FDA-AACR Non-clinical Models for Safety Assessment of Immuno-oncology Products Workshop

Transcript: Afternoon Session

Schneider: 01:23:51 Good afternoon, everyone. I'm Julie Schneider from FDA, and I'm very happy to be announcing the speakers for the afternoon. So, I think we're a little bit behind time, so we're just gonna dive right in, keep going through the agenda. So, I'm happy to introduce Dr. Danuta Herzyk who is Senior Scientific Director and Immunology and Oncology Therapeutic Area Leader for Merck where she's responsible for non-clinical safety assessment strategy and management.

Herzyk: 01:24:28 Thank you very much for this opportunity to speak, and we'll be talking about non-clinical safety and drug development. I like to start with few publications that are, I think, pertinent to our discussions in this workshop. So, we've already discussed that we are living in different era in oncology and the adverse effects are immune related now. And, as described in this paper by [DeLillo and Ravidge 01:25:03] it's really because similar mechanisms contribute to both efficacy and toxicity, and that's our challenge.

Another paper, recently published is about toxicity observed with clinically approved checkpoint inhibitors. It's a meta-analysis data, and mostly focused on anti-CTLA-4 and PD-1/PD-L1 blockers. These immune related adverse effects are quite broad in term of the target organs and they affect mostly skin, gut, endocrine system, lung, and musculoskeletal. So, we have a lot of these different -itis-es. The majority of these effects are mild to moderate, and clinically manageable. We've heard that typically there is a delayed onset of these adverse effects and prolonged duration. We have greater severity with anti-CTLA-4 than with anti-PD-1 therapies. And, toxicity varies between different diseases and therapy settings, especially between adjuvant and metastatic diseases.

Also, there was pointed out this morning that cancer type may be a big factor in triggering certain adverse effects in these patients. For example, with pembrolizumab, we see pneumonitis mostly in lung cancer patients, while uveitis is mostly seen in melanoma patients. So, the patient factor is important. And, the third paper already mentioned is the one published by the co-chairs of the workshop. This paper focuses on non-clinical data, including toxicology data, especially in monkeys, and there is a statement that toxicity in monkeys was minimal and did not predict the clinical adverse effects seen in patients. But, I want to make a distinction between two very different groups of immune-oncology drugs. The ones that target co-stimulatory or co-inhibitor receptors, like checkpoint inhibitors, and require concurrent antigen-specific T-cell receptor signaling for their activity. For this group of drugs, indeed, there is low incidence of toxicity and I would argue in both animals and patients in general. While the other group of drugs that target T-cell recruitment to tumor cells, like the [inaudible 01:28:14] and so on. They are independent of the antigen-specific T-cell signaling and typically are associated with very strong cytokine-mediated responses. And, if there is animal model, we see that in animals as well as in patients.

So, let's try to frame the question about the safety, and focus on checkpoint inhibitors where we have immune stimulation in indirect way by restoring active T-cell immune-surveillance and reduction of immune tolerance. Based on this mechanism, the safety risk, actually, can be predicted because we know that this is associated with pro-inflammatory responses. So, the different type of -itis in patients could be predicted even if we don't see

anything in animals in, sort of, general terms. And, as we heard, very careful monitoring in patients has to really take place.

The question I'd like to focus on is: which adverse effects in patient would be of greatest concern? The mechanism-related type of -itis inflammation, or something unexpected or unknown that we cannot really think of based on biology. Another question is there concern about a single immune-oncology agent or combination of, and if it's the latter, which combination? Combination of biologics or combination of checkpoint inhibitors with small molecules? And, I hope we will address this in panel discussion.

In the meantime, let's take a look at the animal data, not only the toxicology data but starting with knockout mice, in which we have the genetic deletion of receptors, so it's the worst case scenario. Nevertheless, we learn something. And, based on the knockout mice for CTLA-4/PD-1 approved therapies, and I also included LAG-3 which is not approved therapy, yet, but quite intensely studied in recent years, we definitely see differentiation between CTLA-4 and PD-1 in knockout mice. We've heard CTLA-4 knockouts are very lethal in term of autoimmune phenotype while the other two are not. There's late-onset and very, actually, minimal changes, mostly lymphocytic infiltrates in the different tissues.

What do we see when we do the toxicology studies in monkeys? Naive, healthy, relatively speaking, monkeys are not free of pathogens as they are exposed to normal bacteria and viruses. Actually, CTLA-4 blockade in toxicology studies with more extensive dosing, like once a week, doses at 10mg/kg, do show toxicity. Monkeys present with dose- and time-dependent incidence of severity of diarrhea and sometimes that leads to moribund conditions, and have large intestine inflammation and skin changes. While in the early days with relatively, I think, lower doses and lower frequency of dosing, a little bit different data were reported for anti-CTLA-4 than what we have today. Another interesting point is that pharmacodynamic response is delayed, relatively, to the dose administration.

On the other hand, PD-1 therapeutic antibodies in monkeys are very well tolerated up to very high doses. Interestingly, their target engagement is evident months after stopping dosing at the high dose. And, despite the prolonged evidence of target engagement, we have very low incidence and dose independent toxicity, not even toxicity. They are findings of expected mononuclear cellular infiltrates in different tissues. And they are often seen as slight exacerbation of the background changes, because such inflammatory changes are often seen in normal control monkeys over large database we have for monkeys. So, it's not toxicity, per se. The other part is that sometimes these effects are seen in animals following treatment-free period, months after stopping dosing. Once in a while we see one animal or two with some changes in this period.

For LAG-3 blockade, again, monkeys tolerate antibody against this target very well. We do have target engagement evident during the dosing period. The findings here are also minimal, but present in dose-dependent manner, as opposed to PD-1, where only singular animals are affected. And, here we have red skin discoloration and some clinical pathology data consistent with inflammation and, again, infiltrates, but here, for some reason, the infiltrates are mostly in meninges and choroid plexus in the brain, which is background finding in monkeys. So, again, this is just a slight exacerbation of background changes, not completely new finding.

Nevertheless, based on this comparison, there is greater toxicity for anti-CDLA-4 than anti-PD-1 in monkeys, and that is confirmed and consistent with the clinical data. There are also more findings for anti-LAG-3 than anti-PD-1.

How about a dual-blockade? Again starting with double knockout mice, we know CTLA-4 knockouts are lethal, so we can only use conditional knockout mice. And for CTLA-4 and PD-1 double knockout, we definitely see increased incidence in severity of the lymphocytic infiltrates and lymphoid proliferation.

Also, for LAG-3/PD-1 dual knockout mice, we have significantly increased incidence and severity of the findings. And there are not only lymphocytic infiltrates, there are also changes to parenchymal tissues and they include thyroid, pancreas, salivary, heart that can progress to fibrosis and myocardial degeneration. So, definitely much more findings in comparison to age-matched single knockout mice.

How about the monkey studies with dual-blockade? In cyno monkeys treated we see increased incidence and severity in gastrointestinal and skin toxicity of gastrointestinal and skin, so that correlates with the colitis and dermatitis seen in the clinic.

For LAG-3 and PD-1 dual-blockade, we also see increased severity and incidence, but the nature of findings, I shouldn't say toxicity, findings are similar. We see them at lower doses, and occasionally, some animals are presented with additional findings, like we've seen one monkey that had diarrhea seven weeks after the last dose, which is more consistent with anti-PD-1 than with anti-LAG-3. Or, we've seen inflammatory infiltrates in thyroid, which is not a background finding in monkeys, eight weeks after the last dose. So, here and there, we have these findings.

So, what do animal data tell us? Well, the knockout mice show generally consistent findings with the mechanism of action. And, also, monkey show generally consistent profile with expected based on the mechanism of action. The findings are related to immune activation. We have a wide range of tissues affected by, but low incidence, usually, and severity of the findings. So, is that very different or similar to clinically observed toxicity? Well, based on monotherapy with pembrolizumab, and a rather large group of patients treated, in patients with melanoma and lung cancer we see that the incidence of findings is very low. The diagnoses are a little bit different, but they're all related to the proinflammatory responses. So, actually, maybe the monkeys are not that different.

What else this data tell us? Well, we do see differentiation of toxicity profile between different antibodies. And combined treatments indicate the increased toxicity, which is anticipated based on biology, in comparison to single agents. So, then, why the toxicology studies viewed as non-predictive? Part of it is that, well we have these findings, and typically in the context of the monkey studies, the monkey health status and the structure histomorphology of tissues and so on, they are not adverse to the monkeys within the study context. So, in conventional risk assessment they are reported as non-adverse, and they should be. But, that's probably the difference from the different era, from cytotoxic drugs and so on, when adverse meant true frank toxicity seen in animals. We don't have that, we have potential gradual change of pharmacodynamic response that may be indicative of potential toxicity. But, in the context of the study it’s not really toxicity. So, we simply need to pay attention to all the findings, and the fact that findings are not adverse doesn't mean they are not relevant to humans, necessarily.

The other part is, well, the immune system is the most heterogeneous organ in both animals and humans. There is very high individual variability in response to the modulation of the immune system, therefore we have low incidence of findings and difficult interpretation of the data. And, we heard this caveat that we have different immune status in cancer disease versus a healthy host. Some postulate that vaccination in animals could mitigate this. I am not sure if that's the right answer. We really don't understand if vaccination actually decreases or increases variability, and, hopefully, that will be discussed later, too.

So, are these toxicology studies in monkeys helpful? Overall, yes. The animal data are helpful, including the fact that we don't see overt toxicity and report no adverse effect level at the highest tested dose, in the relevant species where we do have demonstrated receptor occupancy or target engagement or pharmacodynamic endpoints. They do inform safety, because there is a difference whether we see this at the low dose and high dose and so on. We typically test pretty wide range of exposure. Sometimes, even two logs. That's not typical, but it happened. And, the most important thing, we have insights into expected versus unexpected changes, and I think that is informative.

Herzyk: 01:42:00 Regarding this first- in-human starting dose determination, actually the discussion already happened that we pay attention mostly to pharmacology data for this reason, less so to no adverse effect level and we have to, as I mentioned during the discussion, take into consideration all kind of data, in-vitro, in-vivo, pharmacologically active doses and so on.

What about these pharmacology studies? Again, we heard a lot about these different models this morning. In general, we use the syngeneic mouse tumor models, and they are selected based on feasibility and relevance to cancer type we want to study. The one big point is that tumor growth is very rapid after implantation and survival rate is about three weeks, so as Sarah and others mentioned, it's a really limited window. These models may be useful to evaluate potential antagonistic effect of combined therapies to deselect therapies, but in the presence of effective anti-tumor response in mouse model's safety signals are hardly detectable.

Still, we use these models, we need to start somewhere. Here are a few examples of studies with a combination of a few biologics: anti-PD1 with anti-GITR, anti-PD1 with anti-CD-137 and anti-PD1 with anti-OX40. Depending on the sensitivity of the model, the combination has either very good synergistic effect or just a little bit, as depicted here in the red lines, we usually see some effect, some positive effect, of the combination. And the mice are typically looking well and they don't present any safety signal.

It happened that for some of the combinations, we also have clinical data from early studies and actually for these three, we had very similar results - good tolerability in patients too.

So, we cannot paint all these combinations with the same brush. Sometimes, like combo with anti-CTLA-4 and anti-PD1, it is definitely more toxic than single agents but in other places, that's not the case.

Combinations with small molecules, it's much more challenging and definitely, there are more clinical reports about increased severity of toxicity and usually that leads to cessation of treatment. And we have multiple examples. Here I included two examples for ipilimumab and two for pembrolizumab.

