fulminant hepatitis within 24 weeks of onset. In 7% preexisting liver disease leads to liver failure and/or death.

#13. When we looked at the 68 cleanest cases, where DILI was felt to be the primary cause of the death or
transplant, we saw a couple of different patterns. Again, ALF, death of transplant within 26 weeks of injury onset, was the most common pattern. They tended to be younger patients, with mostly hepatocellular injury, high bilirubin levels. Hy's Law was present in 51%. The range from DILI onset to that outcome was two to 123 days. Then there were the acute on chronic liver failures. Again, there was a little less hepatocellular injury, fewer meeting Hy's Law criteria, and rapid cholestasis, which is interesting. Patients who had cholestatic injury from the get-go, only four, died within six months, and they died a liver-related death.

#14. A new group, chronic liver failure, who died between 6 and 24 months after injury onset, were not hepatocellular. Their bilirubin levels were not necessarily that high, showed subacute course and none met Hy's Law criteria. Deaths can occur as long as a year or later after injury onset. Looking them, compared to people who didn't die, higher bilirubin, INR, lower albumin and platelets were associated with bad outcomes. The MELD score actually did very well in this analysis.

The chronic group of $19$ included those who went on to chronic liver injury (persistently abnormal AST, ALT, alk (phos, or bilirubin if it was normal to begin with), or if they had a liver biopsy more than six months after
injury onset or evidence of portal hypertension by imaging.

In them we found different predictors. Age really didn't make a difference, but race did. African-Americans were more likely to go into chronic liver injury for unclear reasons. Cancer patients were more likely to have chronic phenotype, and bilirubin and alk phos levels tended to be higher at the initial injury onset.

Factors that were independent included African-American race, longer use of the medication, higher alk phos levels, and higher bilirubin when they first presented, although the model wasn't quite as robust.

#15. Now the issue here is, are we just an epiphenomenon. Is this just injury that's going to resolve over time if you follow beyond six months, or do they really have progressive disease? You only know that if you follow them out and we did. That same group going out to 24 months, included 113 patients with chronic DILI. We then saw everyone at month 12, and again at month 24. We noted that the labs were improving in many patients by month 12. So we concluded that if one year after injury onset you still have abnormal ALT or alk phos, most of us would agree is ongoing injury.

#16. We called them persisters versus resolvers if they had resolved between six and 12 months,
It turns out about 75% of patients with injury at six months were persisters at 12 months. When we followed resolvers out to 24 months, they stayed normal. If we look at persisters versus resolvers after the first six months, again we find some differences. The older you are, the more likely you are to have persistent liver injury, and again, the higher the alk phos, those two variables, appear to be predictive.

#17. Here’re the alk phos levels to show you this. They remained higher although you can see there's a slow decay towards improvement over time, but they were still abnormal in the persisters versus those who had resolved. And clinically, what does this all mean.

#18. Is this just a lab phenomenon, is this really meaningful? We did have quality of life assessments. The physical summary score in patients destined to have persistent DILI, actually were worse from the get-go than those who had self-limited liver injury.

#19. That persisted throughout all the times that we looked at that. So these patients do feel worse than those who had resolved liver injury. Now we also have histology in a limited number of these patients. Remember there were 113, but we had 12 who had sequential biopsies. The median time for the first biopsy was 22 days, second biopsy was 446 days. These were all clinically driven
biopsies, but what was of concern is that when we looked at the selected biopsies, 8 of the 12 had Ishak fibrosis progression over about a year's time, which is quite concerning, and 7 actually had progressive ductopenia.

#20. If you look at the individual cases here, these are the individual agents and what the initial pattern was on the liver biopsy versus the follow up one. You can see it generally goes from chronic hepatitis to chronic cholestasis. There was a couple of patients who started with steatohepatitis and it progressed over time although there was one regressor with tamoxifen.

In general, the R-values were pretty low in these patients who had serial biopsies. So you can see significant histologic progression even up to a year after the injury onset in selected patients.

#21. We had a 19% rate in our prospective study. The Spanish group published 493 cases, using a slightly different causality method. They had a much lower rate, about 5.7%. Then we'll hear from Einar, looking at Sweden with a different group of patients to start with; he had a much lower rate. Depending on which group you look at, we had the highest rate at 19%.

I think I've shown you pretty clearly that DILI is a significant concern, associated with about 10% mortality
within six months, the majority of them liver-related. About 20% are due to other causes. And if you assume that the rate of DILI in the general U.S. population is between 10 and 15/100,000 people per year, that means that there're about 50 to 60,000 idiosyncratic U.S. DILI cases per year, with 10% mortality, about 5000 U.S. deaths/year, larger numbers than we would have anticipated. The more severe the liver injury at the onset, the more likely there will be a poor outcome. The really interesting new data show that chronic DILI occurs in 15 to 20%, African-Americans and individuals with higher alk phos were more likely to develop it, and it was progressive in some.

