Thank you very much. I don't know how many of these meetings I have been
to but they are always very enjoyable. And John is just the energizing bunny
to keep this going the way he does.

I didn't choose this title but I think it is not inappropriate. So, in other areas,
there hasn't been much question that idiosyncratic drug reactions are immune-
mediated. But in the area of hepatology, that was not the case. I think more
and more people have decided that these things really maybe immune-
mediated. And certainly, they have the same characteristics as other types of
idiosyncratic reactions, in terms of delay and onset, et cetera.
Evidence that IDR are immune mediated.

- Not much controversy for idiosyncratic drug reactions (IDRs) such as skin rash and autoimmunity, less so for idiosyncratic drug-induced liver injury (IDILI)
  - Characteristic delay in onset, which varies with the type of IDR and even the drug. IDRs can even occur more than a month after discontinuation of the drug. But on reexposure, typically the IDR occurs immediately, although there are many exceptions, especially for mild IDRs.
  - Presence of eosinophilia, fever, and/or rash, but the absence of these characteristics does not argue against an immune mechanism.
  - Presence of anti-drug antibodies.
  - HLA associations
  - Positive lymphocyte transformation test.

So, there are several pieces of evidence that I am going to point out. I can't point to all four screens at one time, so I apologize. But some of the evidence that these things are immune mediated are at first just the characteristics. I mean this is the sort of typical type of characteristic for immune-mediated reaction. The delay and onset, often a rapid onset on re-challenge, et cetera.

There is often the presence of eosinophils, fever, rash, et cetera, that suggest an immune response but even if those features aren't there, it does not mean that these reactions are not immune-mediated. Often we see the presence of anti-drug antibodies. That doesn't prove that it is an immune-mediated reaction. These could be an epi phenomenon but, again, it is consistent with the hypothesis that these reactions are immune-mediated. And unless you know what the reacting metabolite is and can make the appropriate antigen, you can't test for antiderug antibodies. And so the number of drugs for which this has been shown is relatively limited. More recently, there have been HLA associations. And again, that is pretty strong evidence that the reactions involved are immune-mediated. And finally, there are positive lymphocyte transformation tests. So, in this case, you take cells from the patient who has had an idiosyncratic reaction, incubate with the drug involved, and if they proliferate, that means that the lymphocytes have recognized the drug. And that is, I think, very strong evidence that the reaction is immune-mediated. I
used to not understand why this reaction would be positive because, in most cases, we think it is a reacting metabolite of the drug and not the parent drug that is responsible. So, why is the immune system recognizing the parent drug?
What we have seen is that once you get a strong immune response, you get epitope spreading, so that often, the immune system recognizes the parent drug, as well as drug-modified protein. So, even though I think these things are immune-mediated, I would be the first to admit that we do not have conclusive evidence, in most cases. It is just this pattern that looks like an immune reaction. So, how do we really test the hypothesis that reactions are immune-mediated? What we really want to do is test patients but we want to know what happens before the patient gets sick. What are the events leading up to this immune response? And of course, we don't know who is going to have an idiosyncratic reaction. So, that is very difficult to do.

As in other areas of medical research, animal models are very important but we always have to make the link between the animal model and humans. We are really interested in humans, not animals, and unless the characteristics of the animal model faithfully reproduce what happens in humans, they are really not very useful.

Unfortunately, although animals have idiosyncratic reactions, they are just as idiosyncratic in animals as they are in humans. And unless you have a pretty high incidence, it is not going to be very useful. And if these reactions are
immune-mediated, you would think that we could just stimulate the immune system and that would allow us to develop -- easily allow us to develop animal models. I don't know how many, and I mentioned this last year, how many graduate student years of mine and other people, I am sure, have been wasted trying to develop animal models by stimulating the immune system in various ways and it never worked. And this, to a large degree, mimics what we see in humans, that patients with preexisting liver disease and inflammatory conditions like inflammatory bowel disease are not at significantly increased risk. And so, stimulating the immune system, somehow the immune system seems to be able to differentiate the drug from other inflammatory stimuli.
Isoniazid (INH)-Induced Liver Injury Was Not Believed to Be Immune Mediated

- It was shown that acute INH toxicity in rats is caused by bioactivation of the metabolite N-acetylhydrazine.
- This is the wrong type of toxicity in the wrong species.
- Most of the covalent binding in mice and humans involves direct oxidation of INH to a reactive metabolite.