The problem is that unlike for other situations we heard about, where the reversal translation and mechanistic studies and figuring out back was successful, with these toxicities and these combinations, it's very difficult to recapitulate the findings from the clinic in animal studies. It's not because of the lack of trying. Here, we have two examples of two studies. One, in mice, using murine anti-CTLA-4 and Vemurafenib, tried to mimic study in patients, where hepatotoxicity was observed within about 2 weeks after the treatment. We used different versions of murine anti-CTLA-4 for antibodies to accommodate the different ones that are in the clinic and have different interactions and modulations of Fc receptors. All of them had very good exposure, including the small molecule, and we did not see any hepatotoxicity in these mice whatsoever.

Another example is in the dog. Since we do have canine version of anti-PD1 we combined it with Preladenant, which is adenosine A2 receptor inhibitor, and in patients there was exacerbation of hepatotoxicity. Well, we tried to use this combination in the dogs because in the dog we did see hepatotoxicity with Preladenant alone, and we ran three month study, again to mimic the clinical period for which the toxicity was observed, and while Preladenant alone showed liver toxicity again in these dogs, the combination with anti-PD1 did not show any exacerbation, in fact it looked a little bit less hepatotoxic than the Preladenant alone. So, that was a big surprise.

How about the humanized mouse model? Here we used the human anti CTLA-4, Ipilimumab, combined with Vemurafenib. Again, two week study was conducted using mice ingrafted with human cord blood. So, we see some inflammatory infiltrates in the spleen and the liver, but we don't see true hepatocellular injury and hepatotoxicity or liver enzymes elevation. It is a little bit better than normal mice and one can argue perhaps this is a harbinger of the liver findings that could progress later to the toxicity. But the limitation is related to a concern about the graft versus host disease development in these mice over time, which would confound the data.

Overall the challenges with nonclinical combination studies are that small molecule drugs are associated with the traditional off target toxicity, liver, cardiac, renal ... they are metabolized, transported, and distributed very differently than biologics, and they are mostly characterized in rats and dogs. While the biologics have on target pharmacology related toxicity and they are characterized in mice and monkeys.

We thought one of the problems is that we use these tumor models or humanized mice for pharmacology combinations studies, so we are in wrong species. Well, the recent studies with anti-PD1 and Preladenant in dogs, which is the right tox species for the small molecule, did not increase predictivity either. This study doesn't explain or support the hypotheses here. So, as we were hearing all day long, it's very difficult and challenging. I think, overall, we can probably agree and accept that animal data cannot predict which specific “–itis” cancer patients will develop but they provide insight into signals for potential risk and the big point is that patient individual genetics, underlying disease, co-therapies, history and so on are likely contributors to incidents and type of these immune related adverse effects that normally would be probably not seen, and only manifest with the additional therapy.

So, my sort of high level view of this is that safety evaluation of immuno- oncology agents in toxicology studies provides directional risk assessment rather than prediction of adverse effects in patients. And we have many examples of correctly identified risks based on this directional evaluation, as well as, a few examples of unpredicted toxicity in the clinic. And

the main gap in data is for immuno-oncology biologic combination with small molecules, for which severe and acute toxicity seem to be more likely and more problematic to predict and manage than for biologic combinations. And with that, I would like to thank you the organizers and also my colleagues at Merck, who contributed data and information to this presentation.

Schneider: 01:51:51 Time for questions. Anyone with a burning question?

I'd be interested ... I thought the information you shared with the combination studies towards the end of the presentation was really interesting. Are there further studies you are thinking of, or what are you thinking ... Obviously it's very challenging the IO and the biologics but I'd be really interested to hear what you're thinking -

Herzyk: 01:52:16 About the dog study?

Schneider: 01:52:18 Yeah. Or, you know, moving forward.

Herzyk: 01:52:23 We are asking the same question. We thought, well, this is one of the possibilities that we can answer, at least some questions. Again, it seems like patient component is very important, metabolism and the history of disease is one. Actually, we don't have a good answer. We are trying, we are trying all kinds of approaches and don't have a solution yet. I don't know if somebody could help me with this. The humanized mice - maybe we have a colleague who can speak to that.

Saber: 01:53:12 Online question. How can we increase the sensitivity of our traditional animal models to detect toxicity for IO drugs that simulate immune system? Would a TDAR Assay help? Will you add TDAR to your studies?

Herzyk: 01:53:29 Right. No, we don't do that, we don't add TDAR into our toxicology studies. From our experience, actually, if we need to use immunization, we typically use ex vivo models in monkeys and humans. We have pretty good vaccine that works in monkeys and we have monkeys with memory to that vaccine so we use that system which in our hands is more robust than the *in vivo* TDAR, which we tried once or twice, but were not very satisfied with the model. I think Helen would probably address this, I think we do see in monkeys some responses and we do have findings. Just is the matter of how we interpret and view and summarize and communicate the data. It's more important than adding another complexity to the study by, for example, vaccination and TDAR.

Schneider: 01:54:47 Looks like we have a question at mic two. If you could please introduce yourself.

Oropallo: 01:54:51 Sure. Mike Oropallo from Merck and I just wanted to follow up on your question about what we're thinking about in the next steps. So, we're certainly looking more into humanized mouse models, not only the one shown but there's a bunch out there as we've kind of covered today. They're really complex, which makes this really difficult. But as Danuta showed, it does look like we are getting maybe some early single or early t-cell infiltration and so what I think what we're trying to understand is, is that really a sign of toxicity? Can we uncover biomarkers, whether they're being transcriptional or some protein level that would actually help us then translate to what a clinical phenotype would be. And obviously we would look in the dog or any other study as well. So, I think that's our thought. Can we take that information and then further it.

Schneider: 01:55:37 Thank you. Got some ... mic one, you can ... or mic two, I guess you stood up first. Go ahead.

Trube: 01:55:47 Hi Danuta. Very nice presentation. Kevin Truba, Janssen Biotherapeutics. I'm curious, like with IO and small molecule combinations, from a pharmacology perspective, are we really doing a good job of understanding, you know, DDI and changes in metabolism of say the small molecule early. And then, if we do see something clinically, like with PD1 and Thalidomide, you know, analogs, are we trying to understand that?

Herzyk: 01:56:14 Well, I think with the older drugs, there are gaps because they are being combined mostly based on clinical data, so we probably don't and in the past, I don't think the characterization, especially metabolic characterization of these drugs were as extensive as we do today. So, I would think, no, we don't have good understanding of this data. With the novel drugs, we definitely characterize them quite extensively. Of course, we do see differences in metabolic pathways and handling between humans and animals, so again, we can only do so much. We use *in vitro* human systems, as you know, so I think for the novel drugs we, I would say, do pretty good job and have very good handle and characterization but if we go back to older drugs that are being tried in clinical trials with these novel immunomodulator agents, that's probably not very optimal situation. Yeah.

Trube: 01:57:28 Thank you.

Schneider: 01:57:30 Thank you very much. I think we're -

Herzyk: 01:57:33 Wasn't there one more question?

Schneider: 01:57:33 Yeah. One more quick question.

Speaker 1: 01:57:35 I think it's quick. It might actually be sort of a survey. I was curious, I haven't come across of any examples yet of an immuno-oncology drug causing a toxicity that is not immune related adverse event, like an itis of something or cytokine release syndrome. I was just reminded we were talking about lunch about Seldane having, you know, QTC prolongation or Cisplatin having salt wasting nephropathy or other drugs that cause peripheral neuropathy. There's just so many other off target effects. Are there actually completely unrelated effects from any immuno-oncology therapy that we need to be looking for? Or are we just trying to identify which organs could be affected by an itis?

Herzyk: 01:58:24 Well, the beauty of biologics is they have very specific ... So, they not supposed to cause off target toxicity and usually we don't see it. And in combination, I think, we do in patients because of the other components of the other drugs, but if you don't have receptors or molecule expressed in the pathway that we affecting and modulating, I don't think we see these off target toxicities.

Speaker 1: 01:59:05 [inaudible 01:59:05] I guess I'm just getting at the goal of trying to use non-clinical models to predict what happens in humans but it seems like we're trying to predict its uses for different organ systems or potential interactions with other drugs but, in a way, we're ... I'm just not sure that the reason that we were doing pharmacology toxicology was to try to get at these off target effects from other drugs that do cause them, like QTC prolongation or salt wasting, or you know, these other examples that I gave. But it doesn't seem like that process is mapping well to immuno-oncology, so ... It was kind of a comment that maybe thinking about, are we looking for something that we [crosstalk 01:59:45]

Herzyk: 01:59:46 QTC prolongation, I don't think so. We've seen some blood pressure changes and [inaudible 01:59:52] related to cytokine or fever or something in animals, yes, we do occasionally see that if this is involved in the pathway.

Schneider: 02:00:02 Thank you very much. I am very happy to welcome the next speaker up to the podium. Dr. Helen Haggerty is department head of Immuno and Molecular Toxicology and Therapeutic Area head for Immuno Science within the drug safety evaluation at Bristol Myers Squibb. And she provides scientific leadership and direction for all the programs within her therapeutic area. So, thank you very much.

Haggerty: 02:00:28 Thank you and thank you for the opportunity to be here today to speak. So, I'm gonna be talking today about the use of Energen Challenge in our non-human primate models to access the pharmacodynamic activity and translation to the clinic. And Danuta gave a really nice overall presentation of our experience to date with these agents and the toxicities that we see and the challenges that we face in translating the data that we see in animals to those in humans, with regards to be able to predict these immune mediated toxicities. And many of the mAbs that we work with for these co-simulated agents or these checkpoint inhibitors, the only relevant species that we have is the nonhuman primate, with regards to, it's pharmacology. And so, it is the model most used to assess the safety of these agents for human dosing, but as we have discussed, it has to some extent under predicted the toxicity observed in patients and we've had a lot of discussions here about why that may be. And certainly, these are healthy animals, also often very young animals, and their immune systems are much more quiescent than in a diseased animal and so as we talked about, with the diseased population you have the tumor bird and you have [inaudible 02:01:35] medications. So, there are a number of other variables that may be predisposing the humans to these toxicities, whereas, in our monkey models we're not gonna exactly see those situations.

In addition, the targets are often [inaudible 02:01:52] expressed only upon immune activation and so the receptor level expression is rather low. We also can have differences in the FC receptor, binding affinities and our function across species, especially as we start to modulate these receptors mutating them to change their effector function. But what we do know about these agents is that the toxicity's generally are on target and immune mediate do the pharmacology. As was just being talked about, these are very selective agents, and so we do not see a lot of off target toxicities and what we really are looking at is the potential for immune mediated toxicities. Therefore, to assess the safety and aid in setting a safe starting dose and designing clinical trials it's really important that we do understand the biology of our target and then our monoclonal antibody and be able to predict the pharmacological dose and understand that dose response curve to be able to predict what we're gonna see in humans.

So, given that our data is not really predicting the toxicities that we're seeing in humans, the traditional approach of using an NoAL or HNSDD is really not appropriate for these immuno-oncology agents. And rather we have gone to using the MABEL approach or the pharmacologically active dose. The problem there though with the MABEL is that this is a very conservative dose which often leads to one which is adding little benefit to the patient. So we're taking patients who have a disease and that disease is very much a toxicity to them and yet we're going in at very low doses and providing no benefit. So, it would really be great if we could come in at a higher dose that we, of course, would feel comfortable using that would add more benefit, which is the pharmacologically active dose. So, this is

something we all are struggling with because we really wanna minimize the use of very ineffective doses in our patients.

So, how do we determine this pharmacologically active dose? Well, it's really as we've talked about in other presentations, integrations or totality of a non-clinical data. What do we know about the mechanics of action? How well has that been understood and what are we seeing in our animal studies, both *in vitro* and, as well as, *in vivo* and in our tumor mouse models, for example. And in our nonhuman primates, when we look at that data with regards to PK, receptor occupancy and expression, pharma dynamic activity and trying to model all that, in order to get a sense of what that pharmacologically active dose may be, correlating it back to the animal, the *in vivo* tumor models data, and using also human *in vitro* assays to look, for example a cytokine release potential.