#22. Late deaths were uncommon, but we did have a few patients who died after six months from their liver injury. So I think this begets us in 2017 to think that we now know what the natural history is. Should we be looking at patients who have the more concerning biomarkers from the get-go, and consider them potentially for a clinical trial going forward, toward improving their outcomes?

#23. And that's certainly something that we're thinking about. So, obviously this is a huge amount of work and the DILIN network represents many individuals in this room and others around the country.
#24. I'd like to thank all of them for contributing data and analyses and hard work that's gone into making the study a success.

#25. Thank you. (Applause)

DR. REGEV: For the dissenting opinion, we are going to invite Einar Bjornsson, who's a Professor of Medicine and Chief of Gastroenterology and Hepatology at the National University of Iceland, Reykjavik. The question is: How likely is an acute hepatocellular injury; how likely is it to turn into a chronic ongoing liver injury? Einar.

15. **Is chronic liver disease after acute hepatocellular DILI over estimated?**

DR. BJORNSSON:

# 1. Thank you very much, Arie. I would also like to thank the organizers, Arie Regev and Dr. John Senior for inviting me. I really much appreciate that. I appreciate the comprehensive talk by Bob Fontana, and there may be some repetition, but also some other studies.

# 2. Of course, we have the vanishing bile duct syndrome which is outside the scope of my talk on hepatocellular injury. A recently published paper in Hepatology, illustrates this rare but important phenomenon.

We have drug-induced immune hepatitis. As pointed out by John Senior recently in a Webinar, it's difficult to
call it autoimmune because it's a drug involved.

And then we have today’s topic, which is really cirrhosis. That is the injury we should be worried about. And of course we have well documented, hepatotoxicity by methotrexate, which is induced fibrosis. Not really, although methotrexate can induce acute hepatocellular injury, but most commonly it induces fibrosis in the long run. They present with often decompensated cirrhosis after many years of use. Same thing can happen with amiodarone.

# 3. For some reason, I was asked to write a chapter in the distinguished book edited by Kaplowitz. We don't have time for all the details, but I refer to this book.

And these are some cases, some anecdotes, some case reports that a drug has induced chronic liver injury and sometimes with decompensated liver disease.

# 4. It was also mentioned by Bob Fontana that the first study on chronicity was by Guru Aithal, who, at then was in Newcastle, U.K. He found a very high proportion of chronic abnormalities in liver tests and/or on imaging a few years after recognition of DILI. And I think this was clearly an overestimate, because those patients were identified in a histological database. Those who undergo liver biopsy tend to have slower resolution of the liver tests. We are also sometimes skeptical. We are not sure if it's a DILI or
Anyway, Raul Andrade has done a huge amount of work in this field, as you are all well aware. In their Spanish hepatotoxicity registry, approximately 6% have persistent abnormalities. As been convincingly shown and also in this study, those with cholestatic pattern are significantly more prone to chronicity than hepatocellular type.

When I was in Sweden, we found exactly the same proportion of patients who had chronicity, which were published an a separate study, And then we got them to come in again, and measured the liver tests. Interestingly, two patients who were diagnosed years before were actually on the same drug when they came that had been unidentified as the cause of DILI. They didn't take it much as prescribed but had abnormal liver tests.

So it's really unclear whether these biochemical and/or histological abnormality will lead to liver-related morbidity and mortality. That is the big question.

So in a paper I published in Hepatology more than 10 years ago, I identified patients, like those reported by the Swedish authorities, of suspected DILI who had also jaundice. The aim of the study was to validate Hy's Rule or Law or whatever our friend Zimmerman said; we demonstrated that he was not.
absolutely right about the serious matter of hepatocellular jaundice.

# 9. Anyway, I wanted to look at those patients who had survived the initial injury. We were able to link 685 patients from the former study to the Swedish Hospital discharge registry, which is very complete.

#10. We found that out of these patients, 3.4% had been hospitalized for liver disease after a mean follow up of 10 years. Five had a liver-related mortality. So 8 of them developed cirrhosis, mostly decompensated, and 5 died of liver-related mortality. These 5 had cryptogenic cirrhosis and our interpretation was that DILI might have played a role. Of course, we don't know. I'm convinced and many agree, that chronicity is a good way to get papers published. But I don't think that's the main goal of science anyway or even though it gives us increased quality of life if we get a paper published. (Laughter)

#11. Anyway, we found some cryptogenic cirrhosis and also, of those identified with DILI from many years before, they have other chronic liver diseases like autoimmune hepatitis or alcoholic liver disease.

#12. So only a few of these had cryptogenic cirrhosis, identified from the early 1970s to 2004. And I think that was before we knew that cryptogenic cirrhosis is related to the non-alcoholic fatty liver disease that some of
these patients might also have had. It's difficult from a retrospective study to determine it. We could also see that duration of therapy was longer in patients with liver-related morbidity and mortality. Those who had slow resolution had been treated longer. This was a long term follow up. We could see that 6 out of 7 cholestatic pattern had normalized their liver tests.

