So I am going to get the session started. I am Mark Avigan. I work at the FDA with John on critical path issues and I have a background in both hepatology and molecular biology. So, I am kind of an eclectic guy, but I am delighted that everyone is here and that we have an opportunity to talk about these very important issues.
My talk today is about drug-induced immune injuries, why these are important. And of course, we are talking here about different kinds of injuries, different mechanisms, both with regards to liver as well as other organs.
And what prompts our attention to this as hepatologists and liver injury people with regards to drugs is that there are now new drugs coming online? We will see more of these in the oncology space.
immune injury and then turn my attention to talk about autoimmunity and autoimmune hepatitis, in particular, with regards to accounting for the diverse phenotypes and mechanisms, both with regards to particular drugs and individual patients who are susceptible. And I am going to then introduce the topic of the cancer drugs and we will hear more about this from David Berman and then talk a little bit about the challenges with regard to causality analysis where the RUCAM, as a tool, needs some work. And we are working with our colleagues on the NIH on this question.
So, with regard to immune injury to the liver or other organs for that matter, there are, again, different molecular targets that come into play that incite these reactions that are either drug-associated or altered self-antigens, as we heard. From the point of view of classification in simple terms, although there are commonalities between these broad pathways, there are two broad groupings of immune reactions or immune damage, immunological damage prompted by drugs.

One group of reaction pathways is immunoallergic pathways. And characteristically these have an onset within a few weeks of treatment. They can be very short. Multiple organs can be affected. And again, we think of these as the classic hypersensitivity reaction. So, we are talking about different mechanisms within this group of reactions. Fever and rash are not uncommon. We have heard about eosinophilia today as an example and re-challenge has significant risk.

On the other side of the coin are the autoimmune reactions. And again, they have some similarities in terms of what incites them. But autoimmune reactions are different in that typically their onset occurs after a more prolonged treatment. The type of injury that you see is
more subacute or chronic. Again, there are characteristic ranges of affected organs and these can depend on the specific drug and the specific drug signatures. We will come back to this point. And then for some, there are characteristic autoantibody profiles for certain drugs but this is not always the case. And there are some notable exceptions. From a public health perspective, there has been, of course, longstanding concern with regards to hypersensitivity reactions from drugs and these can be serious. These can be life-threatening. They can kill. We just had a conference a couple of weeks ago -- last week, on Stevens-Johnson syndrome, where patients end up -- these are very reactions, end up in burn units and have terrible reactions. But these are often discovered or determined, identified in the post-market phase because they are quite rare. So, there has to be large treatment exposure before you start seeing these reactions.
And clearly, in this snapshot of safety alerts between 1996 and 2014 from the FDA, you can see that significant regulatory actions have been taken with regards to drugs and withdrawals and so on. Some of these regulatory actions have taken place after replacement of the problem drugs with drugs that have safer profiles.
And likewise, there is a sizeable number of drugs that are labeled by FDA and then of course by the sponsors for autoimmune reactions. And this is just a very partial list, just to give you a sense of it. And different kinds of autoimmune reactions are relevant here.

There are lupus-like syndromes, drug induced lupus erythematosus. I will refer to it as DILE. There is autoimmune hepatitis. And again, there can be disability associated with these kinds of reactions and, in some case, they can be, of course, life-threatening as well.
So, optimizing our risk assessment and case management for this kind of problem is very important. And again, to have an optimal approach in the face of this diversity for risk assessment and also to be able to learn more about them in research, we really need to have -- we need a number of things to set of place. We need to have a universal categorical criteria of reaction types. We have to have a nosology. We have to have a classification scheme that makes sense not just for the experts in pathology, in the pathogenesis but also for clinicians to identify patients, recognize them and so on. We need protective procedures to monitor patients and manage immune reactions. We need to have effective post-market surveillance strategies to tell and evaluate events when they occur, especially since many of these events are rare, so they will occur and be seen in the post-market. And we need adverse event descriptions and instructions to manage risk in labels and other tools with communication that are really optimal.