So, it's really integrating all that data and building a wade of evidence as to where that pharmacologically active dose, what that dose response curve might look like, and what we should be using to justify where we're gonna enter into the clinic with our starting dose. So, in order to assess the PD in our monkey studies, we need to incorporate biomarkers of immune activation. This will allow us to improve the translatability by correlating our exposure receptor occupancy PD, all in one species and also be able to assess that any impact that the AD may have on that data. And it will help us establish a pharmacally active dose and dose response cause sometimes we see a sigmoidal dose response curve but in immune ... We also often see a bell shaped curve and that's important to understand. And also these data can help support the relevance of our non-clinical model, especially in the absence of toxicity and potentially help identify clinical biomarkers that can be incorporated into clinical trials.

So, with our air agents, efficacy is also demonstrated in mouse tumor models, and as we talked about, it's often using surrogate antibodies. So, to test the clinical candidate, you know, it would be nice if we had tumor brain monkey models but that's really not feasible. An immune system in healthy monkeys, as we often talk about, does not provide the appropriate context to fully characterize the IO activity, biological activity. So, one approach that's been taken is really to use immunizing, ... challenging the animals with an antigen in order to give us something to respond to so that you can then assess that any alterations in the immune response based on the treatment of your drug. And to correlate that with various exposure, receptor occupancy, expression, and toxicity findings when they observe.

So, the approaches we have taken in our non-clinical, nonhuman primate studies is to incorporate various antigen challenge and one approach is T-dependent antibody response which was a question that was brought up earlier, in which you can immunize your animals with an antigen, collect serum and then evaluate the antigen specific antibodies. And there are many different immunigens, the vaccines that can be used to elicit this antibody response. One that we have used a lot is KLH, it's a very multimeric immunogenic protein, can be used to look at primary and secondary responses. It's commonly used to assess immune suppression, so we have a lot of data in that area but to use it to assess immuno enhancement, you wanna really use a suboptimal dose so you can see that enhanced response.

Another consideration is Tetanus Toxoide, which will allow you to look at recall responses, cause animals, as well as, humans have already been immunized most often with this vaccine. The thing about the T-dependent antibody response is it's really a CD4 mediated

response and we know that in the fight against cancer that cytotoxic t-cells or CD8 cells, play a very critical in the control of cancer and that would be really great to have a way to assess the CD8 mediated immune response in our nonhuman primate models. And so we know that the viral immune responses are CD8 mediates, so we've taken advantage of this particular approach by using adenovirus 5 in coding proteins which express, so you express a viral protein in a replication in competent vector, which is then very safe to use in your vivariums. And so we immunize our animals with Adenis 5 and coding gag and SIV peptides.

Now, to have this work, this is a class one mediated response, and so you need to MAC haplotype the animals in order to select for monkeys the specific alleles that will recognize the immunodominant epitopes. But fortunately for us, the Cynomolgus monkey of Mauritius origin, which is the one we use in our programs, over 80% of those monkeys do express this allele, so it's not difficult to be able to find these monkeys to incorporate into our studies. And so what we can do then is immunize with these viral particles and then approximately three weeks later we take blood and we can analyze them for antigen specific t-cells using viral peptide tetramer analysis.

And so what am I gonna do in the next couple of slides is present to you a case study in which we incorporated into this case study into a IND toxicology, IND enabling study, the vaccination with both the KLH, as well as, the gag nef and how we looked at these responses with various different assays. And I [inaudible 02:09:17] this particular case study because we did get very nice responses to all of the endpoints that we evaluated. Although, I should say, that that is not always the case. Very often, we may see only one or two endpoints being affected so not all of our agents respond this strongly. And also, I've chosen this particular case study because I'm sure you're all familiar with the target. We've talked about it several times today and that is anti-CTLA4. But in this case this is an anti-CTLA4 non-fucosylated antibody. And by non-fucosylating we enhance the Fc effector function. So, the reason for trying this approach is that the binding portion still blocks CTLA4, similar to ipilimumab to help stimulate t-cell activation and proliferation, but the Fc binding is hanced to the Fc receptor Gama on multiple immune cells increasing the immune cell infiltrate to the tumor.

In addition, it increases inter t mel treg depletion in the tumor micro environment. So, in our study, we wanted to understand how this mutation and this enhanced Fc effector function would affect or pharmacode dynamic activity, as well as, the safety of the molecule and compare that to what we see with ipilimumab.

So, here is an example of the study, the study design that we conducted with our CTLA4 NF molecule antibody, in which we dose at doses of three 15 or 75mg per kg. It was administered weekly to three to five monkeys per sex per group and then we necropsied on day 30 and then we had a recovery period of six weeks. And on day one, we immunized with the KLH, as well as, the Ad5 gag and Ad5 nef effectors and, as you can see, we incorporated a number of endpoints in order to assess the impact of this drug on the immune response. As you can see here, we looked at anti-drug antibodies, we did immunophenotyping to look at tb and nk cells, as well as, memory subtyping, looking at memory, effector treg, populations. We also looked at the t-dependent antibody response. We looked at antigen specific t-cell responses to the viral peptides. As well as, overall t-cell activation and finally we also looked at the ex vivo recall to our immunization.

And so the next couple of slides I'm gonna kinda quickly run through what we saw with these PD endpoints. This is the t-cell dependent antibody response and, as you can see, we had a very robust response to the KLH, both looking at IGM, as well as, IGG. And demonstrating that we had a very nice enhancement of the CD4 mediated immune response. But we also immunized, and as I mentioned, with the viral peptides and in this particular assay, we immunized with Ad5 gag and Ad5 nef and then on week three, we take blood and we immunostain with t-cell specific markers, as well as, fluorchrome conjugated MHC1 tetramer loaded with gag or nef peptides. And then we can analyze by flow cytometry and measure for the antigen specific CD8 t-cells. And what you see here is the data from this and what we observed was an increase in the percentage of both gag, as well as, nef specific CD8 t-cells. So, this is your vehicle and these are your dose treated monkeys. So again, we were able to demonstrate a really nice robust CD8 mediated response.

In addition, looking at the specific antigen response, we looked at the overall ... the fact on the overall t-cell rapidshare, by looking at t-cell activation and proliferation of the overall t-cell response, by taking t-cells and staining them for both t-cell markers, as well as, Ki-67, which is a nuclear proliferation antigen and being able to assess those cells that are proliferating, as well as, with activation marker, in this case CD-69. And again, we saw a very nice robust increase, a dose dependent increase, in the proliferating t-cells, as well in our activated t-cells. And this is looking at t-helper cells, but we saw a very similar response to our CD8 cytotoxic t-cells.

We also looked at immunophenotyping. Particularly looking at special subtype populations and, as you can see, we saw a nice dose dependent increase in activated C25 positive CD4 cells. A nice really increase in the CD4 memory cells, dose dependent increase and that was reflected and correlated within a decrease in the naïve CD4 cells. So you can see, we're pushing these naïve cells to memory cells. And we also, again, saw very similar effect in our CD8 population.

And so finally, the last assay I'm gonna show is an ex vivo recall response. So by incorporating the ex vivo recall response into the, say we can look at both the primary and memory responses. And in this assay, we looked at the activation [inaudible 02:14:57] cells following ex vivo stimulation, with neo antigens, so the antigens that we immunized with on day one measured by activation markers CD-69 and cytokine interferon gamma and TNF alpha expression. Three weeks following immunization, we take blood and we isolate out the PDMC's, we then stimulate ex vivo with antigen of a choice, whether it's KLH, gag or nef, as well as, co-stimulatory molecules and then we assess, at four hours after treatment, we treat with Brefeldin to inhibit cytokine release, and then 20 hours later we do flow cytometric analysis, looking at CD-69, interfering TNF alpha and CD4 CD8 t-cells.

What you can see here is what we observed is an increase in the percentage of activated CD4 cells, only responding to the KLH, which I said is the CD4 mediated response. We do not see any CD4 increase in response to the gag or nef proteins. But in contrast, in the CD8 t-cells ...

Haggerty: 02:16:00 we do see ... we don't see an increase to the KLH, but we do to the Nef, and, particularly, to the Gag protein. So, as I mentioned, one of the objectives was to compare the response that we saw with our CTLA-4 NF with ipilimumab, but we didn't do them in the same study, but in a separate study that we conducted relatively recently with ipilimumab at 10 and 50 mgs per kg. We were able to compare across studies, looking at the two responses. And in the

blue is ipilimumab at 10 and 50 mgs per kg, and in the red is the CTLA-4 NF at three, 15, and 75 mgs per kg. And you can see, we clearly have a shift in the dose ... in the degree of the response with the CTLA-4 NF being much more potent in eliciting this robust antibody response. And again, this is 50 vs 15, so you can see there's clearly a shift in that dose response.

So to summarize what we saw in this study, clearly the CTLA-4 NF caused a dose-dependent increase in both CD4 and CD8-mediated responses to KLH and Gag-Nef, respectively, in multiple endpoints. And it was enhanced relative to that of ipilimumab, and we did look at many of these other of these other endpoints with ipilimumab and we saw the same shift, more or less, in that activity.

But with regard to toxicity, which I have not yet discussed, we also observe toxicity at all doses in this study. And this was characterized by moderate to severe clinical observations and dose-dependent increase in the incidence and severity of lypmhocytic infiltration in numerous tissues. So clearly, we saw toxicity by mutating this NF and enhancing the Fc effector we saw a clear increase in toxicity, as well as in our former dynamic activity. And when we compare this ipilimumab; as I mentioned, we recently did a study with ipilimumab as well at 10 and pushing the dose, as Danuta had mentioned, to 50 mgs to kg. And while the 10 mgs per kg was a relatively clean dose, we clearly did see toxicities at the 50 mg per kg dose. So, if you push the dose, you can start to see immune-mediated toxicities with ipilimumab.

So what did we learn from incorporating these PDM points into this study? Well, it really helped to enhance our understanding of what that mutation in the Fc and enhancing the Fc effector function had on the activity, as well as the safety, and for setting the first inhuman dose ... starting dose ...this is really done based on a comparison of ipilimumab with the PTA ... the former dynamic data used as a benchmark to compare to ipilimumab to help gauge the difference in potency. This is along also with what we saw in our tumor models, so using both of those datas allowed us to better understand what that shift in potency was.

Sorry. Am I going the wrong way here? Yes I am, Alright. Okay. Sorry, here we go. So, I wanted to give a few more examples of how incorporating these biomarkers of immune activation into our studies in our nonhuman primate has allowed us to learn more about our molecules. These are three examples of costimulatory agonists, in which we did studies of a similar nature, and we immunized with different immunogens. And the first example is a monoclonal antibody in which we immunized with KLH, and in that study we observed an increase in the TDAR to KLH, as well as an increase in the ex vivo recall response in our CD4 cells, and an increase in the Ki-67 CD4 positive T cells. And then based on this study, we actually incorporated the Ki-67 CD4 assay into our clinical trials as a marker of PD in the clinic.

In another study, with another costimulatory agonist monoclonal antibody, when we immunized with KLH, we saw a decrease in TDAR to KLH. This was a very unexpected finding, because we were expecting to see an enhancement of that response, and interesting, we also saw absolutely no anti-drug antibodies, which is very unusual for these studies, because generally we do see anti-drug antibodies being generated. But that's, again, very consistent with suppression of an antibody response. In addition, we also, as expected, saw suppressed ex vivo response to KLH. So what exactly was going on here? Well, our hypothesis was that the excess MAB was abrogating the Fc medium crosslinking,

which was very important with these costimulatory molecules, and is essential for their activation. In addition, the MAB blocks the [inaudible 02:21:08] ligand binding, and so with those two things happening, we believe that it is leading to the suppression of that response. So these data really allowed us to have a greater understanding of the biology and had an impact on how we were going to dose escalate.