#13. Only one of these patients had abnormal liver tests after long-term follow-up. Our conclusion was that development of clinically important liver disease after severe drug-induced liver disease is associated with jaundice, rarely, but it can develop in some patients.

#14. We don't know if it's related to DILI but we cannot, of course, exclude it. Bob Fontana has already mentioned his paper so I can go really fast over these slides. So 18.9% met at least one of the 6 protocol defined criteria for chronic DILI. What is in agreement with our study was that duration of use was significantly longer in those who developed so-called chronic liver injury.

In that study, no decompensation was reported and that's the greatest concern for people who have symptomatic liver injury.

#15. Another study came recently published from the Spanish hepatotoxicity registry, that 92% of those with acute liver injury resolved within one year. They found in
the chronic cases that they were more likely to be older age as shown in the DILIN study. They had dyslipidemia but we don't know why, and they had severe DILI at onset.

#16. As you can see here that 25% of the patients ahve at one time after DILI recognition some chronicity, but after 3 years only 12% are still chronic.

#17. mSo if you follow the patients long enough, most of them will have resolution and 75% of those identified at one year, had some abnormalities at three years.

#18. And they found a little difference in this study if they had persistent transaminases, bilirubin, or ALP, more than ULN. They were identified as chronic.

#19. This was so also on histology and imaging.

#20. Main drug classes in the Spanish study were statins and antibiotics. And in this study there were histological data that showed that two cases had ductal lesions, some kind of vanishing bile duct syndrome, 7 had had cirrhosis. No decompensation was reported.

#21. So if I'm allowed to quote the authors, they said in the conclusion of the paper, aside from a small number of cases of early onset cirrhosis which became quiescent, gradual resolution of one or three years or persistent of borderline laboratory abnormalities beyond three years is seen in a very small percentage of cases. The persistence of these very mild abnormalities is of uncertain
significance but does not appear to be an important clinical problem. Hence, the term chronic is somewhat controversial as there are chronic DILI patients, that eventually resolve the liver damage.

#22. So they concluded that the one year was the best cut off and statins were those who were mostly related to chronicity.

#23. So my next to last slide is that, yes, chronic liver disease after acute hepatocellular DILI is overestimated. During long term follow up, most people normalize their liver tests.

Decompensation has anecdotally been reported and evidence for clinically significant liver-related morbidity due to chronic DILI is weak but it has to be said, but there are examples of methotrexate induced-liver injury is rarely an acute hepatocellular DILI. It's more a presentation of cirrhosis after years of use, also true for amiodarone. They were the only two patients in the recently published paper on line by Skip Hayashi and co-authors that didn't survive chronic DILI.

#24. So the liver is a forgiving organ.

Thank you very much. (Applause)

DR. CHALASANI: Why don't we go to the next speaker, Dr. Jim Lewis, who is Professor of Medicine and a longstanding
investigator in the field of DILI. He's going to talk about estimating DILI risk in patients with advanced liver disease and decompensated cirrhosis.

16. Detecting, Evaluating DILI with Active or Advanced Liver Disease

DR. LEWIS:

# 1. Thank you, Naga, and thank you to all the organizers. I titled this with a little help from Yogi. I don't know cricket players with whom probably most in the room are familiar, rather than baseball, but just like John Senior says, he's just just an employee over at FDA. Yogi was just a ballplayer and if you believe either of those -- okay. (Laughter)

I wish I had the IQ pre-meeting from yesterday that probably answered a lot more questions than I will today, but I'm going to try and cover it from a clinical standpoint as someone who adjudicates cases in the clinic, for different companies. We're often faced with somebody who has chronic liver disease. How do you tell it's the drug and not the disease?

# 2. And as John Senior had said several times, the whole point is to see, when we talk about Hy's Law, we have to go through a large causality assessment to determine whether we're dealing with this drug or not.
And I'm just going to go over the tools that we currently have available to us. I'm not going to read all Yogi's quotes. Most of you, if you've ever read them before, know them. But I thought it was apropos for today's topic. We're lost but we're making good time in figuring out how to diagnose DILI in chronic liver disease.

# 3. So there are lots of potential settings where chronic liver disease and DILI will overlap and this is just a list of some that we already know and we deal with every day. A number of drug trials incorporate patients who have these underlying conditions.

# 4. We have PBC and NASH and chronic viral hepatitides, and then of course, we have patients in clinical trials who have underlying liver disease, and we have to decipher what's going on with those individuals as well. Patients who are in diabetes trials, many of them obese, may have NASH. How do we figure out what's going on in terms of drug safety?

I'm going to touch at the end on the issue of hepatobiliary and pancreatic malignancy, because when we see patients so diagnosed and they present with jaundice, we automatically discount the drug.

# 5. In most but not all cases, we're probably correct, but maybe we need more evidence for it. A current issue
in diagnosing DILI and chronic liver disease is that most trials exclude patients with severe chronic liver disease or cirrhotics. We now have a couple of NASH trials with decompensated patients. We've got hepatitis C trials with decompensated cirrhotics. That may be changing.

