

**FDA-AACR Non-clinical Models for Safety Assessment of
Immuno-oncology Products Workshop
Transcript: Morning Session**

Leighton: 00:01:38 Are we good to go now? Well, good morning and welcome to everyone. And welcome to all of those who are on the webcast. I would first like to thank the AACR for all their help in planning this very important and timely workshop which has been about a year in the planning, and my colleagues at the FDA, particularly Haleh Saber and Julie Schneider who assisted in planning this workshop. I would have to mention that from the AACR that we have over 700 registrants for this workshop and that's a record for the last couple of years. This is an attesting to the importance of this topic.

So, about a year ago the results of two clinical trials were reported and these trials are on the slide, in which it was noted there was an increase in deaths in patients being treated with multiple myeloma, patients being treated with pembrolizumab, an immunomodulatory drug either lenalidomide or pomalidomide. At that time, Dr. Pazdur, who's head of the Oncology Center of Excellence, turned to us on the nonclinical side and asked what models we could use to interrogate this toxicity, the increased deaths. We, unfortunately had to tell him at that time that we didn't think that the models would be all that informative, particularly for drugs given in combination. So, at that time, Dr. Pazdur reached out to AACR and he wanted a workshop on this topic. So, here we are.

In addition, last September, the NCI had a think tank on immuno-oncology models and it was the opinion of the FDA attendees at that time that the discussion was not going to be useful for us in terms of trying to understand the toxicities, in some of the questions that we had. So, in developing the concept of this workshop, one goal was to bring together the various stakeholders; the FDA, the NCI, industry and academics in one room to discuss model development. Particularly, what's the state of the art and where are we going?

An additional rationale for this workshop can be shown on this slide. The start dose based on a Mabel approach which most immuno-oncology drugs, the Mabel approach is the basis for the start dose, this is not a very efficient way to set a start dose. It's an *in vitro* approach. The Mabel approach was developed after the TGN incident about a decade ago in which six health volunteers dosed with TGN 1412, an anti CDH monoclonal antibody, suffered severe adverse consequences as a result of the infusion of this antibody. The fall out of this low start dose is inefficient trials, sometimes taking two to five years. This is not an efficient process compared to the six or seven months for most small molecules first in human trials.

As I mentioned earlier, in 2016, the FDA published a paper which surveyed the current approaches to developing immuno-oncology products. What we did was, we identified some data gaps and I also note that additional work on immuno-oncology products along this line has also been published by several different groups. As I mentioned, in 2017, the NCI Injection Lab sponsored a think tank on immune interventions in oncology. What they did was they focused on modeling opportunities in mouse and human specimens. They discussed the use of limitations of these mouse models and the need to develop biomarkers and other research needs. What they did not talk about at that meeting was regulatory science data gaps, which is where we come from. I also note that FDA is researching humanized mouse models on IO products and this work is ongoing.

Some of the immuno-oncology models for immunotherapy include mouse xenografts, syngeneic mice, genetically modified mice, humanized mice, companion animals and organs on a chip. Some of these topics will be touched on by speakers today. Some of the review challenges that we face in reviewing these IO products, the definitions of pharmacologic activities in Mabel are vague and can vary widely from cell line to cell line. These are generally derived from *in vitro* data. There's also a lack of transparency in translating functional assays, at least from our perspective. When we review an application, there's a lack of transparency. We don't know how sponsors are getting to their proposed recommended first in human dose. Receptor [occupancy 00:07:40] seems to be lack of transparency. Not all sponsors use the same approach and the details in applications are sometimes lacking. So, we want to become more informed and have this dialogue as to what we're looking for in an application.

This challenge is in integrating NHP, PK and add me data and xenograft data into first in human dose selection and in terms of the safety assessment. There are challenges in using other relevant factors such as receptor turnover and tumor expression in the first in human dose selection. Some of the topics we hope to touch on today include the current challenges in developing the nonclinical aspects of IO products, how to fill in the data gaps and should we use an existing paradigm? Or, do we need to develop more innovative approaches, to particularly around setting the first in human start dose.

So nonclinical models for IO products, can we advance the field with improved models? What is the utility of these models in better predicting the clinical outcomes for both safety and efficacy? Will one model be sufficient or will it be a case for all targets or will it be a case by case and a different model will have to be developed for each new target. What's the pro's and con's of using different pharmacodynamic models to evaluate the activity and safety for IO products? And can these models be adapted? They're not currently being used but can they be adapted for first in human dose selection. That's the last slide. So, what I'd like to do, is invite my colleague, Haleh Saber to come up to introduce the morning session. Thank you very much.

Saber: 00:09:37 Good morning all and thank you for coming to this exciting workshop. As John mentioned, we have close to 800 people who are registered for this workshop. Most of them are online. Some few things to mention before we get started. If you have your cell phone, please turn it off or turn off the ringers. We have restrooms on both sides. Each speaker has the allocated time but at the end of each talk, there would be a five minute for question and answers. But we also have panel discussions, two panel discussions today. We hope to make this an interactive workshop. And those online you can also provide your comments and ask your questions. For the speakers, you have the timer here and you'll see the timer there. If it's yellow, you have two to three minutes left. If it's red, you're done and then it's time for the five-minute Q&A. I will be introducing the morning speakers but please read the bio's. If I would read the bio's it would be long and these are rich and extensive. Just to make sure that we are on track for all the speakers, we'll be very brief in introducing the speakers.

Our very first talk we are very pleased to have Dr. Maus here with us. Dr. Maus is the Director of Cell Immunotherapy at the Mass General Hospital Cancer Center. She's an Assistant Professor at Harvard Medical School. And again, please read the bio. She has an extensive background. Welcome Dr. Maus, please.

Maus: 00:11:34 Thank you very much. Good morning everybody. I hope everyone has had their coffee. Despite my last name, I'm actually more of an expert in human trials than mouse studies, but here I am. I've learned a lot in the process of preparing this talk. I'm going to talk about current nonclinical models for immuno-oncology products but I will caveat that most of this talk is about T-cell products, so cellular therapies as opposed to check point blockade.

So, I'm going to start with a few statements of the obvious and maybe they're obvious to me but I think they should be obvious to everyone. And that is, that there's no animal model that can fully predict the safety based on either on target or off target effects of a particular new drug in human patients. So, we're obviously talking about modeling and trying to predict. But there is no animal model that will fully do that. I think immuno-oncology products in particular that attempt, because they attempt to modulate the interplay among various different immune cell types, so, T cells, B cells, neutrophils, et cetera, and the tumor, and normal tissues, it's much more complicated to do this. Obviously because no animal has a fully human immune system, a fully human tumor, and normal human tissues. And so, you're always going to be sacrificing one or more of those in order to try to attempt to study one aspect. Immuno-oncology drugs also don't get cleared by the liver and kidney with defined half lives in kinetics and so the idea of a PK or the idea of a dose and a dose finding study gets a lot more complicated, because you're unleashing an immune system where you're giving a living drug that expands and contracts based on how much antigen there is, based on the cytokine milieu, based on whether the patient has had chemotherapy before or not.

So, the idea of trying to apply first in human dosing in PK and PD studies, to immunotherapy products and mapping that from small molecule drugs is really kind of an impossible task in my opinion. The other thing to keep in mind is that patients are typically ill with a disease and have had prior treatments, whereas animals are asymptomatic and get only first line treatments. So, all the animals have a performance status of 90% or more whereas oftentimes first in human studies we're sort of pushing the envelope with can we get with a performance status of KPS of 70% or 80% and sometimes they're even treated in the hospital.

So, what are the goals for animal models for immune therapies? I think that we have to think about the goal in terms of deciding what the animal model is meant to do. If we're thinking about pre-clinical pathways and I'm going to focus most of the talk on this, the idea is to try to establish a path to get to an investigational new drug application that is not on clinical hold until one can proceed with first in human testing in humans. There are some aspects to this of course that are important to keep in mind. If you are studying the human T cell and the human T cell is the drug, that makes the human T cell the investigational product, which means almost by definition that you can't put it into an immunocompetent animal because the animal will not take it. It will just reject it based on the fact that it's human.

Typically, the studies focus on efficacy. So, do we get an anti-tumor effect? It's very difficult to study toxicity. It's possible but this is much less developed and they have to be very, sort of contrived models to get any ideas of toxicity. And, keep in mind, that according to the guidance of the FDA, animal models are not required for most T cell products on the path to I&D. But sponsors and investigators do often use them and I think they are preferred.

In the post clinical setting, so meaning a phase one study has already been done, a toxicity has been observed or a particular phenomenon has been observed, then we can also try to use animal models to try to model that effect and then perhaps test new drugs to ablate that toxicity or understand in more depth what the adverse effect is and what the mechanism of action of that is. Now these, I think, have had a tremendous amount of momentum in the last couple of years and I'm gonna go through some of the papers that have discussed these new models. But they've all been done on the post clinical setting where we already knew what the toxicity we were trying to model was, and then developed an animal model to do that.

And then, finally, and I'm gonna really not talk about this very much and I know that there's other speakers who are going to mention this, is basic science needs. We want to understand mechanisms. We want to model the tumor microenvironment. We want to model immune system interactions. We want to understand basic immunology. Or, we want to model a particular human disease such as autoimmunity or transplantation and cancer. And I would say this is most of what the basic science and immunology and in cancer biology has been about. But it's not necessarily part of the path towards an I&D. Having it in the literature helps but it's not necessarily something that a sponsor has to do in order to understand how a drug is going to behave in a person.

I would say that right now the standard for a new T cell product getting into first in human testing is the NSG. Now, I say standard but please understand that I'm an academic liberal with language, so I'm not quoting FDA terms on this is required or anything like that. This is just my disclosure. NSG mice are non-obese diabetic background mice with a SCID background and also a common gamma chain knockout. The advantage is they can be xenografted with human tumor lines, either cell lines or even primary patient tumor samples. It allows us to study the investigational agent which is the human T cell. The advantage is that it's a very well-established model. They're easy to buy or breed from a couple of different sources. Now, NSG in particular is Jacques but there are alternatives to that. And the path to IND is feasible. One can buy 40 mice and do 40 mouse experiments and you can have all the controls necessary. And they generally behave in a predictable way. They also sustain engraftment of both the human T cells and the tumor cells and they can discriminate anti-tumor efficacy of different T cell constructs, by changing the dose of the tumors, the dose of the T cells and the timing in between injections of both of those.

The limitations of ... so if you have a product that doesn't really work in an NSG mouse or you have three products and you're trying to do a bake-off as to which one is going to be the best one, the NSG mouse can help you with that in terms of which one has the strongest anti-tumor effect. The limitation is that it doesn't model any on target or off target toxicity, so there are no other tissues in the mouse that expresses the target antigen. Occasionally you have a cross reactive one, but sometimes how they're distributed, and it can be a cross reactive one that's not actually an on target, so it would be an off target. So, some protein in the mouse kind of looks like the human one but it has a distribution that's different than what a human would be. It doesn't model cytokine release syndrome or neurologic toxicity or macrophage activation syndrome, and I'm gonna get back to that later in the talk. These are the toxicities that have been observed in T cell therapy products and hematologic malignancies. And it also is very difficult to model any long-term efficacy. Because what happens, is that the NSG mice engrafts so well with human T cells and the T cells will

recognize mouse tissues. And so, then you go xenogeneic graft versus host disease. So, the mice start to get sick because the human T cells start to reject the mice.

And that's completely independent of what particular receptor or re-direction you're trying to give the T cells. So, you're really limited to a six-week window, which in some ways is a benefit, because that means that you get your information faster. But it's really not an appropriate way to try to study a long-term cure.

What about other kinds of mice? I'm going to have a table on the next slide, but just to give you the brief overview, there's also SCID based mice that have some NK cells but they do have much better macrophages than NSG mice. And because of the small population of NK cells and the better macrophages, they tend not to engraft with human tumors or T cells as well because they'll reject them. Athymic nude mice are even worse in that sense because they are more immunocompetent and these have good NK cells and functional macrophages. So many tumors and human T cells don't engraft at all. So, for this purpose, they're not used very often.

People always asked but syngeneic mouse models. There's a few that are available. There are CD19, obviously mice express CD19 for example and you can make transgenic mice that express the human protein as well. Tom Tedder has done some of that where it's actually expressed in the B cell compartment. There's also one for UBM but in general making a transgenic mouse just to express the protein of interest or the target of interest, will also bias your study because then you're over-expressing it at high levels and not in a particular tissue distribution that you're interested in. Because they're immunocompetent, you have to use mouse T cells, mouse signaling, mouse antibodies, mouse tumors, and so you really are no longer studying the human T cell or the investigational product that you're trying to give. So, although you can learn from it and you can model the tumor microenvironment and you can study mechanisms, it doesn't really inform you at all about how well the human T cell will behave. It won't tell you anything about which signaling domains to include in a human T cell for example. And these mice also don't develop the clinical toxicities that we have observed in human T cell trials. You're not really modeling toxicity. What you're really modeling is a tumor microenvironment or interplay in the immune system.

Just to kind of give you ... I went back and dug into the literature, how did we get to the two approved cellular therapy products? This was a paper by Jim Kochenderfer and Steve Rosenberg and their colleagues. This is in 2009. This is their preclinical evaluation of the CD19 CD28 product which is now called axi-cel or axicabtagene ciloleucel. It was actually all *in vitro* data. So, they showed that they could transduce cells and express the CAR and stain it with an anti fab ... anti-mouse antibody. The T cells produced interferon gamma in response to stimulation with a specific antigen. They were able to kill *in vitro*. This is not an I&D so I don't know if they presented other data to the FDA but at least in the paper, this is all that we see. Then, of course, there were clinical trials and I'm not going to go through all of the story, but obviously now this is an approved product. And it became approved in 2017. From the time of publication to the time of FDA approval was about eight years.

How did we get the other FDA product tisagenlecleucel? This was a paper by Mike Malone and Carl June and colleagues that showed that CAR 19 cells with a 41BB signaling domain had antigen dependent and antigen independent activity and they were the ones who used a mouse model here. So, this was an NSG mouse injected with human primary ALL and also

in other figures with leukemia cell lines and then they injected the mice with human CAR-T cells and they were able to show that the tumors, the mice had improved survival and that the ALL got better with the CAR-T cell injection by quantifying the amount of ALL. And so now we have a mouse model that predicted efficacy with this. But again, nothing about toxicity. And this was published in 2009, and again, about an eight-year window.

So, then what happened? It turned out that CAR-T cells in humans were much more potent than expected. It turns out it's actually much harder to cure mice with these cells than it is to cure people. The long-term remissions were seen in the first five patients. This was done at multiple medical centers and they all saw long term remissions in the first five patients. And the toxicities of cytokine release syndrome and neurologic toxicity became apparent and obvious at about patient four in all of the studies. At Sloan Kettering, patient four actually ended up dying from a sepsis like syndrome, which in retrospect may have been cytokine release syndrome. At Penn, this was the first time that we started to see, you know we're really seeing some things that we have to manage. And patient four was Emily Whitehead, who you may have seen videos of who was in the ICU and got the first treatment with anti-cytokine therapy. You pretty much know the rest of the story and I'm gonna focus on the humans, on the mice, I'm sorry.

Why wasn't any of this predicted? So, it turns out that cytokine release syndrome and neurologic toxicity require a functional myeloid compartment. IL-6 production by monocytes and macrophages which are not in NSG mice, at least not in human ones and endothelial cell activation in the brain, blood brain barrier which is also different in mice than in humans and is activated by the cytokines produced in the myeloid compartment. And these interact with human activated T cells. So, none of this would have been predicted, obviously from the data that you saw because there was only one NSG mouse model.

Then we go into the post clinical setting and people are trying to say, well what is the mechanism of this? How can we understand it better? How do we know which drugs are gonna be useful interesting his beyond what was done empirically? People ask a lot also about humanized mice, so we really need to have a mouse that has a myeloid compartment in order to be able to study the interplay between human T cells and a human myeloid compartment. And this is where the humanized mice come in. I'm sorry that this is a bit grayed out. This is from the website www.Taconic.com. So, I started to make my own table and it was late last night and I decided to copy the table, so excuse my laziness. They compare here, not just iconic mice but three different kinds of humanized mice which are in the literature. One is the NOG mouse. The NOG mouse is very similar to the, it's an NSG background, it's also got a SCID mutation. But it has a truncated version of the common gamma chain receptor. It functions a little bit differently than the completely null version which is what the NSG mouse has.

In the NOG background, in the NSG background, what they've now done, is they've made these mice in addition to having these mutations to make the immunodeficient. They also express human cytokines constitutively. So, IL-3, I have them here. I may have deleted them. IL-3, GM-CSF and in the case of the humanized, MISTRG mice, they also have GM-CSF, M-CSF, TPO and serpin alpha. These mice actually have a much more robust myeloid compartment. But they're not naturally born with a human immune system so they all have to be injected with cord blood derived CD34 positive human cells. They graft through for different lengths of time. So, the Taconic mouse seems to engraft for the lifespan of the mouse. The NSG

SGM3 mouse, which is the Jacques version which also has, sorry, I forgot to mention the human stem cell factor transgene. Those grafts tend to be lost after three to four months.

And the MISTRG mice, we don't really have as much of a sense for how long it engrafts for. Because they have to be injected with human cord blood, it also means that it's difficult to get matching human T cells to go with them in some cases, or you can grow them up in the mouse but then you have to sacrifice the mouse to get out a few T cells which then you have to culture ex vivo transduce and then inject into other mice. So, you can imagine that this could get quite difficult to do if you're always having to collect splenocytes from one group of mice that have been injected with human cord blood and then you have to wait for those T cells to mature, collect them, modify them, and then give them back to the litter mates. The other thing is that they're not always available. These are available both for commercial and academic use. These are also, but they are very difficult to come by. There's a long wait list. So, right now, oh I don't know, somebody from Jackson, Karolina Palucka is here, so maybe she'll tell us a little bit about it. But there can be wait lists to be able to get sufficient numbers of mice. And the MISTRG mice are not available at all for commercial use and they're only available to academics under MTA's.

To do this, it's not a mouse that can be bred and used sort of at will to study, none of these are bred and used at will to be able to study these effects. But they are available and one can do very interesting science with them. And in fact, in the last couple of months, there's been some very high-profile papers that have really highlighted what can be done with these mice. Attilio Bondanza recently published in Nature Medicine in May 2018. They used the SGM3 mice which are the NSG mice transgenic for human IL-3, GM-CSF and the stem cell factor, but in order to get this to work, they had to do a sublethal irradiation of newborn mice, so even a time delay of two days abrogated the effects of engraftment in the myeloid compartment and the cytokine release syndrome. They then injected them with human cord blood hematopoietic stem and progenitor cells. And then they wait. The mice do reconstitute hematopoiesis with human B cells, monocytes, T cells and myeloid cells. They're all chimeric so they have a mouse compartment and a human compartment, at least for the myeloid cells. And they do have some immune deficits with a human SGM3 mouse T cells are hyporesponsive to antigen. But they also respond to allogeneic to the mouse T cells and xenogeneic antigens.

It means that you basically need, what I explained before, you need T cells from the same cord or from another mouse injected from the same donor in order to be able to get the "autologous product." And so, the experiment that was done kind of looks like this. What they show is that they can inject these SGM3 mice with hematopoietic stem cells. Then they take a certain population of these mice that have been injected with cord blood. They grow out the T cells. They culture the T cells, they transduce them and then they give it to the litter mates who are injected with the same cord blood products. And then they were able to give these mice the CAR-T cells, and they used CAR 19 and also one that they were interested in which was CD44V6 which is also on monocytes and has potential use in AML.

And what they found is that these mice do get human B cell aplasia. The CD44V6 also lowers human monocytes, the CAR 19 does not. And the mice get cytokine release syndrome. So they have weight changes and they produce human cytokines, and they have a human cytokine profile where they elevated their IL-6 levels, they get fevers and they have the mouse inflammatory marker which I forget what this stands for because I'm here and it's

not enough coffee, is SAA which is the equivalent to our C reactive protein in mice. So, these mice do develop a cytokine release syndrome in response to CARs and there's human T cell, human myeloid cell interaction. And then what they go on to show is that they cytokine release syndrome is dependent on the monocytes because if you can ablate them with clodronate, and so then you get improved survival of the mice because they don't die of cytokine release syndrome. And you also get less clearing of the ALL, suggesting that perhaps some of the cytokine release syndrome is necessary to get a complete eradication of the disease.

They can also test different drugs to try to abate the cytokine release syndrome and what they found is that Anakinra is an IL-1 receptor blocker and Tocilizumab, which is the IL-6 receptor blocker worked for cytokine release syndrome. We already knew that in people but they were able to show this in mice. They also were able to show that only anakinra really ablates the neurologic toxicity. And there they just have percentages of what mice have lethal neurologic toxicity or lethal cytokine release syndrome.

So, this is helpful because it allows us to understand mechanism. But you can see that it's not something that's easily done even in multiple academic labs and it's certainly not easily done in the tremendous amount of industry and commercial development that's going on. I'll just mention that just where I live, in Boston alone, there's over 40 cell therapies that are trying to get from an *in vitro* or a construct into an I&D. So, there's just no way that this could all be done in humanized mouse models like this.

The other paper that came out, and these were back to back, was from Michel Sadelain group. This was a CAR-T cell induced cytokine release syndrome. It was also negative by macrophages so a similar mechanism. But they used a different mouse background. They used SCID beige mice. As I mentioned, these mice actually have better NK cells and better macrophages than NSG mice and because there is a little bit of cross reactivity with mouse macrophages in the human T cells, they were able to observe the cytokine release syndrome.

So let me just walk you through how they did the experiment. What they had to do, is they injected large amounts of human leukemia into the peritoneum in the mice. They had to do this because it allows for a very large tumor burden before the tumor actually makes the mouse sick. So they can allow for very large numbers of tumors. But this also means that they required very large numbers of T cells. So 3 million raji cells given intraperitoneally, then they had to wait three weeks. Normally one would give something like 1 million and wait one week. And then they had to give 30 million CAR-T cells intraperitoneally and usually we give something like 1 million or...

Maus: 00:33:00 maybe 5 million. And so their larger doses. They're not a log-fold higher, but they're still a lot larger. And thus the mice develop cytokine release syndrome two to three days later. And the way that it's defined in the mice is reduced activity, malaise ... not sure quite how you define malaise in a mouse, but they just didn't look well ... piloerection, so goosebumps, because changes in the fur, weight loss, and mortality. And they had this increased mouse serum amyloid. And they had a mixed human-murine cytokine profile, because these are chimeric mice, so they have a little bit of mouse macrophage, but then they also have all the T cell, all the cytokines are coming from the T cell compartment.

And so the degree of the cytokine release syndrome really kind of depends on which ones of the T cell cytokines produced are going to be able to activate the mouse macrophages. And so it isn't just a matter of what the profile of cytokine production is in one compartment or the other. The syndrome will depend on which ones are actually cross-reactive. So, it adds another layer of complexity to this.

And what they showed is that, for example, human interferon-gamma and GM-CSF, which are the two that we think are the most ... two of the most important that mediate the cytokine release syndrome in humans, actually don't cross-react with mice. But they did. So it was really not clear which of the cytokine profiles from the human T cells were the ones that triggered the mouse monocytes and macrophages, but they did find that treatment with human, I'm sorry, with anti-murine IL-6 receptor antibody prevented mortality.

And so that is useful. And here, this is just the schematic of their data showing that they have a murine IL-6, murine TCL-2, murine G-CSF, murine CXCL9. And they also have a profile of human cytokines that are different. Some of them come ... these all come from the human compartment and the reds come from the mouse compartment. And so it's an interesting paper, but again it required these large doses. Not clear that it could be done in the subcutaneous tumor model, not clear that it could be done with any other model other than the one that is explicitly described here, but I think that remains to be seen.

What was interesting, too, in this paper, which allowed them to study mechanism is that they also found that the monocyte in the IL-1 receptor, and IL-1 was sort of upstream of IL-6, and that it was a local effect. So if they looked at the peritoneal myeloid compartment of the [inaudible 00:35:30] cells, macrophages, etc., they were able to see that there was more IL-1 receptor 1, which is the one that's functional. Whereas in a distant compartment in the spleen, there was actually not this IL-1 receptor 1, and in fact it was only this inhibitory IL-1 receptor 2 that was up-regulated by [RNA-seq 00:35:49]. And they also found that blocking IL-1 via the Anakinra drug, or by expressing a mirroring anti-IL-1 receptor alpha, they were able to abrogate the cytokine release syndrome and mortality from cytokine release syndrome.

So again, things that we already sort of knew from the clinical setting, but are being applied in a humanized mouse model to be able to study which drugs could be effective.

In the last one minute, I'm going to talk about other non-mouse models. So just briefly, some people have used dogs, so canines. There's limited reagents though to study and grow canine T cells. They are out-bred, and so ... and they could be useful for veterinarians and pet owners, so there's a whole approval process for that. But the questions remain about whether we should use canine signaling domains, canine viral vector envelopes. So what's been done is it's been shown that canines can be transfused with retroviral vectors, and there was actually a first-in-dog clinical trial with CD19CARs, but these were with ... not with viral transduction, but instead with RNA electroporation. They were able to show that the dog also needed to have sustained engraftment in order for this to work, so there weren't a lot of anti-tumor responses, and no toxicity.