And for our third example, this was another costimulatory agonist where we immunize with KLH as well as Gag-Nef and tetanus toxoid. And what we observed in this study was a loss of receptor expression shortly after treatment. So we saw a profound loss of the receptor expression; it was very sustained throughout our study. And this resulted in a decrease in the TDAR response to KLH, so again we saw this loss of activity rather than enhanced activity, and a reduction ... a corresponding reduction in the Gag and Nef-specific CD8+t cells, so affecting both the CD8 and the CD4-mediated responses. We also observed a decrease, or no enhancement, in proliferation of CD4 or CD8-t cells, respectively, and we saw, interestingly, no effect on our recall response to tetanus toxoid, so that was not impacted. So again, this data gave us a greater understanding of the impact of this sustained receptor internalization and its impact on our biological activity, and it highly impacted our clinical trial design with regard to dose escalation. Okay, why am I going the wrong ... alright, thank you. Okay, so just to conclude, so as we said the nonhuman primate is really the model where we have to do our safety assessment. And we really don't see a lot ... often don't see a lot of toxicities in these models, but by incorporating these antigen-challenging and incorporating these PD markers into our studies, it really allows us to better understand the assessment of the effect of a mut-function with these molecules. And by using different types of antigens, we can look at both CD4-mediated responses, as well as CD8-mediated responses, and we really believe that the CD8-mediated responses are very critical in understanding the activities related to cancer immunity. And so by incorporating these data, these PD assessments, and then correlating with exposure, receptor occupancy and expression, as well as toxicity when it's observed, we really can further understand our knowledge of the biological activity of our monoclonal antibodies, help define the pharmacological active dose and dose response relationship, to help in the translation into aiding in the clinical trial design, as well as it really helps demonstrate the relevance of our model, especially in the absence of toxicity, and at times can help to identify clinical biomarkers.

So I'd just like to, in the next slide, let's see if I can actually do it, alright; go back one more please ... acknowledge there's so many people who have been involved in the evaluation of the number of different compounds here. I would like to recognize the people in my department who have done many of these assays, as well as many people throughout the entire Drug Safety Organization, including Mike Graziano and Todd Bunch, and for the many people in our discovery organization, PCL and Translational Medicine, particularly Alan Korman and John Loffredo, who is very involved in the development of the Adenovirus Assay. Thank you, and I'm happy to answer any questions.

Sarika: 02:24:50 Hi, I'm Sarika, AbbVie, I had a question about your ... first of all, great talk. I had a question about your last ... not your last slide, the one before that where you had the table with the different monoclonal antibodies, one, two, and three.

Haggerty: 02:24:50 I don't know if I can do it...

Sarika: 02:25:04 That's okay... I can just ... so there you actually showed that there was an increased TDAR response to KLH and you were able to measure Ki-67 as a PD marker. I was curious, did you actually do something without the TDAR and see an increase in Ki-67? In other words-

Haggerty: 02:25:21 Yeah, if you're asking me did we do equal studies without immunizing. Is that what you're asking me?

Sarika: 02:25:21 Correct, correct.

Haggerty: 02:25:26 Yes, so no, we did not ... these are monkey studies, so obviously it's very difficult to do a lot of different permutations, and so we just choose to go ahead and immunize to try and help facilitate our understanding of the molecule.

Sarika: 02:25:39 Okay. And the reason I'm asking that is we're trying, all of us here collectively, to understand how can we improve the interpretation without necessarily going and doing too many studies, and so I think that might be a useful bit of information to have, to see if it's really useful in all cases.

Haggerty: 02:25:58 Yeah, so I think the challenge is that you're not going to do multiple studies, so this does allow us to look at antigen-specific responses. And so I think ... I don't think it makes our models less sensitive, so I think that it adds value when we incorporate that.

Sarika: 02:26:20 Okay, thank you.

Saber: 02:26:24 Thank you, great talk, Helen. So it seems like, for this case, your first inhuman dose selection was based on [AP 02:26:30] and the comparative studies that were done, so ... and we've seen that before, and we accept that doses based on other products. I like your slide 2, when you present the totality of data for first inhuman dose selection and you mention receptor occupancy and activity ... *in vitro* activity, and PKPD. You also mention PAD in animal pharmacology data, so my point was that that is missing in the totality of data right now. Very few people use that in the totality of data for first inhuman dose selection. And I think we need to see more of that.

Haggerty: 02:27:11 So you're saying people aren't using the tumor model pharmacology data, or the-

Saber: 02:27:16 They're using it as activity only-

Haggerty: 02:27:19 Okay.

Saber: 02:27:19 But not when it comes to a first inhuman dose selection, and if they include that in that, we might be able to get a little bit higher on high dose.

Haggerty: 02:27:28 Yeah. I think people tend to be a little too conservative, I think, and I think we need to think about these clinical trials. These are patients with disease; the TeGenero incident occurred in a healthy volunteer patient population, which really received absolutely no benefit from that drug. But these are cancer patients who are dealing with cancer, which in itself is toxicity. And so, I think we need to not be so conservative in our ... as we go into the clinic with our doses.

Schneider: 02:27:57 Okay, I think we can take one more quick question.

Maier: 02:27:59 Thank you. Curtis Maier, GSK. Helen, I really liked your rationale for including the antigen challenge in the monkey studies. And one other thing that we've thought about, perhaps, is you might also make the model more sensitive for detecting toxicity. And I'm wondering, say, with your ipi in particular, when you went back in with your vaccine model, did you see any difference in the profile of toxicity, or toxicity at lower doses?

Haggerty: 02:28:34 So, I'm trying to think back when we did the toxicities with ipilimumab; Alan, maybe you remember, was there vaccine in those six-month tox studies that were done?

Korman: 02:28:45 Not in the six months; they were in the cellular vaccines, [inaudible 02:28:49], hepatis B series.

Haggerty: 02:28:52 Yeah, so there were ... we did have some vaccinations in those models. But I actually can't say whether that does make a difference on whether it's more sensitive or not. I can't believe it makes it less sensitive because you are upregulating expression, you would think? But we haven't really done those studies to really make that case.

Schneider: 02:29:11 Excellent, thank you so much, we have ... that was a great mini session, I guess. We have time for a break now, I'm looking at my colleagues; Sarah, we're still okay for 10 minutes? Yeah, I think we've got ... we're a little behind schedule, but we've got a little bit of buffer zone, so right now it's 3:30, so if everyone could come back at 3:40, then we'll continue with the rest of the session.

Haggerty: 02:29:33 Thank you.

Schneider: 02:29:37 Okay, hi everyone, I hear the bells outside, so we're going to reassemble. And actually we're going to have slight shift on the agenda. We're going to switch around the next two speakers before the last panel session. So I am next going to welcome Mariam Eljanne, who is a program director at the Division of Cancer Biology at the National Cancer Institute, and she focuses on programs relating to the translation of basic science into pre-clinical and clinical research, and she is going to be talking about some of the NCI funding programs that support the development of cancer models. And as you've heard in the introductory section, some of us attended a workshop that she was involved in last fall that really kind of got us thinking about some of these issues and helped us formulate the idea for this workshop, so we're very happy to have her here today.

Eljanne: 02:30:39 Thank you to the organizers for inviting me to speak here. Alright, let's see. Alright, what am I supposed to press? Okay. Alright. So, I'll be talking about two initiatives that the NCI is leading. The first one is the Human Cancer Model's Initiative, or HCMI, and the second one is the Initiative to Enhance Applicability of Mammalian Models for translational research. The first initiative is housed in the Office of Cancer Genomics. Both of them are at the NCI, but in divisions, and the second one is housed in the Division of Cancer Biology. So the HCMI is led by Dr. Daniela Gerhard, who I borrowed all the slides from.

So, the reason behind this initiative is that looking at the molecular characterization of cancer has identified cancer-relevant mutations that range between 1% to more than 50% in population and frequency. Of about a thousand cell lines that are commonly used in labs or in different applications in the research, most cannot be compared to the primary tumor.

The clinical and the outcome data is not available, and they do not represent most of the ... certain molecular subtypes, and also pediatric cancer subtypes. And also they don't represent the common combination of lesions. Also, the cell lines do not represent very rare cancers. And they also do not reflect the ethnic and racial subpopulations, and they do not ... one of the important things is that they do not recapture the relationship between the tumor and the microenvironment.

And to tackle some of these shortcomings of these cell lines that are available in the community, different organizations came together ... wrong? ... to form a consortium, The Human Cancer Models Initiative Consortium, that is composed of the Office of Cancer Genomics, which is at the NCI, the Sanger Institute, the next one is the Cancer Research UK, and also the Hubrecht Organoid Technology Organization, which is a nonprofit. These organizations came together and they formed this consortium to ... and the goal is really to collect different cancer biopsies and reculture them, collect the clinical data, take normal tissue and of course planting tumor, and develop the models, determine the genomic data of which includes the whole genome sequencing, whole exome sequencing, RNA-seq from the normal tissue from the tumor, and from the model that is developed, deposit all the data in the data bank, and share it actually with the community, with the industry, and academia.

And this is showing the pilot model of this initiative. And the goal is to really develop about a thousand models from different cancer types, including rare cancers and pediatric cancers, and also cancers that don't have targeted therapy. The core principles of this initiative is to really have it open to the community, an open resource to the community. These different organizations came together and they developed a lot things that are shared, such as informed consent form that allows the development of the model, collection of clinical data, characterization of the different tissue, the normal and the model and the tumor, the linking the data from the personal identified information, distributed academia industry, and all the information collected and available, it will be available through the NCI Genomic Data Commons, the Office of Cancer Genomic website, and the European Bioinformatic Institute. The distribution of this model ... not just the distribution, the models will be validated and quality controlled by ATCC and distributed, of course, and all the protocols will be shared through the Office of Cancer Genomics website and European Bioinformatic Institute.

So as I mentioned, these tissues, the tumor, the normal and the model, will be characterized molecularly using the whole genome sequencing, whole exome sequencing, and the RNA-seq, and that will be done also by the Wellcome Sanger Institute and the Hubrecht Organoid Technology Institute. They will be using most likely different technologies. And they will be shared, of course, by the different websites. And they have a steering committee that's selected 727 data element, which will be part of the Human Cancer Model Initiative searchable catalog. This is just showing how the process of that starts with the biopsy, developing the model, the quality control, validating the model, and the distribution of the models. I'm going a little fast. So, the pilot ... currently the pilot includes different cancers, such as the breast, colorectal, so I'm not going to go through all the names that are listed there. So they are targeting certain cancers as a pilot for this initiative.

And then the second initiative that I'd like to make you aware of is the Research Project to Enhance Applicability of Mammalian Models for Translational Research. So, just to make certain thing clear, the first one is an initiative that was already ... it's funded, and it is ... the US site has already awarded two contracts; one contract is the Cold Spring Harbor, and the

other contract was awarded to the Broad Institute. These two institutes have the contract and they are working on developing these models, and the goal is to really constantly [inaudible 02:38:09] *in vitro* modeling, so none of the models are *in vivo*, they are in culture, and they are mostly organoids, cell cultures, CRC's, neurospheres, so they are not *in vivo* models. This initiative, the goal is to really ... it's *in vivo* and *in vitro* models.

So what we are ... with these funding opportunities that we have on the streets, is to really invite investigators to ... let me just go over ... first, the rationale behind this funding opportunity is, I think most people are aware of the Mouse Consortium that started in 1999 until 2014, and from that experience, the NCI determined that the partnership within and the cross-research communities helped to ensure model validity. And also, multiple viewpoints foster development of better models for a specific purpose. The models to explore basic disease mechanism may not be sufficiently complex to support [inaudible 02:39:32] research. And none of this is new to you. Everybody knows that.