We have to understand the natural fluctuations of the liver tests in these chronic liver diseases so that they can interpret the nuance changes that sometimes occur. We have to determine what kind of monitoring interval we need with our liver tests and until we get that biomarker that's going to specifically tell us that DILI has occurred, we're still dealing with our, you know, our standard liver test, ALT, bilirubin, the rest of them.

How often should we be monitoring patients who already have underlying liver disease? Should that be just biochemical monitoring, or clinical monitoring? Isoniazid for example, uses just clinical monitoring.

And we know how fraught with danger it can be when patients meet the stopping rules but they don't stop, as shown by the DILIN network. Do we need new stopping rules for patients who have chronic liver disease?

# 6. Is a higher threshold, lower threshold, absolute values, correct?. When the FDA put their regulatory guidance together almost 10 years ago now, they highlighted the fact that if you had underlying liver
disease, the rules might be different.

And there was no well-established reason that patients couldn't be studied in clinical trials, but most times exclusions were done to avoid confusion. And now we're in an era where we're studying these patients and we need, perhaps new guidance, which we'll hear about throughout the meeting.

# 7. Some of the causality assessment questions still need answers for patients with chronic liver disease. We heard Dr. Reddy talk about flares in hepatitis B in different scenarios. We have to be able to distinguish those from acute DILI. Are there any animal models of chronic liver disease for the drug under study? I'm not sure that we have that.

Should we use a modified RUCAM score? We didn't really talk about causality assessment much yesterday in the pre-meetings. The RUCAM is used widely around the world in conjunction with expert opinion in many instances. Do we need a RUCAM score that would include chronic liver disease? The Hepatic Adjudication Committees that many of us sit on, strength in numbers, needs to iron out discrepancies in the interpretations.

# 8. And we're going to hear from Dr. Chen tomorrow, on his Rule of 2 and other pharmacologic drug property risk factors which could be useful, especially in patients with
chronic liver disease.

This is from his paper and I'm sure he's going to illustrate this tomorrow, but as you know, the Rule of 2 with high drug dose being over 100 mg and high lipophilicity, and you can see here with the new hepatitis C medications, how they were able to, how the model predicted which of these drugs, in fact, would be hepatotoxic. And this is one of the things that we might be able to incorporate into our clinical discussions when we're adjudicating these patients with underlying liver disease.

# 9. Is there guidance from the literature on how to do this? In clinical trials, we can look at underlying liver disease patients, and try to figure out what the normal fluctuations might be. And we're beginning to see a number of trials where we might actually be able to use those kinds of numbers as the new baselines that we're looking for. In the DILIN network, about 10% of those patients had underlying chronic liver disease.

#10. This slide shows a few studies from the literature trying to understand what the normal ALT values would be in patients with different chronic liver diseases.

Alcohol has always been important because the ratio there is very different than the ratios in ALT and AST that we see with any other liver disease. The AST is
always higher and it's never above 300, and the ALT is never above 100. And this can help in alcoholics who develop ratios or absolute numbers that are far different than the numbers and ratios that we normally see.

#11. Is it acute DILI or is it the underlying liver disease in just its natural progression? So if you have a patient with hepatitis B and the ALT rises with an undetectable DNA level, well, that might be DILI because if it's a flare of ALT and you're also seeing perhaps a flare in viral load, that would tell you that it's probably the underlying disease although in the case of the DAAs for hep C, maybe it is drug related.

With NAFLD and acute ALT rise, would it be something that we would not expect in someone who has a generally stable ALT value throughout the trial.

In PBC, if we see acute rise in any of the enzymes, that might signal that it could be DILI as opposed to some of the mild fluctuations which come out of some of the PBC trials.

In cirrhosis, if the ALT goes higher, that's unusual because most cirrhotic patients have AST be higher than ALT. So if you have a reversal of that ratio, it's something that we can use to say that something else is going on.

#12. But it's not liver tests alone. It's a gestalt that
we all employ with this, and it's looking at the clinical context, the concomitant medications that have already been mentioned. Many patients are on multiple drugs, and it's not easy all the time to figure out whether it was the drug or something else.

So the baseline values in the PBC trials, the alkaline phosphatase is expected to decline on therapy, and if you have an acute rise during treatment, that's going to alert you that something else is going on and could it be the drug under study.

#13. This is from the POISE trial and it shows the normal fluctuations in alkaline phosphatase and that there is a downward trend. So if you were to see someone who has an acute rise during the trial, it would alert you that something else is going on.

It may be the drug under study. It might be a concomitant hepatitis or something else, but it's information like this that I think we can start to use that if we understand the normal fluctuations, we might be able to do a better job clinically with our assessment.

#14. Oncology trials are tough. Hepatic malignancy, what happens to the liver tests in patients who have liver mets, for example. And there are not too many trials that I could find in the literature, some of which suggested that ALT and AST elevations or alkaline phosphatase
elevations in patients with liver metastases, are actually not that common.