A classic drug that was not believed to be immune-mediated is isoniazid. And part of this was based on classic studies done almost four decades ago with isoniazid. And it was shown very clearly that in rats, when you gave a really high dose of the drug, you got acute toxicity that was mediated by a metabolite of acetylhydrazine. But it is the wrong model in the wrong species because that is not the sort of toxicity that we see in humans. It is always delayed in onset. And when we looked at the metabolism, in fact, in the upper right-hand corner, you see so that we developed an antibody that recognizes with isoniazid bound to protein and in four different mice you see covalent binding to a range of different proteins. On the left you see the same immunoblots from control animals that weren't treated. So, you can see that the antibody is quite specific for recognizing isoniazid-modified proteins. It's bioactivation of the parent drug, not acetylhydrazine in these mice, that is leading to the covalent binding.

If you compare mice and rats, there is a little bit of covalent binding of the parent drug in rats but much less than in mice. And if you look at human microsomes, you see covalent binding of the bioactivation of the parent drug. So, we more like mice than we are to rates.
And in collaboration with Will Lee, we took sera from quite a few patients that had isoniazid-induced liver failure and we see a pattern, a different pattern in different patients of antibodies that either recognize isoniazid or autoantibodies that recognize one or more of the P450s that form the reacting metabolites.

Again, this isn't proof that it is immune-mediated but certainly consistent with that hypothesis. And we needed to know what the reactive metabolite was, in order to be able to test this hypothesis. But still, when we treat mice with a reasonable dose of isoniazid that would give comparable to therapeutic concentrations in humans, we don't see any toxicity. So, we don't have an animal model.
And so why is it so difficult to develop animal models of idiosyncratic drug reactions? Well, they may have the wrong MHC repertoire or T cell receptor repertoire. But if you remember that immunoblock that I showed you with covalent binding of isoniazid, it looks like a coomassie blue stain. It is binding to any protein that has a lysine on it. And each one of these proteins is processed to several peptides. So, there ought to be some MHC T cell receptor complex that would recognize one of those peptides. Another possibility is you don't have sufficient activation of antigen presenting cells. But again, we tried to do that and at least the ways that we tried to do it didn't work. We have also tried to increase the formation of reactive metabolite, to deplete glutathione, to do all sorts of things and none of those methods work.

And it appears as if, especially in the liver, the default immune response is immune tolerance. That is the key, I think. So, of course you are familiar with the fact that if you give a whole bunch of people isoniazid, in most cases, nothing happens. So, if you consider Homer normal, that is the result.
In a study that I will show you in a minute, up to 20 percent of the patients will have a bump in ALT but you can continue to treat with isoniazid, the ALT comes back to normal, nothing happens. That is adaptation. And only the rare patient, less than one in a thousand, develops liver failure. Now, if the injury is mediated by the immune system, this adaptation must be immune tolerance. And a good example, I think, of that, Paul mentioned this yesterday with lumiracoxib, it is associated with a specific HLA genotype that is pretty good evidence that it is immune-mediated. And it is the same HLA association for the mild toxicity as it is for the severe toxicity. So, again, if that reaction is immune-mediated, that adaptation must involve immune tolerance.