And also, with these new funding opportunities that we have on the streets, there will be a constant influx of ideas of how to develop, or how to improve models, and that will help make the models much closer to ... they will never be the perfect ... but they will be much better in presenting the human disease. So from that experience, it was determined that also having a platform where scientists can share ideas, can share models, can share issues will help, actually, improve the models and speed up the progress in the field. And with that, it was determined that we need a forum for the community, which will be used as a locus to new create cross disciplinary groups around either specific technical, scientific, or informatics challenge to improve or expand translational use of oncology models.

Also, it will promote effective communication and collaborations among different communities of research, especially those who generate or identify models that ... and those that need to use the models either for drug screening or for prevention for different goals. And also, it will be very useful in disseminating well-validated translational models and their associated data. It will be also a very great ... it is currently, because it is open to anybody that wants to access it and look at the data, is to share a large data, such as imaging, and by informatics data that you cannot email to each other. So this would be a great platform to share that kind of data. And so I already mentioned that. Access to ... also, the forum will include protocols, because it will make things a lot easier if people don't have to reinvent the wheel every time they want to create a model, or they want to improve a model. They can learn from other people's experience. So that's the hope, that people will be sharing protocols and people will be learning from each other.

So, the purpose of the ... these two, I call them the PAR, these 17-244 and 17-245, are funding opportunities to invite either expanding, improving, or transforming the utility of mammalian cancer, and tumor model for translational research. It is also to show that translational mammalian models are suitable for use in pre-clinical and co-clinical setting. Develop and characteracterize mammalians with cancer as representative models of human disease, and those who to demonstrate that mammalian models or their derivatives; as I mentioned, this is not just *in vivo*, it's also *in vitro*, either organoids or whatever *in vitro* system that you think you can develop or improve that will be able to help screen a drug, or test any kind of clinical question, are welcome into this funding opportunity.

Also, to demonstrate how to overcome ... if you have an idea to help overcome translational deficiencies in mammalian oncology models, you're welcome to apply. The [inaudible

02:43:21] use of mammalian models or their genetics or unexpected translation challenge. Also, advance the standard practice for use of translational models and test approaches to validate and credential models. And also you can challenge the current practices of how models are used in the translational research. So, these are just some of the goals of these two funding opportunity announcements. They have been on the street since the fall ... I think the spring 2014, and they have been renewed, and this ... the last ... this current funding approach is, 17-244, 245; the last submission date is March 2020.

So I just want to give you a quick idea about the two different funding opportunities. The 17-244 is a collaborative RO1 project. This ... it is up to five years, and really, most of the projects are three years, but you have to show that the two additional years are really necessary for the project to be successful. It is up to ... the funding is up to 450 direct cost per year, per project. So this ... per project. So when it says collaborative, that means there can be one or two more RO1's that are coming together asking for funding. But sometimes not all of them are successful; only one is successful. Doesn't mean that it will fail; one RO1 out of the collaborative three groups can be funded even if the other ones do not make it through the review process. So each one of those RO1's that are supposed to be collaborative will be reviewed independently by a peer review and either all three can be funded, which hasn't been the case so far; we haven't had any of the collaborative, really, all three funded. So we had some ... like one out of three that were funded, so it has been very tough to fund the collaborative.

They address the technical and experimental parameters that ensure effective translational use of mammalian models. They can identify and propose the means to tackle [and match 02:45:54] translational requirements or extend the range of insights; similar things that I mentioned in my previous slide. The difference with the 17-245 RO1 is that these are individual, independent projects. Also, they can be funded up to five years with 450,000 direct cost per year, and their scope is much more narrow than the collaborative RO1's, and they can address one or more of the technical and experimental parameters that ensure effective translational use of mammalian models, identify and propose one way to address an [inaudible 02:46:37] need of translational research. And, if possible, they can use the Oncology Model Forum. For the collaborative, they have to use the ... because it's a collaborative project, they have to use the forum. But for the independent RO1's, they are encouraged to use the forum so they can collaborate and learn from other people's experience, and share their experience and their data.

Schneider: 02:47:06 Sorry, we're a bit over time-

Eljanne: 02:47:08 Yep, so this is ... I'm not going to go over all of this because it's already in the funding announcement that is on the street. You can just Google the two different numbers, the PAR 17-244, 17-245, and you'll find a list of projects that are accepted for the announcements. This is just a snapshot of the Oncology Forum, and if you have any questions, I'm not sure if Nancy is still here? Yep, she is in the back if you have any questions about the oncology forum, you can talk to Dr. Woodrow who is Program Director in the Division of Cancer Biology. And the next slide is just showing the link to resources on the Oncology Forum. Happy to take any questions. Sorry to go-

Schneider: 02:47:57 No, that's ... thank you!

Schneider: 02:48:03 I had a quick question about the PAR's, the enhancing ... sorry ... Applicability of Mammalian Models for Translational Research. Do you have a sense of how ... I know, I can't remember exactly how long they've been funded, but do you have a sense of how many of the models are being used for, kind of, safety, toxicity, questions versus efficacy questions?

Eljanne: 02:48:24 So far I didn't see any that actually ... because I'm sure they were proposed, but the funded ones, none of them are actually addressing the toxicity.

Schneider: 02:48:33 Okay, so the ones that got funded so far are all-

Eljanne: 02:48:36 They don't have any toxicity questions, they're not addressing any toxicity questions.

Schneider: 02:48:40 Okay.

Eljanne: 02:48:40 I think there were some that came with such questions, but none of the ones that were funded since the beginning of this PAR are funded, no.

Speaker 2: 02:48:49 Okay.

Schneider: 02:48:51 Yeah, Haleh, go ahead.

Saber: 02:48:56 Haleh Saber, FDA. When you fund a program, or research, do you ... or, how much attention is given to basic science versus regulatory need and translational science? Do you... I mean, I'm thinking about this eventually coming to the FDA. Is that part of the decision-making for funding the program?

Eljanne: 02:49:23 Well, the goal of the program is to really develop, improve, validate models. So there are applications that come with ... this is a model; we want to improve the model to test these drugs or these small molecules. So yes, those applications do come in. But then, this funding opportunity is not for ... it's for mechanistic; for research. Definitely those are ... I didn't put it there, but any application that comes with a mechanistic question will not be accepted in these PARS. It's really creating…

Eljanne: 02:50:00 but mostly improving, validating, showing that the model you have or the model you are developing is really representing the human disease, or can it be used to test certain therapies. But not, anything mechanistic is not really accepted in this FOA.

Schneider: 02:50:28 Great, thank you. Any other questions? Okay, well thank you so much.

Eljanne: 02:50:33 Thank you.

Schneider: 02:50:34 Excellent, so our final speaker before the second panel discussing is Doctor Robert Li who is a senior toxicologist and pharmacology sub-team leader in safety assessment at Genentech and he supports non-clinical safety assessments of both small and large paramedicals across a range of therapeutic areas including oncology, immunology, and cancer immunology. So thank you, and thanks for your flexibility in terms of switching the presentation order.

Li: 02:51:07 Sure, thanks Julie for the introduction. And also like to start by giving many thanks to the organizing committee for putting together this really great workshop, and also giving me

this opportunity to share this case study, which I personally think is a very interesting case study about a safety risk medication strategy for the immunotherapy combination about atezolizumab and vemurefanib.

So moving on to this slide, let me first give you a quick overview in terms of non-clinical profiles of both drugs. So as many of you are familiar that atezolizumab is the humanized anti-PDL1 monoclonal blocking, monoclonal antibody. It's a biologic. And vemurefanib is a small molecule selective BRAF enzyme inhibitor. In terms of the non-clinical pharmacology or efficacy assessment, we used a [inaudible 02:52:02] model for atezo. And we used a xenograft tumor [inaudible 02:52:03] models for vem. So fundamentally those two models are very different and Danuta already briefly touched on that. One is immunocompetent model. The other one is immunodeficient model.

In terms of the tox species, we mainly used a xeno for atezo even though we did do a short-term two-week non-GLP mouse study at the beginning of the program. But again, most of the studies we used are xenos. And in comparison, we used the rats and dogs for vem-related toxicology studies. In terms of the major target organ findings, atezo-related findings seems to be very limited to arteritis. So peri-arteritis in several different organs. But these findings did not seem to cause any functional impact. For example, in the liver we did not see any hepatic cell necrosis or degeneration and/or associated liver enzyme changes.

In contrast, the liver was also identified as one of the target organs for vem, but the findings appear to be very different as compared to atezo findings because we did see the hepatic cell necrosis or degeneration and also we saw the associated liver enzyme increase, such as ALT and AST. And bone marrow is also identified as another target organ for vem in the toxicology studies. Another important part I want to emphasize here is that in the non-clinical studies with either molecules we did not see any skin-related abnormalities. The reason I emphasize that because in a clinical study with both molecules, we didn't see a skin-related adverse event, which I'm going to come back to that in a minute.

But generally speaking, if you look at this non-clinical safety profiles of the liver ... basically the non-clinical safety profiles suggest the liver may be the only potential overlapping the target organs for this type of combination even though we do understand that the mechanism of this kind of action for single agent-related effects in livers appears to be very different. But a key question here is whether or not atezo treatment could potentially exacerbate the initial effect caused by vem in the liver.

So with that, moving on to the next slide, let me just switch gears a little bit to tell you and give you a little bit of context in terms of the scientific rationale why we're trying to combine these two agents together to force enhanced anti-tumor activity in the clinic. So the first important piece of evidence is that more than 50% of melanomas are known to carry this specific V600E mutations in the BRAF oncogene. And that caused the constitutive activation of a very important signaling pathway for cell differentiation and proliferation, which is the [inaudible 02:54:42] pathway.

And the second piece of evidence is that it has also been recognized that BRAF inhibition can lead to generally enhanced immune response within tumors such as increased tumors within a tumor marker environment as you can see from this IHC staining for CD8 T cells in post- BRAF inhibition treatment versus the pre-treatment. You can see that there's a clear increase of CDT in the tumor marker environment.

So basically with all this supporting evidence together ... So we're moving into the phase one trials. So first you've evaluated the tolerability and the toxicity of this type of combination. And it's worth mentioning that when this combo trial was initiated, vem has already been approved for market use with well-defined patient safety profiles established already. And atezo treatment had been tested in about 30 patients around that time. In terms of clinical AEs, vem-related AEs are mainly related to fatigue, nausea, liver injury, an incidence of about 30% with minor severity up to grade two. And also the skin rash was a little bit higher incidence at about 40% to 50% of the incidence, really depending on which trial are you looking at. And with a similar minor severity up to grade two. And importantly, a skin rash and liver sickness are all self-limited and they're going to resolve over time without even having any dosing holidays or adjustments in most cases.

And in terms of the atezo treatment in those 30 patients, it was generally well-tolerated with manageable AE profiles, which seems to be very consistent with the major pharmacology of atezo, which is immune activation. So together, you're looking at a non-clinical data and the phase 1A data from the atezo, it did really suggest a minimum overlap the safety issues with the vem-related safety profiles, except for skin rash, diarrhea, and uveitis. And the atezo-related skin rash occurred in about a similar incidence of 30% and also a similar severity as we saw from the vem-related findings. And importantly I want to point it out that in the 30 patients we did not see any hepatic AEs around that time. So basically in addition to the potential overlapping liver toxicity as suggested by the non-clinical data, there's clinical data to really suggest that skin rash could be the second overlapping toxicity for this type of combination. That would bring us to the same question: whether or not atezo treatment could potentially exacerbate vem-related skin rash?

So with that in mind, let's move on to the next slide. Let me walk you through the phase 1B study design step by step, and also share with you some preliminary very high-level data in terms of the safety and efficacy. So we started with the first cohort, 30 patients. And you can see from here it's a very standardized study design. So we started both drug treatments, vem and atezo, at the same time with different dose levels. We completed the drug treatment until we really hit the significant safety signals or we lost the clinical benefit.