And pancreatic cancer causing obstructive jaundice can be confusing when the bilirubin goes up, the alkaline phosphatase goes up. So we need more information, I think, especially as we have more oncology trials that are being done, and want to make sure that the drugs are safe.

#15. I mentioned RUCAM at the beginning. RUCAM was designed for acute liver injury, not for patients with underlying preexisting chronic liver disease.

#16. Gaby Danan and Rolf Teschke have updated the RUCAM. For those of you who haven't read their paper that came out last year, they're making suggestions on how RUCAM can be strengthened as a general causality method.

#17. When it comes to chronic liver disease, I think we still need expert opinion, and you know, whatever that entails, because RUCAM by itself, I don't think is ready for chronic liver disease.

Will Lee and the late Rolf Olsson and some others from AstraZeneca and I attempted to use a RUCAM scoring system a few years ago with Exanta, ximelagatran. And we had a number of patients who had elevations in ALT at that time, and we gave them possible or probable scores here that were on the low side.

#18. And it pointed out that RUCAM was just not ready for
a clinical trial where you really don't know anything about the drug historically. So I don't know that RUCAM is going to be very helpful at the moment, but there are clearly elements in the RUCAM scoring that are the basis of all our causality assessments. How long was the patient on the drug? What happened when the drug was stopped? Can we exclude alternative causes? Other medications? Rechallenge, which we don't do much anymore, was the basis for how RUCAM was validated, but we no longer do it intentionally in most instances.

So I'm not sure RUCAM is the answer. How best to use expert opinion to distinguish acute DILI from underlying chronic liver disease? We can look at the injury pattern. Is that similar or different than the typical pattern that we normally see? Are there any signs of immunoallergy or hypersensitivity that have developed? What about the co-morbidities, heart failure, hypoxic hepatitis or shock liver, gallbladder disease? It's not easy.

#19. Some of the additional tricks of the trade, that we use: it's very rare to have DILI after an acute dose. The ALT/AST ratios I've already mentioned, the time course to improvement or not, the dechallenge test that we do, the rechallenge. Is the work-up sufficient to exclude everything else? That's always difficult.

#20. So expert interpretation is needed if we think that
there might've been drug tolerance or adaptation. None of the scoring systems will really allow you to say that there was adaptation going on, but clinically we can make that determination.

There are cases of DILI where the drug has been discontinued and there’s a delay of several weeks. Many of the antibiotics are associated with that, augmentin, in particular. How do you factor that in? RUCAM won't help you there. What about the published literature? We're starting to accumulate more information about normal levels in chronic liver disease in trials.

Do we take the absolute height of the values or do we use a fold-increase of some type? It's not settled, as you heard earlier this morning. So there are challenges.

#21. Does the latency change for drugs causing acute injury if they're given to a patient with already underlying liver disease? I don't think we know. Is alcohol or fatty liver a risk factor for DILI in these cases? Is MELD score going to be useful? If it goes up precipitously in somebody in a cirrhosis trial, is it a clue that there might be DILI going on?

And you have to look at all the other things that can happen to that person with cirrhosis. Have they developed SBP or sepsis or have they developed HCC as a result. so the causality assessment is always key.
So here's an easy one. Okay. Here's somebody in a clinical trial. They have at screening, their ALT is about twice normal, AST about the same, twice normal. And as they go along week four, there's an abrupt rise in ALT. It's now 156. The AST is also up. The bilirubin stays okay. The alkaline phosphatase doesn't change. And a work-up is started. And you can see that all the typical markers for viral hepatitis and autoimmune hepatitis, everything else is done. They even checked an acetaminophen level, and those are all negative. At week five, one week later, the patient comes back and the numbers are even higher. And so the drug is discontinued at that time. And then over the course of the next several weeks, everything reverts back to the baseline. Is this the natural history of that chronic liver disease, or is this DILI, or is it something else? Now to the extent that we've excluded all the normal things, this would be considered at least possible or probable DILI for that particular drug.

Here's another one. This is a PBC trial. baseline alkaline phosphatase 345, about three times normal. Minimal elevations in ALT and AST. Normal bilirubin. They've been on Urso at the same time for the past several years. Week 60 of the trial, more than a year later, the patient develops acute epigastric and right upper quadrant
pain, nausea, vomiting. They're diagnosed with biliary colic, gallstones are seen on an ultrasound, there's mild dilation of the common bile duct. An ERCP is performed, and a biliary stent is placed despite the absence of stones in the bile duct. Okay. Augmentin is then given for five days. The study drug is continued and you can see the numbers on admission to the hospital and then six weeks later, despite continuing the drug, things improve because this was probably gallstones and not DILI from the drug.

#24. Stopping rules. Do we throw the baby out with the bath water? Do we use fold elevations or do we use absolute values? I think we can have a good discussion on it.

Does Hy's Law still apply? We discussed yesterday that Hy's Law probably does apply because it's the same principle.