Now, in the face of these needs, we have to reconcile these real important challenges. And we go through some of these challenges. But one of the challenges, of course, is that some drugs actually can cause more than one kind of reaction. And we heard today about minocycline as an example of a drug that, in some individuals caused an immunoallergic reaction but in other people, they get a more classic autoimmune picture. So, different individuals can have...
from the same drug, different reactions. So, that has to be somehow -- that is one of the challenges that has to be incorporated in how we communicate risk.
There are also variable temporal features of severity and affected organs for the same type of injury type. So, that is another layer of diversity and complexity that has to be communicated. So, for example, minocycline autoimmunity can include drug-induced lupus erythematosus. It could cause autoimmune hepatitis. It can affect other organs such as thyroid, where you can get thyroiditis, other endocrinopathies and so on.

Another example is lamotrigine which can cause hypersensitivity of different organs, skin, liver, meninges, in different people, presumably with common mechanisms of injury.

So, another challenge in this group of challenges is that there are inter-individual differences which are hard to predict. So, there are co-determinants of risk that are idiosyncratic. They have to do with the HLA polymorphisms. They have to do with pre-existing antigen exposures that might have been primordial but have sort of set into motion a recognition of an antigen as foreign or an altered self-antigen and then the danger signals. That is, the concomitant, which we will come to in a moment.
So, now I am going to focus more of my attention to the autoimmune side of that ledger that I showed you before and, of course, there are classic -- there are manifold manifestations of autoimmunity from drugs and there are some classic presentations which overlap, to some extent, but not completely with what we have referred to as idiopathic autoimmunity.

So, in the case of drug-induced lupus, these signs and symptoms that I have listed here arthralgia, serositis, and so on are subacute and chronic cutaneous SLE, these are classic for drug reactions but, notably, many patients with drug-induced autoimmunity do not have some other features that are seen with idiopathic lupus, such as renal involvement for many drugs, CNS involvement and very serious, life-threatening skin reactions.

So, another feature of the drug reaction is that it is slow to onset after initiation of the drug. It is slow to resolve often, unless you intervene with steroids so that the clinical syndrome is a little bit different than what we see in the idiopathic from. And also, sensitization is not easily seen. Sensitization was seen with immunoallergic reactions but not with these reactions.
So, whether the liver is the target organ or you have other organs that are affected, there are certain common pathways across these different kinds of autoimmune injuries that come into play. So, there is a triggering mechanism that we heard about this morning, very nice presentations that initiate the reaction either through haptens of drug metabolites or through an alter self-antigen and there is a fair amount of data that we have heard about in previous meetings about the secondary stress signals that are the so-called danger hypothesis where concomitantly there is an infection or a heightened inflammation which, somehow, changes the regulatory network and makes susceptibility to initiation more complete.

And then the reactions through drug effects can be driven or sustained through drivers, through driver mechanisms, which I have listed here. And these include drug effects on a variety of steps in immune homeostasis. And notably, one of the ones that we heard about today, which is a very important one to learn more about is the issue of tolerance. Certain drugs actually can change or perturbate tolerance and I will come back to that point later. But also but these pathologic steps actually afford an opportunity for interventions and prevention in certain individuals who are susceptible and need certain drugs. So, this is something that we had asked about before and requires more research.
So, there are now over almost 100 drugs that are associated with lupus. The most well-known ones are, of course, procainamide and hydralazine, where the rates of these reactions is extraordinary, particularly in patients who are slow acetylators or have certain HLA isotypes, the classic DR4.