So, although it is difficult to do prospective studies in humans, we did it with isoniazid because the incidence of mild injuries, actually pretty high, up to 20 percent. And what we found is that in those patients that had a mild increase in ALT and the ALT just went from what is it, 18 to 93, I think only one of the six patients that had an increase was over 100 and they continued on treatment and it goes back to normal.
In those patients that had an increase in ALT, you see an increase in Th17 cells. That is in the upper right-hand corner, this is one example but all six of them had an increase -- what did I say -- all those that had an increase in ALT had an increase in Th17 cells, which are proinflammatory cells but they also had an increase in T cells producing IL-10, which is an immunosuppressive cytokine. So, even in these mild injuries, we are seeing a risk immune response. With isoniazid, we don't see any liver injury in mice at a reasonable dose of the drug.
But with another drug that causes both liver injury and agranulocytosis, amodiaquine, here is a metabolic scheme showing the formation of the reactive metabolite. We do, in mice, see mild injury. So, there is an increase in ALT. We continue treatment with the drug, and then you get adaptation. Again, we believe this is immune tolerance. So, if it is immune tolerance, one possible way to overcome that immune tolerance is to immunize. We know what the reactive metabolite is. We can bind this molecule to protein. The immunized mice with amodiaquine-modified hepatic proteins, along with adjuvant, and then we wait a couple weeks and then we treat with oral amodiaquine. We should now get a much stronger immune response.
And it may be hard for you to see but the bars that are elevated are the ones that were not immunized. We get an increase in ALT. But in those that were immunized, that immunization, instead of making a liver injury worse, it was actually protective. It was a paradoxical response.
If it is difficult to develop animal models because the default response is immune tolerance, it might be possible to overcome tolerance by immunization

• Amodiaquine alone causes mild immune-mediated liver injury that resolves despite continued treatment.
• Immunization with amodiaquine-modified hepatic proteins followed by treatment with oral amodiaquine should lead to severe hepatitis.

And if you look in the liver of these animals, you see an increase in myeloid-derived suppressor cells and T regulatory cells. So, this immunization actually induced immune tolerance, even though we used adjuvant to the drug-modified proteins.
So, another strategy, if the dominant response is immune tolerance, maybe if we block immune tolerance, we could get more injury. And as you probably know, there are a lot of drugs being developed now to block immune tolerance for the treatment of cancer. And it is a very promising area of research. And two of those molecules are PD-1 and CTLA-4.
And this is a complicated slide but you see in wild-type animals, again with amodiaquine, there is an increase in ALT but, despite treatment, the ALT goes back to normal.
If we co-treat with anti-CTLA-4, we get a stronger immune response and more injury, but it still goes back to normal, despite continuing treatment.

If the dominant response to drugs that cause idiosyncratic liver injury is immune tolerance, it might be possible to develop an animal model by inhibiting molecules involved in immune tolerance.

- PD-1 is expressed on activated T cells, B cells, and macrophages. It negatively regulates TCR signals.
- CTLA-4 is expressed on T cells and competes with CD8 for binding to CD80 and CD86 on antigen presenting cells to inhibit immune responses.
- These molecules are being targeted to treat cancer.
On the right side, these are PD-1 knockouts. Again, we get a stronger immune response and injury but it resolves, despite continued treatment.

But if we co-treat these animals with anti-CTLA-4, now -- and the scale is different here, now it doesn't resolve and we get histopathology of piecemeal necrosis that looks just like what happens in humans with severe liver injury. Now, despite the fact -- and the ALTs are not that high but, as you know, clinically, I would much rather have a high ALT from ischemic liver injury than a sustained liver injury over a long period of time. And we do see an increase in bilirubin in these animals, along with the histopathology but we don't get overt liver failure.
AQ treatment increases the number of PD-1\(^+\) and CTLA-4\(^+\) T cells and in PD-1\(^-\) mice cotreated with anti-CTLA-4 increases cytotoxic T cells.