#25. We just have to sort of move up and down, with the causality assessment to determine if it is a Hy's Law case implying that there could be acute liver failure on the horizon.

#26. And I think that's really the definition of what Hy's Law implies, even in chronic liver disease. This was, can you use an elevated baseline, elevation. This was a trial we did for a statin, and we allowed people with chronic
liver disease and ALTs up to five times normal into the trial.

#27 And you can see here that it was the placebo patients who had most of the liver abnormalities develop a doubling of their baseline ALT compared to the statin. And that's part of the statin story that they seem to be pretty safe.

#28. Just to finish up. The non-DILI causes of jaundice and other events are more common than we think. Remember, DILI is uncommon. It's a rare event, but so many other things are more common. People get viral hepatitis, they have gallstone diseases, they have all of the other things that are listed here, all of which confuse someone who's looking at abnormal liver tests.

#28. They're on number of medications. You saw that herbals and dietary supplements are now the second leading class of drugs causing DILI in the DILIN network and around the world.

#29. Do we know if that pancreaticobiliary malignancy is drug related or not? If it's diagnosed within weeks or months of somebody being in a clinical trial, the answer is no. But if they're on a drug for years, and they develop a malignancy, are we absolutely certain that the drug had nothing to do with it. And you know, we discounted out of hand almost when we adjudicate these
cases. We say, oh no, they have obstructive jaundice from pancreatic cancer and I'm just wondering if we're always correct, especially if it's a long period of time that they've been on the drug. But were there any signals of malignancy in animal models or something that we might go by? The ultimate diagnosis is probably going to be a biomarker.

#30. I hope we hear more about those today because it ain't over til it's over. And I thank you very much. (Applause)

DR. CHALASANI: Bob, can you come up. Einar, can you come back up here as well. So, Jim, excellent talk. All three talks were great. Jim, the latency in chronic liver disease, I think, the 2015 Gastro paper from DILIN, I think there are a couple of tables. Basically I don't think there is a relationship.

In other words, in patients with underlying chronic liver disease, the latency wasn't any different. That's one. And there was another table looking at long and short latency cases.

Once again, there isn't any clustering of chronic liver disease in either extremes. So from DILIN prospective study, I don't think we're seeing a signal for shorter latency or rapid onset of DILI in patients
with underlying liver disease.

OPEN DISCUSSION, Session IB

DR. REGEV: I have a question, while we're waiting for questions, regarding the debate on delay of chronic liver injury after acute hepatocellular injury. Certainly there were a few patients with elevated liver tests after one year in the DILIN study. I'm wondering at that point, was there another causality assessment done? Did we go through all the hoops to make sure we understand why they have elevated liver enzymes? We're assuming it's related to the drug.

DR. FONTANA: In DILIN everyone is followed prospectively for six months before the causality is done, so we already have six months to know if there was something else sneaky going on. I don't recall that there many, if any, cases of some alternative diagnosis becoming apparent at one year or two years. So, no we don't really see competing causes emerging late.

DR. REGEV: Just based on the data you showed from Skip's study, you had 22 die of cancer at the end of the follow up. It's a significant group that had other diseases that can cause increased liver tests.

DR. FONTANA: Yes, but they died of the malignancy
without clinically significant liver injury at the time of their death.

**DR. REGEV**: They still had DILI.

**DR. FONTANA**: -- so they died but it was not due to DILI.

**DR. REGEV**: It was still there. Wasn't the malignancy associated with elevated, those elevations?

**DR. FONTANA**: No. They died of progressive cancer.

**DR. CHALASANI**: But they had DILI while getting treatment for cancer. There's a question of --

**DR. FONTANA**: Right.

**DR. CHALASANI**: And they still died.

**DR. FONTANA**: -- which we're seeing more and more of at this stage.

**DR. REGEV**: The question is was it still DILI after a year? That's my concern.

**DR. CHALASANI**: Other comments.

**DR. BJORNSSON**: If I remember correctly, in univariate analysis, the patients with malignancy had more commonly chronic liver injury than the rest of the patients. So there's a point there. I don't know if it's associated with malignancy or not.

**DR. CHALASANI**: Let's move on. Herb.

**DR. BONKOVSKY**: Herb Bonkovsky from Wake Forest and UNC Chapel Hill. Maybe Skip will want to comment on that last point because you reviewed that most thoroughly and
recently.

**DR. HAYASHI:** I don't have anything to add. Basically in almost all of them, liver enzymes had nearly or fully resolved. And then they went on to die of their malignancy later on. We followed them out for two years. That's why we're picking them up.

**DR. BONKOVSKY:** But my main question was to Dr. Bjornsson and Dr. Fontana, about these chronic cases. So there seems to be quite a difference in the prevalence if one looks at DILIN versus the Swedish registry. The latter is nationwide, whereas DILIN considers probably more severe patients that have been referred to centers. But could you comment, Dr. Bjornsson about long term liver biopsies in those patients because, you know, are they really resolved or is there significant fibrosis that is going to eventually, perhaps lead to a shortening of overall life expectancy. And the same question for Dr. Fontana.