But other drugs as well are associated with lupus more rarely but they need to be labeled and they need to be communicated and recognized by clinicians when they occur. And interestingly, as we heard about before, there is a gender predisposition in females more than males but it is less pronounced in the idiopathic variety. We see these drug reactions more in older people but maybe that is because they are on polypharmacy.
And here is a very partial list of drugs that have been linked to autoimmune hepatitis. And again, what is interesting is some of these drugs actually are more specifically reported as predisposing to autoimmune hepatitis rather than DILE. And some of these drugs have been removed from the market because of this effect or have not been introduced into the market.

Currently, we heard that the drugs that are being marketed currently that have this issue that is recognize, minocycline and nitrofurantoin but this is a moving target because now we are having these new oncology drugs coming online. We have more biologic agents, like the TNF-alphas that we heard about, where this kind of problem has been recognized. So, the complexion of the drugs that are causing this problem over time will change.

### Drugs/biologics & risk for drug-induced AIH*

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<tr>
<th>CAUSAL LINK</th>
<th>DRUGS</th>
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<tr>
<td>+++</td>
<td>Tienilic Acid, Oxyphenisatin, Metyldopa, Dihydralazine, Minocycline, Nitrofurantoin, Clometucine, Propylthiouracil, Diclofenac, INH, Infliximab, IFN-α, IFN-β</td>
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<tr>
<td>++</td>
<td>Fenofobrate Statins, Etanercept, Adalimumab, Indomethacin, Meloxicam, Terbenafine, Imitinib, Atomoxetine, Pemoline, Phenprocoumon, Doxycyline, Germander, Morinda Citrifolia</td>
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* Representative (Incomplete) List; X. Xiao & C. Chang, J Autoimm. 2014; RL. Rubin, Toxicol. 2005

19 Mar 2015   Annual Meeting on DILI

FDA/C-Path/PBRMA/AASLD
And there has, of course, been a long-standing interest in genetic susceptibility markers, not surprisingly. Of course, if they have utility, if they have good predictive value, not surprisingly, some of these as we heard very elegantly from Dr. Czaja, that some of these are overlapping or at least the HLA loci are overlapping with those that are implicated in the idiopathic forms of autoimmunity and autoimmune hepatitis. But there are specific isotypes for certain drugs, where there is an HLA connection having to do with antigen presentation. For example, minocycline and nitrocine polymorphism at the 30 amino acid position of the first open reading frame. A slow acetylator status rings the bell with regards to hydralazine. It has a similar pathway, actually, as INH. Again, so how some of these things have to do with the metabolism of the drugs, we don't completely understand.

And then of course, if we want to develop genetic susceptibility markers as clinical tools, as risk management tools, they have to have good predictive value for us.
And so far, with maybe a few exceptions, individual loci, as markers or enrichment for risk have very small effect sizes. So, that their contribution of overall risk is relatively small. So, this is a kind of stumbling block.

But what we don't know actually, is how to compute the combinatorial effects of multiple interactive genetic loci, as well as other effects as well. So, this is difficult to study but it is an open question. And the modeling of risk effects of multiple loci and genetic loci, as well as non-genetic factors, and the challenges, the experimental challenges of how to do this is nicely captured by this diagram that was published a number of years ago by Teri Manolio, who is at the NIH who we had this conference with the other week.
And basically, there are two important factors or two important variables that determine risk for a genetic locus. One is the effect size of the locus on risk and the other is its frequency in the population. And so you can from that make a diagram. And on the right side of the diagram are the kind of common variants that we often will determine by GWAs. They are frequently expressed in the population and they often will have a small effect size. So, on the one hand, they are easier to discovery in a case-controlled study design but they also are disappointingly, they have small effect sizes to be used as these single markers of risk.
On the left side of the diagram are the rare alleles that are inherited in more of a Mendalian way and they may have high risk but they are hard to discover because you have to know where in the genome to look. You can't just have a pangenomic system method to discovery because there is a lot of false discovery in that method.

This kind of a diagram highlights the experimental challenges of determining the biosystem genomic regulators. What are the tradeoffs from an FDA perspective, from a regulatory perspective of when we consider the utility of markers and when they might enter into a label or into an instruction to clinicians.