Non-human primates. I won't be able to go through the pictures because I spent too long on the other parts. They're obviously very expensive. They're limited. Poor tolerance of chemotherapy. There has been a paper by Mike Jensen and Leslie Kean recently that

showed that non-human primates can be injected with CAR T cells to a different antigen, to CD20, and they do develop a cytokine release syndrome and neurologic toxicity. And they do give it after a lower dose of cyclophosphamide. But these are non-tumor bearing, non-human primates. And they were able to show that the non-human primates get a cytokine profile that's similar to the human one with inflammatory markers.

This is in [NF-4 00:37:40], because they're so expensive. So we've now treated hundreds more humans than we have non-human primates. But what you can do with non-human primates and what they did is they were able to sacrifice two of them to study what happened in their brain. And what they found is a diffuse ... so they did find T cell infiltration into the CSF, but what was different is that they also found that there was diffuse T cell infiltration, both CAR positive and CAR negative, into the brain parenchyma, which is not what has been seen in humans. And so although it modeled some of it, what we see in humans, it's really not exactly the same.

My blue sky thinking a year ago was that there'd be NSG mice engrafted with a panel of viable human tissues. That hasn't really happened yet. And this would be to model on target or off target toxicity. My blue sky thinking at the time was that there'd be a mouse that develops macrophage activation syndrome. So check, that's been done now. So now my new blue sky thinking is that there's some humanized mouse that is in-bred, that is either serially transplanted and that's for sale, so that each individual investigator doesn't have to find the cord blood and figure out how to get autologous T cells for it. And so that it can all be matched and autologous with other immune cells, but that all be done somewhere where they can breed a lot of mice and get a lot of cord blood to do that.

So in preparing for this, I came up with this tremendous statement. Who knew that modeling the human immune system and immune therapies and animals could be so complicated? I think it really is. There's going to be very difficult ... it's going to be very challenging to be able to attempt completely model these human systems in mouse models. And with that, I know I've gone over by about a minute, so thank you for your attention and I'm happy to take questions.

- Saber: 00:39:28 Thank you, Dr. Maus. We have time for two questions only. If you have any question from the audience ... I have, I do have one question, I'll start. You mentioned neurotoxicity in the animals. One difficulty that we have in analyzing the results from the INDs and toxicology studies was that it was difficult to correlate the neurotoxicity from the animals to humans, because often times humans are dizzy or speech problem-
- Maus: 00:39:55 Aphasia-
- Saber: 00:39:56 Yes. So we tried the best to do it through histopath, and it's not a direct correlation. So how was the ... what are the endpoints that you use for neurotoxicity?
- Maus: 00:40:07 In humans?
- Saber: 00:40:08 In animals.
- Maus: 00:40:09 In animals. So there's been two or three, really. And it has, like slow movements, seizures, and lethargy have been the three, I guess, three things that I've seen that were described in

the non-human primates and in the mouse models. Again, I think in humans, it's much more subtle. So we see things like difficulty with handwriting, which can't honestly even be tested in pediatric patients all the time. So it's very subtle. But I think the other thing to keep in mind is in humans, in the vast majority it's reversible within a day or two. And so I think if our ... we do much worse sometimes with chemotherapy. We do much worse sometimes with other drugs. And so if somebody has bad handwriting for a day, I think that that's tolerable. I'm not sure that there's necessarily a need to try to figure that out exactly. Ideally, scientifically, one would want to, but sometimes the testing and the diagnostics is more challenging.

Saber: 00:41:11 Thank you.

Question from the audience? Okay.

Maus: 00:41:20 It's too early for my-

Saber: 00:41:22 [inaudible 00:41:22].

Maus: 00:41:22 ... in the morning.

Saber: 00:41:23 Thank you. Thank you very much. One question, okay.

Maus: 00:41:28 Thank you.

Speaker 1: 00:41:30 For the neurologic toxicities that you're talking about, how well do they correlate with something like a really bad illness? Are you seeing similar kinds of things, if someone has a horrible case of flu or something? Or are you seeing ... or is this really different from that kind of syndrome?

Maus: 00:41:47 It's ... yeah, it's a good question. The question is, how well does the neurologic toxicity that we see with CAR T cells correlate with just sort of acute illness. I think in the ... before we got to patient 4, I think that was sort of the idea, is like, well, you know, when people are septic, and they have no blood pressure and they have a fever of 106, it's okay to be a little confused. And that's not unusual. But I think what's really emerged is that there is a particular neurologic profile with these patients. And I didn't go over all of it, since it's human data, that's been characterized in detail. There's a couple of EEG changes, like diffuse slowing, and this poor handwriting, difficulty with word finding, sort of amnesia for the whole time that this happened. And obviously I'm not trying to minimize the neurologic toxicity. It can be quite severe. It's just that there's a range. And many patients, and depending on which product and so on, will have a very mild neurologic toxicity. And other patients can have a lethal neurologic toxicity. What's, I think, emerging is that from the human data, trying to predict who's going to do what, and then trying to intervene earlier is mostly is the goal. But I think that there is a particular syndrome that is special.

Now we also talk a lot, of course, to our neurology colleagues, and they find it special, but not necessarily that special, either. So there are other syndromes, but they can be bad. Like meningitis, for example, can give people confusion and things like that. But those typically have a different course. So there's a little bit of overlap. Or sometimes, patients when they have surgery of the posterior fossa, they can have the sort of 24 hour syndrome of aphasia

and word-finding difficulty that is similar to what we see in CAR T cells. But that's really like a completely different process. I think what's emerging as a final common pathway is just disruption of the blood-brain barrier. And it's not necessarily that specific. There's a lot of things that will do that. But this does it in a sort of reliable and predictable way. And it may have to do with neuroanatomically which areas are more susceptible to this cytokine mediated disruption.

- Saber: 00:44:00 One thing I learned from your talk is that we can incorporate some safety endpoints, very simple, such as you mentioned body weight loss, clinical signs of neurotoxicity. So that's ... because I heard before that it's not possible, it's not done. But it is done at times when ... it could be done if the model is relevant.
- Maus: 00:44:22 Yes.
- Peter: 00:44:27 If you will recall, during the early stages in the development of the Hepatitis B vaccine, you had a similar problem where the mouse model was used, everything was fine, but in humans, they had deaths. Fortunately, during the same period, there was an animal, woodchuck, somewhere in Virginia, it had similar viral infection, and it was a [inaudible 00:44:52] model for the human trials. Is it no possible to have a database of like immune responses, because it looks like everything is [inaudible 00:45:03], central immune response. I don't know whether such a model can be created. So that in clinical trial, you have to look for the particular animal which-
- Maus: 00:45:18 Can I ... I'm having a little bit of trouble understanding. Can I repeat parts of the question, and then ... sorry to interrupt.
- So, in Hepatitis B, you're saying that mouse models didn't-
- Peter: 00:45:25 Yeah, in the early stages they had a problem whereby using the mouse model, everything was perfect, but in humans, they encountered deaths among the subjects. It was found out that in the cell, they had empty [vacuoles 00:45:39], which was not possible with the mouse model. But within the same period, the woodchuck is a kind of animal-
- Maus: 00:45:50 The woodchuck.
- Peter: 00:45:50 Yeah, woodchuck. They were having the same ... some had similar viral, Hepatitis B viral infection. When the model was used, it was better than lots of the mice. They were closer to the human ...
- Maus: 00:46:09 Right. That's a very interesting idea, to suggest beyond the model, something like the woodchuck or some ... I'm not sure that for testing human T cells or human immune system that it would necessarily replicate, or be any better. It's possible. I'm not sure exactly where one starts in identifying a new species for this sort of thing. But I think it's a very ... it's an interesting concept.

This is a little bit different in that it's not an infection or it's not a particular protein. And because CAR T cells are a platform for so many different diseases, I think it would be challenging to try to identify different species for each different target, if that were to be necessary. But I think it's a really ... it's an interesting concept. I have not heard of a

particular species that has a human ... an immune system that resembles human more than either mice or non-human primates or canines.

Saber: 00:47:04 So thank you very much. In the interest of time, I think we're going to move on to the next speaker, and there's going to be lots of, plenty of question, interesting questions that we can always ask during the panel discussion.

Our next speaker is Dr. Javaid. Dr. Javaid joined Merck in the genetics and pharmacogenomics department in 2015. And she currently leads a team that interfaces with oncology, immunology, and pharmacology. And again, please read the full bio. Thank you, Dr. Javaid.

Javaid: 00:47:36 Yes. Thank you. And thank you for giving me this opportunity to present. I will be talking about Keytruda, specifically the clinical experience we had with multiple myeloma and the utility of preclinical models in this setting.

So Keytruda, just to put everybody on the same page, is a potent antagonistic antibody against PD-1. PD-L1 and L2 engage the PD-1 receptor on T cells and inhibit T cell activation. Keytruda blocks PD-L1, L2 binding to PD-1 and reactivates T cells, so they can destroy the tumor.

Since the talk is specifically going to be focused on multiple myeloma, I'm going to give a really quick overview. The phase one monotherapy study with pembro, the phase one combination study of pembro plus Len and Dex, as well as the phase two combination study of pembro plus Pom and Dex, and relapse in a refractory multiple myeloma, identified no DLTs. However, the phase three combination studies, these were randomized, both in keynote 183 and 185, keynote 183 being pembro plus Pom and Dex, versus PomDex, keynote 185 being pembro plus Len and Dex, versus Len and Dex, was put on hold after an imbalance due to the number of deaths in the pembro arm. All of the subjects discontinued Keytruda.

In the Merck's analysis of the data, not one specific adverse event led to the distinct discontinuation of death. There were a variety causes of deaths without a unifying root cause, along with the confounding medical history. And the full safety and efficacy data was presented and published earlier this year at ASCO.

I'm not going to do a deep dive, but I'm going to go quickly over some of the adverse events that were identified in keynote 23, as well as in keynote 185. Just to quickly reiterate, keynote 23 was a phase one study of pembro plus Len and Dex, and again, no DLTs were identified. In keynote 185, which is the phase three randomized study of pembro plus Len and Dex, versus LenDex, this was put on hold.

This is the Kaplan-Meier curve for overall survival in keynote 23. So, if you look at the curve for all patients, versus your Len refractory patients, you can see that the one-year overall survival rate, as well as the two-year overall survival rate, is actually very similar between the two groups.

If we now look at treatment-related AEs, you can see, looking at any grade greater than 10%, or grades three to five, the AEs are consistent with individual drug safety profiles for

approved indications, as well as the AEs associated with pembro and relapse in refractory multiple myeloma are similar to those of other indications. There were two deaths that did occur because of treatment-related AEs, and three patients discontinued because of the treatment-related AEs.

If we now specifically focus on the immune-mediated adverse events, there was no treatment discontinuation that was required for management of the reported immune-related AEs, as well as there were no cases of pneumonitis or colitis that were reported. And no infusion reactions were reported also.

In the phase three study, the randomized keynote 185, again this was pembro, Len, and Dex, in newly diagnosed multiple myeloma. The primary endpoint for this study was PFS. There were 301 out of 640 patients that were enrolled. 151 in the pembro arm, versus 150 in the standard of care arm. The overall response rate was 64% in pembro, versus 62% in standard of care. But there were 19 deaths that occurred in the pembro arm, versus the nine in the standard of care arm. And both the PFS as the overall survival curves are shown on the right-hand side. In terms of the treatment-related AEs, I'm not going to go into this in detail. This was published earlier at ASCO this year. But the main point that I do want to make is that there was no specific safety signal that was detected. There was a greater incidence of death, that was from a diverse set of events, and this was not due to any specific safety signal. And there was a greater incidence of death in the pembro arm that may have resulted from a higher proportion of older patients, that a high-risk disease among those that did die. The one caveat I do want to make is that immunotherapy does exhibit delayed survival benefits. So early termination of immunotherapy trials may lead to reduced statistical power to detect differences in survival rates between the two groups.

So we had different results in the phase one and phase two, versus the phase three studies. So what did we learn from the preclinical models?

To give a quick overview of some of the preclinical models that we're utilizing at Merck, we use the syngeneic tumor models. There's the [GEMS 00:52:35] humanized mouse models, as well as human tumor histoculture. I'm not going to focus on the GEMS or the humanized mouse models, or the human tumor histoculture. For this talk, it's mainly going to be focused on the syngeneic tumor models, specifically subcutaneous or orthotopic injections.

We are using the murinized DX400 clone. This is an anti-mouse PD-1, IGG-1 antibody, which is used as a surrogate for pembro. For the rest of the talk, if I'm talking about syngeneic mouse models, I'll be saying anti-PD-1, but just keep in mind that it's actually the murinized DX400 clone that I'll be utilizing.

The information that's coming from the clinical, as well as the preclinical setting, is being used to fuel new hypotheses. In terms of model generation, we're looking at expression profiling, so this is whole tumor, as well as other tissues, so spleen, lymph nodes. We're looking at bulk, sorted cells, single-cell RNA sequencing, 10x genomics. We're also utilizing multiomics techniques, so think [RIPSEEK 00:53:35], where you're now looking at transcriptome [inaudible 00:53:37] in every single cell, as well as whole exome sequencing. We're doing immunophenotyping, so clonality, histology, flow, as well as mass cytometry. We're also looking at cytokine and chemokine profiling.

For the examples of syngeneic tumor models, I'm just giving you a list of the 11 tumor models that are our work horse models that we typically use. These models all have different responses to anti-PD-1 treatment. The one fact, the couple of facts that I do want to add is, that so far, since we have three or four different ... three strains listed here, or four, I guess I should say ... none of these strains seem to correlate with response to anti-PD-1. These models are derived from tumors that are originated from specific tissues. But we're not looking at them in terms of an indication-specific response. This is indication nonspecific subcutaneous models. Also do not recapitulate indication specific histology and physiology. And the important information that we're learning from this is the interaction of the tumor with the host microenvironment and the immune response, and how IO therapies affect this interaction.

Using our anti-PD-1, we have differential response to anti-PD-1 treatment, red being the highest, to black being the most non-responsive models. So we can bucket them in terms of your high responders, your partial responders, and your low responders. I have spider graphs on the right hand side indicating an example of a high response, partial response, as well as a low response. The blue is your isotype control, red is your anti-PD-1 treatment. And what you can see is, if you're looking at an MC-38 model, and you give an anti-PD-1, almost all of the mice respond.

In your [RENKA 00:55:24] model, you can see half of the red overlap with the blue, so half the mice respond and the other half don't. Whereas in your B16-F10 model, none of the mice respond at all. So now if you're looking at combinations for example, you may want to pick something that's a partial model, so you're looking at synergy or additive effects.

Immunophenotyping is also critical for looking at preclinical model selection for IO. This is a paper that came out of MedImmune in 2017. And depending on what IO programs you are actually planning on looking at, and if they're targeting specific immune subsets, you can use the immunophenotyping information to actually figure out which model is the most relevant. Here's an example of CD26 [rankin 00:56:08] 4T1, which are all of your biopsy models. And then you have your MC38-LL2 and B16-F10, which is your C57 [black 6 00:56:17]. So if you're looking at a model that needs to be really [inaudible 00:56:20] infiltrated, B16-F10 is actually a really good model for this. For CD26, if you're going after an NK target. 4T1 if you're interested in for [inaudible 00:56:30] MDSCs. MC-38 if you're interested in a large number of monocytic MDSCs.

We can also use some of the clinical information and reverse translate this into the preclinical setting. This is an example of a T cell gene expression profile signature, or GEP, which correlates with PD-1 response in tumor models. The signature, which is an 18 gene signature, was prospectively tested and validated in melanoma, and it's predictive of response and resistance to pembro across multiple tumor types. If we now take it back into the mouse models, again, these are bucketed as low responders to PD-1, your partial responders to PD-1, and high responders to PD-1. This is post PD-1 data after two doses of PD-1, which were given at day 0 and day 4. What you can see is, that your high responders tend to have a higher T cell gene expression signature, relative to your partial responders and your low responders.

So what about the characterization of our [heme-onc 00:57:31] models? There are really limited number of heme-onc models that are being used by the academic, biopharma, and

CRO research community. And it's even less so in the syngeneic model setting. Most researchers tend to test this in a subcutaneous format, which may not represent the biology of disseminated tumors. Fewer mouse than human heme-onc cell lines exist. And there's actually really limited information on how these the cell lines actually align with human lines or clinical disease. And to fully investigate the heme-onc cell lines and response to therapy in the preclinical models, we need to have some type of marker, so either endogenous or labeled. Some of the ectopic markers, for example, GFP or Luciferase, can be immunogenic, and this can impact cell inputs, proliferation, [inaudible 00:58:22] growth rates, as well as your response to therapy.

At Merck, we have characterized nine or so models. These are across different tissue types, as well as different strains. They've been tested both subcutaneously, as well as systemically by IV. They have been luciferized, and some of them are still in progress. And you can see where that is listed. They've also been sequenced for both RNA-seq and whole exome, and the idea is, how does it align to the human cell lines, as well as the disease, and that analysis is ongoing. And then we also have sensitivity to PD-1, plus ... the four pluses being the most highest sensitive to PD-1, minus being least sensitive to PD-1.

The other fact that I do want to add is that safety is not being monitored, except for body weight loss. In terms of anti-PD-1 treatment, all models did not show any significant body weight loss.

This is a very similar heat map that I showed for the solid models. And again this is differential response to anti-PD-1 treatment in the heme-onc models. Again, red being maximum response, black being least response to anti-PD-1. And you can see I've bucketed them in terms of high responders, partial responders, and low responders. Again, the survival curves or the BLI is on the right hand side. You're seeing an example of a model where C1498 is a high responder to PD-1, black being isotype control, blue being anti-PD-1 treatment, 5TGM1 being a partial response, and J558 being a low response model. Apologies for not having the same curves. I was making this a little bit last night also.

In terms of what we actually used, in terms of supporting the multiple myeloma trials for pembro, the pharmacology studies that were run was a combination of anti-PD-1, Len, and Dex. We used the solid tumor model, MC-38. Again, this is a high responder to anti-PD-1. And we used two heme-onc tumors models, the 5TGM1, which is a partial responder to anti-PD-1, and J558, which is a low responder to anti-PD-1.

So if we look at the MC-38 model, again this is a high responder. This was looking at anti-tumor efficacy and tolerability, and this was done in simultaneous combination with Len and Dex. The group sizes are 12, and you can look at the different treatment groups, as well as the dose and the dosing schedule that was used.

If we now look at the survival curve, your x-axis being time, y-axis being tumor volume, what you can see is your black is your isotype treatment, green is your Len and Dex. You can see that there's very little effect in the MC-38 model when you give standard of care. Blue is your anti-PD-1 treatment, MC-38 being a high responder to PD-1. You can see almost all of the mice respond. However, when you combine anti-PD-1 with Len and Dex, you do get combination efficacy. And you can see Len and Dex does not antagonize with anti-PD-1 treatment. Again, no change in body weight was observed.

If we now look at the 5TGM1 model, again this is a partial responder to model. This was done by IV. Again, the group sizes for each of the different groups were 10. They were treated at different ... they were treated exactly at the same time, and they were given different routes of administration depending on the type of drug they were being given.

If we now look at anti-tumor efficacy and tolerability in this model, we're now measuring BLI, so this is looking at luciferase by imaging. Again, what you can see is black is your isotype control, but now red is your standard of care. This is Len and Dex. You can see again in the 5TGM model, there is very little response with your standard of care. It looks almost identical to your isotype ... or your vehicle control.

When you now give your anti-PD-1 treatment, which is in blue, you see a really nice response. And then when you give your combination of anti-PD-1 plus Len and Dex, you can see again, you are not antagonizing Len and Dex with anti-PD-1.

If we look at body weight, you can see all the lines are right on top of each other, and no change in body weight was observed. This was tolerated really nicely.

The last study I'm going to show is J558. As a quick reminder, this is a low responder to PD-1. And again, we were looking at anti-tumor efficacy and tolerability. Again, this is simultaneous combination of anti-PD-1 plus Len and Dex.

If we now look at BLI, as well as your tumor growth, what you can see is a slightly different scenario than what I showed earlier in the first two studies, in MC-38, as well as the 5TGM1 model. In J558, if we look at the vehicle, and then your single agent PD-1 treatment, you do see a slight response with PD-1, but again, this is a non-responsive model to PD-1, so you're not seeing the full efficacy, or the partial responses you were seeing in the earlier models.

If we now add Len and Dex as a single agent, you see that there's no change, it looks very similar to your vehicle control. However, the combination of Len and Dex with PD-1, again, there was no change and the error bars do tend to slightly overlap. And then the final tumor volume is shown over here. And there was no significant differences that was observed in the BLI signal at the study termination, which was at day 58. This is now looking at the individual tumor growth kinetics. So you have your vehicle, your PD-1, LenDex, and then your combination. And what you can see is your vehicle, Len and Dex, as well as your combination, look very similar to each other. The BLI is shown in the bright blue color. You can see there is a slight response with anti-PD-1, relative to the other groups.

Again, there was no change in body weight that was observed with LenDex and and the anti-PD-1 treatment. All of them were extremely well tolerated. You can see that over here if we're looking at body weight. And then the percent survival is shown over here, very similar to the BLI graph that I'd shown two slides ago.

The clinical endpoints that required removal from the study was body condition, as well as paralysis. And just as a quick comment, about 40 to 50% of those that survived in vehicle, Len and Dex, and the combination groups, and then there was an 80% survival in the anti-PD-1 treatment group.

So in conclusion for this study, the phase three studies that were put on hold due to increased mortality observed in the investigational arm that was not observed in phase one and phase two trials combined, indicated that factors related to patient population played a role in different safety outcomes of these studies. There are multiple syngeneic mouse models, both solid as well as heme, that are being utilized to characterize support of our IO combinations. However, these mouse models are confounded by the use of specific strains and tissues, and this is independent of anti-PD-1 response, regardless of whether you use solid tumor models or heme-onc models. Syngeneic models also have limitations for the ability to evaluate safety. Body weight is being measured for tolerability and the studies are also being done for a finite amount of time. And in the preclinical setting, preclinical pharmacology models can potentially be leveraged for the detection of strong synergistic toxicity...

- Javaid: 01:06:00 but are unlikely to predict AE's that are observed in the clinical setting. So, thank you.
- Saber: 01:06:21 Thank you, Sarah. Questions? And please introduce yourself when asking the questions.
- Maus: 01:06:28 Hi, I'm Marcela Maus. Great talk.
- Javaid: 01:06:31 Thank you.
- Maus: 01:06:32 It strikes me, so I have two thoughts. One is that as oncologists, I think we've often thought that an immune therapy, particularly anti-PD-1, will be better tolerated than chemotherapy. And so I wonder if these changes in the patient population were a bit of, as you mentioned, perhaps patient selection in terms of giving this to patients who are older, sicker, or less performance status than you saw when you had very strict eligibility criteria in the phase one, phase two. So I'm going to assume that that is ... do you agree? Is that part of it?
- Javaid: 01:07:07 There was a trend for it, but that's all, but the data still-
- Maus: 01:07:11 That's not totally what the-
- Javaid: 01:07:13 Yes, yes, it was a trend. It was an observation that we made but that was ...
- Maus: 01:07:17 And so then I wonder from that then if one of the things to test in your models is mice that are older ...
- Javaid: 01:07:24 Yes.
- Maus: 01:07:25 ... and/or mice that have been through chemotherapy because chemotherapy has, I don't want to use like ... it decreases performance status oftentimes. The more chemotherapy you've gotten, the less organ function you have, the less tolerability ... we talk about people having, you know, just gone through a lot.
- Javaid: 01:07:49 Yeah. So the first part of your question is actually really relevant. We're actually going and starting to look at mice that are older. It just takes a lot longer because when the mice come in, they're typically 6 to 8 weeks, and then you start to give therapy. But when you have to

wait a year to actually start, it takes a year to start those studies, so some of those studies are ongoing at the moment.

In terms of your looking at preclinical models with chemo as well as with anti-PD-1, we have done those studies, and we do see that the mice are able to tolerate it. And we do get really nice response with chemo plus anti-PD1. We tested an [enChIP cytokine 01:08:24] in our [inaudible 01:08:25] solid tumor model, which was only one of the models. I'm not showing the data here, but we're going back and starting to test across a couple of other chemotherapy regimens also. The other caveat that comes is, is the dosing, right? I'm doing simultaneous dosing so do you have to stagger them or exactly what do you have to do? Those are things that are still being discussed and still has to be planned out.

Hartke: 01:08:47 Hi. Good morning. Jim Hartke.

Javaid: 01:08:49 Hi.

Hartke: 01:08:51 When Marcela gave her talk, and this is a general thing that we discussed, is that we were able to look back at the cytokine release syndrome and some of the neurotoxicity to find models that replicated that. When we're talking about the clinical findings here, we don't really understand what happened there to look back and do an animal model. Do you have any insights about that? And I presume that a lot of this combo work was done prior to the trials rather than afterwards.

Javaid: 01:09:19 Yes. The data I'm showing you is historical data that was done prior to that, but in terms of the cytokine or anything like ... we did not see anything. The models were extremely well tolerated ... yes ...

Hartke: 01:09:34 Yeah, but we don't know even clinically what we would want to reach back into an animal model to ... Yeah, so that makes the model really hard to-

Javaid: 01:09:42 It does. Yes, I completely agree with that.

Hartke: 01:09:44 Yeah. Okay, thanks.

Javaid: 01:09:44 Yes.

Kalamegham: 01:09:46 So, the gentleman before kind of asked the question I had in mind, but I do want to get-

Saber: 01:09:46 Could you introduce yourself?