There is decreased function but not overt liver failure. And we also see, and again, this, I am sure, is difficult to see but in the wild type animals, there is an increase in T cells that express PD-1, that express CTLA-4, et cetera. In the PD-1 knockouts, there is an increase in Treg. So, even though we are getting a strong immune response and liver injury, there is still -- the immune system is trying to down regulate that immune response. In the lower quadrant here, you see also an increase in cytotoxic T cells. These are CD8 T cells that express granzyme B and perforin. And so this suggests that injury may be mediated by cytotoxic T cells. And there is evidence clinically that some of the most severe liver injury is mediated by cytotoxic T cells.
So, what we did is deplete CD8 T cells and sure enough, it totally protects these animals from liver injury.
So, how about other drugs? And Arie was very enthusiastic when I presented some of this data last year with a different way of trying to block immune tolerance. We weren't seeing injury with isoniazid, so I was a little hesitant at that point. But when we used the same system with isoniazid and I say here it increases liver injury, that is actually a misstatement because without using PD-1 knockouts and anti-CTLA-4, we don't see any liver injury but in that model, we do see liver injury.

The same thing happens with nevirapine. We don't see any liver injury in wild type animals but, in this model, we see liver injury with nevirapine. So, it looks like blocking immune tolerance is exposing the potential of a drug to cause immune liver injury. And there is another drug that I can't tell you about because of the confidentiality agreement but a drug that is used to treat cancer by modulating immune response, we are seeing the same picture. Now, there are a lot of different cells and molecules involved in immune tolerance.

And Lance Pohl has a paper that has been accepted in Hepatology, where he looked at it from a different perspective. Lance did work with halothane some three decades ago, that actually convinced me that these events were immune mediated. And Lance, for three decades, has been trying to develop animal...
models without success. But finally, he succeeded. Unfortunately, he had a stroke and has had to close down his lab. But instead of going after immune tolerance with PD-1 and CTLA-4, he depleted myeloid-derived suppressor cells and he gets liver injury with halothane that looks very similar to what happens in humans. There are multiple mechanisms, redundant mechanisms for immune tolerance and any one of these can have an effect. The other interesting point is that some of the most severe liver injury, I think, is mediated by CD8 T cells and we showed that we could block that in the amodiaquine model, in his model, it looks more like halothane. He sees eosinophilia and if he blocks CD8 T cells, it doesn't protect but if he blocks CD4 T cells, it does protect. These drugs are causing immune responses that damage the liver but the immune response can be different with different drugs and even the same drug in different people.
And how about biologicals? It is not surprising that drugs like interferon alpha would cause autoimmune hepatitis. It is stimulating the immune system. What is more surprising is that drugs that are supposed to be immunosuppressive like infliximab also can cause autoimmune hepatitis. TNF alpha is doing more -- it is more complicated than just that this is an immunosuppressive drug. And not only can some of these drugs used to treat cancer cause liver injury but they can interact with other drugs. So, for example, if you co-treat with ipilimumab, and I am not that familiar with that drug, but the drug can cause an increase in ALT but you combine with anti-CTLA-4 and it markedly increases the risk of severe liver injury. So, as we develop these drugs, we are going to see drug interactions with other drugs because it uncovers the potential of the drug to cause liver injury.
And I will go through this quickly because it is not liver and I need to go through it quickly. We developed an animal model for nevirapine-induced skin rash. Now, it is a lot easier to induce an immune response in the skin than it is in the liver because the liver, the default immune response is, again, immune tolerance.

We have succeeded in developing an animal model of an IDR that is very similar to the IDR in humans without manipulation of the immune system.