There are different factors at play. And on the right side are the factors that favor a marker as a clinical tool, when the allele is common, when the test has a high positive predictive value, when the result strongly implicates treatment benefits versus risks, when there are few and expensive alternate treatments and when this adverse event is severe and will kill you if you get it. Those are the kinds of things that you would say hey, let's test for this.
Now, it ends up that we do have some labels where we actually recommend genomic marker testing, but there are some nuances to this. Even with demographic groups, there can be variability in the frequency of an allele, which then impacts the value of testing. An example is HLA-B*5701 for the abacavir hypersensitivity reaction, where the marker actually is very frequently expressed in Caucasians, in 5 to 8 percent of the population. So, you just have to test 20 people to prevent one bad reaction. That is a no-brainer. But if you go to East Asia, you know, Korea and places like that particular allele is very rare. So, you have to test over a thousand people to prevent one reaction. So, there is some variability in the utility of the marker, based upon allele frequency.

Autoantibodies, are the sine qua non biomarkers of autoimmunity and drug-induced autoimmunity as well, they are not necessarily the mechanisms by which tissue injury occurs but they are manifestations of the dysregulation. Why there are different cellular components and isotypes among different autoimmune drug reactions is really not fully understood. We have heard a little bit, we got some inkling of this this morning but it is still not completely understood.

Another frustration is that high versus low titers of drug-induced autoantibodies do not predict clinical significance of severity or injury. So again, the titer or the
concentration of the antibodies don't really correlate with injuries. So, these are biomarkers but they are challenged in terms of what they really mean.
The key point is made in this slide, which is that different drugs have different risk profiles for different kinds of autoimmune reactions, based upon what has been reported in the literature. So, for example, procainamide is very tied to DILI, as is hydralazine, less so to autoimmune hepatitis. But some drugs are connected to both and this makes it more complicated. Some drugs are connected to one target form of injury or one syndrome, even though they are connected in terms of their presumed pathologic pathways.

Another interesting point is that different drugs actually have different characteristic autoantibody profiles but these are not entirely specific, so that commonly the ANA, which is an immunofluorescent test and this has a homogeneous pattern, often what it reflects are autoantibodies to histones. And they are seen in many drug reactions with DILI, not necessarily all.

Some drugs give other characteristics of autoantibody, such as double-stranded DNA antibodies with minocycline or perinuclear antineutrophil cytoplasmic antibodies, which actually reflect antibodies to myeloperoxidase. Infliximab also gives a kind of particular set of autoantibodies, including DNA antibodies, cardiolipin antibodies.

<table>
<thead>
<tr>
<th>Drug</th>
<th>DILE Association</th>
<th>DILE Ag/Auto-Ab</th>
<th>AIH Association</th>
<th>AIH Ag/Auto-Ab</th>
<th>Other Rx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procainamide</td>
<td>+++</td>
<td>histone, histone-DNA, cardiolipin</td>
<td>-</td>
<td>N/A</td>
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</tr>
<tr>
<td>Hydralazine</td>
<td>+++</td>
<td>histone, ANCA histone-DNA</td>
<td>+</td>
<td>N/A</td>
<td>Vasculitis</td>
</tr>
<tr>
<td>Minocycline</td>
<td>++</td>
<td>ANA, pANCA, cardiolipin, ds-DNA, Ro/SSA</td>
<td>+++</td>
<td>ANA, pANCA</td>
<td>Thyroiditis</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>±</td>
<td>N/A</td>
<td>+++</td>
<td>ANA, SMA</td>
<td></td>
</tr>
<tr>
<td>Methyldopa</td>
<td>++</td>
<td>ANA, histone</td>
<td>+++</td>
<td>ANA, SMA</td>
<td>Hemolytic Anemia</td>
</tr>
<tr>
<td>Infliximab</td>
<td>+</td>
<td>ds-DNA A (IgM, IgG), cardiolipin</td>
<td>++</td>
<td>ANA, SMA ds-DNA</td>
<td>Vasculitis ILD Nephritis</td>
</tr>
</tbody>
</table>