So from the safety perspective, what we found is even though we did not identify the DLT from the first cohort, but what we saw is we saw a clear increase in terms of both incidence and severity of two major clinical AEs, skin rash and a liver enzyme increase. As you can see, that incidence is bumped up from original 20% to 40% for single agent to 100% here. And the severity went up from less than grade two to grade three. So when we had this kind of finding, the team had to really pause around this. So really thinking about what is a major cause for this kind of increase in both incidence and the severity? And more importantly, what can we do to manage these clinical AE profiles?

And a few things we do understand around that time, number one: for example, the vem-related skin rash, even though that time we still did not understand the definitive underlying mechanism for skin rash. But some cases do suggest involvement of some immunological components. And more importantly, those kind of involvements suggest that those findings could be [inaudible 02:58:58] exacerbated in the presence of immunostimulatory agents such as atezo treatment. And more importantly that those findings could resolve over time in most cases without even having any dosing holidays or adjustments.

So based on that kind of thinking, the team's proposal is "Why don't just give patients vem treatment first?" It's kind of giving them the opportunity to acclimate to the vem-related AE findings, and then you initiated the atezo treatment. So hopefully through that kind of modification we can lower the probability of having the more severe reaction as we saw from this cohort 1. So with that in mind, so why do the cohort 2? So as I said, we modified the study design by switching from concurrent start to start the patients on vem treatment first for 56 days. Then follow it by the atezo treatment. And this type of modified study design demonstrates to be very effective because it did lead to a pretty clear decrease in both incidence and severity of both AE findings.

So as you can see, as summarized in that table great success. So based on that, we took a further step to modify the study design in cohort 3, but that's mainly based on the efficacy point of view consideration because we're trying to understand what is the optimal [inaudible 03:00:18] window period? Because remember one of the scientific rationales for this kind of combination is vem could potentially prime the immune system, getting the immune system activated and then followed by the atezo treatment, hopefully that could lead it to the enhanced anti-tumor activity.

So from the fundamental immunology perspective we certainly don't want to wait too long for [inaudible 03:00:39] to be primed. Otherwise, they're going to get exhausted and lost a regional kind of anti-tumor activity. So based on that kind of thinking, by moving to cohort 3 we shorten the vem run-in from 56 days to 28 days. And the rest of the study design is pretty much the same as seen in cohort 2. And this kind of further modification did not change the safety profile as compared to cohort 2. As you can see from that table as a lower bottom table, the skin rash and liver enzyme changes in both instances, the severity appears to be very comparable to cohort 2. Much better than cohort 1. So safety issues seems to be managed pretty well based on this kind of modification, but another equally important question is what about the efficacy?

So moving on to this slide, which gives you the quick snapshot in terms of the objective response rate, as you can see that briefly, compared to cohort 1, cohort 2 and 3 did not compromise the clinical efficacy but in fact they demonstrated even better the response rate as compared to cohort 1. So which was first supported by this increased CDT cell staining in post-vem biopsy samples as compared to pre-treatment, which I don't have time to really go through the details.

But let's take a little moment to quickly summarize what we learned from this phase 1B trial. So we did not identify the DLT. But we did see the clear increased safety signals in terms of the incidence and the severity of two major AE findings: skin rash and the liver enzyme increase. So that does represent a potential hurdle for continuously advancing the clinical trials. What are we going to do? We've got two potential options: one, we go to the pre-clinical space. We try to do develop a model, which may take a long time or we're not able to do that. Or we look at a totality of data to formulate a scientific sound rationale to be able to manage the clinical design directly. So we chose the latter one. So this modified study design seems to be very effective to manage these clinical AEs because we did demonstrate a very improved tolerability for this kind of vem run-in period, this kind of design regardless of 28 days or 56 days as compared to concurrent start. And that kind of modification did not lose the clinical efficacy. In fact, it demonstrated even better clinical efficacy compared to cohort one.

So with that in mind, moving on to my second-last slide, let's take a look at a few points. Some of the points that Danuta already touched on, that to really look at those points that are related to the very important question, which is what we can do to really predict or better predict or manage the clinical AEs or toxicity associated with the immunotherapy combinations, particularly for large molecules plus small molecules. So we know that is very difficult in doing that using the current non-clinical model, not as largely because of the non-fundamental differences in the current non-clinical model that are normally used for evaluating the two types of molecules, fundamentally from those in pharmacology and the toxicology perspectives, they're very different: immunocompetent versus immunodeficient model. For biologics, we used the xenos in most cases. And in contrast, we used dogs as a [inaudible 03:03:53] species for small molecules most of the time.

So based on this we understand that developing a good, clinically relevant non-clinical model to harmonize those differences can be very, very challenging for these types of combinations. So in addition to these kinds of non-clinical efforts another way, an alternative way we may want to consider is why we don't just look at the clinical data? What a totality of data we have to directly modify the clinical study design. And the best timing of doing that is this phase one stage. So you look at the totality of data you have, target the biology, pharmacology, single agent related toxicity and the AE profiles. And really to try to formulate a scientific and sound rationale. In some cases, you may not have enough to be able to do that. In this case I just went through, fortunately we do. So when you formulate those kinds of scientific rationale you could have used that to guide your clinical design modification. And that can be very effective in some cases such as the one that I just shared with you guys.

So with that, I'm going to stop here by acknowledging many people who made a good contribution. Again, folks from Genentech: Rod, Mark, [inaudible 03:05:02] for their great supporting on these two programs. And also the FDA workshop organizing committee. And also the [inaudible 03:05:10] leadership. So I'd be happy to take any questions.

Saber: 03:05:28 This is an online question we had this morning. But perhaps you can tackle this because it's regarding combination therapy that involves immune checkpoint inhibitors and stimulators in combination with sting agonists. And these are very toxic drugs. Any recommendations? Any experience?

Li: 03:05:28 You talked about combining two biologics?

Saber: 03:05:52 Well, it could be a small molecule sting agonist. So it's a biologic with a small molecule, but the small molecule in this case is a sting agonist.

Li: 03:06:01 Oh sting agonist. Again, I guess when I was looking at this my personal view on this is really you have to start from each single agent. So if for example, if the sting agonist for this one specifically has already been tested in the toxicology, so what is the findings? What are the major target organs? What is the most sensitive species? So that could consider it as the species in my view to moving forward, if I have to do the combo tox studies. And to understand that another immune checkpoint inhibitor's biologics has crossover activity in that species or not. So and then try to optimize your study design to be able to answer the question before you even take these combinations to the clinical trials. Or if that sting agonists are in the clinical trials, look at what you found from patients. How does that really informs you in terms of potential safety and liability for those specific combinations? I think

it really depends on the totality of the data of you have, it depends on the stage of the molecule you have.

Schneider: 03:07:04 Thanks. Please introduce yourself.

Speaker 3: 03:07:06 Yeah, I am [inaudible 03:07:07] and I'm from Beijing. I now have two questions. And the first question is regarding the atezo. And a lot of speakers have mentioned about it, it's really hard to identify the toxicity with immunomodulators. So with the atezo you already in the ... In the monkeys, I think, you see some kind of immune-related toxicity. Also the liver toxicity. So I was wondering what's your consideration among the different products like the PD1, PDL1. And why some products can identify this kind of toxicity. Is it related with animals? Or is it related with different products?

Li: 03:07:49 That's a very good question. Number one, I need to clarify that. That's exactly what I mentioned with only some arteritis in those many different organs. But that finding did not cause or was associated with any functional impact. I cannot call that as adverse. That's exactly what we said that is, as I think I Danuta pointed this as well, based on pharmacology you would expect those kind of itis findings in humans as well. But the real question is: why in that specific organ, either in animals or humans? Why in the liver? Why in the lung? Why in the specific individual patients? To answer that question, you've got to really factor in the multiple, individual related factors genetic background, microbiome differences. Diet differences. I think that's why we have that kind of workshop today.

Speaker 3: 03:08:40 Okay. Thank you. So another question is regarding the combo status. So especially for the small molecule and that molecule is really hard to find the right model to evaluate the safety. So that is maybe a standard question. And what is best way to evaluate the safety prior to the combo status in clinical trials?

Li: 03:09:08 Right. So again, back to the fundamental question we've been asking and also we've been trying to find the answer to is how are we going to really harmonize that? What we're not sure about is pharmacologically or toxologically relevant. Will you find a model to harmonize the crossover activity. And that's even a big challenge for step one. So if you start with small molecules, for example in this kind of case either rats or dogs, we used the major tox species for vem, which is small molecule. Again, if I have to do 10 years back, I happen to do a combo tox studies, especially try to answer the liver tox liabilities for this combination, I may want to choose dog because dog demonstrated liver toxicity while rat did not. That seems to be the more sensitive species. But if I throw atezo into that, it does not cross with dogs. How are we going to solve that problem? Or are we going to have go back to the research people to ask them to generate dog surrogate antibody, which may take a while? So that's the hurdles we're having in step one.

Schneider: 03:10:13 Okay. I think we have time for one last question at mic two. Please introduce yourself.

Trube: 03:10:17 Thank you. Kevin Trube, Jannsen. Hey Robert, really nice presentation again. Quick question: you saw a rash in both non-clinical assessments, right?

Li: 03:10:27 No. We did not see rash in non-clinical studies.

Trube: 03:10:29 You did not see it. Oh you saw the liver tox-

Li: 03:10:31 Exactly.

Trube: 03:10:32 But you saw the rash. So when you saw the rash in both and the exacerbation based on the first clinical paradigm, did that give you a sense of pause to think maybe that there's something that's predictive here and we should go back in the xeno and look at the combination to understand longer term potential toxicities in human that could have a commonality? Do you know what I mean?

Li: 03:10:58 Well, let me try to answer your question. For number one that again, we saw the rash with both agents in the clinical study. So by the time we reach the combo tox studies, we finished all the chronic tox studies for each single agent. So we first did a 26 week tox study and then followed up with a 13 week study. And we did not see any skin abnormalities in those findings, in that kind of toxicity studies. We saw the skin rash, vem-related skin rash at a pretty high incidence, like 40% or 50%. And in the beginning as I said that we really look at that and try to understand what could have been the main driver of this kind of skin rash. Is that gonna be the immune-mediated effects or likely immune-mediated effects? And now you have this well understood pharmacology of atezo to immmuno-stimulating. Could that be exacerbating the effects? Is that scientifically sound enough to move forward along with a clinical study? Or it's not sufficient. You have to go back to your animal studies, try to collect it, try to collect additional information to give you this kind of mechanistic insight.

That's my point that this is the alternative way, which turns out to be working perfectly well in this combination. But it doesn't mean it's going to be effective in other combinations because you may not have enough data, and again from fundamental biology or clinical data to really give you the mechanistic insight about what's going on to guide you to do your clinical trial modification.

Trube: 03:12:33 All right. Thank you.

Schneider: 03:12:34 Thank you so much. Great. So I think we're going to move into our final panel session. So I will invite the afternoon speakers to come and join me up here at the table as well as my colleague, Dr. John Leighton. Also joined by one additional panelist, Dr. Alan Korman, who's vice president of immuno-oncology discovery at Bristol Myers Squibb. So we're very happy to welcome to him as well. So if you could all come and join me under the very bright lights, that would be great. Dr. Korman, since you haven't really had a chance to speak yet, I thought maybe you might want to just introduce yourself and kick us off and share some impressions since the others have had a chance to speak.

Korman: 03:13:37 Yeah, my name's Alan Korman. I'm VP of immuno-oncology discovery at the discovery site in California. So we develop the drugs that we hand to many of you. I did have a number of points that I wanted to make from the earlier discussion section, which is we often overestimate our knowledge about the mechanism of action of these drugs. So while it's very clear that PD1 can activate CD8 cells, PD1 is expressed on about a half a dozen different cell types. And we don't always know exactly which cells we're impacting. Certainly similar things are true for CTLA4. But every so often there are surprises like the study that Helen described where when we enhance the binding to the FC receptor, we actually did see toxicities that we hadn't seen as impactful with ipilimumab. Again, as my colleagues know, we still don't understand the mechanism of action of that. So I think more emphasis on understanding what cells we're impacting, I think will have a lot of importance going forward.