**DR. BJORNSSON:** In the Swedish study, all the patients at the onset had jaundice, so these were severe cases. These were not minimally elevated liver tests, but I think you should also compare it to the Spanish hepatotoxicity registry. These were also quite sick patients.

But liver biopsies, I don't know. As everybody is aware, there's a lot of sampling variability in liver biopsies.
I think it would be worthwhile doing FibroScan in these patients. And you don't do liver biopsies if it's not clinically indicated.

**DR. BONKOVSKY:** I certainly agree with you. And of course, MRI and other methods of trying to assess fibrosis. Dr. Fontana, any comment.

**DR. FONTANA:** My point is that study designs are very different. We're prospectively following from the injury onset for two years and Einar's were patients who had it in the past and then were, through the medical records system, found to have been rehospitalized or had apparent decompensated cirrhosis. So he has a much longer follow up of hard major events retrospective. We have a shorter follow up of less severe liver injury. So they're different study designs giving you different pieces of information.

As I said in our study, we had a limited number of patients who had serial liver biopsies. So there's, and as I showed, most of those had histologic progression, but again there's probably selection bias with who we're re-biopsying because we're worried about them.

We don't biopsy people who resolve, so I would agree with Einar that prospectively following these patients out with some non-invasive tool would be attractive and we're
certainly doing that now, but we don't have the data yet.

**DR. BJORNSSON:** Just a short comment. I mean the design is very similar to the, Rolf's study of the hepatotoxicity. That's a prospective.

**DR. CHALASANI:** Can I make a point that in some of the studies there is a relationship with chronic DILI as we define it and statin usage. I think some of that should just be underlying fatty liver.

In a lot of them, you don't have baseline and I think the FibroScan part of the DILIN prospective study may shed some light, but otherwise I think some of the low estimations just could be underlying --

**DR. FONTANA:** We did look. I presented a lot of data. But if you recall the study of the 113 patients who were followed out from six months to two years, in about 75% were persisters and 25% were resolvers. We actually looked at the lipid profiles in those two groups and they were not different. So it wasn't just that the persisters were all the hyperlipidemic patients with obvious metabolic syndrome. So I agree with you. Prospective assessment of the liver would be better. These are all indirect markers, at best.

**DR. REGEV:** Mark.

**DR. AVIGAN:** I enjoyed those talks. I'm concerned about the idea that we're setting up a straw man perhaps, and
that we have some recognition bias based on cases, especially that we collect these cases after they present clinically.

So there's under the radar screen and then there's in the radar screen. And one the questions that comes up is, the level of injury that is necessary to see a case to call it a potential DILI case and then work it up versus a subclinical case that may smolder on. And then the consequence of a longer term duration of treatment versus shorter until the time when you actually identify the case. The question then is: what about the actual bias of recognizing DILI and then determining from that bias what actually is the risk for chronicity and a long term kind of complication of chronic liver disease of fibrogenesis, when there's the potential of such a bias? I want both of you to comment on that. For example, statins are often used chronically so that by the time you may see a case, it turns out to be that it's associated with chronic liver disease, but the use of those drugs often, there's a prelude of long term use before you recognize potential cases. So that's the question.

**DR. FONTANA:** Maybe I can go first, then Einar can. Clearly DILIN is a referral-based study. It's not population based at all. So I think that's an important point. And I showed you what the entry criteria were,
which aren't that severe, ALT five times, alk phos, et cetera. So we are getting referred cases and we then follow them up. Whereas Einar's paper from Gastro was a population-based study of all comers in Iceland, and I don't recall if you had the rate of chronicity in your 100 cases. I can't recall.

**DR. BJORNSSON:** No.

**DR. FONTANA:** No. It would be the difference that minor DILI should resolve and be a nonissue and therefore the overall rate may be lower.

**DR. AVIGAN:** -- or a smoldering subclinical over a very long period of time may tend towards a more chronic effect in the long term without an acute very, sort of --

**DR. FONTANA:** Yes. So in terms of the specific agents, minocycline comes to mind, right. That you can be on it for a year and a half and you have chronic hepatitis with fibrosis by the time you present. And we're not seeing just minocycline cases or drugs that have a long latency in the chronic cases. In fact, some of them were on antibiotics for three days. So it's not quite parsing out that it's drugs that are given chronically that cause chronic disease up front, I guess.

**DR. BJORNSSON:** Yes. I'm not so sure as it's ss simple as Mark says, but, because in the Spanish study, the severity of liver disease predicted chronicity.
Severity, not only the longer duration. So also older age and things like that. It'll be interesting to ask Will Lee about the acetaminophen cases. Those who survived the acute liver failure, if they have a chronic, if that has been looked at.

DR. LEE: So we have not seen cases. What we're seeing now interestingly enough, is what we're calling the APAP frequent flyer. The person who comes in with multiple acute episodes. We've had up to six or seven episodes. I think it's because people are getting hydrocodone-acetaminophen combination on the street and they're ingesting it, and then coming in but they do this repeatedly. We haven't seen any chronicity to acetaminophen. I think that's the experience that Roger Williams reported, you know, 30 years ago.