Kalamegham: 01:09:52 Oh sorry, Rasika Kalamegham. I do wanna ask your thoughts on how your experience with non-clinical safety models with immuno can go on to inform what is absolutely necessary as we now approach the age of immuno combo.

Javaid: 01:10:16 So, in terms of that question, I think for the pre-clinical models and looking at IO and IO combinations, you have to pick the right models. You have to know where your target is expressed. You have to know what the different populations are. The dosing also becomes extremely important. You can do all the different simultaneous versus combination dosing.

But if you have a good way to start and you know what your PKPD is and what your target engagement is, I think all of that will help.

Kalamegham: 01:10:48 I think what I was really getting at was most patients in the clinic who are gonna end up getting a combo are usually exposed to monotherapy before they get to the combo.

Javid: 01:10:58 Yes.

Kalamegham: 01:10:59 So how do we factor for that? Because it's really going to be very different if you just start off with a naive mouse, so to speak.

Javid: 01:11:08 Yes. So typically, we don't go and treat mice with chemo and then we go right into IO and IO combinations. These are mice that are naïve, and also keep in mind that we are treating them typically at 100 millimeter cubed. So by the time you have to take them down due to IACUC regulation, it's 2000 millimeter cubed, and your time window is less than 17 days before you actually hit that. So, by the time you actually start your treatment to the end of treatment, you have 17 days give or so.

So I think it's gonna be extremely difficult. I think you have to try other models. GEM's being a really good example where the disease progressed for a longer amount of time. But again, those models are really hard to set up. I think this is something that has to be ongoing, and there have to be models that either we can extend for a longer amount of time that we can actually see whether it recapitulates human disease. But in terms of the syngeneic mouse models that I presented, that's not possible at the moment just because of the narrow window of time we have.

Kalamegham: 01:12:08 Thanks.

Saber: 01:12:10 I wonder before going into combo therapy, do we have even good models of safety for predicting what happens in humans for monotherapy, let alone combo.

Javid: 01:12:23 Yes. And that's something we have to work, yes, that's something we'll-

Saber: 01:12:25 Yes, would be a good discussion for the panel. Thank you very much.

Javid: 01:12:31 Thank you.

Saber: 01:12:38 Our next speaker is, Dr. Palucka. She could not be here in person, but we will have her on the line, and we'll see her slides. Dr. Palucka, I've been told that we can hear you. Dr. Palucka is a Professor and Associate Director of Cancer and Immunology at Jackson's Lab. And she is a professor in the Department of Immunology at the University of Connecticut School of Medicine.

You're on Dr. Palucka. I will let you know when your time is up.

Palucka: 01:13:13 Thank you very much. Good morning. Thank you for this opportunity to speak in the meeting. I have to say I will not talk about the toxicity or adverse effect. I don't study that, although following the talks of my two colleagues, I am quite inclined to start looking at

that, so thank you for this. I will rather tell you about how, over the years, we have used different models of humanized mice to study human immune system interface. And in this context, I hope that this may give you the ideas perhaps how the models can be used to this effect. I have two disclosures. The next slide is going to show ... I have grant support from Merck to study the effect of anti-PD-1 in humanized mice. My second disclosure is I really agree with Bob Weinberg that just because the models are imperfect, it does not mean that they are wrong. I think my colleagues in the two earlier talks already made these points [inaudible 01:14:25] I come back in conclusion. The next slide is, you are all very familiar with this, it is just to give you the rationale of how we have ... why we started using humanized mice and little bit ... we know now that cancer immune landscape is complex and heterogeneous, involves numerous immune cell types that can impact the responses.

The next slide illustrates there are different approaches to this categorization. The hot tumors, cold tumors, etc. But I personally like the best approach, this [inaudible 01:15:09] current opinion immunology from the French group where we realize that the hot tumors from the standpoint of the [CTS 01:15:19] is only one component, and the cold tumors can actually have different immune compositions all the way from the immune ignorance and immune neglected tumors towards inflammatory tumors which are very infiltrated. These are not cold tumors because of the absence of the immune cells. They just have the wrong immune cells.

The question is how to study this and how to model the ... perhaps even predict the response to immunotherapies with this in mind. Of course, there are models, mouse models, syngeneic models, and GEMs that support this kind of studies, and over the years, we have learned a lot.

However, as the next slide illustrates, there are some substantial differences between the mouse and human that can actually impact the conclusions that we are making in the field. Perhaps the adaptive immunity, the key features are quite well conserved although even there are differences in the affinity of T cell responsive, in the longevity of T cell responsive and the type of antigens and the cross presentation, etc. But the differences in the [inaudible 01:16:44] are really quite profound.

They have been summarized in review during our immunology that you can see on the slide, but just a few examples. For example, the TLR9, TLR8, inflammasome components, the number of molecules that in pathways that are involved in the innate immune sensing that might be really critical for the immunotherapy mechanisms as well as efficacy differ substantially.

Personally, I think that that might be one of the reasons why we have such a discrepancy between mouse and human in cancer vaccines. As you all know, there are numerous phase three trials and different approaches to cancer vaccines that failed. Really other than the [Dendreon 01:17:39] vaccine, most of the phase three trials are negative. Let's keep in mind that all of these approaches were tested extensively in the mouse models and were efficacious. So it's really important to keep in mind when extrapolating this, and that was the reason why we have decided many years ago to embark upon using humanized mice however imperfect to try to model this interaction.

So the next slide illustrates the different models that we have been using over the years. On the left is the recent model that is based on adoptive T cell transfer. These are not SCID mice that are deficient in the mouse that are two microglobulin. We want to remind you that these mice exist because they have much less [GDHD 01:18:34] because of the instable mouse MHC. When transferred it with human hematopoietic progenitor cells, these mice generate human B cells, human dendritic cells, some very limited other myeloid compartment. They do not generate T cells, but if you have T cells from the donor of CD34 cells, you can do adoptive transfer of mature T cells at the later time point.

In fact, we have been using these mice for studies of influenza virus and influenza vaccines and the biology of the lytic cells as well as on the studies in breast cancer, and I will show you couple of later. More recently, we have started using the mice where the human T cells develop indigenously, and of course, that has a lot of limitations, and I will come back to this.

Nevertheless, these are the two models that ... actually, one is MISTRG and the other one NSG-SGM3 mice, both of which provide some substantial human myeloid compartment and is the thing that [inaudible 01:19:49] over other models.

So one example of how we have developed programs from patients to the humanized mice and back to patients is breast cancer many years ago, and that is on the upper left panel. We found the presence of mature dendritic cells infiltrating breast cancer tumors. We found that these red mature dendritic cells are in very close interaction with blue CD4 T cells. We have analyzed T cells in the tumors from patients. This is all patients, and we found this presence of the IL-13 producing, interferon-gamma producing, IL-10 negative inflammatory Th2 cells.

So we wondered what is the impact of these cells on the tumor development and that's where we used our first model of humanized mice that I explained to you in the prior slide. In the upper right panel, you can see that when we are adoptively transfer CD4 T cells, that resulted in the profound acceleration of the breast cancer development. These are all breast cancer cell lines. Then when we analyzed the CD4 T cells in the model, tumor infiltrating CD4 T cells in the model, this is the middle panel in the lower level, you see that we found the T cells with very comparable phenotypes to that found in the patient's IL-13 producing interferon-gamma producing cells. That led, of course, to the very logical experiment blocking IL-13, and that is shown in the lower right panel when the mice were treated with agent blocking IL-13, this accelerated tumor development was inhibited.

So then we spent several years trying to identify the absent signal that could drive this. We have published extensively on this. I'm not going to be [inaudible 01:21:45], just to say that this process was dependent on the human dendritic cells that were activated to maturation, skewed to maturation promoting stage two responses through TSLP, and then we found that actually the TSLP interaction can be mediated by IL-1 data.

So in the next slide, we have treated these humanized mice with IL-1 receptor antagonist, anakinra, that was mentioned earlier today in the context of CAR T cells. That is shown in the upper panel when you look at the tumor development curves in our model, mice that are treated with anakinra, which is green, do not experience this accelerated tumor progression. When we analyze this-

- Saber: 01:22:40 Karolina, we have to stop you for a second. You're on which slide? We're a little bit behind. What is the-
- Palucka: 01:22:45 I'm on the slide number nine.
- Saber: 01:22:49 Slide nine. Okay, good. Thank you.
- Palucka: 01:22:52 Shall I follow my slides or shall I follow what shows up on the WebEx?
- Saber: 01:22:58 WebEx.
- Palucka: 01:23:00 Okay. So as you can see in the upper panel, the green curve shows that mice treated with anakinra, and even when we delay the treatment with anakinra on few days, do not experience accelerated tumor progressions. When we analyze the T cells from these mice, at the site of the tumor implantation, you can appreciate in the bottom panel that there is inhibition of the IL-13 production in the mice treated with anakinra, and substantial increase in the production of interferon-gamma suggesting that this block of this inflammatory response not only controls tumor progression, but also allows reprogramming of the tumor micro-environment from the inflammatory pro-tumor [phase two 01:23:52] phenotype to anti-tumor interferon-gamma phenotype.

So with this in mind and with data in hand, we went back to the patients, and we looked whether these mature dendritic cells that I showed you in the prior slide, and hopefully, the slide is going to come up soon, it will be on the upper left plot. We found that these dendritic cells are actually full of IL-1 data. We then measured IL-1 data in the tumors from over 150 patients by now. I think these are all patients with breast cancer, primary breast cancer with different stages of the disease.

We observed that there is enhanced IL-1 data production in the tumors according to the histological state. So the more invasive the cancer, the more IL-1 data. Of course, we don't know what is the cause, what is the effect. Nevertheless, that and many other studies that I don't have time to discuss prompted us to actually design a clinical trial, so really from the patients to humanized mice back to the patients, to design a clinical trial where we combined the anakinra IL-1 receptor antagonist with chemotherapy in patients with metastatic triple negative breast cancer.

The way this trial was designed was that we had run in with anakinra, so the anakinra only for two weeks, and then it was followed by anakinra in combination with chemotherapy. These were all patients that were resistant to all prior therapies. So the endpoint was not clinical. It was the blood transcription to understand whether there are changes in the blood transcriptional signature.

As illustrated in the heat map on the right side of the slide, the yellow is the baseline for the patients. The blue, the first line, is the two weeks of anakinra. Then one, two, three, four, treatment with chemotherapy. You can see that there is down regulation of inflammatory signature and a lot of IL-1 pathways in the bottom part of the heat map, and then up-regulation only ordered by anakinra only, and then especially in combination with chemotherapy when probably some of the antigen is released. Up-regulation of a lot of

transcripts that are related to the T cell function including [inaudible 01:26:36]. So this reprogramming of the tumor environment can also be detected in the blood.

The next example of how we are using humanized mice to study the basic immunology and relay it back to the patients will be discussed in the next couple of slides. That is now in the metastatic melanoma where we found that unlike primary breast cancer where, of course, there are macrophages, but there is quite substantial infiltrate of dendritic cells. In the metastatic melanoma, we find a lot of massive actually infiltrate of CD14 positive cells as illustrated here, which are mainly macrophages, and which are mainly around the tumors, surrounding the tumor. Some of them are [intratumor 01:27:31].

If you look at the T cells, which are here in yellow, the T cells are predominantly engaged by the CD14 positive cells. They are very rarely moving into the tumor. So it is important for us because this balance between macrophage and dendritic cells as you all are familiar with can really dictate in the very simplistic way as illustrated in this slide, it can dictate the fate of the antigen in the tissue, which may be critical for efficacy of immunotherapy.

So in the very simplistic way, the macrophages are thought as mainly antigen degrading cells, and dendritic cells as antigen presenting cells.

So for some reason, we went back in this slide. But to try to study this *in vivo*, we are using two models of humanized mice. One of them is ... I'm sorry. I'm not sure which slide you're seeing because I see we went back. Hello?

Saber: 01:28:42 The heading says *in vivo* models of human myeloid cells and human melanoma, MISTRG.

Palucka: 01:28:47 I'm sorry, there is a delay. I apologize.

We have used these *in vivo* models, two models. One is MISTRG discussed already earlier. The other one is SGM3. The major difference ... there are many differences, of course, between these models, but the important difference is that the MISTRG mice had to start with the CSF-1 dependent myeloid compartment, and they are not transgenic, they are not in models. The SGM3 mice are transgenic, so they have uncontrolled expression of the human cytokine. They do not have genetic, at least these models, there are now ... there is development, of course, to express CSF-1, but in the data that I will show you, we do not have CSF-1 modulation. So we are looking at the CSF-1 independent myeloid compartment.

So MISTRG mice, we had wonderful collaboration with the [inaudible 01:29:51]. We were able to study the interaction on melanoma, and as the slide shows, on the upper left, there is a brief comparison of the classical NSG mice, MISTRG mice, and the [human blood 01:30:11]. Not surprisingly, MISTRG mice had many more myeloid cells. When we put melanoma tumors in this [inaudible 01:30:21] melanoma cell line into this mice in the subcu model, on the upper right panel, you can appreciate the infiltrate of the CD14 positive [inaudible 01:30:32] tumor macrophages where in NSG models, of course, we do not have this infiltrate. In MISTRG mice, there is quite substantial infiltrate, a little bit less than human patients but quite comparable.

You can see on the lower left panel how the tumor appears. In the MISTRG mice, there is increased vascularization and also increased melanin concentration in the tumors. That

really prompted us to test the efficacy of Avastin anti-VEGF not because it is approved treatment in melanoma but to test whether this involves actually this increased vascularization whether it involves VEGF and whether this model could be used to study drugs.

As you can see in the lower right panel, when we treat the mice with Avastin, there is substantial inhibition of the tumor development at the implantation site. So since then, we have been working very hard trying to develop autologous humanized mouse where we have collected over the years from patients tumors and hematopoietic progenitor cells to construct the mice with the ... because that's potentially one of the problems of all the humanized mouse models based on the HPC transplant. They are allogeneic to the tumors that we put in.

So this slide shows you that even with the MISTRG, we can actually obtain the engraftment of the human immune system when putting very low numbers of human hematopoietic progenitor cells from the G-CSF mobilized blood. When the MISTRG mice are ... I'm sorry, there is some confusion because the slide doesn't show up. I don't understand why. When MISTRG mice are enriched by expression of human IL-6, this engraftment is much improved.

The next slide shows that actually we were able to, in this fully autologous system, we were also able to observe accelerated tumor growth.

I apologize. I need to interrupt again. It says this slide has been [inaudible 01:33:07] by presenter. I cannot [inaudible 01:33:10].

Saber: 01:33:12 It says humanized MISTRG6 mice support autologous to more development and drug response.

Palucka: 01:33:18 Thank you. So I see something different. Okay, so I will go after my slide.

So what you can observe here on the right side of this slide is that in three patients that we were able to do this experiment with, in all three patients, there is engraftment of the human hematopoietic progenitor cells, and the autologous tumors grow much faster or much bigger, maybe that's a better explanation, they're much bigger in the mice that were engrafted with HPCs versus the non-engrafted mice.

Then in patient number three, we were able to engraft larger cohort of mice than in the patient one and two, and so we were able to do some preliminary experiments with treatment. So we first did Avastin treatment similarly to the cell line and the allogeneic model that I showed you before. There is control of the tumor development. We also treated the mice with pembrolizumab, and as well, we were able to see the inhibition of the tumor development. So we are studying this now in depth to try to understand what are the mechanisms of this and how this model could be used farther.

So we were also interested to understand and capitalize on the advantage of the NSG-SGM3 mice, whether the CSF-1 independent myeloid compartment would have similar effect on the development of melanoma tumors using the same cell line. So on the left is what I have shown you earlier, and on the right, what you see is the development of melanoma tumors

implanted subcu in the humanized NSG and SGM3 mice. As you can see, there is no difference between the two strains.

So, of course, these experiments are not done side by side, so of course, there is a possibility of numerous confounding parameters, but we think that the main difference is that the CSF-1 dependent, what it suggests is that CSF-1 dependent compartment is critical for the development of the subcu tumors.

But then we also wonder that maybe there is another explanation and maybe there are some metastatic tumors that actually would use the cell and perhaps this is a consumption effect rather than the different cell types. So we looked at the later time points. Here we are looking at almost two months after the tumor implantation. We found that there is substantial spread of metastatic disease in the humanized SGM3 mice as opposed to humanized NSG mice. And there is, as I showed you, no difference in the so-called primary tumor on the implantation side. But there is a substantial metastatic spread both in the spleen and in the liver. This is very highly reproducible, completely independent on the donor. In the next slide, what we ... to cut the long story short, I'm showing you that this metastatic spread actually is dependent, is mediated by human CD33 positive myeloid cells that develop in this SGM3 mice. The experiment is such that we construct humanized SGM3 mice, we purify human CD45 positive cells from spleen and liver, and then we sort them into myeloid and non-myeloid compartment by the CD33 expression. Then we adopt it. We transfer them into non-humanized SGM3 mice, which are implanted with the tumor.

The lower left plot shows you that if we put melanoma tumors in non-humanized SGM3 mice, we have very sporadic metastasis. When we transfer adoptively human CD45 positive cells, that [inaudible 01:37:31] pro-metastatic activity. But within the CD45 positive cells, the pro-metastatic activity, if mediated by the human CD33 positive cells and not by the human CD33 negative cells. So then what we wondered is whether this has anything to do with what can be seen in the patients. What we did was the myeloid [nanosting 01:37:53] analysis of the human CD33 positive cells purified from [inaudible 01:38:00] SGM3 mice, so the pro-metastatic cells.

On the tumor samples from patients with metastatic melanoma, in what we saw by immunofluorescence, there is CD33 infiltrate, and I just showed you earlier, massive CD14 infiltrate. We found that on nearly 80% overlap in the genes between the patient and the model suggesting that this model may offer us the clues as to what are the completely human dependent. Please keep in mind, the mouse cells are there. The tumors are not metastasizing if there is no human myeloid cell. So this is really completely human dependent metastatic [threat 01:38:45] of the disease.

Then again coming back to the Avastin experiment that I showed you we have done in MISTRG in 2D cell model, we were wondering whether that would be also part of this tumor development, and what you can see in this slide...

Palucka: 01:39:00 on the left, is the tumor growth at the implantation site. So you can already appreciate that even though there is some statistically significant [inaudible 01:39:13]. On the primary implantation site, it is nowhere near compared to what we have found in Mr. G.

I'm finding that there might be different mechanisms and that the seeds of one [inaudible 01:39:28] control, is different than the use of one independent control. And then when we look at the metastatic side, we found that the least inhibition of metastases, these are all macro-metastases, of metastatic spread to screen, in the mice treated with Avastin, but there is no in fact on the liver. Suggesting that, and it is not surprising knowing what we have already learned from the mouse model, that different metastatic sites may be controlled by different mechanisms. But, what I think is important here is that we can now appreciate it in the human micro environment, and analyze what are the different pathways depending on the [inaudible 01:40:13].

And in the human [inaudible 01:40:15] that I have led, I want to now, it'll take a little bit of the work that my colleagues at the Jackson Laboratory are doing in terms of evaluating some little mass if it's in humanized mice models. This work is done mainly by [inaudible 01:40:32] in the *In Vivo* Pharmacology campus of Jackson Sacramento. We had been corroborating with a little bit just on the standpoint of the city piece.

So, of course there is a big interest, and again we had fair talks this morning, in understanding can we use humanized mouse models to at least gain some insight into mechanisms of how these drugs are working, and maybe perhaps even some impact on how the combinations would be working. And here is one example of such experiments. This is done with humanized NSG mouse, where we are using breast cancer PDX2 mouse. These are not alive. Here what I'm showing you, this is PDX model from triple negative breast cancer. And the mice are either untreated or treated with platinum in green or treated with Keytruda in red, and the dosage is indicated. And then they are followed for up to 21 days, I believe. And what you see here are the tumor growth curves. So there's clear inhibition of the tumor growth, both in the platinum and Keytruda. And then if you look on the right panel you can see mean two more volume over time of the experiment, so there is still important impact of platinum but also of Keytruda.

The question that comes very often in these discussions, of course, is first of all the efficacy of the engraftments, and then this is allergenate model. The hemato particles lining their cells are from different donors. And in fact because of the limitation of the carbon black HPC's, usually the mice from different donors are mixed in the experiment in a way that is controlled and balanced, so that the cohorts are with the same, in this case, from three different HPC donors.

The engraftment was over 25% in the blood and the tumor expressed PDR1. And then in the table, the sleeper table you can see the level of the match, the HLA match between the tumor and the donors, and in this experiment there was match with all three donors and different lot size. And part of this work was recent establishing passive donors. One of the questions of that time was whether this is the only PDX model and the only type of the tumors that cannot follow the responsive to these treatments in the humanized energy mice.

And so the experiment was done also with the lung cancer model as I'm showing you here. Also with bladder cancer, PDX's and again, there can be treatment response in this case, in the black is again saline control. In the green is Keytruda alone, and in the red is Keytruda combined with Paxil. Growth curves and main tumor volume.

Interestingly, in this experiment there was no match with one of the donors of the HPC. And I think that reflects what the group has seen in other models as well, including bladder cancer. The data is really, in the main MHC, LFI, we were not able to identify the need for the match. We don't know what happens with the minor [inaudible 01:44:32] compatible with the antigens, and there may be other non-classical MHC molecules that are at play, we don't know that yet. But, it appears that when looking at the measurement side for the presentation, there is really we cannot pinpoint a correlation between the HLA match and response to anti-PD1.

There is however, variation between the donors, and it's not clear yet why, and what the explanation. Perhaps, non-classical MHC or minor [inaudible 01:45:10]. But as the slide entices of donor variation show, in the same lunk as PDX model, in some experiments there is treatment response as shown in the right panel. But in some experiments there isn't. And the way that we address that at this stage is quite laborious and expensive I have to say, because what we need to do is to conserve the cohort with several donors and we do not know purely which donor will be responder, and which donor will not be a responder, and the mechanisms of this differences are. As I mentioned, that's one of the questions that we are pursuing.

However, we were able to address some of the questions of what are the mechanisms that underline if there is some present, and that's illustrated in the next couple of slides. So what you see here is first a question of the mechanism. We wanted to know whether the response was but dependent on the humanization, and in fact it is because if you look at the left panel, again the lunk PDX model, the black are the vertical controls and now we show you each mouse in the cohort, and the green is some cyclophosphamide. Majority of the mice treated with cyclophosphamide, complete inhibition of tumor progression at the source implantation site. When as all of the control mice actually experienced tumor progression.

When we do the same experiment in the non-humanized NIG mice, with the exception of one mouse, there is no difference between the vertical and cyclophosphamide, suggesting that indeed the presence of the human cells is critical to these responses when they happen.

That prompted the question whether this is T cell dependent, and to do these experiments we have used breast cancer cell lining, that has easiest [inaudible 01:47:29] as well as potentially genetically edit, to go deeper in the mechanisms. That is illustrated in the next slide where we used MDA and B231, the cell line which is one of the classical and widely used breast cancer cell line from people living with this cancer. And the mice were constructed and then treated with cyclophosphamide, and with cyclophosphamide and anti CD8 antibody.

What you can see on the left is that in the cyclophosphamide group, the target molecule is actually mutilated because we cannot detect PD1 staining in the blood, in the human CD45 property cells. So the drug engaged the target. And when we look at the right panel, black is the control, the red is treatment with cyclophosphamide. There is treatment response, although not as profound as we have seen in the earlier PDX model. And then when we treated the mice with cyclophosphamide and with anti CD8, anti-human CD8 antibody, we completely abolished this treatment response, suggesting that actually the human CD8 cells

that develop in this model, however imperfect and immature they are, they do contribute to this terrible effect. So to further gain some insight into how these T cells behave, the next slide will show you the immunofluorescent staining of the hog tissue staining, where we labeled T cells with red and blue exactly showing the tumor. And that's from the same type of experiment from the MDA 8231 cells. And you can see in the control tumor, the T cells are on the peripheral of the tumor, and very few of them are able to infiltrate the tumor. Whereas in the remnants of the tumor that are present, as I showed you in the prior slide, it's not complete response, not like many of the PDX tumors that we have seen. There is still some tumor detectable, but even when we look at what is left there is different structure of the tumor very close to the AP cells, and also different localization of CD8. Which now really can deeply integrate. We don't know what is the cause, what is the effect. Are the T cells there because they could infiltrate and that's why it works, or are the T cells there because the tumor is being killed and that makes more room for them to come in? These are some of the questions that we are trying to address in this model.

So, to summarize a little bit, what are the challenges and the opportunities of the humanized mice, I tried to point at some of them during the talk. I think one of the major opportunities as we found in our metastases study is that it may offer as the possibility to look at human-human interactions. This is not to say that maybe the mouse models do not have similar pathways that are engaged, but clearly the mouse to human is not faithful signaling, because in this model melanoma does not metastasize in the absence of the human myeloid cells.

But of course there are numerous challenges, which also are also opportunities for the development of new models. One big challenge is the interaction of human hematologic progenitor cells, especially from the patients. There is a lot of research going on in a number of ways, how to derive HPC's from the IPS, and I think in the next couple of years a lot of these are going to be solved.

Another big issue is the mouse host. There is mouse myeloid cells are present. We do not know yet, how they end up here. It's quite likely that they do, and we are developing models where we try to delete mouse myeloid compartments. And it was already earlier mentioned that GBHD is the problem. I'll show you a slide to illustrate how this could be especially controlled, but as I mentioned in the large scheme better to micro globally mice. This is less of the problem because of the instability of the mouse. MAC class one.