Leighton: 03:15:11 Yeah, I'd like to ask a question to the audience as well as to the fellow panel members. We heard this morning about option one and option two. And I'm also aware that there's a lot of pressure that once you get into a clinical trial that you keep on going. So what would trigger someone to stop a clinical trial? To go back and interrogate the clinical models along the lines of option two? Is that really realistic?

Haggerty: 03:15:47 I can speak to our anti-CD137 where we saw liver toxicity that was quite severe and really put us on clinical hold. So we did go back and try to do a lot of investigative work in the mouse model where we did see liver toxicity, although it was very low grade. So we did a ton of mechanistic work to try to understand the cell type, to see if we could enhance it using LPS or using a liver toxin like acetaminophen. So we did do a lot of work to try to work that out. Eventually we got back in the clinic. It was more related to changing the dose and dropping that down. But we certainly did try to have a better understanding of that.

Leighton: 03:16:27 So would it be fair to say the trigger would then be a clinical hold?

Haggerty: 03:16:31 Well, in that case it was. In fact, we did see the liver tox in our studies but it was very low grade. And we didn't see it in the monkey models. So now you're trying to understand is this rat or mouse-specific finding? What does this mean? Of course it did lead us to monitor for the finding.

Schneider: 03:16:49 Actually kind of related to that, did you feel in that case that you had the models that you needed to be able to ... was that a challenge?

Haggerty: 03:16:57 Well, we felt that the best model we had was the mouse. But we also didn't feel like no matter how hard we pushed the mouse, we could never get the degree of toxicity we saw in the clinical space. So we never really felt 100% like that was the model, but it was the model that we had.

Herzyk: 03:17:17 I'd like to add to this. So in the case I described with [inaudible 03:17:22] in anti-PD1, we've seen this exacerbated liver toxicity. And based on the data we went back and started doing non-clinical studies and we were not put on clinical hold. It was just clinical observation and of course, this was viewed as unacceptable toxicity for this combination but that triggered additional work. It wasn't very successful but nevertheless it was pursued.

Schneider: 03:18:01 Do we have a question at mic two?

Sung: 03:18:04 Sung from ImmuneOnc Therapeutics. I'm interested in general how people are using pharmacodynamic data from your monkey studies if you have any, in combination with other data to come up with a first human dose. Specifically from the BMS speaker, I'm interested in knowing whether you use your TDAR data from the xeno study to come up with a different dose or similar dose with your fucosylated anti-CTLA4.

Haggerty: 03:18:35 So I think when you look at these first in human dose projections, you're really taking all the data together. And sometimes that may not be the most sensitive model or value in order to pick that dose. But it certainly is factored into the overall assessment.

Sung: 03:18:57 So did you have to go lower dose with your non- fucosylated CTLA-4? I guess that's specific.

Haggerty: 03:19:02 No. And so for that particular molecule, because it was ipilimumab modified and we had a lot of data with ipilimumab in the clinic, we were able to actually use the PD data more to benchmark what is the shift in potency between the two molecules. And then use that, both from the tumor model data as well as from the monkey data and then apply that shift in our starting dose.

Korman: 03:19:31 Yeah, I'll add something on that. So again, with respect to the mouse models, we showed that mouse isotypes varied in their potency with the depleting isotype being more potent than the partially depleting isotype. And we were able to model that using FCR gamma transgenic mice as well. However ... And we did see a difference in potency that may reflect the adverse event potency of NF versus IGG-1, the isotype of ipilimumab. But it's also important to point out that using that molecule, the analogous surrogate in mouse, we didn't see those toxicities in mice. So the mouse still is less reflective of the human case.

Herzyk: 03:20:30 I have a follow-up question to this about comparison of potency between different molecules. Did your data from *in vivo* in vaccinated monkeys correlate with *in vitro* potency data with human systems?

Korman: 03:20:50 So as you're probably aware, there aren't a lot of good *in vitro* assay systems for CTLA4. PD1 is a much more potent molecule *in vitro*. But there is a super-antigen induced stimulation of PBMCs. And indeed the non-fucosylated form is superior to IGG-1 or a non-FCR binder. But again, using that that's a modest but detectable difference. Not really very quantifiable.

Herzyk: 03:21:29 So I think that's a very good point that CTLA4 is quite unique in terms of the utility of the vaccination model because it works very well. And for this purpose I think it works and the use is very helpful. But for many others it doesn't. So I think we have to probably have to make this the word that this is not universal. It doesn't work all the time with all the targets. And actually CTLA4 is quite unique.

LaBranche: 03:22:05 Tim LaBranche, Blueprint Medicines. I have a question along the vaccination lines as well. For those working with small molecules in the IO space where you have the benefit of the binding domain that's homologous between species and you don't have to go to the monkey, and you can use rodents, rats in particular. And if you're working on novel IO targets and you're trying to relatively screen a variety of targets or a variety of compounds for their "efficacy", the TDAR assay comes up again. And there's a [inaudible 03:22:44] that offers a low dose TDAR assay so if there's enough room that you can show an enhanced response. The point that was made earlier about CD4 versus CD8 is a good one. They tend to go well together. But in cases where they may or may not, you may want to look at CD8. And does anyone have familiarity with a model or an assay where they have something comparable to that adenovirus, that replication-deficient virus that was used in the monkey that you talked about that you can use in the rat?

Herzyk: 03:23:17 We used hepatitis surface antigen vaccine in mice once. Yeah, we have the model for the mouse.

LaBranche: 03:23:29 So the MHV vaccine for the mouse?

Herzyk: 03:23:32 Yeah.

Haggerty: 03:23:33 I mean, there's certainly a lot of T-dependent antigens that you can use in a rat model. Now I don't know necessarily of a CD8-mediated model necessarily.

LaBranche: 03:23:45 Is it necessary? How often do you see for the TDAR in the monkey being not tracking well with your CD8 assay?

Haggerty: 03:23:58 Well, we haven't done enough of them at this point.

Korman: 03:24:01 I was gonna say, in the mouse you have the alternative of adoptive transfer of antigen-specific T-cell and then you could immunize as you wish. For example, Ova reactive T-cells could be adoptively transferred, you could follow those.

LaBranche: 03:24:23 K, thanks.

Schneider: 03:24:26 Question, mic two.

Naraina: 03:24:29 Thank you very much. So, this older workshop about the non-clinical studies to assess safety in immuno-oncology studies is very interesting. And my question is more like, generic question basically, it's a general question. It is about, because it is all of goal of all the health scientists globally to improve efficacy and safety with minimized clinical studies and minimized animal studies, if possible. So, where do we stand in terms of minimizing animal studies? Like, so if you see the trend from the year 2010 to 2018, so are we increasing the animals studies, especially in the oncology? Because oncology is such a field that we require the physiological ... would be required to mimic physiological modeling as well as the [inaudible 03:25:29] modeling as well as the systemic modeling basically, like we need to take care of everything in oncology modeling. So but I'm still curious that are we ... do we have any focus in terms of minimizing clinical studies and minimizing animal studies so as to achieve maximum efficacy and very good safety at the same time? So it is a question of curiosity, basically. Thank you.

Herzyk: 03:26:03 Well we definitely trying to use different systems and one of the systems in oncology is tumor, human tumor histoculture system that we use for screening and [inaudible 03:26:18] so that's one in-vitro and actually has growing application and use. Whether we can totally go away from animal models, I don't know how soon that could be done, if ever, but we definitely try to use more and more *in vitro* systems.

Saber: 03:26:44 Can I address that also, with biological products, we don't always have relevant, pharmacologically relevant animal, so we usually use less compared to the small [inaudible 03:26:59] and because we have many more biological products today, the trend has been less, not because we try to use less, or the policy says to use less but because we use them when it's relevant and ... if it's not relevant, we don't use them.

Li: 03:27:18 Another comment I want to add on to this is, and this is something that Genentech has been trying to do is try to really minimize animal studies. Thinking about transitioning from non-geo-P to geo-P studies, right. So many times you identify the hazard from your non=geo-P studies and also based on a target expression based on a mode of action and whether or not you need to repeat a study, exact same study design but just in a different geo-P manner versus non-geo-P manner. We made a great success- very recent case study which is about T-cell dependent by specific antibodies we only did it the non-geo-p study and we used in-

vitro access to set up the starting does for humans and we did not do any geo-P studies. Unless it was a lot of animals used.

Haggerty: 03:28:04 I also think we could, and I think we talked about this at lunch, but I really don't think that the three month repeat dose studies are really adding a lot of value and we could probably consider whether those were worth continuing.

Naraina: 03:28:18 Thank you very much and sorry ...

Leighton: 03:28:21 Thank you very much, Helen.

Naraina: 03:28:24 Sorry, I forgot to announce myself and my name is Naraina and I am from the FDA. Thank you.

Leighton: 03:28:33 One of the reasons why I asked Amy LeBlanc to come today was to present perhaps an alternative way of thinking about using animals to ... as part of the safety and efficacy assessment. It's something to continue to think about it. It's an evolving field with using comparative oncology and I think it is getting better, the tools are the reagents are getting better and more available. So I think it's something to continue to think about. I would also maybe put a note of caution, drug developments worldwide and why FDA may be accepting of some of these alternatives or alternative ways of thinking, the rest of the world might not. And I'd like to ask Robert a question. I see with Itolizumab, you did six month toxicity studies. Is there a reason why, when the guidance calls for three months? Why did you do six months?

Herzyk: 03:29:35 It was us ... timing.

Leighton: 03:29:37 No, I think it was Itolizumab, right?

Korman: 03:29:37 Otezla.

Leighton: 03:29:43 Yeah, and I think-

Li: 03:29:43 You talking about the Otezla [inaudible 03:29:44]?

Leighton: 03:29:43 Yes.

Li: 03:29:45 Yes, we did do the six-month study and that's probably because of the antigen challenge. We tried to build into it and try to intercept.

Leighton: 03:29:50 Yeah.

Li: 03:29:54 Oh that's because of antigen challenge, we try to build into it to really get a chronic read out into some antigen response and also the potential reversibilities on those kind of things.

Leighton: 03:30:03 Yeah, the comment I would have is not so much about the antibody challenge, but that something we often see is that a follow-on products will often follow what the first product did so that if ... [inaudible 03:30:20] did six month studies, everybody's going to do six month studies thinking that that's what the FDA told them to do and that might be a

misconception. We might, but often times sponsors will just do a six month study and the rational is not clear to us and everybody following on so it's something to think about as you think about your individual development program for second or third in class.

Haggerty: 03:30:49 So I think for Ipilimumab we also did a six-month study but I think there was a shift in the guidance time when it was done versus when that changed.

Herzyk: 03:30:57 For Pembrolizumab the rational was very different, we were thinking about developing this for infectious diseases before we [crosstalk 03:31:06].

Li: 03:31:07 Yeah, another-

Herzyk: 03:31:07 So it was discovered based on HIV in [inaudible 03:31:12] so the six-month tox study was because at that time, we didn't even know which indication would come from. We knew it worked somewhere.

Li: 03:31:23 Yeah. Looks like the line it was [inaudible 03:31:24] another one an important reasons we did a six month study for Otezla is because we also targeting for the evidence error. Right? So that really requires a chronic dosing- that's before the guidance shift. That's what happened.