- Nevirapine causes an immune-mediated skin rash in Brown Norway rats similar to the rash that it causes in humans.
And again, we have found that in rats we get a skin rash that looks very much like what happens in humans and this table lists the different characteristics; it is very similar between rats and humans.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Humans</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash</td>
<td>mild morbilliform to TEN</td>
<td>mild to severe but no blisters</td>
</tr>
<tr>
<td>Plasma levels</td>
<td>1-10 μg/ml</td>
<td>20-40 μg/ml</td>
</tr>
<tr>
<td>Time to onset</td>
<td>1-3 weeks</td>
<td>2-3 weeks</td>
</tr>
<tr>
<td>Dose response</td>
<td>incidence increases with dose</td>
<td>incidence increases with dose</td>
</tr>
<tr>
<td>Sex dependence</td>
<td>incidence greater in women</td>
<td>incidence greater in females but probably metabolic difference</td>
</tr>
<tr>
<td>Low dose pretreatment</td>
<td>decreases incidence</td>
<td>prevents rash</td>
</tr>
<tr>
<td>Rechallenge</td>
<td>immediate onset and more severe</td>
<td>decreased time to onset and decreased dose required</td>
</tr>
<tr>
<td>Histology</td>
<td>little data, lymphocytic infiltrate</td>
<td>T cells and macrophages</td>
</tr>
<tr>
<td>T cell dependence</td>
<td>incidence low if CD4+ count low</td>
<td>depletion of CD4+ T cells is protective.</td>
</tr>
<tr>
<td>In vitro lymphocyte response to NVP</td>
<td>produce IFN-γ</td>
<td>produce IFN-γ</td>
</tr>
</tbody>
</table>
Nevirapine is oxidized to a free radical intermediate that partitions between oxygen rebound to form the 12-OH-metabolite and loss of another hydrogen to form a quinone methide. The quinone methide binds to hepatic proteins and is presumably responsible for hepatotoxicity. The 12-OH-metabolite is sulfated in the epidermis and the sulfate covalently binds and causes the skin rash.

And we were able to show that there is a reactive sulfate formed in the skin that is responsible for this skin rash.
12-OH-Nevirapine Sulfate Formed in the Skin is Responsible for the Rash

- Topical administration of a sulfotransferase inhibitor prevents covalent binding and the rash where it is applied.
- The required sulfotransferase is not present in mouse skin, and we have not been able to induce the rash in mice.
- This is the first time that a reactive sulfate formed in the skin has been shown to be the cause of skin rashes, and there are several other drugs that probably cause a skin rash by a similar mechanism.

And then the next question is, because we could prevent the covalent binding and the rash with a topical sulfotransferase inhibitor, the next question is how does covalent binding of this reactor metabolite that we showed clearly is responsible for the rash, how does it induce this immune response that leads to the skin rash?
And it was known that chemically reactive agents applied to the skin -- poison ivy, or dinitrochlorobenzene -- cause contact hypersensitivity. And it is known from that literature that animals that are deficient in the inflammasome apparatus are resistant. And although we were getting a reactive metabolite formed in the skin from a precursor that came from the liver, otherwise it should be a similar mechanisms to contact hypersensitivity.

How does the reactive metabolite of nevirapine cause a skin rash?

- Chemically reactive compounds applied to the skin cause a skin rash (contact hypersensitivity).
- This is known to involve inflammasome activation because ASC- or NALP3-deficient mice (ASC and NALP3 are components of the inflammasome) have impaired responses to contact sensitizers.
So, maybe activation of inflammasomes is an important early step in the induction of an immune response. And this is just a pictorial of the inflammasome. It is a complex structure. What is important is that procaspase gets activated to caspase 1 and that converts pro-IL-1 beta to active IL-1 beta. And if something increases the level of IL-1 beta, and you can block it with a caspase 1 inhibitor, that means it must have come from an inflammasome.
So, we looked at pairs of drugs that caused idiosyncratic reactions, one of which is much safer than the other. So, we compared telaprevir with boceprevir. Telaprevir had a black box warning because of severe skin rash, boceprevir doesn't. Dimethyl fumarate is a drug being developed for the treatment of multiple sclerosis, is associated with contact hypersensitivity and a bunch of adverse reactions.