And then another very big idea of a lot of development going on is how to improve the lymphoid [inaudible 01:52:44], and dorian function. One of the challenges is that time off is the mouse, so these cells are not selected on the human MNHC. But I have to say, even in the BAT models where the mice are transplanted with the certain organs, such as fetal liver and fetal [inaudible 01:53:05], they improve a little bit over the timing selection, and these models were used quite a lot in the HIV field. But it is not yet satisfactory so, again there is numerous efforts going on to improve this, as well to improve the lymph node development and eventually [inaudible 01:53:28]. And it may be critical as we do not know the nature of the mechanisms that mediate some of the toxicity that we see in the clinic, that it may be, in my opinion, critical to develop these improved models as they may offer a better insight, at least in the mechanisms.

So one example of mice that are actually available-

Saber: 01:53:54 Two minutes, Karolina.

Palucka: 01:54:00 I am finishing. The host as you think, mouse plus one is unstable, and just to show you the saliva credit of the mice after the injection of the mature PBMC's, there the NRG mice are gonna die eventually and when they are edited to delete the class one, the survivor is much improved. The next slide just summarizes what I already told you. The numerous ideas in which a lot of research going on how to improve the models.

So next to the last slide, entitled NRG model for Human Immunotherapy, I think that the papers that studied the toxicity of cat T cells, actually illustrated this best. The clinic is developing so fast that I think it will be very difficult for any model, whether it be mouse or humanized mouse to predict, I think the best that we can do and that we can do actually a lot of good, eventually, is to add that demouance to the question that we learned from the clinic, and then develop the models to study the mechanisms, and then eventually go back to the patients. I think the error where the movement was from the mouse to the patient, [inaudible 01:55:23] thankfully over, and there is quite recently an amazing paper published showing the new cell type in the brain that has unilateral function and that is not even present in the mouse. So we have to be very aware, that even with the best models we will only see part of it, but this part can be critically important, as for example the part that the papers showed.

I also think that the genomic engineering have an ability of increased power and novel methods to do genomic and genetic engineering will change the field and in the next few years we are going to have much better model.

And to finish, I just want to thank my lab, and the people in my lab, and Jan Max Phoenix, Julie, and Tina are the main people involved in the work with humanized mice. I want to thank our patients, of course founding [inaudible 01:56:25] collaborators, my colleagues at the Jackson Laboratory, as well as wonderful collaboration with Dr. Flavel Antoval, and I'm sorry, Michael Turoti, I don't know if he's onto this slide. And then for many years I have been collaborating with Jack Panchera. Thank you for your attention.

Saber: 01:56:51 Questions for Dr. Palucka. Again, please introduce yourself and mention your affiliations.

I'm going to start with the first question. How long did these mice live, what is the survival?

Palucka: 01:57:07 Which ones of them?

Saber: 01:57:08 The humanized.

Palucka: 01:57:11 So that will depend on the model and the longevity of the mice, especially after treatment. Those that had myeloid compartment, is a factor. In our hands the LGMC mice can live up to six, seven months of the experiment, but eventually they are going to succumb. Partially to the disease, the original disease, partially to the GBHZ. The not Keytruda microglobulin mice can live much longer. The NSG mice when not treated can live even up to nine, eight months. Then the problem that you face is that, and perhaps the newer models which have the combination of growth factors that support hematopoietic presenter cells, maybe Mr. G 6, will help solve this partially, is that it is not yet clear what signals are needed for the long

term repopulating hematopoietic preventative cells. That's independent of the humanized mice.

I don't think, there is somebody from the field, the person of which I apologize for my ignorance, but I am not aware that we already know what factors we would need to express in order to be able the long lead hematopoietic pro genial cell, that could be serial if transplanted. There are directions towards this, that definitely NSG mice, CPO, are six of the factors that support this. So I think in the next few years that will be resolved, and that is critical because currently even if you have a long term mouse, the myeloid compartment will go down with time because of the inability to renew. And so you end up having mouse with human lymphoid cells, but very little myeloid cells.

Saber: 01:59:15 Thank you, and we had a question in the room.

Bunch: 01:59:17 Hi, yes, Todd Bunch, Bristol-Meyers Squibb. You mentioned some data and you showed some data, on human variability, you said there was donor variability. I'm curious if you could comment if you've seen that variability across multiple tumor types, in your models. And from the slide it looked like the differences across the two donors weren't severe, so could you also comment if that's translated into even non-responsiveness in some cases?

Palucka: 01:59:51 Yes, so depending on which questions we are asking in the model. For example, in the Mr. G mice, or in the LGM3 mice in the metastatic model, we have not avoid this in length, either breast cancer cell lines or melanoma cell lines. We have not observed between donor variability. Sometimes, it varies variability must be driven by something good, because regardless of how many donors of HPCs we use, we have very robust clinical and immune cenotype.

In the experiment, dependent on the CD8 T cells, we do see this variability, we see across the models, even be it cell lines or PDXs. I really don't know what the underlying cause is as yet. I think that it may have to do with some, either minor histocompatibility antigens or non-classical antigen presenting molecules, because these are T cells that are genetic, invoking the same conditions from mouse to mouse. I'm certain that between different animal groups and different animal classivities there is different microbiomes. But within the same groups and the same mouse rooms, this variability still exists so I don't think that microbiome would be the only answer, it may be some contributing factor, but I don't know that yet. But there is this variability in the response to anti PD1, it's across different tumors, and PDXs all said that I have tumors is not unique to either one.

Saber: 02:01:49 Okay, thank you very much. We are on time and this is your break, 15 minutes. Please be back here at 10:15 sharp. We'll start at 10:15. There is coffee for everyone so go help yourself. Wake up and we hope to get more questions from you. ...

Okay, it's 10:15 and we are ready to continue with the next speaker. And if the next speaker is here, we'd like to welcome Dr. Beatty. You're here. Yes please.

Dr. Beatty is an Assistant Professor of Medicine at U Penn School of Medicine in the Division of Hematology and Oncology, he's a Chair of PanCan Precision Promise Immunotherapy Working Group, among all other roles that he has. So welcome, and we look forward to your talk.

Beatty: 02:04:32 Great, thank you very much and thank you for the invitation to come speak today. I think this is a really important subject matter, as surely all of you do, as well.

My goal is really to try to dig into some of the syngeneic and transplantable immuno competent mouse models that are available learn about immunotherapy oncology toxicities. I'll share with you a little bit about some of those models, but also some of the lessons that may have arisen already from those models. These are just disclosures.

So as we all are quite well aware there is an ability of immunotherapy to provoke a wide range of immune related toxicities. These are signs and symptoms on the right hand side that can emerge and really inflammation in just about any organ within the body. Of course the type of intervention can really lead to a preferential display of distinct toxicities.

And I think as we think about trying to model this biology in mice, we need to be grounded in how closely does the mouse immune system mirror what we see in humans. And this was touched on a little bit by Dr. Palucka in a review article that was presented several years ago. The human blood and the mouse blood is not the same. There are clearly distinctions there. Neutrophil biology is not the same, arginase biology is not the same in mouse and humans. Hematopoietic cells are not the same, in fact there's a whole range and this table's not meant to be read, it's meant to make the point that there are quite a few differences in the immune system between the mouse and the human, even though they are really quite conserved in their genome, which was studied five or so years ago, presented in Nature.

And just to highlight a couple of these, Dr. Palucka had highlighted her favorite ones, these were two of my favorites. CD40 on endothelial cells is not expressed in the mouse, but it is in humans, perhaps that's one of the reasons for cytokine release syndrome that is more apparent in humans as it is in mice. TH expression of IO10 is different in mouse and humans. Certainly IO10 biology is not clearly the same in mice and humans. We usually think of it as immunosuppressive in the mice, and yet it's not being used as a super antagonist in humans. So there's clearly a distinct biology.

Nonetheless, I would say that overall the structure of the immune system of mice and humans is quite similar. And so that is one of the major reasons we all are using mouse models in order to confirm human biology. Now there are several distinct types of immuno-competent mice models that can be used. There are transplantable tumor models, and I listed two examples of this. And I just want to highlight the fact that these can take place either subcutaneously, you can put into the organ of interest, you can inject it intravenously, there's multiple different way, and peritoneally, that you can deliver these tumor cells to try to mirror some pathology in distant organs that's the same.

Spontaneous models are of course tumors arise in a stochastic manor, they're longer. Mouse models, there's models of pancreas cancer, breast cancer, there's models of lung cancer, glioblastoma, others. And then of course there's also non tumor models, right? And whether or not they may actually hold merit in understanding the biology that occurs in immune-oncology and I'll show you some of those examples.

Now there are advantages and disadvantages to each one of these, right? Transplantable models you can put the tumor in, you get a tumor very quickly, the cell lines can be genetically modified so you can understand tumor intrinsic aspects. You can modify the

mouse, you can understand host aspects. Those types of things are much more difficult to do in spontaneous models, which are more costly, there's a stochastic tumor development, but they do recapitulate somewhat faithfully. The tumor micro environment that we see in humans, and there's also the emergence of new tolerance mechanisms that occur, and so this allows for an adaptive immune system to the presence of a tumor.

And finally there's non tumor models, and this was eluded to earlier, that one of the challenges with these transplantable models is that you got a 17 day window in which to look for your toxicities, yet in patients these occur over weeks to months. So these non-tumor models actually offer that opportunity for repeated dosing, and I'll show you some examples of that.

Of course there are disadvantages to each of these as well. Implantation into a mouse is vaccine, and it induces an immune response immediately. There's a danger signal that occurs because of the dead cells and the inflammation of the injection. There's a limited time window for monitoring, and of course just using mouse models in general, there's the challenge of whether or not there's a good mouse surrogate to the human drug that's being used.

Spontaneous models, costly. There's a tumor latency issue. Tumor heterogeneity can also be a very much challenge. Each one of these tumors that emerges in the model can be different, and that makes it very challenging to understand what you're looking at. And then of course the non-tumor models, you can't examine the effects due to the tumor being present.

So how should we then incorporate preclinical models into the study of IO toxicities? So I put together this model, I think it really formulates the two pathways that exist. The first is that we have option one. We can try to learn about this Biology in the lab, right? Either through cell lines, mouse models, and then translate those toxicity findings back into the patient in order to form how we translate and how we monitor patients. And I put in here that the work that's done in cynomolgus monkeys is all done in non-tumor bearing monkeys. And maybe that's an important point to make as we go forward here.

The alternative of course is to, what I think most of the speakers thus far have already made the point of, is that we identify the toxicity profiles in patients being very cautious about moving our drugs into patients, and then identify the models to be able to study those in the mice, and other preclinical models.

So there are of course advantages and disadvantages to each of these. Option one, mechanistically we might learn quite a bit of information, and may inform toxicities, and change the way in which we model or monitor patients. Of course, this requires more extensive preclinical modeling, it's not quite clear the toxicities that you find in patients are actually going to mirror what you see humans.

Which models do we use? Should they all be standardized? Probably not, most likely not. And the heterogeneity that you might see between models. You might find outcomes in valve C mice, but not actually in black six mice. And so does that mean it's not gonna be an absolute finding in patients.

The alternative is that we can be more scientifically focused. We identify the toxicities, we move those into the mouse models. But which models do we use? Is there of course this issue of a human, a mouse surrogate for the therapy that's being tested? And what about these issues of human and mouse system biology not perfectly being matched.

So I wanted to, as I was putting this together-

Beatty: 02:12:00 to walk through three examples. And so, just so you know what the examples are upfront, the first one will be C24, the second one will be PD1 and the last one will be CD40. And some of the lessons that we've learned along the way. So, certainly, I think we're all pretty well aware of the fact that C24, and I just picked out Adalimumab, in this case, can cause various immune toxicities. And I think there's a couple points to make. It can involve multiple different organs. It can have its onset of toxicities in a delayed setting, right? And the treatment of choice is to discontinue the use and to incorporate steroids. And so, is that the best way? It works in some case, but is it the best way. So, I think there are some major questions that are now in the field, which is the predictability. How do you know which patients are gonna have these toxicities? How do you when, what the timing is going to be for those toxicities? What's the mechanisms underlying the pathology that is behind those toxicities. And, are there they better treatment or prevention, even prevention ways, in which to incorporate in here?

So, what have we learned in terms of pre-clinical modeling in terms of C24? Well, if you go back to the very early studies in 1995, you knock out C24 genetically, which you find causes lympho-proliferation, and mice die at very young age. Right? But that's not what we see in patients. And, of course, there was a series of studies that were done to show that C24 could unravel auto-immunity, in this case, encephalomyelitis, in this case diabetes. In this case colitis. And these findings were all present prior to moving C24 antibodies into the clinic. And so, the first set of investigations took an approach to, in a few patients metastatic melanoma, to look at C24 antibody in patients. And basically, it was pretty well tolerated, actually. If you go back and look at this early paper. Grade 1 rash, some constitutional symptoms, but really no major toxicities that would have been appreciated. And so, if you read closely in the patient-in the paper, this was the comment that was made in terms of how to move this drug forward based on all this auto-immune toxicities that were seen in the mice and even the lethal toxicities seen when you knock out C24. It was said that before clinical use, MDX010, anti-C24 antibody, underwent extensive evaluation in cynomolgus monkeys. Again, these are non-tumor bearing monkeys, right? And did not cause any notable critical or pathological toxicity. I repeated IV doses from 3-30 mg/kg in acute and chronic toxicology studies. This data wasn't presented here, but that's what was found.

If we move on to the next study, you start to, you move past the N04, right, as Dr. Mass was saying you start to see new toxicities, right? Now you start to see dermatitis Grade 3-4, hepatitis, hypophysitis, colitis. So now these toxicities are starting to emerge. And as, of course, you move to a larger patient population, these now 511 patients, these toxicities have remained. So, and I would say, and I think Dr. Jim Allison would also say, that we still don't quite understand the biology of C24. There's still a lot to learn. In fact, if you conditionally knock out C24 in adult mice, you don't see the same effects that you saw in the... in knocking it out at birth.

And so, this raises some very important questions around the biology. And I think what we've learned, is that transgenic mouse models targeting the same molecule can produce pretty widely diverse and pathological outcomes. Conditional during deletion during adulthood vs. genetic deletion at birth can have different outcomes, right. And, but also, that just knocking it, uh blocking C24 can unleash non-malignant inflammatory conditions. And so, whether or not to have a tumor present or not, to study immune toxicities is, I think, an open question.

So, I think this also makes the point of a conceptual model, which is that perhaps one of the reasons why the cynomolgus monkeys perhaps, the reason why our mice, when we deliver C24 antibodies don't have any avert toxicities is because they haven't been conditioned yet. They haven't had that predisposing agent. Our patients are on multiple medications. They get viruses. They're not in barrier facilities. And so, this may be the underlying reason as to why we see these toxicities in patients.

So can we uncover that type of biology? Well, I'm going to show you a couple of examples here of this in C24. This was an animal model of hypophysitis in which they took mice and they treated them with anti-C24 antibodies. This is the clone out here, it's hamster anti-mouse antibody. They deliver that on days 0, 1, and 3 for five weeks vs. control. And then they provide a little bit of inflammation as well, with complete [inaudible 02:17:20] And what they find when they look in the pituitary gland is actually an increase in infiltrating mono-nuclear cells, in the setting of treatments. And that these mono-nuclear cells were actually CD45 positive cells. And they didn't find any inflammation, actually, in the thyroid, the liver, the colon, or the skin. It was actually pretty specific. And they went on to try to understand this. And what they found was that there were serum antibodies that were produced in the setting of the C24 treatment, and that they could overlay these antibodies on prolactin expressing pituitary cells, as well as ACTH hormone expressing pituitary gland cells. But they couldn't see any of this no green here, in the control, IGG. And then they went back to the patients, and they looked at the patients that had evidence of hypophysitis, all of these were melanoma except for one. And they found that seven of the seven patients had pituitary antibodies in their serum. There was 13 of 13 patients without hypophysitis, lacked pituitary antibodies. And they found that a subset of these pituitary glands expressing prolactin also expressed C24.

And so they went on to propose that C24 antibodies bind directly to cells within the pituitary gland and mediate an activation of complement and actually a T-cell independent mechanism of toxicity. This was thought more to be related to a myeloid and innate cell biology. And so, you know, as this- this is a model, and as I walk through each of the models, I'll share with you some of the limitations.

Of course, in this model, they didn't show that there was direct evidence for C24 binding to C24 in the pituitary gland. And, of course, it's also unclear if this secondary hypophysis model accurately mimics what's seen in humans. Nonetheless, it is model that has reproduced the biology.

Another example is colitis. So, this is a different model in which regulatory T-cells identified by these markers: CD4, high levels of CD25, and low levels of CD45RB, are adoptively transferred into immuno-compromised frag 2 knockout mice. And then later, this is a mouse... an Hepatic, sorry a um, similar to H. Pylori, a mouse pathogen called H. Hepaticus is

injected into the mice, and the mice then receive C24 either early or late. And what they found is that there is an increase colon thickness. So, there's evidence of colitis and infiltration of leukocytes when the mice are infected. But if they adoptively transfer in their regulatory T-cells, that this is blocked. But, one can uncover this biology, which I should that the bacteria is present in both these conditions, so it hasn't been depleted, and now by blocking the C24, one actually sees an emergence of this colitis again.

So a subclinical pathology, perhaps, that is unmasked by delivering C24 and, of course, if you didn't have the bacteria present, you don't get this colitis, effect like you do when you have it present.

And so I think there's a couple points here. Certainly, that the C24 blockade led to inflammation and that these mice that had this predisposing condition, despite an accumulation, actually of Fox P3 cells in the setting of C24 blockade was not a depletion. And they could also reproduce the same biology by delaying the treatment of C24. So this provides another model, perhaps, of being able to study the colitis outcomes that are seen.

Of course, it's an immunocompromised model, and hepatitis near an [inaudible 02:21:20] hepatic pathogen. It is similar to H. Pylori, but it's not a major pathogen that we see in humans. So, I want to return to this conceptual model, because what we're starting to see is perhaps there's some sort of pre-disposing condition that might be allowing for C24 to uncover a biology. And another example of this is in the liver injury model. Amodiaquin is not a medication that we typically give to our patients with cancer. It's an anti-malarial drug, but it's been used as a means of creating a kind of subclinical hepatitis. And in this model, what the authors have done is to take wild type or PD1 knockout mice, and they treat them with C24 antibodies, and then now deliver this liver injury causing agent. And what they find, actually, is that if you just deliver C24, C24 by itself in mice doesn't really produce much in the way of a transaminitis. Whether it's in the wild type mice, or in PD1 knockout mice over many weeks of treatment. Alright?

So, just what we've, you know, typically seen, we can't reproduce this biology in mice. But as soon as you start to deliver something that injures the liver a little bit, now you start to see just with the drug itself, a transaminitis, but it resolves. But in the setting, now, of PD1 blockade, it no longer resolves. Right? So causing this kind of pre-disposing effect is now unveiling, or unleashing a biology that we wouldn't have otherwise appreciated. And what that histologically looks like is this massive infiltration of mononuclear cells that you don't see if you don't have an liver injury. Right?

So, of course there's challenges to this model as well. Does PD1 Blockade recapitulate the findings that you would see in a PD1 knockout mouse? We don't know the answer to that. And, of course, amodiaquin is not a common drug used in cancer patients, but cancer patients are on a lot of drugs: statins, and other things that can cause a little underlying liver inflammation, and so is that one of the predisposing conditions in our patients.

Okay, so Example 2, then, is around some anti-PD-1. So we've seen a range of toxicities in the clinic. These differ a little bit in terms of their frequency from what we see with C24 blocking antibodies. And of course, one of the major ones that has emerged, and one of the most prominent one is rash, even though many of us talk about pneumonitis as a big one. And so, what have we learned over the years in terms of PD-1? Well, we certainly know that

aged C57 Black6 mice, in which PD-1 has been knocked out, will develop an arthritis, and lupus-like proliferative arthritis. They'll develop glomerular nephritis in the kidney and inflammation and this appears to be due to a IGG deposition. And PD-1 can also be involved in regulating graph vs. host disease.

And so what these authors concluded, based on this early study in 1999, was that PD-1 is involved in the proliferal self-tolerance by serving as a negative regulator of immune tolerance. So that's what we know. But then, if you look in a different mouse genetic background Bal C mice, and if you knock out PD-1, you get a different set of findings. So now, these mice developed dilated cardiomyopathy, they have congestive heart failure. Again, there's deposition of IGG on the surface of cardiomyocytes. And these authors concluded that PD-1 is important in the prevention of auto-immune diseases. Okay? So then, if you take a different mouse model, now a nod-mouse model, these mice are in the pre- they are pre-diabetic, in the age of 1-10 weeks, and they found that blocking PD-1 led to diabetes in these mice. And, of course, it was more pronounced in older mice, and so they concluded that PD-1, PDL1 pathway has a central role in the induction and progression auto-immune diabetes in non=mod. Mice.

And then finally, if you go back to the Black 6 mice, but now actually create a little bit of a liver injury, in which they do this by injecting Con A so this is an experimental auto-immune hepatitis, what they find is that there is an accumulation of CD8 T-lymphocytes in the liver, even in the mice that don't receive Con A, but it's not pathologic. But now, when they deliver Con A, what they find is that there's increased liver damage, and they conclude that PDL1 may regulate inter-hepatic CD8 T-cell accumulation, and how it controls inflammation on immune disorders and tolerance in the liver.

So, I think what I'm trying to highlight here is the fact that each one of these is a different strain of mouse. Each one of these, then, reveals a different set of immune toxicities and auto-immunity. And so, is there a role, now, for genetics and pre-disposing insult and inflammation and driving this phenomenon that we see with PD-1/PD-L1 blockade in the clinic.

So, I want to take you through a clinical case cause these are all mice. And show you a different model here looking at rash. So, this was a fifty-eight year old woman- sorry fifty year old woman, stage 4 melanoma BRAS mutated, that was a clinical case presentation by several authors just last year. And what they reported on was that this patient had received a combination immune checkpoint therapy. And, not too far into the treatment, they-after the first cycle, actually- they found that there was Grade 2 rash that was forming and was quite a significant amount of pruritus, and so [inaudible 02:27:29] was discontinued. And so the patient went on and received the PD-1 blockade and the eruption on the skin slowly progressed. And so after each cycle, the patient receives steroids, and there was partial improvement.

Another cycle was given of treatment, and the eruption continued to progress and so high dose steroids were started at this point and a biopsy was done. And the biopsy showed a myelin interface dermatitis with rare necrotic keratinocytes. And it was also found, interestingly enough, that PD-L1 was expressed on the keratinocytes. And so this patient, not too long after this, was then admitted to the hospital tachycardic, afebrile, plaques

covering 90% of the body, and desquamation. And you can see an example here of what that looked like. Pretty severe, right. Not completely common, but it can happen, right?

And so, a biopsy, then, showed toxic epidermal necrolysis with interface dermatitis, and a full epidermal thickness necrosis. And the patient eventually died six days later after being admitted due to septic shock and multi-organ failure. So, pretty dramatic, right, for this particular patient. And there have been attempts to model this. Again, this is in a non-tumor bearing model. This is taking now OT-1. So this are mice that are TCR transgenic mice. They have CD8 T-cells that will recognize a peptide called Sinfecal and the chicken ovalbumin protein, it's a very strong antigen. And what they have done is to engineer mice to express ova under the control of the keratin 14 promoter. So, these mice have ova sitting on their keratinocytes. Right, and now they adoptively transfer in these ova TCR cells and what they find is that when they tweak the system a little bit and essentially if you adoptively transfer in T-cells that are from PD-1/OT-1 knockout mice, sorry OT-1/PD-1 knockout mice, you find that these mice rapidly lose weight. And there's about an eighty percent death rate. Right?

Whereas the mice that received wild type OT-1, they all lived. So, there's a massive necrosis within the epidermis that occurs. So that recapitulates what was seen. This is not due to fast. They had other reasons for looking for that. But they did show that the keratinocytes in this model express PD-L1 and the up-regulated. Even in the setting even of having the ova present in the mouse. And, when they adoptively transfer these cells, they see this really massive serum cytokine response within interfering gamma produced, and they report but don't show the data that this can, uh, this effect can all be blocked in the interferon IL-6 and TNF knockout mice.

And of course for our patients, the patient receives steroids, Infliximab, azolitmin blocking the antibody and also, IVIG, intravenous immunoglobulins. So, if you come back to this patient as well you can see this pathology and interestingly enough there was PD-L1 expression within predominantly the keratinocytes. So whether or not this is exactly mirroring this biology, is unclear. But, the model has its strengths, and of course it also has its limitations. Although if it's a strong antigen, it's not certainly a tumor antigen. It's not clear what the keratinocyte antigens would have been. Was this predisposed by another drug that the patient received, and the PD-1 unmasked that effect, which can happen with TEN. It's also not clear in this mouse model whether or not PD-1 blocking antibodies would reproduce the same biology that was seen with genetic deletion.