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And when we look at drugs that induce autoimmune hepatitis, again, some of them are very heavily weighted towards autoimmune hepatitis and not to DILI and they have characteristic profiles of autoantibodies, which we heard about today, that fit into either the so-called Type 1 autoimmune hepatitis category or the Type 2 autoimmune hepatitis category. The first four drugs on this list, actually, they were so tainted with risk for autoimmune hepatitis that they have either been removed from the market or they were never introduced to the market. We heard about tienilic acid before, and they make these characteristic antibodies, which you can determine in vitro or in cell staining from liver or from kidney in the microsomal traction. They turn out to bind to CYP2C9, one of the cytochromes and the dihydralazine CYP1A2, which there was a nice review, brief review that Paul Watkins actually wrote a number of years about the CYP1A2 antibody.

And then ipilimumab which we will hear about later is a drug which revs up T cells but doesn't really create any antibodies that are characteristic, at least so far, that we haven't discovered.

So, why do these particular drugs pick these particular cytochromes? We heard a little bit this morning about this idea of epitope expansion. Some of these drugs have a stop step where they meet the cytochrome in their metabolic
clearance and so there is a physical proximity in the metabolism of these drugs with these cytochromes. And whether that has an effect on how the immune system ultimately decides to actually make antibodies against the enzyme, rather than drug is an open question but it may have something to do with this idea of expansion of the epitopes.
And other findings with regard to autoantibodies is that they are often detected in individuals without liver injury. Procainamide patients have an extraordinary rate of developing ANA, antineutrophil antibodies, even though many of them don't have a clinical syndrome. Likewise, infliximab, in a study of RA patients, 15 percent of all RA patients treated with infliximab actually have been found to develop double-stranded DNA antibodies. And 55 percent with IBD develop ANA. So, of course, most of those patients do not have a clinical syndrome.

And we talked about, on the other hand, the point that autoantibodies can pick out, they have characteristic signatures for certain drugs which I have listed here. So, when we see a clinical syndrome and we see these autoantibodies, it is the circumstantial evidence that the drug is somehow tied to the reaction, but it is not foolproof. With regards to checkpoint inhibitors, as we will hear, there are autoreactive T cells that come into play. And perhaps in the future we will have good assays, not to measure autoantibodies, but to measure T cell reactivity in the presence of certain clones of T cells that are responding to particular drugs. And that might be the clinical assays of the future.
Now, because autoantibodies are limited, we want to look for other potential dysregulated mechanisms as potential biomarkers. And there is a lot of literature about mechanisms that come into play. One is the inhibition of DNA methyltransferase by certain drugs, procainamide and hydralazine, for example, which then basically unleashes gene expression through hypomethylation of a certain gene regulatory regions and then the expression in those T cells of certain molecules that enhance activity of the T cells. TH-2 cells are the ones that drive B cell autoreactivity.

We heard a little bit today about this idea of reduced apoptosis, a defect that has been proposed with regard to clearance of cellular debris and perturbation there. And we heard a little bit from Jack about this idea of disruption of tolerance.

One of the mechanisms with regard to procainamide hydroxylamine which was nicely reviewed a number of years ago by Jack in one of his reviews, shows that there is a perturbation in a mouse model for positive thymic selection so that the T cells that are selected to be kept and recirculated are defective in some way and they don't tolerate. They somehow activate. They don't have an energetic reaction.
So, I am going to close by just making a few points about these checkpoint inhibitors as a prelude to David Berman's talk. And I just point out that we are beginning to see more of these kinds of drugs at FDA, and we will see more of this in the future. I listed some of the molecular targets for these inhibitors. Because of the nature of how they work, they are linked to a high-risk for autoimmune organ injuries because they basically soup up autoreactive T cells and perhaps NK cells. That is how they work but they can also cause autoimmune injuries and we see a lot of them.