Ethacrynic acid is an old drug. It is also a microacceptor. If you are a chemist, you know what that means. If you are not, you probably don't. But these drugs are chemically reactive but ethacrynic acid, although it is known to covalently bind to protein, forms a glutathione adduct, I went through the literature and I couldn't find one report of an idiosyncratic reaction to ethacrynic acid. I don't know why.
So, when we looked in in vitro assay of the ability of these drugs to activate infflammasomes, so this is a dose response curve, telaprevir activated infflammasomes. We could block it with an caspase inhibitor. Boceprevir didn't significantly activate infflammasomes. A different scale here, dimethyl fumerate really activated infflammasomes and ethacrynic acid, not a bit, even though it covalently binds to protein.
One thing that I have been interested in for a long time is clozapine and olanzapine. Clozapine causes agranulocytosis, as mentioned yesterday, can also cause liver injury. In most patients treated with the drug, there is an increase in IL-6, neutrophilia. It clearly causes an immune response. Olanzapine does not cause a significant incidence of agranulocytosis in humans, and it does not cause neutrophilia in rats even though both are oxidized to a reactive nitrenium ion by myeloperoxidase.

Contrasting Clozapine and Olanzapine

- Clozapine causes agranulocytosis with an incidence of about 0.2%
- It causes an increase in IL-6, other cytokines, C-reactive protein, fever, and paradoxical neutrophilia in >50% of patients.
- Clozapine also causes neutrophilia and an increase in alpha-1-glycoprotein (equivalent to C-reactive protein in humans) in rats.
- Olanzapine does not cause a significant incidence of agranulocytosis in humans, and it does not cause neutrophilia in rats even though both are oxidized to a reactive nitrenium ion by myeloperoxidase.
But in terms of inflammasome activation, at the same concentration, clozapine activates inflammasomes and olanzapine doesn't. So, there is some other difference than dose between these two drugs. I don't know what it is but it clearly shows up with inflammasome activation.
Amodiaquine, the drug that we used for the liver injury model, it also activates inflammasomes. So, this may be a biomarker for the ability of a drug to cause an idiosyncratic reaction. Now, with drugs that are intrinsically reactive, that is easy to test. Even with clozapine, there is enough mild peroxidase in these THP-1 cells, we get bioactivation and covalent binding. I didn't show you the data but we did covalent binding of clozapine to the THP-1 cells. But if the drug requires P450 bioactivation, these cells don't have a significant amount of P450.

My best guess, and it really is a guess, is that maybe the hepatocytes make a reactive metabolite. It is known that hepatocytes release exosomes, or microvesicles, or whatever you want to call them. These would be taken up by antigen presenting cells, Kupffer cells, and other antigen presenting cells and proactivate them. And so we have started studies looking for this. Unfortunately, in the way that we isolate them, it is just killing the THP-1 cells. So, I think we have to go back and not use a simple way to isolate them but use a more complicated way.

Am I running out of time? Yes, okay.
Inflammasome Activation as a Biomarker of IDR Risk

- These results suggest that the ability of a reactive drug or reactive metabolite to activate inflammasomes may be a biomarker of IDR risk.
- The assay is simple for drugs with intrinsic chemical reactivity or are easily oxidized by peroxidases, but it is more of a challenge for drugs that require bioactivation by P450.
- Possible approaches include co-cultures and in vivo studies of inflammasome activation.
- The release of microvesicles containing molecules such as HMGB-1 by hepatocytes is an attractive mechanism by which inflammasomes may be activated in the liver.
Immune Responses to Drugs That Cause IDRs

- Drugs such as clozapine clearly cause an immune response in most humans and rodents treated with the drug.
- A careful study of the immune response to drugs, especially by phenotyping mononuclear leukocytes, may provide important mechanistic clues and ultimately provide biomarkers of IDR risk.
What are the IDR risk factors in Humans?