So, this is the final example, which is looking at CD-40 and some of the lessons that have been learned here. So, CD-40 is a member of the TNF receptor superfamily. It regulates immune activation, and can actually mediate tumor apoptosis directly. It's expressed on dendritic cells, B-cells, monocytes, but it's also on other non-hematopoietic cells. It can be expressed on endothelial cells, platelets, even some fibroblasts. CD-40 activates antigen presenting cells, and the dogma in the literature has always been that it bridges innate adaptive immunity, and allows for better activation and priming of T-cells. And in the clinic, it's been found that when one uses an agonistic CD-40 antibody to drive a positive signal into the cells, that there is cytokine release syndrome in patients, there's hepatitis, so the inflammation in the liver, and then also there can be seen as thrombocytopenia. And this is just an example of the first in human study that was done showing that there is an transaminitis that can be seen as one increases the dose of the CD-40 antibody. It occurs quickly, and then eventually resolves, and that's in both the AST and ALT.

So, this has been investigated in the clinic as the, a little bit of the biology that's behind this. This is one example. This now combines a tumor-bearing model. It's a Black 6. They're using the Lewis lung carcinoma cells. They inject them intravenously into the mice. And then they come in with CD-40. Not quite typical to what we do in the clinic, of giving repeated dosing every day. But, I can tell you that the half-life of this antibody is about seven days in the mice, and so it doesn't really matter. They just overdosed. And, they also introduced IL-2 in the same setting. So it's not just CD-40 but I think there's good lessons to learn from this.

The reason why they did the study initially was because they wanted to find, to figure out whether or not there was an impact of age. So, we all use 6-8, 10 week old, 12 week old mice, which are kind of like college teenagers, and you know, just out of graduate school students. And most of our patients are 60, 70, 50. They're older patients. And so, they wanted to know what would happen if they delivered this treatment. And what they found, unsurprisingly was that when they delivered this treatment to aged mice, 22 months of age, that the mice all died. And that was pretty traumatic for the, right? Whereas the young mice did just fine. Right?

So, they wanted to understand why that the case. Well, they looked in the mice and they looked at the liver, and they found that in young there's a little bit of hepatitis that occurs in the liver. But when you get in the aged mice, this is really quite significant. And it recurs, occurs quickly, within 40, 24-48 hours you see these findings. And so they went on to show that, yes, you get a trans-aminitis. This occurs when you give the combination treatment, and if you do this in the aged mice compared to the young mice, you see that there's much more TNF that's actually released. So, contrary to what we think that older immune systems might not be as responsive, this is actually showing that an older immune is actually more responsive, right, in terms of cytokine release.

And they went on to look at this more closely, they could show that if they blocked macrophages, so they deplete macrophages now using quadrinate encapsulated liposomes. So, if you're not aware of how that works, liposomes are taken up by macrophages because macrophages just eat them. And the quadrinate is toxic to the macrophages, and so it kills the macrophage once the liposome has been eaten. And you can see that the histopathology score is decreased in the liver when you deliver these liposomes, and trans-aminitis is decreased as well as the TNF.

So, much of this, not all of it, because there's still a difference here, is being driven by macrophages. And, you can also see that in terms of the trans-aminitis, that this necrosis marked by arrows is decreased now in the mice with the quadrinate. And so mice, also, now live. So, by depleting the macrophages, now, one can improve survival. And so, the authors went on further to look at how this might be working. They took bone marrow-derived macrophages from mice and humans, and human monocytes-macrophages derived from monocytes, and they stimulated them with LPS in-vitro. And they found that the aged macrophages, both mice and humans, make more cytokines, in this case TNF, but also IL-6 was looked at.

And, they went on to then show that by blocking this toxicity using Tenoreceptor, which is a TNF blocking antibody, they can ameliorate the trans-aminitis, improve the histology score without impacting negatively the therapeutic effect. And so, I think that's the, one of the major important points there.

Now, we in the lab have also looked at this in a different scenario. There's been much work about putting chemotherapy first, and then giving a CD-40 agonist. And one of the things that we had learned along the way, in the clinic and in mice, was that chemotherapy can be mildly suppressive and that may eliminate some of the positive effects that you would get from a CD-40 agonist. So we flip the sequence. We gave CD-40, and then we gave chemotherapy. And as we did that, we used the tumor model, the pancreas tumor model and we found that when you give just chemotherapy, you really have little effect on tumor necrosis or proliferation in the animal model.

But when you give CD-40 first, and then give chemotherapy, CD-40 alone at this time point doesn't cause these effects, but the chemo, but adding in the chemotherapy now leads to much more necrosis and much more prolifer-and decrease in proliferation. The problem was the mice lost weight. And in many cases the mice died. And so, this was challenging. But, if you flipped the timing to now 5 days later, the mice don't lose weight, and all of the therapeutic efficacy is seen.

And so, in that's just shown here. So the timing of delivering the second drug can potentially ameliorate the toxicity that we otherwise saw. Now it has gone on to look at what might be happening here. And, of course, the liver has been thought to be a major player. And if you flip this circuit of giving CD-40 then Gemcitabine, you actually see these necrotic regions within the liver, and there's a trans-aminitis that occurs. This is all at Day 2 and not at Day 5. And you can block this by, in tumor-bearing mice, It's not so clear you can block this in tumor free mice here. But in tumor-bearing mice, you can block it using a CSF-1 receptor blocking antibody.

So, I think this points to the liver. In one sense that the liver is a metabolizer of drugs, delivering chemotherapy at the wrong time in the sequence with immunotherapy could lead to toxicities. It turns out that our rheumatologic colleagues have thought about this quite a bit, particularly in the setting of IL-6, and what happens with cytokine P-450, which is an important mediator of metabolism within the liver.

They've shown that IL-6 reduces P-450 levels, where that will increase the bioavailability of drugs. So you can imagine inducing inflammation that's IL-6 dependent. And in mice we saw that Card T-cells, will that lead to increased exposure to the drugs. The concomitant drugs that the patient is receiving. And the rheumatologic community tells us that [inaudible 02:39:30] and IL-6 receptor blocking antibody actually decreases the bioavailability of Simvastatin by increasing P-450 levels. And also omeprazole. So, that also changes the dosing sequence, right, of how you would deliver drugs.

Tumors can also modulate immune biology. And that's been shown that pancreatic cancer exosomes can activate Cuyper cells. And they can activate hepatic Stellite cells, leading to deposition of fibrosis in the liver. So that the liver is not normal in our patients with cancer. Right?

And, even this paper looking at tumor-induced IL-6 in reproduct, programming host metabolism, it actually was that there was impaired ketogenic potential. So, in the setting of starvation or choleric insufficiency, these mice that are cachectic will increase their production of glucocorticoids. And that may have impact on biology.

And then finally, we have also recently presented an abstract from at this point, that's tumor-derived IL-6, can also activate hepatocytes, and they become staph-reactivated, there's contrived a recruitment of myeloid cells and fibrosis in the liver. And so I think all this is pointing to the fact that the liver is not normal. Right. At least in these mice, and we've also seen that in patients and data that I'm not showing here.

So, IL-6 can regulate drug metabolism in the liver. Tumor-derived factors can regulate liver biology. Tumor-derived IL-6 alters liver metabolism, and even tumor-derived IL-6 can alter hepatocytes. The parenchymal cells in liver, which can impact immune and fibrotic contexture in the liver.

So, I think the implications here are that changes in immune homeostasis in patients, induced by immunotherapy may significantly impact drug availability leading to drug toxicity. With drugs that are infected by IL-6, or actually sit-3A4, statins, anti-depressants, calcium channel blockers, antibiotics, warfar- it sounds like our patient population, right? Each one of these drugs, and so I think this is an important thing to consider.

Now, there are, of course, challenges with modeling immune-related adverse events, and I've alluded to some of these. There's the lack of co-morbidities, right? Non-Alcoholic fatty liver disease is pretty common. In adults, 30-40%. Obesity, at least to IL-6 levels. Heart disease, diabetes-our mice don't have any of these-right? The genetics are vastly different. The diet and microbiome changes. It varies from patient to patient. Our mice, we try to keep them the same. Medications-our mice get first in human, first in mouse treatments. First line treatments. So, they don't have all the concomitant medications, the statins, the Warfarin, etc. And of course they're not exposed to community acquired infections which could uncover pathology.

So, I want to return in the last minute or so here to this model that I put up initially, so we had option one and option number two. I would actually argue that I think that based on the most efficient path forward is to move our drugs so as we are, understanding the fact that if there is a toxicity that is seen early on, that we need to understand it. But much of this is going to emerge in the patient population. And that then needs to move back into the clinic, with the, or sorry to the mouse clinic with the appropriate models.

So, just as I said a summary, key take home point, I think pre-clinical immunocompetent mouse models can inform a mechanism of immune resistance and or immune-related adverse events, but there's no perfect model. So I don't think it's gonna be feasible to standardize models. I think they need to match the question to be addressed. I do think there's something to the sub-clinical pathology if either from genetic predisposition or environmental insults that predisposes to these events. These things can be measured in mice. You can look at hepatic enzymes, weight loss, organ pathology, serum cytokines, even if there's an absence of overt symptoms. Immunotherapy may alter the tolerability of chemotherapy. I think that's an important one to keep in mind. And changes in the liver biology. I think that's an important one to keep in mind. And changes in the liver biology that occur in the setting of tumor development, as well as immunotherapy, may impact the PK profiles for concomitant drugs, which is not something that we necessarily look at.

Most current models, though, do not incorporate sub-clinical pathology. One might argue that this might be an advantage of the spontaneous models, where you would see this type

of biology. Mechanisms regulating may be different between tumor free and tumor bearing mice. We do have some data to suggest the CD-40 chemo effect is not ameliorated by CSF-1 receptor blocking antibodies in tumor free mice.

Age is important. Mirroring toxicity may not negatively impact efficacy, so it might be a good thing. And finally, I think that pre-clinical models are not absolute predictors, that, but, if you find toxicity in the pre-clinical setting, it would justify investigation. And, that's all I had. Thank you for your attention.

- Saber: 02:45:05 Great talk. Thank you. I'll start with the first one. So our thinking at the FDA is usually it's good to do a toxicology studies in healthy animals and we like them to be free of viruses etc. and now you're making me think that that may not be always good. Maybe it's good to have dirty mice and not so clean, or maybe it's a good idea in some settings to have some exposure to previous agents to cause some injury, organ injury, but this isn't perhaps a discussion we can have also at the panel.
- Beatty: 02:45:44 Yeah. I would just say that my intent for showing that is not to complicate matters. I think that one doesn't know exactly what level of dirtiness or what dirtiness looks like, and I think standardizing that is going to be impossible, and so I don't think that that necessarily is the right path forward unless there's some biology to suggest that that might be something worthy of looking at.
- Saber: 02:46:18 How about this standard model of exposing animals or a mouse to certain agents to cause damage to certain organs?
- Beatty: 02:46:33 So I think it's difficult because then what organs do you go after? Do you go after all of them? I mean with immune-
- Saber: 02:46:43 Open question.
- Beatty: 02:46:44 Yeah, immune toxicities can happen anywhere, and they're not going to happen of all patients, and the PD-1 knockout, you might have thought that all the patients were going to get glomerulonephritis. Maybe they have some subclinical level of that and we don't actually look because we're not doing biopsies on all those patients, but it's not manifesting as something that's of concern. So I don't think that's ... I think it's a challenging question. I think there has to be some sort of driving force for wanting to do that. Maybe there's already clinical data to suggest that your new drug needs to go through that process. And an example of that may be in the 4-1BB agonist setting where hepatitis was seen. But of course, then in the clinical setting, the newer ones haven't manifested that way. So there can be just even drug biology that may determine whether you will see the pathology or not, so it's challenging. It's not straightforward.
- Saber: 02:47:49 Please introduce yourself and your affiliation.
- Chen: 02:47:52 Cindy Chen from [inaudible 02:47:53] and thank you so much. This is really interesting that you show the immune related adverse effect could be that out serve in the patients could be actually characterized in the animal models to mimic that predisposing condition in these patients. So I'm just wondering, have you actually tested several different doses? Do you expect there's a doses related or dose proportional that is related to the immune related

adverse effect? So if so how would you think that would be translate into a dose selection of these immuno-oncology agents? Thank you.

Beatty: 02:48:37 Are you specifically asking about CD40 or any of the ones that were-

Chen: 02:48:42 Maybe it's more like in general.

Beatty: 02:48:51 In general. Okay. So there's an easy way to answer that question, which is that the studies that we've done in the lab, we've looked at CD40. We have tested multiple different doses. We selected doses based on biological activity because there's not overt toxicity can be seen in the mice until you start to deliver other agents at the right time. How do you translate that into the clinic then? Not so easily done. I think you're asking the question of really, "Do you dose the biological activity or do you dose the toxicity?" Maybe that's one way of rephrasing the question. I think my preference, my opinion on this is that one doses the biological activity and not the toxicity with I-O drugs. That's of course, challenging depending on the location of where you expect your drug to be acting. I think that if it's a drug that needs to be acting within the tumor micro environment, you're going to need biopsies to be able to look at that, and that's quite challenging. So having surrogates in the blood is, of course the easiest way to do that. So I think those are some answers to the questions, but it's not an easy thing to answer. I don't know that dosing equivalents are going to necessarily be the same in mice and humans.

I think you need to explore that and find where you would see the minimal biological activity, and perhaps go one lower to start if it's a first in human drug.

Chen: 02:50:30 Thank you.

Beatty: 02:50:31 Yeah.

Saber: 02:50:31 One last question.

Steeves: 02:50:34 Hi. Meredith Steeves, Eli Lilly. I very much agree with your point that standardizing pre-clinical models is probably not going to work. My question is, is the value here more involved in understanding mechanistically what's going on in the clinic and then being able to apply that? So in the case of the patient where you had that grayed fiber ash, does that warrant saying, "Well, maybe we should use cytokine blockers in clinical cases?" Maybe that is nor regular done. I don't actually know. Looking from mechanistic insights from pre-clinical models to help inform clinical management.

Beatty: 02:51:11 Yeah. I completely agree with you. I think that's the place to ... these will be the most impactful. Yeah.

Steeves: 02:51:22 Thank you.

Beatty: 02:51:23 Thank you very much.

Saber: 02:51:31 Moving onto our next speaker, Dr. Amy LeBlanc. Amy is a board certified vet oncologist and the director at the NCI, Comparative Oncology Program. We look forward to your talk.

LeBlanc: 02:51:49 Thank you. I know I'm standing between you and lunch so we'll get on with it. So it's a pleasure to be here. I'm just make sure it's working. Yes. So the program that I run is the Comparative Oncology Program. We just for organizational orientation sit within the Center for Cancer Research, which is the intramural program of the NCI. The parent branch that we belong to is molecular imaging and that has mostly to do with my scientific background prior to coming to the NCI. But I've outlined the Division of Cancer Therapy and Diagnosis because they are one of our major collaborators within the NCI system. But what a little unusual about us is that although we are an intramural program, we have a very extensive extramural presence and I'll walk you through a little bit of that in the beginning of this talk, and then give you three specific examples of where studies that our program has initiated or are planning to initiate have specifically addressed where the tumor bearing dog can inform immune oncology drug development. So, I guess this is probably a good place to start, for those of you who are maybe not all that familiar with the concept of comparative oncology.

Basically, it's centered on the premise that the tumor bearing pet dog that lives with you in your house, and is a member of your family, and is exposed to all the things that you eat and drink and breathe develops cancer naturally. I'm sure those of you who have pets have known about this concept. This is not a foreign one to you. So these are naturally occurring tumors that grow spontaneously and they recapitulate the gamut of human tumors. There are solid tumors, hematologic malignancies, etc. leukemias, lymphomas. The dog, of course is an immune competent host and these tumors that develop in these dogs generally have, sadly for them and their owners, a very compressed disease progression and fairly short survival. Some of that may be because dogs that are presented to their veterinarians for veterinary care may be present in very late in course. So for example, in the case of a dog developing a brain tumor, there may be really subtle signs that an owner's not picking up on, and if the dog doesn't write with a pen or read a newspaper, there's not going to be a lot of early warning signs, and they may not present for veterinary care until they have a very large invasive tumor in the brain.

And so oftentimes, when we are managing veterinary patients in the veterinary clinics, a clinical setting, we are talking about survival in the range of weeks to months. These are not dogs that we typically cure. We have a good track record of managing disease and maintaining quality of life, but these are generally not patients that are cured. At least not all that often. Of course since the tumor is developing naturally in this dog, there's a nice company-evolution of the tumor and its stroma with a lot of inherent heterogeneity. And then again, in the clinical setting, a huge advantage is that veterinary oncologists, for the last 35 years have been applying chemotherapy, surgery, and radiotherapy therapeutic strategies as both as single agents and in combinations. And a lot of what we have done has been modeled very much on how human medical and radiation and surgical oncology has been introduced and practiced for all those decades as well, and so we have a lot of experience and knowledge to know what the sort of "standards" that we have in the veterinary clinic can provide tumor control and tumor maintenance and good quality of life. Despite all that, though, the dogs that we manage with cancer do develop resistance to therapy.

Their tumor metastasize, and in many, many cases, the pattern of that resistance and metastasis is very similar to what happens to human patients. And another major advantage is that there are no "standards of care." So if you, as a pet owning public have a dog with cancer and you seek veterinary care, you are not beholden to do anything really. There are

certainly lots of palliative care and end of life support strategies that veterinarians offer, but you can really do whatever you would like and whatever you feel is appropriate. So there are many situations where we have conducted clinical trials where we have had an experimental agent that has gone directly into a treatment naive dog. But we can also have heavily pre-treated patients enter these trials, and so we have the ability to really interrogate a lot of different background situations in these patients that allows us to have a lot of flexibility and insight, and not just be beholden to having a heavily pre-treated patient with a poor performance status come onto a trial.

So we maintain that the tumor bearing dog has a lot of value at multiple different stages in drug development, and that we can specifically apply the dog as a model for asking very distinct questions about PK/PD relationships, looking at efficacy signals, and defining responding histologies, and looking at opportunities to define biomarkers and deeply interrogating dose and regimen prior to or even after human trials have been initiated. So these are the people that we work with in our extramural consortium, so my job at the NCI is to link sponsors of these trials to access to the patients, these dogs that are presented to veterinary schools across the US and Canada, and in doing so we have a lot of their access to really important trial supporting mechanisms that we have, access to proteomic and genomic databases. We have a pharmacodynamics core laboratory that operates out of Colorado State University that maintains a menu of dog friendly, correlative biologic assays and other key readouts that are important for understanding and interpreting the data that's generated within dogs. And then of course always trying to make sure that we advocate for the appropriate application of a dog with cancer to drug development activities.

So I agree with everyone today that there's no perfect model for anything. But I think that our approach and our platform here is to say that we believe the dog can and has strongly complemented these activities that are carried out in mice, and in humans, and in non-human primates as well. So this is a snapshot of the various trials that have been conducted since the Comparative Oncology Program was initiated in 2003, so we're about 15 years in. I'm going to show you specific data and examples from two of these trials that have evaluated immune based therapies for cancer so the COTCO10 trial and also an open trial that was underpinned by some prior work and that's been published looking at an oncolytic viral therapy platform, and I'll also tell you about a quick study that we're planning in the next couple of years that will address a novel photoimmunotherapy approach for bladder cancer. And this slide just also is a reminder to all of you that within one dog patient, there lies many, many opportunities to collect high volume, high quality biologic specimens over a repeated sampling schedule, so tumor bearing dogs that enter onto the clinical trials that we conduct very often have repeated tumor and matched normal biopsies taken within the course of a drug exposure along with a full complement of biologic specimens that are very valuable for biomarker hypotheses and clinical laboratory monitoring.

And that's also just a reminder too that there is a huge amount of expertise looking at hematologic biochemical and immunological readouts for toxicity in the dog, so you can look very clearly at immune cell subsets in the peripheral blood of dogs, and you can look at neutrophil function, and there are multiplex cytokine kits that are validated for the dogs. You can look at cytokine release syndrome and characterize the kinetics, the scope, and the extent of that. So there's just a lot of opportunities that are available just in one particular

patient. Their physical size allows us to repeatedly sample these things over the course of a clinical trial schema. So I'm going to just give you quick three examples, specific examples and I'll start off with canine melanoma and an il-12 based immuno cytokine therapy. So just a note about dogs with melanoma, so this is again, a naturally occurring disease in the dog. In the canine patient, these tumors generally develop in the oral mucosa or around the nail beds. This is not a dermal disease. It's not UV induced. We are learning more and more every day about its biology. It appears very much that there are some shared pathway activations and loss of P16 and P53 in these tumors.

There have been a couple of really nice publications recently looking at the molecular landscape of this disease and trying to understand comparatively the genomics of the dog melanoma in light of human melanoma genomics. It is an extremely aggressive disease, so once it is noted in the mouth of this dog on the upper left there, it's very common for there to be lymph node and lung metastases, and those can also occur in other distance sites, like spleen, liver, and brain. We've known, again, in the veterinary world that this is a relatively poor responding tumor to cytotoxic chemotherapy, so I think that matches again, also the human experience. But that it's oddly and interestingly very responsive to immunotherapy. And so you can sort of see where the parallels could be very attractive for looking at melanoma in the dog as a model for immune oncology development, so I tell you that because the trial that we conducted, which was published a few years back now with nhs-il-12, the published data was very much focused on the data derived from dogs with melanoma that entered this trial. And so this was ... and again, right here you see the sort of best efficacy signal that came out of this trial.

It was a dog that had a greater than four centimeter really ugly melanoma in the maxilla that had pretty much a complete visual response to therapy, repeated administration of a fully human antibody il-12 conjugate, and this dog received repeated administrations of this agent over several months. And so I'll get into some of the details here and show you why this is probably, as we stand here today, the best example of where evaluating this drug in the dog is a really exciting opportunity to see the value of studying dogs with naturally occurring cancers for immune oncology drug development. So if you read this paper, you'll see that this is the schema, and so this was a dose escalation and a subsequent cohort expansion of this drug administered subcutaneously in tumor bearing dogs. And show you this because this table captures the richness of the samples and the observations that occurred during this 29 day study protocol, and dogs were eligible to have repeated administration of the agent if they showed any sort of recessed based response at day 29, and so that's why there were dogs in this trial that had repeated drug administration despite the fact that it's a fully human antibody and il-12 conjugate.

And so it also just goes to show you that in veterinary medicine, those of you who aren't aware, there's a significant amount of sophisticated imaging and assessment of performance status, and so it's not just, "Did they lose weight?" It's, "How are they feeling at home? Did they finish their meals? Did they require intervention in between study visits that occurred with repeated frequency?" So the owners are amazing and they bring their dogs in religiously for these study visits, and they are as ... you as pet owners would know, you know your dog better than anybody. It's like a child, so we can pick up on very subtle issues that relate to tolerability of an agent during the course of this observation. And so this study is a nice example of most of our studies that look like this where there are repeated assessments of tumor and of the patients as a whole. But then also deep investigation of

responding biomarkers, immune cell infiltrates and routine hematologic and biochemical analyses.

So from this paper, I can also show you that dogs receiving nhs-il-12 subcutaneously achieved measurable drug exposures across multiple dosing cohorts. So this study had five planned dosing cohorts, and what's interesting about this trial at a huge departure from how I think the majority, if not all human studies are designed is that this was a single patient escalation trial, so one dog was enrolled at .4 and then we immediately went to .8 and so on and so forth. And I don't have time to get into all of these details but this trial's a good example of where when we did see issues that related to fairly serious adverse events, we were able to expand cohorts and de-escalate and more deeply interrogate the dosing cohorts to identify really where the MTD would lie for this agent. And we were also able to demonstrate that dogs that receive this agent have a nice P-response that is emulated by interfering gamma and il-10 release, so this just gives you an idea of where the interfere on release occurred post drug administration across the dosing cohorts and how the release was proportional to the exposure, and similarly with il-10. So I think the key points to take away from this is that this is an example of where a fully human igG1 antibody il-12 conjugate was safely and repeatedly administered to tumor bearing dogs. We were able to define the relevant PD bio markers as induction of interfering gamma il-10, and that also the repeated tumor biopsies that were taken within the context of this trial. Also demonstrated that CD8 positive tills were all correlated nicely to exposure to this drug, particularly at the MTD, and that there was an efficacy signal identified in two of the seven dogs on this trial that had melanoma. The adverse events that we saw in this trial were obviously related to the cytokine release syndrome so, and impact on other systems, so there was self-limiting lymphopenia fever, hepatic enzymopathy that were all characterized with standard biochemical assays, and that dogs that received above 1.6 mgs per meter squared did have grade four or five events. In a couple of instances they were fatal events, so we observed disseminated intravascular coagulation linked to significant thrombocytopenia, vascular leak syndrome, etc. and that when we were able to expand a cohort at the NTD, which was .8 mig per meter squared, which translates to about 26 micrograms per kg, which was dose level two, allowed us to then confirm tolerability, PK/PD relationships and the efficacy signal that was seen in melanoma.

What's really important, though, I think, about this work is that are prioritization of this agent occurred after the canine data was received in 2010, so we did this study back in 2007, 8, and 9, so it was actually, there's a lot more that we did that I don't have time to get into today, but when EMD received this data, they were then able to internally reprioritize the advancement of this agent, and opened then an IND at the NIH in 2011, and it really garnered sufficient enthusiasm for an IND to be sponsored. So just a couple weeks ago, the phase one work that was conducted under Dr. James Gulley at the clinical center was published in Clinical Cancer Research, so seven years later and 59 patients, the MTD was defined, so remember in the dog it was about 26 micrograms per kg. The MTD in the human patients in the phase one setting was to find as about 17 micrograms per kg so pretty similar to the dog and it took them eight dose levels to get there. And the PD data that was collected in the human patients was strikingly similar to that which we had published years earlier in the dog, so interferon gamma il-10.