DR. REGEV: Years ago. So this is, by the way, even in a frequent flyer, six times acute events and then resolution does not turn into chronicity.

DR. LEE: We haven't biopsied them. We did look at their huge hospital bills though.

(Laughter)

DR. ROSENBERG: Yes, Amy Rosenberg, FDA. So forgive my immunologic vent, but are none of these chronic DILI cases attributable to an immune response to drug-induced injury. If they are, if there's some immunologic
component, are we looking at HLA haplotypes and could that explain differences between findings in different studies where HLAs clearly --

**DR. BJORNSSON:** Can I stop you? One of our interesting findings after long term follow up in the paper of Journal of Hepatology was that 6 patients in the total cohort, 6 of the 23 patients, developed autoimmune hepatitis in the long run, which is clearly higher than the incidence in the general population. So something happened. Even though they recovered clinically, they developed autoimmune hepatitis. But we didn't have any control group.

**DR. FONTANA:** I can tell you in DILIN, we've done extensive GWAS, genotyping in all the cases and looked at different outcomes, survival versus not, chronicity versus not. We're not finding a GWAS-wide significant association thus far with the chips and things that we've been using. Now that's not to say that it's not immune mediated. But it seems to me that there’s a common theme here between the studies is older individuals perhaps may be more prone and perhaps if you're more cholestatic to start with. There may be some host factors that predispose to chronicity and it may be that once you've initiated it, you can't turn off the injury. Beyond that, we've not
found HLA or genome-wide significant associations.

DR. REGEV:  Skip.

DR. HAYASHI:  Skip Hayashi, UNC.  This is for Einar.  And I think you answered it, but I just wanted to push you on what you said.  So in your population-based study, this later one where you looked at incidence which is very strong because it's population-based, don't you have that data for the cases who didn't resolve, because you had to adjudicate them and part of that is the washout? Didn't you have some or you must have looked at some of those cases that didn't resolve their enzymes completely?  Do you not have that or, because I don't remember that in the paper?

DR. BJORNSSON:  No. I think it was very rare. Maybe one or two cases at one year, but they resolved when we followed them. They were not as severe cases as in the DILIN study. I mean, only 30 percent had jaundice. We didn't, for recognize any bile duct injury. So we didn't have any chronicity in that cohort, no.

DR. HAYASHI:  But I would argue that that's probably your strongest card to play, frankly, because you have a population-based study and it's unbiased. Bob just said, DILIN is biased. We had severe cases. That's my only comment.

DR. REGEV:  Yes.
DR. MEHTA: Ruby Mehta, FDA. Thank you for the great talks. So did you notice any difference amongst the patients who had DILI and specifically the ones who had immunoallergic DILI, if they were treated with corticosteroids and had resolution slowly, the ones who went into chronic drug-induced liver injury had higher chances of resolution compared to those who did not. Was there any relationship to corticosteroid use or not?

DR. FONTANA: I think it was in one of my slides on chronicity, and it's published, that about 30% of both groups who either went on to chronicity or didn't, got steroids, and it wasn't apparent that the steroids made any difference. So, I can't say it did. And in terms of immunoallergic features, we just don't see that much. You know, we don't see many eosinophilia in these patients. So I can't really comment that the steroids were or were not beneficial.

DR. REGEV: We have time for two last questions before our lunch break. So, go ahead.

MS. MARQUEZ: Loretta Marquez, Janssen. And the question is for Dr. Fontana. In the DILIN investigation, have you considered investigating genetic polymorphisms of the metabolic enzymes as a factor for chronicity in addition to the HLA and all that.

DR. FONTANA: Sure. We've published a couple of papers
already on GWAS of the overall cohort for both susceptibility as well as clinical outcome, you know, across all, you know, million SNPs, and not found a whole lot there.

MS. MARQUEZ: Okay. Thank you.

DR. OMOKARO: Stephanie Omokaro, FDA. Two quick questions; One, what percent of DILI related deaths occurred in patients that were still on the suspected drug? Secondl: is there any available information on the potential for reinjury in patients with a prolonged resolution period of DILI?

DR. FONTANA: To answer the first question, patients that are better phenotyped in Skip's paper were invariably hospitalized for liver failure and/or getting transplant or dying. It wasn't that they were just continuing to get the drug. In fact, most of them had stopped everything. So that wasn't a risk factor. I'm sorry, what was the second question?

DR. OMOKARO: The potential for reinjury, any available information on that? In patients in that prolonged resolution period of DILI?

DR. FONTANA: Yes. Good question. So we've not been seeing lots of patients getting a second injury, if you will, during their prolonged follow up. Yes.

DR. REGEV: Okay. I would like to thank the speakers and
thank the audience, and we're having a lunch break right now. (Applause)

lunch break 11:30 -12:30