- Genetic – all known strong associations are with HLA genes, but with the exception of abacavir, even if a person has the required HLA gene they are still unlikely to have an IDR to the drug – there must be additional factors. Many IDRs do not appear to have a strong HLA association.
- T cell receptors – formed by random gene recombination
- Activation of the immune system/inflammatory condition – with some exceptions - this does not appear to be important.
- Deficiency in immune tolerance – although most patients do not have marked deficiency in immune tolerance, immune system-related genes are associated with IDR risk.
- The immune system is a product of everything it has ever been exposed to - some patients have antibodies against drugs that they have never been exposed to, which is presumably due to cross-reactivity with some pathogen.

So, what are risk factors in humans? Genetic factors are, obviously, important. T cell receptors are formed by random recombination events. So, even identical twins have different T cell receptor repertoires. I talked about activation in the immune system and, again, clinically, in the ways that you might expect preexisting liver disease, et cetera, that doesn't seem to be important. Deficiency in immune tolerance, the patients that have idiosyncratic reaction do not have the degree of immune tolerance deficiency that these animal models do. So, I think we are uncovering something but I don't think that is a major issue in humans, although polymorphisms in IL-10 can affect the type of immune response you get and the mortality of DILI. It doesn't seem to affect the risk.

One point I would like to make is I think the immune system is a product of everything. It is like the brain. It is a product of everything it has ever been exposed to and so different people are going to respond differently.
Treatment of Serious IDRs

- If there were an effective treatment for serious IDRs it would markedly decrease their significance.
- Many serious IDRs appear to be mediated by CD8+ T cells.
- The usual care for toxic epidermal necrolysis is IVIG; steroids do not appear to decrease mortality.
- Liver injury is often treated with steroids, but with the exception of idiopathic autoimmune hepatitis, efficacy is unclear.
- An effective treatment for aplastic anemia, whether idiopathic or drug-induced, is anti-thymocyte globulin.
- The most serious cases of IDILI appear to be mediated by CD8 T cells and depleting CD8+ T cells prevented liver injury in our model.
- Targeting CD8+ T cells may be a more effective treatment; various treatments should be studied.

We'll pass over that one.
So, I think valid animal models are important. There is compelling evidence, I think that most idiosyncratic reactions, including idiosyncratic DILI is immune-mediated, genetic factors play a role but there are other factors that are important. I think, again, environment, you know it is nurture-nature issue again. I think environmental factors important but we don't know exactly what they are. They are not the obvious environmental factors. I think prior exposure to different pathogens set how our immune response responds.

And finally, the most severe reactions are ones that persist after you stop the drug. And if you know what the mechanism is, whether with some of the most severe, it is due to cytotoxic T cells or with other ones that have a more immunoallergic type. I think we have an opportunity window to treat these patients, so that they don't develop overt liver failure, so they don't die or require a liver transplant. And if we could treat them better, I think it would be much less a serious problem. In other fields of idiosyncratic reactions, attempts are made to do this but, for some reason, although patients are often treated with steroids, there has been no good trials to see what works in treating these patients.

Conclusions

- Valid animal models are important for mechanistic studies, but they are difficult to produce; most animal models of IDR do not represent the same mechanism as human IDRs.
- There is compelling evidence that most IDRs are immune mediated, and reactive metabolites are responsible for initiating the immune response; however, not all reactive metabolites carry the same risk.
- Genetic factors play an important role in who is at increased risk, but other factors are also important.
- Mild IDRs are always more common than serious IDRs, and many resolve despite continued treatment. If they are immune mediated, this adaptation must involve immune tolerance.
- One mechanism by which drugs appear to induce an immune response is through activation of inflammasomes. This may represent a good biomarker to predict which drugs that form reactive metabolites are likely to pose a significant risk of serious IDRs.
- Serious IDRs persist after the drug is stopped. If they are immune mediated, there could be a window in which serious IDRs could be effectively treated.
And finally, I want to thank the people that actually do the work, not me, and I thank you for your attention. And I'm sorry I went long.