They of course had added findings from distinct immuno score assessment of PBMC subsets and TCR sequencing. So there was a much rich availability of regions to study the immune

response at that point in time. But the point here is that the canine data was comparable on many important levels to the human data. Same agent, similar MTD, similar AE profile. And we did this work cumulatively in the clinical trial setting for less than \$300,000 in about 18 months' time, so it's really the first example of where you can really directly compare the process of evaluating an agent in dogs and in humans in this setting, so we're really excited about that outcome. And if anyone's interested, this is the screen capture, so this paper literally just came out a couple weeks ago ... a month ago, okay. Next I want to walk you through a canine T-cell lymphoma and oncolytic virotherapy optimization project that we're involved in, so oncolytic viruses are obviously continued areas of research and a very active area of research for many. We are specifically interested in a replication competent attenuated strain of VSV Indiana that was developed primarily through the efforts of many of my friends and colleagues at the Mayo Clinic, so Steve Russel, [Coli 03:10:37] Pang, Glenn Barber, Shruthi Naik, and others.

Originally this platform was developed and is continued to be assessed in patients with multiple myeloma and this particular virus has been attenuated through the addition of two specific trans genes, so interferon beta and the sodium iodide symporter that allow this virus to have direct antitumor effect, but also through the activity of interferon beta, some protection of normal tissues and cross priming of T-cells. The NIS allows imaging of viral trafficking to occur through nuclear medicine techniques, and this platform, as well as many other VSV platforms are currently under study in human patients. This one is being assessed in intertumoral and IV dosing in patients with solid tumors, myeloma, leukemia, and lymphomas. This is just a schema of some of the pre-clinical development that underpins the use of this construct, and this shows you where the peak of the ... and this expression occurs probably within 48 to 72 hours after exposure, and so that can be captured and quantified with technetium 99 uptake in this mouse model. So prior to joining the NCI, I was at the University of Tennessee at the College of Veterinary Medicine there, and I had a joint appointment with a medical school and had access to the necessary resources to do these two studies with my Mayo Clinic collaborators.

So we initially said, "Well, we're interested in understanding whether or not you can assess this virus in dogs period, because dogs develop multiple myeloma and many other solid and hematologic malignancies that are relevant to humans." So we went through a rapid single dose escalation study in beagles and identified the systemic administration of VSV that expresses human interferon beta ... and that should say ncss, not NIH ... at a wide variety of dosing cohorts. We characterize the toxicity immune response and shedding in beagles, and that work enabled the pet dog clinical trial that the Morris Animal Foundation sponsored back in 2012. And so that work, we published in molecular cancer therapeutics and it was relevant because it now supports the work that we're doing from the NCI's perspective also with this same group of collaborators more deeply interrogating the activity and the immune response in toxicity and other factors relating to VSV application in dogs with cancer this is also what's really important about this. The clinical trial that was sponsored by Morris was that two of the dogs that were enrolled in that trial, both boxers ... this is Beasley and Roxie ... had high grade peripheral T-cell lymphoma that veterinary oncologists have many, many years noted that these are dogs that typically have a very aggressive clinical course through recent investigations into their molecular profile.

We know that these are dogs that have classically MHC class two low expression on the tumor cells that are CD-4 and CD-45 positive and both these dogs had a rapid reduction in

tumor burden within seven days of virus administration. So this is a full systemic dose of virus given to these dogs. This is 10 to the ninth TCID50 per meter squared, so a boxer is usually about 65 to 70 pounds. They're getting a significant systemic dose, and then we were able to assess not just the response to the drug, but also the immune related consequences and other toxicities that occurred. So this is the schema of how that prior work now has rolled into a full NCICOTCO24 trial where we have a schema that is geared towards not just a dose escalation of the systemically administered viral platform, but also we're deeply interrogating the idea of multiple dosing strategies, so not just one systemic dose of virus, but multiple dosing carried out within a short period of time because dogs do develop neutralizing antibodies within about 14 to 21 days, and we have the ability to, again, look at viral shedding and disease burden, and viremia, and clinical pathology within the context of a 28 day exposure, and understand really how this data can inform a parallel human trial.

So the deliverables of this, I think are pretty clear in that we have been able to demonstrate that you can in fact safely administer both systemically, and we actually had a small cohort previously of intertumoral treated dogs with this virus, and it allowed also us to explore not just the impact of the human interferon beta construct, but also a canine specific interferon beta construct, so through the miracle of viral engineering, you can actually switch out the interferon to be species specific. And then this expression facilitates molecular imaging of viral trafficking *in vivo*, which we also had some previous data that I don't have time to show you today, with how that works really well. Adverse events associated with systemic administration of this virus are what we would expect, which are hepatic DLTs that look very much like a significant yet reversible endomopathy and cytokine release syndrome. Again, these were fully characterized and attributable within the context of our clinical trial schema, and the efficacy signal that occurred within those two dogs with T-cell lymphoma spurred on now additional investigations of this agent in humans with T-cell lymphoma, and the work that we are currently doing is linked to an open IND for this agent, so it allows us to, in real time inform what happens on the human side with respect to looking at different distinct tumor types and various dosing strategies.

Next, I want to tell you quickly about a study that we're planning in the next couple of years that evaluates how the dog with invasive urothelial carcinoma can be a nice model for EGFR based photoimmunotherapy. So a colleague of mine in molecular imaging, Hisataka Kobayashi has developed near infrared photoimmunotherapy as a treatment strategy for humans, so this is where you take a specific antibody that binds a cell surface receptor like EGFR, and you link that to a photo reactive dye, and then with exposure to near infrared light, you induce cyto toxicity. They have already embarked upon a clinical trial phase one, [inaudible 03:17:35] trial with this agent in head and neck cancer patients that are EGFR over expressing, and they've had a really nice outcome, so they've had no severe adverse side effects with this agent, and they've had a really nice assessment of response. And so, the question then becomes, "what else could we think about of this agent being assessed in human patients and where can the dog inform that?"

LeBlanc: 03:18:06 When we think about the types of canine tumors that over express EGFR. The one that comes first in mind is canine bladder cancer. Really I should be calling it invasive urothelial carcinoma. The folks at Purdue University's veterinary school have done a really wonderful job of making the study of this disease and the dog their life's work. So Debbie Knapp is a veterinary oncologist there that has published extensively on this disease in the dog and its

similarities to muscle invasive urothelial carcinomas in humans. Some of her work I've captured here just to show you.

From a gene expression stand point there are similar patterns as dogs and humans cluster into both the luminal and basal subgroups. And so you cannot just look at them clinically and see how they invade the bladder and metastasize to surrounding structures. But they also have a molecular landscape that appears similar.

It turns out the dogs with naturally occurring urothelial carcinoma are ideal participants in clinical trials. It has mostly to do with the fact that veterinarians have known for a long time that there is a strong breed predisposition for this disease. So, the Scottish Terrier is unfortunately sort of the poster child. The Sheltie, the Beagle and other small terriers are known to be anywhere from ten to sixteen times more likely to get this disease than other pure bred or mixed breed dogs.

Again, sort of the same theme: dogs present with very advanced disease typically, by the time that their owners are made aware of the diagnosis. It's muscle invasive from the beginning, at least from where we capture the clinical assessment of the disease in dogs. We've also known for some time that herbicide exposure increases the risk. So, Chem lawn and other lawn treatment programs. We've seen that epidemiologically you can link exposure in the yard to the dog. And you can recover these chemicals within the urine of dogs that have bladder cancer who live in situations where their lawn is treated frequently.

What's really interesting and a recent realization is that dogs with bladder cancer, about eighty five percent of them have a BRAFF analogous mutation so it's the v595e mutation. Analogous to the v600e mutation in human melanoma. Which is interesting and no one really predicted that would happen. And also, I think important in that veterinary medicine has not really done a great job of managing this disease in the dog. Currently the medical and surgical therapies that we have available to us are pretty unrewarding and fraught with side effects that are not amenable to a good quality of life. And so typically when a dog is diagnosed their progression free interval is somewhere between a hundred and two hundred days. We're not really doing a great job and so there's a lot of interest amongst the dog owning community to look for new therapies. And this disease does spread, predictably to local regional structures as well as distant sites like lung, liver and bone.

So enter an amazing opportunity, which is the production and availability of a canine specific anti EGFR antibody which is can-225. This was developed through a group at the university of Vienna and published in 2014. And through caninization they have created this antibody and assessed its binding properties and its effect or function in cell lines. This is published literature just showing you through assessment of this antibody's interaction with EGFR over expressing an EGFR negative human and canine cell lines. This is mammary tumor cell lines that were assessed in this paper. That this agent does reduce viability and proliferation and also induces ADCP. Not ADCC but definitely does have a phagocytic function as far as tumor cell killing goes.

We reached out to this group and said we're interested in trying to connect photo immunotherapy in the EFGR over expressing human patient to your antibody and trying to figure out if we can create an analogous canine product. So taking the CAN-225 antibody, linking it to the photoactive near infrared dye. And then trying to understand if we can

effectively translate this new agent through a bladder cancer trial in dogs to identify a new place to apply this technology to a human patient population that has significant underserved needs.

I don't get into the underserved needs of human bladder cancer patients, but they are significant. Just as they are in dogs. And so we recently published a paper where we took canine bladder cancer cell lines that are known to have a dynamic range of EGFR expression and basically were able to show that in a xenograft setting you can apply photo immunotherapy to xenografted mice bearing canine TCC lesions. And you can induce tumor cell kill and improve survival.

Next what we want to do is assess, through a proof of concept study in a relatively small number of dogs, we want to look at single dose safety tolerability and efficacy. We want to recruit dogs with bladder cancer and conduct a trial that has serial cystoscopy biopsy and histopathology of the tumor and surround "normal bladder". And really look at not just single dose EGFRIT. Dogs would receive a systemic dose of antibody and cystoscopy guided laser directed therapy of the tumor would be conducted and we can evaluate also expanded cohorts once we define an MTD or really what I should say is a biologically effective dose. And then also consider combinations of this agent with other immune active agents in the dog. We're excited about the future of this in the coming months, years. Last, in just the handful of minutes I have left, I want to run through the recent knowledge that's been garnered about checkpoint molecules and correlative assays in the canine space. So this is just sort of a laundry list of what we know about canine immune checkpoints and checkpoint inhibitors.

Speaking specifically about PDL and PDL-1 we know those genes are conserved 100% amongst dog breeds. Because that's one of the major concerns that people raise when we get into comparative oncology and looking at companion dogs is that they're not just Beagles that are two years old that have been in BSL2 housing their whole lives. These are a wide variety of mixed breed out bred populations as well as pure bred dogs. So, how do you capture the key information out of a really diverse population? Luckily, at least in this particular aspect of checkpoint inhibitors they are conserved. Recombinant canine PD-1 and PDL-1 proteins have been constructed.

We know they bind to one another and that an anti PD-1 antibody blocks soluble PD-1 binding. With canine PDL-1 in a dose dependent manner and through a variety of other experiments that involve fresh canine tumor biopsy explant cultures and access to canine PBMCs and these anti PD-1 reagents. We can look at changes that interfere in gamma production and we can then roll into looking at collections of naturally occurring canine tumors that have been collected from patients and placed in bio-specimen repositories to ask questions about density of T-cell infiltration and what the landscape of those cells looks like and so on and so on.

And actually, there's also a clinical trial that has been studying the effective and anti PD-1 antibody, a canine specific anti PD-1 antibody, through Mark Animal Health. We should actually be seeing the results of that later on this year.

I'll just show you a couple of snapshots of papers that you can pull out and read for more detail. So this one was published a couple years ago looking at just IHC assessment of PDL-1

expression in canine cancers and then they specifically drilled down again in melanoma to look at TILs expressing PD-1. Just a couple of figures from there.

To give you an idea of the landscape of the type of tumors that were assessed. Melanomas were most common, but you get an assessment of what we commonly see in the veterinary cancer clinic. So melanomas were by far the most proportionally positive. PD-1 expression was seen on TILs from dogs with melanoma. And this paper looked at immune regulation of canine tumor and canine microphage PDL-1 expression. So all fourteen canine cancer cell lines in this paper had constitutive PDL-1 expression. And that you could up-regulate that after exposure to interfering gamma and to light receptor 3 stimulation. And then checkpoint molecule in B and T cell lymphomas was also assessed here. B cell malignancies tended to have a higher PDL-1 expression than normal B cells. And that both normal and malignant T cells were somewhat lower.

TILs that were identified in both the Bad T cell lymphoma patients had increased expression of PD-1 and PDL-1 when you compared those cells to B and T cells that were obtained from healthy animals.

And then finally a Japanese group recently did a study with a canine specific PDL-1 monoclonal antibody. And through repeated dosing actually identified an efficacy signal in two dogs. One had melanoma and the other had a poorly differentiated oral sarcoma, shown here.

Last couple minutes I'll just show you a few of the NCI sponsored activities that give you an idea of what the NCI is doing to help support the research and expand the knowledge in the comparative immun oncology space. So these are all extramural DCTD sponsored initiatives that take the form of granting opportunities. So there were a series of P30 cancer center supplements awarded in 2016 to look at mutational load and characterize new antigens across a spectrum of canine cancers. These are the folks who won those awards and the types of tumors that they're assessing. I'm happy to point you toward Connie Summers who's in the audience if you want more details about these awards.

And then also, there was a cooperative U series grant opportunity that was issued in 2017 to really ask some deep questions about the various opportunities in canine immune-oncology clinical trials that were being conducted out in the veterinary community. Assisted by a U24 coordinating center which is at the University of Pennsylvania. And so those awards were given out to these individuals. And again you can see the types of tumors that they're studying and the approaches that they're taking which are really diverse and exciting. So everything from onco lithic viro-therapy to an IBO inhibitor to a CB200 project, etc... So, I think really exciting broad swathe of opportunities there. We're excited about seeing the data that come out of these trials in the coming years.

My program assist along with Connie and Toby from DCTD. The Penn coordinating center is at the center of this as well.

The last exciting part is that a canine genomic data commons is coming. Through the initiatives sponsored through the DCTD we are working very diligently on trying to create and stand up a canine genomics data commons that would be patterned off the human GBC. The idea here is that it would be completely comparative from the beginning. So, if you

were interested, in say, canine melanoma and its similarities and its differences to human melanoma or any other human tumor you would have a platform to assess the available data. In a single portal that would support those types of questions. And what lies ahead for us. There's a lot of reasons to be excited about what dogs that live with us in our households can offer and the complimentary opportunities and value they add. I think its really important for us to focus on the scientific dialogue and make sure that everything we do is geared towards the absolute highest standards for clinical trial design, execution, and reporting. And there needs to be a continued effort towards developing canine specific reagents and correlative assays to support these trial efforts and really enhance human translation.

Thanks to my group and many, many people within the NCI, the NIH and beyond that have helped make all this happen. And I'm happy to take any questions. I'll try, at least.

- Saber: 03:31:49 Thank you, please introduce yourself.
- Haggerty: 03:31:51 Hi Amy, Helen Haggerty, Bristol-Myers Squibb. Really nice presentation. And I know you and I talked about this before, but one of the issues in working in the canine model is the limitations in the reagents that are available to really interrogate the immune system. And I know you mentioned that there are a number of people who have been working on and there are more and more reagents that are becoming available all the time. So, it'd be really great if they could become commercially available and if there was some kind of a database as you talked about with the genomics in which we could go to, to find these reagents.
- LeBlanc: 03:32:19 The NCI, I think has acknowledged that and is putting efforts towards developing not just reagents and molecules and antibodies and things like that, that could be potentially transferred out to the community. But, equally important are the correlative assays. So when you're looking through a trial it's not sufficient at all just to look for efficacy. There needs to be a really deep mechanistic understanding of the good, the bad and the ugly.
- Speaker 2: 03:32:50 Thank you, my name is [inaudible 03:32:53] I am from ABDIA office of [inaudible 03:32:56] and the talk is very impressive and my small questions is, [inaudible 03:33:02] curiosity. Like, before you go for clinical studies, do you think you have any need to go for humanized animal studies after these outcomes. Do you anticipate any humanized animal model studies in addition to what you have conducted so far before going to the clinical studies. That's my question
- LeBlanc: 03:33:24 Looking at the opportunities or the need for humanized models to compliment what we do? Is that your question?
- Speaker 2: 03:33:32 Yes, in order to assure additional safety and efficacy, do you anticipate any humanized animal studies? That's my question.
- LeBlanc: 03:33:40 I think if there were specific questions that we could not answer in a dog. For which we didn't have a reasonable way of looking at, particularly, like toxicity. If there was a target, or for whatever reason there was a syndrome or a protein that we couldn't effectively assay in the dog, that was critically important to the forward movement in humans. Then, of course. I think that the main take home here is that this is a complimentary model. This is not a situation where we at all believe that the tumor bearing dog is going to replace any

significant portion of all the modeling that needs to be done. Because this is an incredibly complex field. I think we all acknowledge that.

I think that the opportunity, particularly when I showed you the NHSIL-12 is more to say that you can distinctly ask some really excited questions in the dog and cumulatively take the package forward to humans. Perhaps a little quicker, or at least with better knowledge of what to anticipate in a human. And there may be the opportunity to let the dog inform the starting dose. We reached the MTD in the dog really, pretty quickly. And if there is an opportunity for us to help that data informs the first in human dose. That would be pretty exciting and would maybe save a lot of time and money and patient accrual. Maybe it wouldn't take 59 patients to reach and MTD. Maybe it could reduce that.

That's really my hope is that we become part of the puzzle. And maybe simplify it if we can.

Speaker 2: 03:35:14 Thank you.

Abbey: 03:35:17 Hi, Sirica Kella Abbey. Related to this previous question and what you just commented on: Are you suggesting that, and especially with your first example with IL2 combo. Would you expect these, in a situation where the molecule cross-reacts in a canine species, that this could actually replace the pre-clinical, traditional, GLP non-human primate study.

LeBlanc: 03:35:41 Ooh, that's a dangerous one.

Yeah, I don't know that I am comfortable with the word replace. I think that maybe a better word would be like "refine". Maybe help, in certain situations, maybe not use as many animals. Or at least help guide the structure of those studies. Because, I don't think in the current landscape we have... this is one example. I don't have 50 examples, I have one really good example that literally took eight years for us to see the full trajectory of where the dog data matched the human phase one data.

I think as we learn more, it would be my hope that we could help refine and speed up some of the preclinical work that precedes a first in human trial. But, I think it's really premature for us to use the word replace.

Speaker 3: 03:36:33 You mentioned the example with PD-1 inhibitors. Do you have also examples of other checkpoint inhibitors or stimulators. CD4[crosstalk 03:36:42]

LeBlanc: 03:36:41 Not yet. Those things are coming, though. There was already a nod to the PEN trial of the CAR-T cell project in dogs with lymphoma. That's just one example of where I think the literature will really expand in the coming years. And that's really a credit to the NCI funding these types of activities through the cooperative use series grant.

Speaker 3: 03:37:02 It would be nice to know in the future if they predict toxicities into humans, because right now this is what is lacking. Animals do not predict.

Leighton: 03:37:13 Hi John Leighton at the FDA. Dr. Beatty spoke about the aged immune system. And I was just wondering whether or not these models. Presumably these dogs are older when they get

cancer. I wonder whether or not these models can be used to interrogate the difference in the response of an older immune system.

LeBlanc: 03:37:35 There's actually a decent amount of information about the canine immune system and how it changes through age. And how you can characterize immune cell subsets, presence, function and activity across neutrophils and all the various other important immune cell subtypes. I think that it's critically important. The average patient that comes onto our trials is 10. A 10 year old dog is a pretty decent correlate to a 70 year old human. And they have experienced life and they probably don't have normal immune function anymore.

We actually know that age impacts neutrophils function. We've known that for 10 years. And we also know that their bone marrow ages and doesn't have the full complement of expanded marrow compartment anymore. Really as they approach age seven, the marrow compartment starts to wane. And they probably don't have fully normal kidney and liver function based on routine hematologic and biochemical testing. So there's a lot of opportunity I think to get into what pre-dates, and what sort of baseline assessment you can get off of a tumor bearing pet dog. And how that could be relevant to any other pre-existing [inaudible 03:38:58] that occur in human patients that might be relevant for toxicity. Or at least prediction of whether that would be an issue or not.

Saber: 03:39:06 Because you will not be here for the panel, I want to ask you one more question. I'm guessing that after today you will have many more clients coming to you. And I wonder if there are enough dog patients to accommodate all these drugs coming on board.

LeBlanc: 03:39:22 So that's why we have 24 sites in the US and Canada. And we maintain that clinical trial consortium for that very reason. We need to have a multicenter presence to have access to sufficient patients for entry into these trials.

I also want to emphasize that not every trial. They're all different, because they're asking very distinct biologic questions. Some of these trials have 12, 15, 18 dogs. We have open osteosarcoma studies that have 300 dogs in them. It really, I think, is dependent on what the sponsor wants. What specific questions we need to ask and answer. Whether or not they're feasible to be asked and answered in pet dogs. And whether or not we have access to significant tumor varying patients through our network. Typically, that's where the conversation starts.

Any of you who are interested in following up with me, happy to do that. I have to run, but I'm easy to find because I'm a government employee.

Saber: 03:40:22 One last question.

Peter: 03:40:24 Peter [inaudible 03:40:25] I have a question in the wide revealed cases for a particular period for malignant melanoma in humans. One of the side effects was [inaudible 03:40:39] and the issue was it due to the medication or due to environmental factors. From your own experience, since you mentioned you have similarities between the disease in dogs as well as in humans. Do you [inaudible 03:40:54] my analysis?

LeBlanc: 03:40:56 Maybe this will help answer. When we have adverse events that occur during our clinical trials, we have a system for attribution and grading of those adverse events. Typically, what

we try very diligently to do, is talk with the investigator at the site who is appreciating these clinical trials, and hear from the pet owner what happened and get distinct details. So we can try to attribute it to disease or to the research itself. So, if it's an infected incision, was that from the fact the we took a biopsy there? Or is it from the agent? And what we know about the agent from other animal model studies that we have access to prior to initiating a canine trial.

We're not always able to 100% fully attribute these things but we really do our best to categorize much like any other trial would: probable, likely, definite, unrelated, those types of categories.

Does that answer your question?

Peter: 03:41:52 It does.

LeBlanc: 03:41:52 That's a tough one.

Hart: 03:41:56 Hi Amy, Tim Hart for GSK. One question.

As you start thinking about going into these trials, what are some of the expectations or requirements for safety testing prior to going into the canine trials. So you can either accelerate selecting a starting dose for the canine trials versus how quickly you can escalate.

LeBlanc: 03:42:17 Sure, I think the oncolytic viro therapy story I told you is a good example of where we went into tumor bearing pet dogs with a Morris- Hamill Foundation funded trial off data that was collected from 9 Beagle dogs. And that was enough.

We did single dose escalation. We knew we were working with a virus that... We had abundant mouse data. But, the shortcoming of the mouse data was that the folks at that time didn't really have a good feeling of why some of the adverse events were occurring in the mice and whether or not it was related to tumor lysis syndrome. Or whether it was related to the actions of the virus. We initiated a pet dog trial with a very small amount of Beagle data. And I would say that it's really important for us to do the minimal amount. Mostly because I don't want to go through lots of Beagles. Because, I'm a veterinarian and we're big on not wasting that life.

Hart: 03:43:17 Were those non- Beagles a terminal study? Or were they just non-terminal evaluations.

LeBlanc: 03:43:23 That particular study about half of them were terminal because we needed to understand the disposition of the virus in target organs. So, we needed to get the kidney out and say: Is there still live virus in this kidney? Is there live virus recovered from the spleen, or the liver, or the testes, or the brain? We needed to understand that. But we only had to sacrifice a handful. And the rest of them were treated and observed and held in isolation and then they were adopted out.

There was a mechanism for them staying in close contact with the hospital, as far as being able to monitor them. But, they were maintained within a research pool or they went home as a pet. I think the message here is that I don't think that we need a full GLP or GCP

compliant in 36 Beagles to a pet dog study. In fact I'd argue against that. We just need enough to get an idea of what we need to expect as far as toxicities. Because, these are peoples pets. We owe them some information so they can have an informed decision on whether to enter the trial or not.

- Hart: 03:44:35 And one follow-up on that. The dose scaling factor was mgs per meter squared for that one compound. Is that your standard scaling factor you use?
- LeBlanc: 03:44:47 No. That's obviously very much guided by the toxicology and pharmacology groups that come from the sponsors that we work with. Usually have some idea of how we can allometrically scale and the appropriate way to do that depending on the kinetics of the agent and the adme features properties and all that. Typically actuals it's a mg per kg, not a mg per meter squared basis. That just happened to be how we did that one particular agent because of other issues.
- Hart: 03:45:18 Thank you.
- Saber: 03:45:20 Thank you very much.

So, we had a very good productive morning. Thank you to all the speakers. Thank you Amy. And, we will be back here at one o'clock sharp. Lunch is on your own, except for the speakers and panelists there will be lunch provided by AACR.

LUNCH BREAK

- Saber: 00:01:00 It's one o'clock, and we hope to continue with the discussions and the presentations. Now, before we start the next presentations, I would like to ask Dr. Pazdur to come and give few words of wisdom. Of course, you all know Dr. Pazdur. He is the director in OCE, Oncology Center of Excellence, and OHOP, Office of Hematology-Oncology Products. Please.
- Pazdur: 00:01:33 Thank you, Haleh. I'm going to entitle this 'Think Out of the Box,' because that's what we have to do, both in the clinical development of these immunological agents and also the pre-clinical evaluation of the agents.