Korman: 03:31:37 Relevant to this question on the prior question, there certainly is less combination studies and tox studies being performed so we performed a study of PD-1 and C-T ally 4 combination PD-1 and Lag, I believe C-T ally 4, CD-1-37 but now those combinations are really tested in clinical trials with the standard does of Nevo or Prembro and then escalating doses of the new second drug, so I think that's reducing certainly animal use and again maybe less requirements from the FDA.

Herzyk: 03:32:24 But at the same time, other agencies are not necessarily on the same page, something to require, so ...

Schneider: 03:32:35 Yeah, question at mic one.

Van Deen: 03:32:37 Yeah, my name is Steve Van Deen from Persephone Biome. I would like to get you all's thought son safety studies for the use of live microbes in conjunction with I.O. if anyone's given any thought to that and ... how that can be demonstrated when microbes have a very diffuse impact on the immune system that's not very well characterized?

haggerty: 03:33:05 So you're talking about the microbiome impact on the immune system?

Van Deen: 03:33:08 Yeah, basically.

Haggerty: 03:33:09 Take that one?

Korman: 03:33:12 Yeah, I'm not sure what the requirements would be for providing, say a probiotic in combination ... we do, we have recognized that there are differences in activity of a lot of the I-O drugs depending on the strain of mice and the source of those mice, whether antibiotic treatment is used or not, so there certainly is an impact, but I'm not aware of

experiments where, apart from reconstitution, fecal transfer is a probiotic given directly but I think those experiments are in trial but not by us.

Van Deen: 03:34:03 Yeah, I guess I'm wondering if there's any ... you all would advocate any particular safety cautions that need to be taken, there's other than the standard for live bio-therapeutics is really if there's no pathogens, there's really no antibiotic resistance which is very focused on the microbe itself but not on any impact that the two can have in conjunction.

Herzyk: 03:34:26 I would just add to this that ... definitely we think that microbiome has impact and plays a role but as my colleague said, we don't do any systematic studies but even using monkeys with different husbandry practice, you can have different responses and probably that's one factor is in ... so using monkeys from different sources or even different immunization schedule or timing and husbandry procedures may affect the responses to definitely there is an element but within, I think, most big organizations, these practices are sort of established and standardized but between companies, between CROs, that may differ and could have some impact.

Schneider: 03:35:24 Is there a question at mic two?

Rysianno: 03:35:31 Oh, hi, Mike Rysianno from Bristol-Myers Squibb. First of all, I want to commend the organizations- good workshop, and the speakers, we covered a lot of topics today. But my observation is that as much as were concerned about safety, I don't think that's the biggest problem we have. I think it's lack of efficacy. When you look at all the clinical trials in this space and only 20% respond, what's happening to the other 80%? Why is that not- why aren't they responding? I think this goes back to some comments that were made earlier from Ellen and others, we truly don't understand the biology of these molecules very well. That's number one. And number two, the animal models, themselves, are not fully predictive. Not just on the safety side, but on the efficacy side. Or we would have cured cancer. Right? We basically cured cancer in mice decades ago. So, we have some fundamental problems here around the biology- understanding the biology and actually understanding the models themselves and how well they predict the outcome of clinical trials. And I would offer to say that really what we need to struggle with here is the efficacy side, not so much the safety side. So that's my comment.

Saber: 03:36:50 So I think that question is almost an answer to the question I was going to ask, that is, at the FDA, this Haleh Saber, at the FDA, we don't see the other side. What are the issues that you need to be addressed, that you're struggling with, you want to change, or do you want to keep everything the same when it comes to immune check-point inhibitors and stimulator or do you feel like somethings need to be changed and done better and what are the gaps and how would you change that, how would you address those? I guess one of them was efficacy activity, and what are the other areas?

Haggerty: 03:37:36 I think maybe talking about first the human starting dose and trying to get it a little higher. I think we tend to be a little conservative and I'm not so sure that that's always needed. I think as we gain more and more information about these molecules, we haven't really seen any acute serious safety signals ... it's more of a chronic issue. So, I just wonder and then you talked about going back and looking at the data sets and comparing what the doses were, I think that would be a good exercise, to see whether we can change that paradigm.

Leighton: 03:38:12 Yeah, I think Dr. Pazdur talked about it earlier when thinking outside the box, and I think one of the problems we got is we got ourselves trapped in the box with the whole [inaudible 03:38:22] incident and healthy volunteers dosed simultaneously for the most part and I think we need to get away from that, but also I think we need to make better use of the single patient does escalation, interpatient dose escalation, and moving away from perhaps the three by three trial design to get to a recommended phase two dose and perhaps we outta rethink the MTD approach and think about developing better pharmaco-dynamic bio-markers so that we can dose the optimal biologic dosing and perhaps within clinical models could better inform on pharmacodynamic bio-marker and in getting to that dose. We tend to just keep dumping drug in until we go well past saturation and it doesn't always make sense.

Korman: 03:39:25 Yeah, two points here, you know one that's difficult for those of us in discovery is when we don't have a mouse homolog of a protein or a target that we think might be interesting in man so we're sometimes hampered about generated efficacy data in some cases, we don't have that and then again, how do we translate what we can do *in vitro* to those human doses. One thing we haven't heard today is any discussion of intro-tumoral injection, of agents we do that with some known agents but how do we do that with novel agents where maybe we can actually get a handle on mechanism of action with low dose inter-tumoral injection and what are the requirements for delivering something like that into clinical trial. I know there's some papers on phase zero trials but those don't really address biologics as much as small molecules, I think.

Leighton: 03:40:46 Yeah, one of the problems about the phase zero trials is that there is this requirement to stop and then close down the IND and patients want to keep on getting dosed. So, it really hasn't been ... that whole exploratory and IND phase zero trial, really hasn't- we've seen a few of them but far less than, I guess, what the ideal would be.

Korman: 03:41:10 Right.

Schneider: 03:41:13 I think that's Dr. Beatty back there?

Beatty: 03:41:15 Yeah, I just wanted to make a comment around the efficacy, toxicity and those issues. Because, and echo a couple of things, the first is that when you look at some solid malignancies like pancreatic cancer and glioblastoma, yeah we're struggling in terms of efficacy and it's very unlikely that we're going to see activity with a single agent drug that's going to be any form of durability like we have seen and some cases with melanoma and lung cancer. That said, I think the time when toxicity's become more of a focus, is around when you do have efficacy, try to have a drug that works, if it's CAR T-cells and AML, and lymphomas you want to ameliorate, just mitigate that toxicity, right so that you can broaden the impacts and so you're not just selecting a small subset of patients who are potentially going to benefit, you want to be able to get a broader patient population and pancreatic and glioblastoma we're just trying to get some level of efficacy and there the challenge is going to be, in terms of FDA, is not going to be a single drug, it's going to be combinations of drugs, right? It's going to be combinations that are sequenced together.

And how do we rapidly move forward? There's gotta be rapid failures so that we can get to success and not have that long period of assessing a single drug that's just not going to by itself work. And so I do want to also echo the point of single patients learning from them and I think the idea of the run-in is really quite important, right because you have an

opportunity to treat, you get a little response and how you put these things together is going to be really important. I will, I guess, end with one question. Rather than just having a complete comment, which is, in terms of the run-in treatments approach, some of that was fortuitous that you were able to find an approach there that worked and then you worked your way backwards, right? And so I guess, one of the questions is, is there any insights that you can share around how far can you go backwards, right? Because you have done just a one week run-in. Is this a tolerance to an idiosynchractic drug reaction that's taking place in the liver and you just happen to find that right sweet spot? Is that sweet spot going to be different depending on each drug? So, I think those are ... the other way of thinking of it, is liver inflammation a marker of when you should then insert the PD-1 ... so you're seeing a peak and it dropping it off, which you can see around three weeks with [inaudible 03:44:15]. Any insights there I think would be useful in terms of knowing how could you put these drugs together and start to build on them to potentially more, in a faster way, learn how to bring efficacy. Then you can start worrying about more in the way of toxicity. So just, question at the end and a few comments.

Korman: 03:44:40 Was that more about the ... yeah, this came up earlier and you addressed it, Dr. Beatty, with sequencing and I wanted to give the example of we tried to sequence C to Ally 4 and PD-1 in mouse models and in that setting, surprisingly we had to give them together. Just shifting them two days apart in either direction didn't give the same efficacy and we know that that's not the way it works. In man, you can give them sequentially, so again, in some way, failure of the mouse model for at least one aspect, the sequencing, certainly the efficacy combination came there, but those experiments were actually done at at time where we actually didn't understand how some of these antibodies worked in the mouse and with that understanding, I think we have a better sense of how to do some of those experiments and again, with, again focus on the right kind of surrogate with the right kind of FCR, so ... I don't know if that helps, but just another example contrary to what you discuss with Aux-40 and PD-1. Again, which may be very model dependent.

Speaker 4: 03:46:14 Yeah, just I think we are talking about a lot of things. I have two comments, one is regarding the duration and I think you know, because a lot of poor data is already approved and based on in the package sometimes, you know there is a response within three months, some response it is six months, so the follower maybe follow the longer time so I think that is my issue. Another issue is a different agency has a different requirements, sometimes the other agency they may require a longer duration so that it is one struggle we are trying to work with. That is about the duration, another said previously is the tolerability and efficacy, I think once we have very good efficacy in animal model, the tolerability should be there. That is my ... comment about that. And one question is regarding- maybe is not is a question, we are struggling, you know, currently we have one poor data and the binding affinity compared between the human and the monkey is very weak.

It's not completely without any kind of binding but just too weak so in that case, we did the very conservative environment where still did three months, monitoring status, but considering the binding affinities big difference we did the human transcending mass so by the transcending mass we only did one month starting so that is our question, is this kind of worry conservative environment strategy three months in monkey and one month in monkeys, is that ... possible to support the anti- and in the application and all three months monkey is starting if that is very relevant, we can support both anti- and in the application. Assuming we didn't see any very specific issue. So this is my question. When we considering the monkey is not very relevant species but based on the CRO's recommendation, they're

thinking, even though it's not very relevant, but you still can see some sort of off target to check if there is any kind of off target toxicity so based on that kind of recommendation, we did this kind of environment. With one month, the human transcends mass model as a support, we are wondering if that strategy can support both anti- and in the application, yeah thank you.

Leighton: 03:49:05 Time for one more comment.

Leighton: 03:49:09 I don't think we can really, this is not the form to address the regulatory questions like that, IND and NDA, this is more of a sort of basic science, we're trying to understand the basic science of ... and see how the models will develop- whether or not a one-month study or three or six months study is sufficient ... I don't want to really address it right now.

Rysianno: 03:49:36 So, Mike Rysianno with Bristol-Myers Squibb- so I don't want anyone to leave with the impression I don't believe in our animal models, because I do think they're relevant for safety testing as well as efficacy. But I do want to emphasize and re-emphasize the point that was made a couple times that we have to learn from our clinical trials. Right? And I think we have to learn from our, or for our human subjects. And I had a ... I worked for a former department head, I won't name the company who said, you can ask all the questions you want about the most relevant animal models but the most relevant animal model walks on two legs and sleeps on his back so it's the human. So we gotta learn from those studies.

Herzyk: 03:50:19 Yeah, I think we all agree and we do a lot of this reverse translation whole world because of that, yeah. Well, you go ahead.

Leighton: 03:50:37 Yeah, I would like to thank everybody for attending and a great discussion, all the speakers who gave up time to come here. I would also like to mention that there was a pre-meeting blog that was posted to the AACR, there will be a post-meeting blog as well, too. And the intent is that ... it's all voluntary, but I know that the FDA will gather it's thoughts relative to the meeting, Haleh, Julie and myself and will ask the AACR to post our blog and industry and academics I think are welcome to do the same to get the various perspectives from the various groups, what they took away from this meeting and so I really want to reiterate my thanks to the AACR for all their efforts and help in organizing what I think was a very productive workshop. I learned a lot and I have a lot of thoughts going forward so, thanks very much.