It is in the label but it is also in the post-market experience. The most common is colitis, but also hepatitis, liver failure, endocrine effects. And so there is a real risk level for life-threatening AEs, which you can actually see in clinical trials. You don't have to get a million patients exposed before you start seeing them. Within a few thousand patients, you see a whole bunch of these reactions.

So, what we need to do a better job going forward is how to pick out patients to predict who are going to be the bad actors. Who are going to be more susceptible to autoimmune unintended reactions, rather than the reactions against the cancer cells.
So, just to give a snapshot from my colleagues who where working this up from our spontaneous report database at FDA, and this is not expected because these reactions were actually seen in clinical trials as well, is that there is a certain percentage of patients, a certain number of patients in the spontaneous report who have been reported with colitis. The most common known adverse event in this category of adverse events, some with intestinal perforation and also cases of autoimmune hepatitis and hepatic failure.

Now, when we look at the cases with more focus, it turns out that many of the patients who were bad actors actually already have underlying liver disease with, in this case, melanoma metastases to the liver. But there is a very striking temporality between the onset of serious liver function changes and the treatment step itself. So, there is a complexity of underlying cancer in the liver and then addition of a drug that actually, for these individuals, tips the balance not in their favor.
And I just wanted to highlight an example of a case of interest that shows these complexities in the post-market database of a 60-year-old male. He has melanoma metastases with small lesions in his liver. He was apparently a good candidate for ipilimumab. This is the drug that is a CTLA4 inhibitor. And after the second dose, within three weeks, he developed flagrant liver failure with hepatic encephalopathy, hepatic cellular necrosis, very dramatic enzyme increases. And remarkably, because of the nature of this drug, there is no ANA positive -- ANA is not remarkable. And the immunoglobulins are not elevated either. So, this is a particular feature of this kind of autoimmunity. The clinicians thought this was the drug reaction. They put the patient on prednisone and they put them on high-dose steroids. The patient didn't do very well and quickly died.
The question for these kinds of drugs is that new drugs are coming online to treat cancer cells, basically through a therapeutic autoimmunity. The issue is how to find the sweet spot, what I have called an autoimmune Goldilocks zone, where we are actually aiming to find the right level of autoimmunity to deal with the cancer cell but not to harm our organs. And how to do this more elegantly is going to be the subject of more research in the future; how to pick out the patients who are susceptible, how to monitor them, how to early intervene, and modify their treatment course, and so on.
In my last slide, I want to make some self-evident comments about causality with regards to autoimmunity, where we are challenged using an algorithmic RUCAM score. And I just want to make these points, looking forward to perhaps more diversity RUCAM scoring, based on the drugs that are in question. The points I want to make are that the broad range of clinical presentations and timelines challenges the utility of a single algorithmic assessment of causality in these kinds of reactions in autoimmune hepatitis.

Current RUCAM criteria of causality are not in alignment with a late onset chronic autoimmune phenotype of hepatitis. Time/exposure effects, steroid responsiveness, histopathology and serology bear attention for such an algorithm. Matching specific autoantibodies with certain drug-induced injuries as an algorithmic criteria for causality may have utility but requires case and control testing with validation studies.

And finally, in the future, a set of RUCAM-like scales might be established that would be appropriate to align with particularly drug-related AIH scenarios. So, right now, we are sort of left with an expert opinion. But going forward and our colleagues at the NIH and DILIN have been thinking about this, maybe we will have more than one set of algorithmic criteria to employ, based upon the drugs that are suspected.
So, then I am going to finish and go on to our next speaker, Dr. David Berman, who works at BMS. He is an expert immunopathologist who has been guiding different aspects of their program in oncotherapy. And he had a stint at the NIH working with Dr. Kleiner as an MD-PhD and we are very happy to have him.