Far too often we have attempted to take what we do with cytotoxic drugs and put them into a new class of drugs or new class of therapies, and many times that might not be appropriate. I think that this is probably one of the reasons why we're having this or one of the major reasons, is really to take a step backward in the pre-clinical evaluation of these agents and say, "What makes sense? What doesn't make sense? And let's think about bigger picture issues."

This is not only an area for a pre-clinical development, but also, I think in the clinical development of these agents, whether they be a specific checkpoint inhibitor PD-1 therapy, CAR T-cell, whatever. They represent a new class of therapies, and we have to re-evaluate what we do both in the pre-clinical and clinical evaluation of the drugs.

For example, with the PD-1 drugs from a clinical perspective I think we have to analyze our dosing of these drugs. And as you can see, many times there are now supplements coming in, looking at different dosing strategies of the drugs.

Similarly, how we develop these drugs from a clinical perspective in various diseases, does it make sense to follow the kind of classical paradigm that we use to develop cytotoxic drugs of looking at first line therapy, second line therapies in specific diseases. Because these therapies, the PD-1 drugs and checkpoint inhibitors or checkpoint inhibitors, operate under a different mechanism. As you saw we had one example earlier this year, this past year, doing an approval on tissue agnostic or site agnostic diseases.

So, I think there are many questions that we have to step back and take a look at that's simply applying lessons learned from cytotoxic drugs may or may not be appropriate to newer classes of therapy. These include dosing, types of tumors, how we develop these drugs in various tumor types, and more importantly, how we develop these drugs in combinations.

If one takes a look at our classical combination therapies and cytotoxic therapies, we basically took any two drugs that had activity and non-overlapping toxicity and lumped them together basically, and we developed sometimes successfully sometimes curative regimens such as ABVD, MOPP chemotherapy, testicular cancer chemotherapies.

However, with the immunological drugs, it may present a more problematic approach. Just lumping drugs together may not be the appropriate thing to do, and that was seen, I think in some of our earlier experiences that we saw with the combination therapies in multiple myeloma, which made a step back and take a look at what we were doing here. But, I think that is the sobering note that we need to take a look at what makes sense as far as sequencing these drugs.

So, the challenge that I have for you today is really to think out of the box. The other thing that I wanna emphasize and I don't think the FDA frequently emphasizes this enough is that we are in a very special time when it comes to developing these agents. I have been in oncology for the past 40 years, and I have never seen a time of more active investigation in the field. Over 40% of all former related activity now is in the area of oncology.

If one takes a look at PD-1 therapies, as you all know there are many, many PD-1 therapies being developed. And, how we develop all these agents also have to be looked at. But, we are in a very interesting time which makes us need to step back and take a look at what we're doing.

I think many times the American public thinks that drug development stops when the drug is approved. That is not the situation here. When the drug is approved especially with these PD-1 therapies or checkpoint inhibitors, we're really beginning a journey here with these drugs of these therapies. And, we are going to have to have modifications of patient populations that are treated, perhaps they're going to be more limited based on biomarkers. We're probably gonna have to take a look at different dosing strategies. We're gonna have to take a look at different combinations that may be different from as I mentioned before with cytotoxic therapies.

So, drug approval is not the end of the story with these drugs, and really just the beginning. The American public and investigators and the entire oncology community has to realize that we are in this dynamic situation here where there are going to be changes to indications to select more patients, more appropriate patient populations to take a look at different dosing schedules, et cetera. So, this degree of regulatory flexibility is going to have to be recognized by the treating community and our patients. And, I think that this is appropriate to have that discussion with all of our stakeholders here.

We could take a very draconian approach to drug development and say, "We're not approving anything until we have the complete story." However, that would be at the detriment of patients who are obviously facing desperately desperate situations in fighting for their lives in many cases, and need these drugs. And we can't wait until we have the complete story here.

So, I think the emphasis that I'd like to leave you with is think out of the box regarding these drugs how we evaluate them from a non-clinical perspective. That we as clinicians also have to take a look at how we develop these drugs, and we can't just repeat the past history with these drugs.

And, as far as drug regulators, we have to think of novel approaches to getting these drugs to people who need them the most, yet be cognizant of the fact that the whole story may not be there, and we will have to do modifications of product labels, indications and other regulatory avenues that we have, to make these drugs available to the American public. I hope I've summarized my thoughts on these drugs. I am not a clinical pharmacologist or a toxicologist, so I wish you well in looking at these different models. But, I think here again, it is a situation where we have to really think of what is outside of the box, and thinking outside of the box with novel approaches, rather than just trying to duplicate past history that we've had with cytotoxic therapies. Thank you very much.

Saber: 00:08:34 Excellent. Thank you, Dr. Pazdur. And, our next speaker is Dr. Lei Zheng, and Dr. Zheng is a physician scientist of the Department of Oncology and Surgery at Johns Hopkins School of Medicine, and the Sidney Kimmel Cancer Center. He's co-director of the Precision Medicine Center of Excellence for Pancreatic Cancer, please.

Zheng: 00:08:57 Thank you. Thank you for having me here. And, now I know why having me to give this talk, because I'm really not in the box, ever, so I can really think out of the box.

Okay. All right. So, this is a table I steal from Dr. Haggerty who is also a speaker later in the afternoon, and her review is really nicely summarized the FDA approved immuno-oncology agents, and also divide them into a few categories.

So, I use this table as a template, and I also look at each different category of the hem-oncology agent that has already been approved by the FDA, and look at what is the non-clinical animal models I use for a safe assessment. And you can see it's quite limited resource of non-clinical animal models for a safety assess.

On the other hand, even in the old days, we already have quite a variety of pre-clinical model for access to the anti-tumor efficacy. So today, I'm just going to focus on the immuno-oncology agents that CDER is responsible for.

What are the challenges and the lessons we can learn today? So the immune modulating antibodies still is the largest category of the immuno-oncology agents that are currently in development. So, when we talk about the issue we always say, "Species relevance has been an issue." However, affinity of binding to human and a monkey PD-1 has been similar for most products. And the EC50 for blocking interaction between the PD-1 receptor and its ligand is also similar between species.

However, at exposure well above those seen clinically, there was no clear auto-immunity, and Dr. Beatty has given example of CTL4, and in the non-human primate, single agent there's no toxicity. However, auto-immune toxicity were observed in a combination of anti-PD1 and anti-CTL4 antibodies. So BMS has published a very nice study and showed there's auto-immune toxicity observed with a combination of nivolumab and ipilimumab. However, not many of us were for this type of study. But the take home message is, you know, that tells us, for immuno-oncology agents, one plus one is not equal to two. This is exactly what we see in the immuno-oncology combination in a clinic.

Another challenge is the antagonists. We still have difficulty in developing the antagonist antibodies. So clearly, an antagonist immune stimulation is considered high risk, compared to the immunomodulation where antagonists are binding to target receptor. However, the thinking behind this may be too simplified. So, we think because antagonists typically exhibit a linear dose response. For example, anti-CTL4 antibody and anti PD-1 antibody. On the other hand, agonists often are associated with a bell-shaped dose response. Not necessarily always true, but that tells us it's very difficult to predict what will be the response with the next dose level. That is the challenge with the agonist antibody.

So here is some example. So, this is TLR7 agonist from AstraZeneca. The study show nicely this is a bell curve ... Curve response. Look at another example, the anti CD137 agonist from Pfizer. For the induction of the NF Kappa B reporter, it shows a linear dose response, while look at the enhancement of the IL-2 response in a setting of combination with the anti-CD3, it's a bell shape.

Nevertheless, at least anti-CD137 agonist antibody from BMS, the liver toxicity, you really cannot predict by any of the cytokine release assays. While if you look back, we can see that in a mouse model, and there's a marked mononuclear inflammation and infiltration of CD8 T cells. Here, histology shows that CD3 T-cell immunohistochemistry. Obviously, that also tells us pretty challenged to do the immunohistochemistry of the lymphocyte on a mouse tissue. At that time, there's really no available staining method for CD8.

So, *in vitro* dose response is often different from *in vivo* dose response. So, for example the *in vitro* study I just show you, the anti CD137 agonist antibody demonstrate a bell-shaped IL-2 response curve. However, this still could be an artifact that *in vitro* culture system because this was never repeated and noted *in vivo*, in a humanized xenograft mouse model, or even in the non-human primate. So, neither *in vitro* nor *in vivo* cytokine release assay is predictive.

So, a native intra-cytokine release assay may not be reliable use to make assumption about the risk for patients. Dr. Leighton this morning has given the example of a TGN. So, I never actually really dig into this case until a few days ago, when he mentioned to me, and I always think I should study design because I feel it's wrong to enroll a six patients at the

same time, with the same dose level. But in reality is ... Everything is because of the preclinical study and the non-clinical analyses actually give the clinician a wrong assumption.

So, the *in vitro* system and using soluble antibody, there is no cytokine release observed. However later it finds it's not true. It really depends on your assay system. The same assay system, same PBMC, or using the diluted whole blood. But if antibodies are immobilized in one of the three ways I show here, and you see the cytokine release. And there's also low level, the cytokine release was observed in the primate study. But due to the assumption, the drug is safe based on this non-clinical assay. I wouldn't say the first-in-human dose was calculated improperly because there's really no base to calculate the human dose. As a matter of fact, the first human dose used is about 500 lower than the predicted clinically dose. But still actually caused the catastrophic toxicity. And then because of this assumption, no proper interval was left between dosing the first and next patient, and neither were investigator prepared for managing the cytokine release, cytokine storm.

So, we need a special recognition of the immuno target agent. Indeed, FDA actually recognize this for many years. And it was suggested that using the MABEL to help to predict and select the first-in-human dose. However, MABEL is relied heavily on the pharmacology studies. It's also challenged to translate the *in vitro* data into *in vivo* with our immuno oncology products. I'm not saying actual will be more challenging for the I- O combination. So, this is a helpful diagram. So, here's the MABEL dose. It's possible in that TGN cases, which maybe very rare, even if start with the MABEL dose, you already see that ends up the toxicity.

However, in many other cases, if you start with the dose select by MABEL, maybe take many, many patient for a dose escalation before you even reach the therapeutic dose. So, we have to think about it.

The next is I want to say, what can we learn from the T-cell therapy. There's already a lot of the example from the T-cell therapy we can learn from, and for our antibody development, particular those bi-specific antibody, and also for example the TCR mimic antibodies. Both either engage T-cell, or mimic T-cell. So not only for the cytokine release syndrome, but also the toxicity often come from our own target off-tumor toxicity. But if you look at the example I highlight here, the on-target and off-target tumor toxicity often because we're not pay attention to the low level of the expression of the target on the tissue outside of tumor organs. So, I just think back my own study, for how many times we have test our target are interested in all different kinds of tissue? Probably only show a nice figure to compare the tumor versus para-tumor tissue in the same organ. So that wouldn't be sufficient for develop these therapies.

There's also example for the TCRT therapy. Very similar. And even for the cancer to [inaudible 00:19:42], we have to think about the active T-cell still can cross the blood brain barrier. So, then you wouldn't be surprised to see the on-target and off-tumor toxicity in the brain. And also none of the tumor-associated antigen is truly specific in the tumor. And you wouldn't be surprised to see the toxicity due to the low expression level of the cancer-associated antigen in normal tissues. And also there's an off-target, off-tumor toxicities. I have recently done study to elude the peptide from MHC molecule in my laboratory, and I was very surprised to see how many of these peptides could be potentially cross-reacted.

So what factor have impact on the toxicity from the cytokine release? On-target off-tumor toxicity as I just show? Off-target toxicity, but don't forget, there's also what I called on-tumor, on-target toxicity, because it depends on the tumor burden. And that's exactly what we often see in the heme malignancy, because of the high disease burden. It's also associated with the high toxicity because there's the high burden of the targets.

So, this is a challenge, but what's the opportunity? I try to think out-of-the box. We have now not just a few animal models. We have a variety of mouse model, either tumor-free, or tumor-bearing animal model. The previous speakers have already nicely summarized these models. I think we should take advantage of that. And we should add a safety assessment in the tumor-bearing preclinical model, and I'll tell you a few of my points to justify that. That probably will add more safety assessment in addition to the traditional non-clinical animal models.

So, what we can do? First, low level cytokine release in non-clinical animal model, either observe in the non-human primate, or observe just when we're doing an anti-tumor efficacy study, that should prompt more caution. However, traditionally we only test a few cytokine. Now we have more technology to test an array of cytokine. And you can see for different pathology response, there may involve a different cytokine. I really think we should think about not just test a few cytokine, but also test array of cytokine.

Second, the mild symptoms or non-specific symptoms such as weight loss should prompt pathological examination of toxicity, including auto-immune toxicity. A mouse wouldn't speak. However, the weight loss is still very objective. The challenge part is, as you're also seeing at the previous speaker, and in showing that is many study, there's no weight loss, because the experimental course is so short. Usually, particularly in those tumor implant model, the treatment course is only one month. So we should think about using the tumor model, where the kinetic of tumor regression is slower, or spontaneous tumor model to offer long term administration of the immuno modulating agent.

The second is, we should increase the experiment sampling to detect the presence of biochemical or immunity. And offer just one test for those biochemistry for liver damage is probably not be sufficient. And also should have a more immunohistochemistry study on the tissue level. I'm going to talk about later. And also think about at risk, not just checking the traditional cytokine profile.

And again, assess combination immunomodulation, there're strains of the mice that are more susceptible to auto-immunity. In the same token, like Dr. Beatty has shown, we'll probably have to think about if combine with some treatment that may cause the liver injury. I just heard from Chuck Drake, he had an education talk last night, and told about the first case of the auto-immune hepatitis. They recognize after [inaudible 00:24:49]. Again, not just the injury caused by a few beer. What about actually chemotherapy drug that patient has previous exposed. That's also actually should prompt us to think about it.

And the last we should assess auto-immune adverse reaction by histological examination because mouse wouldn't be able to speak, but the pathology, they can tell us. Humanized mouse model provides an opportunity to assess treatment related toxicity in tumor-free and also in tumor-bearing mice. Particular these tumor-bearing mice give you an opportunity to resemble the patient situation and their clinical relevant condition.

I wouldn't want to repeat about the humanized mouse model because this morning we already have the excellent talk. But I want to point out different type of humanized model probably, for different purpose of assessing the toxicity. I wouldn't think the PBMC reconstituted model would be sufficient to test auto-immune toxicity, while those stem cell reconstituted model probably would be a better option for testing the auto-immune toxicity.

I steal this table from a talk, actually also at FDA here, a workshop two years ago from Christina Howard's talk. This is what John mentioned this morning. FDA also conducted study with a humanized mouse to look at auto-immune toxicity. This study's actually very nicely show, even a mouse wouldn't speak, you're able to, by examining the tissue and look at the worse pathology, particularly those sub-clinical change, and you can see really the high rate of adverse reaction at the histological level, can observe in this mice in the pilot experiment.

But this is associated with another challenge, because we have much fewer mouse pathologists than the human pathologists. It's still quite challenged to recognize the auto-immune toxicity at the histology level for human patient. So, the toxicity should be studies in both wild type and also in a tumor-bearing mice.

I want to show an example. It's a lesson we can learn from the oncolytic virus, the T-vac. I feel this is very nice in complying to many other immuno-oncological agents. So, for T-vac assessment, in every step, not only the naïve tumor-free tumor is assessed, but also the tumor-bearing mice is assessed. Obviously, the presence of tumor would affect the *in vivo* bio-distribution, and different route of treatments also will make a difference in terms of the toxicology and also about the pharmacokinetics. I'll argue this will be applied to cytokine and many of the immune agonist ligands safety assessment.

So, I want to give a few story, not try to show anything from my lab. I was surprised actually Greg didn't show this. This is a transgenic mice. My lab and many other labs working on the pancreatic cancer use. It's a very challenge model. It's very hard to breed, and the tumor actually spontaneously develop, can be anywhere between four months to actually as late as eight months. Because it's very challenge, it really takes a lot of effort to complete one study with anti-tumor efficacy study. But at least, I never thought about at the same time to assess the toxicity of the drugs. Probably this model provides me the best opportunity to study that, since I already spent such a big effort.

Another model actually we should think about is orthotopic model. This is a pancreatic orthotopic model. Many of us can do it in academic lab, but it will be very challenge to have it done in a non-academic lab. Pancreatic orthotopic will be something relatively easy from technical perspective, but to implant the tumor to other organs will be quite challenging, particular for a non-academic laboratory. However, the same problem is this model maybe a good model to assess the efficacy, may not be a very good model to assess the toxicity. But we probably also should think about it. When we look at this model, and we probably also should think about other organs in these models.

So finally, this is another animal model that often use my laboratory, a liver metastasis model. By inject the tumor through the spleen and let them form the liver metastasis spontaneously. And now I start to learn something. In our current project, to study into

tumor injection of the immune agonists ended up some guidance. We try to mimic the clinical situation, and also not only look at anti-tumor efficacy or [inaudible 00:30:52] effect, but also toxicity assessment in comparison with other route of drug administration. So, the point I want to argue is, there's still more animal model there allow us to think out of the box. We really should start to think about anti-tumor efficacy, at the same times about safety assessment. So, the last point is, we should evaluate the biology and expression of the target in the intended clinical population and the models. And to look vigorously at the off-tumor target expression and off-target in the preclinical model.

So, the last but not least point is ... The bottom line is we need to determine the first in human dose. So there is a different way to determine a first in human dose. For example, dose response in the cytokine release. However, what challenge is that the receptors of immuno agonists often only transient express on activated T-cell. So cytokine release for agonists may never be predictive. *In vivo* toxicology study, often healthy, non-human primates have negligible activated T-cell due to lack of the relevant antigen. So it's different from the human being because we have already exposed to lots of antigens, have a lot of activated T-cell, lot of memory T-cell in our bodies Receptor occupancy is one way to determine a first in human dose. But relationship between the periphery and the tissue receptor occupancy still not well established. Not same between the receptor occupancy and efficacy and toxicity. And also John mentioned this morning, probably not just the receptor occupancy, but also the turnaround time, is also important. Because the receptor distribute between tumor and non-tumor tissue, so it's also important to establish the relation between the tumor-free model and the tumor-bearing models.

So finally, it's also possible use the minimal pharmacology active dose to determine a first in human dose. But that will be sometimes quite dangerous. First is the difference in the minimal pharmacology active dose between the mouse model and a human patient. If something actually just like the TGN, anti-CD28 antibody, you probably even with the very low dose, and you see in the mouse model, probably will cause unaccepted toxicity. But in often time, the dose that is active in the mice are usually project to a very low first in human dose level, far from the effective dose in a human patient...

Zheng: 00:34:00 So in summary, spatial relevance is still an issue, but not the main issue. At least, not a whole issue. And this is the issue we can never resolve, so I would argue this is not a issue. Neither *in vitro* nor *in vivo* cytokine release actually is predictive. However, low level the cytokine release in non-clinical animal model should prompt more caution.

Increase experimental sampling to detect for the presence the biochemical autoimmunity. Think about it. Take advantage of the transgenic model, which is live longer, and allowing for the multiple dose of treatment. And also assess the more objective factor. For example, weight loss. Or assess autoimmune adverse reaction for [histologic 00:34:57] examination. And what we need actually is more technology to help us to assess the mouse tissue.

[inaudible 00:35:08] tumor models, particularly the spontaneous tumor model I just said, and humanize the model, provide opportunities to assess the toxicity with a clinical relevant condition and also the route of different drug administrations. Evaluate the biology and expression of the target in the intended clinical population, particular the tumor bearing preclinical models.

Finally, for the measurements to determine the first in human dose, think about a combined experience and effort for multiple measurements to determine the first in human dose. Again, no one size fits all okay. Thank you very much. Okay. It's short, probably. I hope to leave more time for the panel discussion.

Saber: 00:36:00 Thank you. Very interesting presentation. If you have questions from the audience, please come to the microphone and ask your questions. I will start with the first question perhaps. You mentioned the path for first in human dose selection, pharmacologically active dose in the mouth. In our 2016 publication, when we were looking at first in human doses, we noted that very few sponsors use their xenograft data or mouse data for first in human dose selection or justification.

And this may not be just a question for you but for the audience as well, and we can continue it during the panel discussion, is that currently a lot of first in human doses are based on *in vitro* pharmacology studies, and even though the mouse studies may not be perfect and still might result in a low first in human dose, perhaps it would be a little bit higher than the doses based on *in vitro* studies. But I don't know if anybody has looked and compared the first in human doses from *in vitro* studies to first in human doses from *in vivo* pharmacology studies to see where we stand. Which one is better, more efficient, or more optimal? Do we have the data? Do you have the data?

Zheng: 00:37:32 You know, I think one example what I saw is using the pharmacology active dose is one of the OX40 antibody, if I remember correct. I think it's the challenge part is traditionally we always start with the cycle and release, the *in vitro* pharmacology and go all the way down to the receptor occupancy. And after we were not able to determine the first in human dose, then go for the ... Look at advocacy dose in the mouse model. I think that is probably the reason, because we're not thinking in the opposite sequence. We're not thinking of them in a big picture all together [inaudible 00:38:27] go through the traditional sequence. [inaudible 00:38:31] we don't see the cytokine release. Don't see the toxicity in the primate. Don't see the other information that can provide to us. And then to go for ... Probably that is one of the reason why there's really very rare example to use at MPD dose to predict at a first in human.

Saber: 00:38:58 So perhaps this is something we can collect the data and compare the doses to the human doses.

Zheng: 00:39:07 Exactly. Yeah. I don't think there's a lot of data at all. Yeah.

Bunch: 00:39:16 Hi. [Todd Bunch 00:39:16]. BMS. I wasn't gonna address that last comment, but I had a different question, if that's okay. You mentioned a danger of a potential for disconnect between peripheral RO versus tumor RO, and I'm just curious if you could elaborate a little bit more. Have you seen significant enough disconnects that that would really impact your dose projection? And also, in your experience, what the drivers of that disconnect might be?

Zheng: 00:39:45 I think your question is about my argument that we need a look at both tumor free and the tumor bearing model, right? I think one of the example is ... I don't think I really have a lot example, and one example I present is the T-VEC study. But really not many other example. But you can think about it with the tumor and without tumor, and the receptor occupancy will be different. And the cytokine distribution will also be different. And also the [inaudible

00:40:30] the target [inaudible 00:40:33] distribution also gonna be different between the tumor and the normal tissue. I think that will be ... You anticipate to see the difference between the tumor bearer mice and the tumor free mice.

Another argument is about the ... We don't see the toxicity probably because of those mice or those non-human primates is not really suspect to the autoimmune toxicity. For example, just ask Dr. Beatty [inaudible 00:41:11] the liver injury [inaudible 00:41:13] pretty was pretty exposed to the autoimmune toxicity. So the tumor bearer mice possibly actually create a situation [inaudible 00:41:20] have some pretty exposed to change in the microenvironment of the organs that suspect to the toxicity. That's my argument. But certainly I have to say, I don't have the answer, because here I'm just raise more questions than the answer. Yeah.

Saber: 00:41:44 Okay. Thank you very much, and please stay. We are having the panel discussion now, and I invite all our speakers to come up. And Dr. Beatty, Dr. Javaid, Dr. Maus.

We do have a question for Dr. Beatty from someone online. From mouse studies using the CD40 example where chemo is delayed five days versus two days, how would one translate that to human testing? And how many patients need to be tested to know if it has an impact?

Beatty: 00:42:53 Sounds like somebody wants to design a clinical trial. They should come talk to me. There's a couple things to make note of. The first is that the ... And I discussed this offline with some during the break. The original study in pancreas cancer using chemo and CD40 paired it such that chemo was delivered first and then CD40 was delivered 48 hours later, and then the next dose of chemo was delivered five days later. It was serendipitous that that was the way in which it was done.

Moving forward, the reason for removing the initial dose of chemotherapy was because of its potential myelosuppressive effects, and because of the fact that CD40 can induce an innate anti-tumor effect on top of the adaptive immune effect that has been the dogma. And so if you deliver chemotherapy first, you ablate the myeloid cells. You lose the potential, theoretically, the potential to see that innate anti-tumor effect.

How would you do that? Well, it would be of interest to study the sequence of how one would deliver chemotherapy and then a CD40 [inaudible 00:44:30] so you could deliver it first and then give CD40, and then you can deliver it, CD40, first, and then give chemotherapy.

There are other challenges in there, and that's why I say if whoever's asking this question wants to know the sample size and that kind of thing to design the trial, should come talk because the other challenges are how frequently to deliver the CD40. And there are reports that if you give it weekly, that's too soon. That you can actually ablate immune responses if you give it every two weeks. We don't know. Every four weeks seems okay. Is every three weeks better than every two weeks? We don't know some of these aspects about how to translate it effectively.

Certainly it's also not clear with newer chemotherapeutics the timing of gemcitabine abraxane, how that differs than a CarboTaxol regime versus a gemcitabine regime and the toxicities. How they would be different.

Saber: 00:45:35 Thank you. I wanna mention again why we are having this workshop before we go into the questions, because we had a comment online and it was more of an intention of this workshop. In 2016, when we published the paper, we noticed that for many of these immune oncology agents, immune checkpoint stimulators and inhibitors, the toxicology studies cannot be used for first in human dose selection, and they do not necessarily predict toxicities in humans.

For two of the products that we examined, they had toxicities in the animals. For some of them, it was incidental finding here and there, that now that we know some of these agents will result in inflammatory response, we dig into the organs and we see an incidental finding here, inflammatory response there, we say, "Oh. Look. I have a response, and here's the toxicity."

But I think nowadays, we pay more attention, and even when it's incidental and not dose dependent, we still look at it and say, "These are related to the drug, investigational drug." But with newer agents coming on board, it's really difficult to distinguish between an incidental inflammatory response versus a drug related response that many of these studies are claim.

And we looked at dose selection base on NOAEL or HNSTD, and for some of these products, the first in human dose was not safe. So the question remains then, how to better use the non-clinical models that we have, because it also came to our attention last year when we went to the NCI and attended the think tank gathering, that there are many non-clinical models out there that are used mainly for activity studies.

But the question that we had was that, can we tweak them and make a better use of them? And so it wouldn't be just for activity. Maybe we can use them for first in human dose selection. Maybe we can throw in some safety end points in them, not to make it full blown talks or [GLP 00:48:04] talks, but maybe just clinical observation. So, get additional information from these pharmacology studies.

So, we are bringing in the NCI together with academic centers and pharmaceutical companies so they can all meet here and have this discussion rolling, and it would be great to know that we can do more with these pharmacology studies, but at the end of the day, if we find that there is so much we can do and not much more to be done at this point, that's also a conclusion. That's why we are having this workshop.

With that said, I'm gonna go. If you have questions from the audience, we'll take those questions first.

Leighton: 00:48:51 Yeah. Hi. I'm John Leighton. We got a question online that was submitted to the AACR that ... the question of whether or not you could discuss the optimal dosing schedule maximally efficacious administration schedule, particularly with combinations, and whether or not the non-clinical models can inform on the number of administrations, time period between administrations, and other factors to have best to use ... to evaluate, use a non-clinical model to evaluate an optimal clinical schedule, particularly for combinations. Particularly around an immuno-oncology drug, checkpoint inhibitor, stimulator, with other immunomodulatory agents.

- Saber: 00:49:43 Any taker? Maybe John. You wanna answer that question?
- Zheng: 00:49:49 [crosstalk 00:49:49] I could go on. You're a panelist.
- Beatty: 00:49:52 I'll make a comment. There's a couple of things. And I don't know that everybody necessarily agrees in the community. I think it's been placed out there, as you've said, that the sequence of immuno-oncology drugs may matter. And one of those examples is some work that was published looking at an OX40 agonist in combination with a PD-1 antagonist. And the data showed that if you delivered the two drugs simultaneously that that was worse than either agent by itself in terms of anti-tumor efficacy, in the breast cancer mouse model that they used.

But if you delayed the delivery of a PD-1 agonist by about a week, that now you saw at least an additive, if not synergistic, effect of the combination therapy. That is a hypothesis that is in the field. Whether or not it will translate to patients is still to be seen. I think that there is some data to suggest that out there, that the combination of one immune therapy drug with another at the same time may negate activity, but we're waiting to see that officially.

There is also some encouraging data, of course, around lung cancer, where you get your chemo and radiation, go to surgery, and then you get your PD-1 antagonist antibody afterward. So that's a sequencing effect, and there were certainly clear benefits with that.

I think that at least addresses a little bit around the sequencing that are maybe some value in sequencing drugs. It becomes very hard moving forward, though, because these PD-1 antagonists stay in the circulation for months. So if you come off one drug, right, and now you go on the next immuno therapy drug, you don't know the impact of having recently seen the previous one. It's not to say that that's necessarily going to be a bad thing in all cases, it just makes it a point that we have to be cognizant of in terms of how we interpret subsequent therapies.

- Maus: 00:52:12 I'm not sure [inaudible 00:52:12]. Can I? I'm not sure this is a direct answer to the question, but I just wanted to echo one thing that Dr. Beatty said, which is how long these drugs stay around is one thing. And even if the drug gets cleared by PK or by any measurable means, sometimes these have lingering effects on the cell, which can stay around for much longer.

And so one thing that we've seen sort of anecdotally in the CAR T field in humans is that sometimes people will have ... They may have had a partial response or loss or relapsed after CAR, and then whatever therapy they get next can induce a sort of recrudescence of the CAR and then they can present with cytokine release syndrome and can have a response that's related to refueling the CAR T cells, so to speak.

And it's been seen with multiple drugs. It's been seen with lenalidomide. It's been seen with anti-PD-1. It's been seen with some small molecule drugs. It's interesting that these ... the combinations and the sequences of the combinations. I think the kinetics of that are gonna be complicated. Hopefully in a good way, though.

- Kalamegham: 00:53:16 Yes. Right. And I just wanted to add to that and say it is even more complicated when you think about the fact that we have data now on tumor evolution and we know ... Again, it's a lot of preclinical animal model data that shows you you have certain mutations that can take

over, and you have both directed and sort of broader evolution of tumors. So sequencing can almost direct tumor evolution in a way, but it also has to account for the fact that there is tumor evolution happening otherwise.

And another layer on top of that, on top of tumor heterogeneity and tumor evolution, is the whole tumor micro environment effect. And how does that impact dosing and sequencing and scheduling? Not that I have answers, but it's just making things more complicated.

Seigler: 00:54:12 Hi. I'm [Sarah Seigler 00:54:14]. I just wanted to ask the panel about your impressions about the starting doses for the CAR T cell trials. They've done a ton of mouse studies, but with few exceptions, the doses, especially for the dose escalation study, seem to be all within the same range, so I'm really wondering what the value of all these mouse studies are as, you know, moving forward.

Maus: 00:54:38 Yeah. I was maybe looking at someone else to take it.

Seigler: 00:54:41 Sorry. [crosstalk 00:54:42].

Maus: 00:54:42 No. No. It's fine. I'm happy to discuss. I think you bring up a great point. There have been a couple of different doses studied in mice, and we talk about Michel Sadelain has pioneered this sort of stress testing where they lower the dose so much to try to see differences in efficacy. And that seems to work in terms of trying to have a bake off of different CARs to see which one is the most efficacious. Sometimes they all work fantastically well if you just give enough of them, and so you have to try to lower the dose to see a difference in the mice.

When it comes to humans, we're kind of boxed into a certain dose range, because giving less than 10 million cells is ... It's basically like giving 10 mils of blood. It seems like it's a very small amount. It's even less than you can probably contain in the bag. And so there's sort of a floor of, let's say, something like 10 to 50 million cells, and then there's a ceiling around 10 to the 9th cells, because that's how many we can manufacture from any particular person.

It is 10 to the 7th or 10 to the 9th, so that's a reasonable scale, but it turns out that most of the dosing ranges that have been effective have been within that window. And so I think it's not really that necessary at this point to try to figure out which dose you're going to use for a first in human study based on a mouse, because there's already been sort of an established standard in the clinical setting that somewhere between 10 to the 7th and 10 to the 9th is about what we can do.

The other thing to think about is even if you get to higher doses than they, when we're giving cells intravenously, they can pool in the lung for a while before they get redistributed. And so injecting a very high dose of T cells has the potential to cause hypoxia and pulmonary edema, not because of on target effects, but just because of sludging and pooling in the lung.

I think the dose range is less meaningful in T cell studies, and because the second reason that they're less meaningful in terms of using these mouse models is that the cells will grow in humans, and so if you half the dose, it's basically like one population doubling. Potentially

it means delaying side effects by 24 hours. But it doesn't really change what the effective dose in the patient is.

Seigler: 00:57:10 Thank you.

Maus: 00:57:11 Thanks for the question.

Peter: 00:57:14 [inaudible 00:57:14] one of the challenges of immuno-oncology [inaudible 00:57:18] is the issue of delayed immune response. We talk [inaudible 00:57:24] how do you address the issue? Do you have the time for the animals to develop delayed immune response or what do you do?

Saber: 00:57:36 One thing we looked at was whether we see any toxicities in one month versus three months versus six months after ... in the short term and chronic toxicology studies. And for PD-1 inhibitors, there was no difference. So even you waited six months, still you get the same results as if you did a one-month study. Whereas in patients, some of these effects are seen, adverse effects are seen after one month or three months, but if you look at the range, some patients experience it after a few days. And in the animals, it was very clean.

That said, that was for PD-1 inhibitors. For CTLA-4 inhibitor, it's a different story. For one CTLA-4 inhibitor, the dose was low and there was no toxicity even in the six-month study. With another one, when they went up to five times that dose, they did see some toxicities. So not all checkpoint inhibitors and stimulators are the same, but definitely there is no clear correlation between animals and humans, and that's why we're having this workshop today with all these discussions.

Peter: 00:58:49 Thank you.

Finkelstein: 00:58:50 Hi. Martin Finkelstein from Pfizer. And it's nice to have a lot of MDs up there, because my take on this is we're trying to look for a non-clinical model, and when it comes to the clinic, let's just take the PD-1s, PD-L1s. What? 20% or so of the compounds work in people. So we don't even know what patients and why it's working. So what are you supposed to model? We don't know exactly what's happening in humans or efficacy, so that's kind of hard to model.

And then when it comes to safety, most of the toxicities are, for grade three or four, less than 10%, right? It's not easy for tox studies to come up with rare toxicities, and the people that have those toxicities, we're not sure why they're predisposed. So I'm not sure how you can come up with a non-clinical model until you know what's happening in the clinic, and again, there's billions of dollars being spent in the clinic to figure out the efficacy and toxicity. And I know there's still a lot of holes there.

So I'm just wondering, what do you think you ... we could do with models that would help, right? I mean, one more thing is this ... It's a big race, right? I mean, Merck, BMS, especially, spending tons of money. Lots of clinical trials. Are people gonna wait and see what this model does to pick the next clinical trial? And if you still had toxicity, are you gonna stop? I'm just wondering, what is the end result? 'Cause I'm not sure I can see it without knowing a lot more about what's happening in the clinic.

Saber: 01:00:15 Yeah. That's a very good question, and what we are hoping to get out of this workshop is the discussion to see can we get something more out of these models? Can we project that slide, please? Can we use them for first in human dose selection?

This is for those of you who attended the APT. Sorry. This is the same slide that you saw before. These are ... INDs are submitted with pharmacology studies, and many of these INDs already contain *in vivo* animal data PD studies. So let's say you have IND 1. You have drug A. This is, let's say, OX40 inhibitor, and you have an animal model for that drug. Drug A. If I can get this to work. Well, anyway, I'm just gonna talk.

A is mouse A. You developed it for this particular drug and this IND. And let's say that you have the doses given to the mouse and this is a patient derived xenograft model for this drug. And the lowest pharmacologic active dose here is one mic per Kg. And let's say now you've gone into your phase one, phase two trials. You know that the recommended phase two dose or the recommended dose or the optimal dose is six mg per kg.

Now you have IND two, and you have mouse two, and a different xenograft, a different mouse model. You have for the ease of this math, I said that the lowest pharmacologic active dose in all of them is one mg per kg. So let's say that for this one, the recommended phase two dose is five mg per Kg.

And for IND three, you have a totally different model and you have your pharmacologically active dose, and you have the recommended phase two dose. Can we plot them here? On the X axis, you have the INDs. On the Y you have recommended phase two doses over the [PAD 01:02:36] in the animals.

And let's look at the margins. So six over one is sixfold. So here's six. For IND two, five over one is fivefold. IND three, this is tenfold. And if they are all above one, you are in a safe range.

So let's plot them and let's see if, for all the different models that we have, are they all safe doses when you compare them to active doses and safe doses in humans? If they are below one, maybe we can introduce a margin.

Anybody has looked at these so far. Should we start looking at these? Because right now, what we are doing is we are using *in vitro* studies, and for some of these INDs, we are at microgram doses, and it takes three to five years to complete the phase one trial to find the right dose. And that's not optimal.

And if we can throw in some safety end points into them, that would be great. I don't think we have the safety end points now, but can we throw in the, I don't know, clinical observation? Body weight? And check that and maybe in the future we can have some safety data added to this. And that's because we recognize that we don't have an optimal studies right now, so we are using these NHB studies, but we are not making the best use of them.

Maybe this would be the future. I don't know. But at least we can start taking a look at these.

- Maus: 01:04:12 Can I comment on that? I don't do as much checkpoint, but if we think about what we learned from Merck and PD-1, I mean, Pembro and anti-PD-1 have a different toxicity profile based on what the tumor is. Right? If I got it correct, that signal was seen in multiple myeloma, but not in lung cancer and not in melanoma, and so it's hard to imagine that any of these mice would have helped figure out some dose or some safety signal given that they ... [crosstalk 01:04:42].
- Saber: 01:04:42 And that's why safety is out right now. That's why I put question mark in front of the safety. But we do have pharmacologically active doses right now. We don't have any safety end points into these right now.
- Maus: 01:04:54 So for the mice, yes, we don't have any safety end points. The one thing I would like to add is, because these mice have tumors in there, we're aggressively treating them with the drug, so just the exposures that these mice are exposed to are not what are exposed in the clinical setting. So you have to be very cognizant of that. So we use *in vitro* as well as receptor occupancy as well as mouse experiments, but with the caveat that we just have to be careful looking at the dosing that we're giving mice, because that's not gonna be easily translatable into the clinical setting.
- Saber: 01:05:31 So you've-
- Zheng: 01:05:32 Yeah.
- Saber: 01:05:32 ... Compared the doses in the mouse and you found them not to be safe even with a [crosstalk 01:05:38].
- Maus: 01:05:38 No. No. They were safe in the mouse, but in the mice, just because we're giving them the tumor and the tumor is so large with respect of a human, just the dosing that we're giving is a little ... It's much higher than what you would give in the clinical setting.
- Saber: 01:05:52 Fair enough. So for some of these, it might be we might be below the line. This is a very optimistic approach, but I think what I'm trying to say is that we haven't looked at this yet. And once we look at this, and if they're unsafe, they're unsafe, we know that we are ... We cannot use these PADs, the PAD models from the mouse studies. But I know that some sponsors are using them.
- Blansit: 01:06:23 This is Diane Blansit from Boehringer Ingelheim. I think one of the issues that a lot of the companies have is we don't generally do extensive dose response testing in these models. So to try to use it the way that you're demonstrating up there, we would have to start doing more extensive dose response in the efficacy models to see where the effective dose actually is. A lot of times we just slam them with a lot of antibody to see whether we have an effect, and I don't know if that's true for all companies, but I think that is probably true for some.
- Herzyk: 01:06:55 Danuta Herzyk from Merck. Actually, to this point, while it's true that usually we use higher doses in mice because of the aggressive growth of tumor and Dr. Maus also talked about it. Mice require higher doses and so on. And usually, they respond at the higher dose, and you can only titrate for ... actually in limited way, because then they stop responding. Depending

on the model, but the careful dose response is not very often achievable, really, in these tumor models. So that's another problem.

And too, at least from my experience, and I'm going to touch on this, we need to use a combination of different data sets for this first in human. So pharmacologically active dose in [inaudible 01:07:58] models, *in vitro* concentration from functional.

Herzyk: 01:08:02 Also, using human cells, and tumor in-vitro, and so on. Then some receptor occupancy in someone, in animal study, either mouse or monkeys. And, typically, yeah, we have very different responses in these different systems. So, we have to extrapolate and, typically, we land somewhere in between. Mice are high in-vitro gives a very potent, usually, response, because it's isolated system, so concentration is low. And, usually, receptor occupancy is something in between. So, all three components are important, and I think they should be, if feasible, taken together. It's not just one system.

Saber: 01:08:53 Right, and thank you. It's important to have the totality of data, and not just to stick to one. That's correct. Totally agree with you.

What else would you do in terms of these models that you have, in terms of making it more translational? Would you just use it for activities? Best just to stick to these models for activity assessment? And, also, I see that sometimes these models are used to interrogate toxicities after a drug has gone into Phase 1 or Phase 2, and then there is use of these models just for interrogation. Do you think that there is something else could be done with these models, or should we stick to what we have? What are your thoughts?

Maus: 01:09:50 I was gonna say, I think one thing that I've learned today from listening to my colleagues and the people here is that it seems to be much more effective to go in the reverse. I think that Dr. Beatty called it option two which is to observe something in the clinic, and then try to design a mouse model that will replicate that. And then, you can, perhaps, test different management strategies if it could be done in a [inaudible 01:10:13] you could say, like in the setting of CAR T-cells, it was, "Is anakinra better than tocilizumab? Is one upstream of the other? Or can you test different potential clinical management strategies without having to do a lot of different clinical trials?" But, trying to model it all in one, and have a mouse predict what toxicities you're going to see, I think, it hasn't worked, and I'm not getting a lot of optimism from anybody that that is something that ...

Javaid: 01:10:43 I would actually agree with Marcela on this. Typically, when we use mouse models, we're looking at efficacy. We're looking at whether we see cytokine and chemokine, and what the mechanism of action is. And, if we have any biomarkers. Toxicity, we start to interrogate them usually if we see something in the Phase 1/ Phase 2 trials, and then we can come back and see where the mouse models ... Were you able to see that in the mouse models? And, then can you go back and predict? But, doing it from the very early on from where you are first trying to look at novel checkpoint agents or looking at new combinations is going to be very difficult, I think, in the mouse models.

Beatty: 01:11:20 Yeah, I'll agree with that. I outlined it here. It's three things. The models are good for biology, mechanisms response, mechanisms of resistance, and hopefully the models can identify biomarkers, particularly those that are in the blood would be the easiest, because you can have an easy marker of response. And, then the toxicities are when you see them in

the clinic, and you use very scientifically focused models to address the mechanism so that you can, hopefully, either prevent, predict, or treat, or at least understand the timing of when they emerge.

Zheng: 01:11:54 I just wanna ... I agree, but I just wanna play devil's advocate. So, I still think we should think about, you know reversal definitely is necessary, because we recognize that the toxicity in the clinic setting, you have to study that. But, I think we still can think about it more proactively. And, I have to recognize that if I ... I only recently had reason to pay attention to these, but if I go back to my lab notebook. A lot of data will never be published, because we see the negative fact. I really cannot tell, sometimes, what I see the combination is due to the toxicity versus inactive combination advocacy. So, I think we have this potential to generate data, accumulate the data ... When we study the advocacy activity.

Another point I wanna make is the different model will probably have a ... you have to think of it differently. For most of the subcutaneous tumor model, the treatment course is very short, and you probably not gonna gain much about learning about toxicities just for a short treatment course. Certainly when you are not using the transaction model, it's a lot of hard work. But, that's probably will be among those model, probably most ideal model.

And, also, something between [inaudible 01:13:42]. In general, the top model will have a longer animal disease course compared to the subcutaneous model. And, also, those model resemble the clinical situation better, so I think we probably should think about when we just make a conclusion, our current animal model may not suitable for the toxicity assessment. But, just think about different model, probably have a different capacity to study that.

Saber: 01:14:18 Thank you. Yes.

Agarwal: 01:14:19 A quick question. This is Vikas Agarwal from TARIS Biomedical. I have a general question on the non-clinical evaluation of all these antibodies. Can you really test the commercial drugs in animal models, and use ... How do you go about doing that? I mean, that's a question that I had. So, let's say Keytruda or nivolumab, if you put that in ... Do you put the same drug in the rat and mouse models, or do you have to synthesize something else?

Javaid: 01:14:50 So, in the syngenic mouse models, you cannot use Keytruda, because you have to use a murinized version of that, so it's not the same drug that you're putting in the mice. It's the murinized version.

Agarwal: 01:15:01 So, would that be generally applicable across this category of checkpoint [inaudible 01:15:05], that you have to synthesize the drugs ...

Javaid: 01:15:07 Yes.

Agarwal: 01:15:08 ... for mice, rats ...

Javaid: 01:15:09 Yes, typically for efficacy unless you have a cross-reactive antibody, but if you don't then we tend to use the murinized version. And, they tend not to give ADA, either.

- Agarwal: 01:15:19 Right, so how do you, sort of translate that knowledge when you ... I know we are having this workshop on that, but how do you translate that knowledge from that specific drug to the humanized version of the drug? So, is there one more factor to be considered, then?
- Javaid: 01:15:36 We use the murinized version to look at the efficacy ... maybe Danuta. No.
- So the murinized version we can use to look at efficacy and tolerability and to see, are we getting the cytokines/chemokines mechanism of action and biomarkers? But, when we get the humanized version we have to go and do the in-vitro experiments as well as the receptor occupancy. So, that's kind of where the translatability is coming in, but it's not going to be the same antibody that you're using in mice that you're gonna be using in humans. So, you have to take that into account when you're doing these studies, that there's going to be differences.
- Agarwal: 01:16:15 Hmm. Okay, thanks.
- Herzyk: 01:16:20 Again, Danuta Herzyk from Merck. I like to back to this question about what can we do better prospectively in terms of the animal models? And, I think there was a discussion and statement that probably standardization of this model was not feasible. On the other hand, we heard, "Well, we need to try harder, and do some, perhaps, more tedious work about qualification of these models." That's the old-fashioned way I used to be doing my lab work, but, well, perhaps we can choose several tumor mouse model and use standard of care drugs. And have better understanding more systematic evaluation because we've seen from Sarah's presentation that, while the same combination gives different results in different models. Do we need to do multiple models, or should we have a few, sort of, qualified type of models with the most commonly used standard of care combinations or otherwise therapies? It sounds very tedious, and not very, I think, attractive. I think it's much more attractive to do the mechanistic studies, and uncover new things, but I don't know. I just wanted to bring this question.
- Saber: 01:17:55 That's a very good question.
- Herzyk: 01:17:55 Is that something for NCI, perhaps?
- Saber: 01:17:59 That's a very good point you bring. So, I really want to ask you all if you submitted an IND, and you used your animal models for first-in-human dose selection, what criteria did you use, because we accept it when you submit it and the doses were safe when we compared them to the doses given to humans, they were like few folds below. So, they were safe doses. So, did you try different models, and you used the most sensitive model? So, maybe that is missing from the IND packages.
- Graziano: 01:18:36 Do we time for another question, or comment?
- Saber: 01:18:39 One.
- Graziano: 01:18:40 Okay, so, Mike Graziano from Bristol-Myers Squibb. Now, let's pretend I'm a new toxicologist, and I'm working for a new startup company, and I have a checkpoint inhibitor I wanna get into clinical trials. I'm gonna hire the four of you as consultants, okay. What are

you gonna tell me to do, what studies, what [inaudible 01:18:59] to convince Haleh and John that this drug is safe?

Maus: 01:19:09 I only make cells, so if you want to make cells, I can help you.

Saber: 01:19:14 Hire her if you want to make cells.

Maus: 01:19:17 I didn't say hire, I said help.

Saber: 01:19:18 Oh, I'm sorry.

Mike Graziano: 01:19:22 I would just say the start, the first challenge will be whether or not the drug that you're gonna put into your pre-clinical model is the same or not as what you're gonna put in the patients, right? If it's different, then you're really gonna be learning about biology and understanding whether or not it's a potentially good target to go after. Of course, you're gonna go through all the standard things of the non-primate studies to show that it's otherwise safe in a very non-tumor-bearing kind of kid-in-the-box/bubble kind of approach. But, a lot of that's still gonna be at the level of putting it into the patients, right? And, being very meticulous about monitoring, and see what you find, and I think that's one of the things that we haven't touched on, but I think it's quite obvious and maybe needs to come to the front is that we need to learn from every patient that's treated, right? And, so, that starts not only at the industry level, but also goes into the academic and clinician side of which you see a patient who has a rash, you biopsy it. And, that doesn't always happen. So, there are other breaks in the chain that do occur. But, I think you have to learn at the patient level, because the toxicology studies may or may not tell you. If you don't see something, it doesn't mean that it's gonna happen.

Maus: 01:20:56 I think what they're asking is, "What do you need ... " And I suppose it's the FDA's prerogative to say, "What do you need to put into the pharm/tox package?" So, I would assume that there's some either PDX model or syngeneic model where you have to do some dose escalation of your antibody.

Javaid: 01:21:14 They would, but keep in mind then that it depends if your antibody is cross-reactive, or not right. So, it would be the same package that we've been talking about with the other checkpoint agents.

Zheng: 01:21:24 So if it's already approved agent for the same target or same category like checkpoint inhibitor, I think that probably will be the easiest way. You have a benchmark to compare you can follow. I think, I would recommend at least to follow, you know, compare the benchmark. That's actually come with [inaudible 01:21:52] you already recognize the toxicity in the clinic, at least. But as same molecule of the same class. I think you should think about using those animal model that's mimic those toxicity in the study your [inaudible 01:22:10] agent. But, what I'm trying to advocate is, for those new class, new target. And, you have nothing to follow and that's challenge. That's why I say we have to think about something more proactive.

Another challenge is the combination, and that's another challenge. So, that's why I advocate about the using non-clinical model for looking at toxicity [inaudible 01:22:40] more proactively.

Javaid: 01:22:40 In terms of the humanized mice, I think it would depend on donor-to-donor variability, and how reproducible the samples are. Because, at least in the settings I've seen it in and at least some of the data that was shown, earlier, also, you could see that in some donors you got really good anti-PD-1 response, and in some donors, you did not. And, that's not something you know prior to before you actually do the experiments. So, I think they're good but they're not the only model that you should be using.

Saber: 01:23:16 Okay, thank you for the panelists. I think we have to get going in the interest of time, please.