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Session 1 – Assessment Challenges with Complex Active Ingredients: Peptides & Oligonucleotides

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- **Immunogenicity Risk Assessment of Peptides: Progress and Remaining Challenges**
  Eric Pang, PhD Senior Chemist, DTP I, ORS, OGD, CDER, FDA

- **Generic Oligonucleotides – Challenges and Opportunities**
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- **Challenges in Immunogenicity Risk Assessment for Complex Active Ingredients**
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- **Research Initiatives for Harmonization of Immunogenicity Risk Assessments for Generic Peptides**
  Andrew Graves, MS, SCYM Director, Immunogenicity Assessment, Teva Pharmaceutical Industries Ltd.

- **Immunogenicity of Oligonucleotides**
  Sudhir Agrawal, DPhil President, ARNAY Sciences; Affiliate Professor, University of Mass.

- **Immunological Challenges Associated with Nucleic Acid-Based Therapeutics**
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Panel Discussion

In addition to moderators and presenters listed above:

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MODERATOR: And I'm happy to be here with my co-host or co-moderator. Yan Wang, who's from. She's a deputy division director in ORS, which is a super office or in the Super Office of OGD. The first session of this meeting is entitled "Assessment Challenges with Complex Active Ingredients: Peptides and Oligonucleotides".

So we welcome you to this session and we hope you enjoy the presentations and discussion. So our session will include presentations on proposed research to improve the characterization of active ingredients, impurities, immunogenicity for generic drugs with complex active ingredients, with a focus on peptides and oligonucleotide products.

So we're lucky to have a panel of experts from the generic drug industry in academia, along with subject matter experts from the FDA who will have the opportunity to interact with our presenters and discuss the research gaps that have been identified.

During the session, we'll welcome public comments to the FDA on the topic of our session. The audience may comment at the start of the panel discussion using the microphones in the room. And that will start after the formal presentations. And those online may submit comments to the public docket, which is on the link that's in the at the top right of this slide. And we'll monitor the docket throughout the session and endeavor to address some of those questions during the panel discussion.

I just want to make this comment the comments are really thought are really more to provide thoughts on research gaps that may exist towards the development of generic drugs rather than ask specific questions for the regarding the presentations.

So without further ado, I'd like to provide a brief introduction to the first speaker about in our session. And so that will be Eric Pang. He's a senior chemist in the Office of Research and Standards in the Super Office of OGD. He's a policy lead. He specializes peptide large molecule drugs and his presentation title is "Immunogenicity Risk Assessment for Peptides: Progress and Remaining Challenges". Thanks.

Eric Pang: Hi, good morning and good afternoon and good evening to everyone. So without further ado, I'll jump right into my talk to conserve time.

The Biologics Price Competition and Innovation Act of 2010 outlined the definition of peptides. Basically any amino acid sequence that is less than 40 or has 40 or less amino acids should be considered as a peptide and those will be reviewed under the follow-on pathway. Which includes ANDA and over the years 130 FDA approved peptide products have been designated as a reference listed drug. The early products include some of the natural extract products such as protamine, corticotropin, but later on with advancements in synthetic and recombinant manufacturing process gave rise to approval and development of many other popular products. Including the very popular GLP-1 product that you have heard earlier today.

This is the guidance that started all that is the ANDAs for certain highly purified synthetic peptide drug product that refers to listed drug of recombinant origin. In this guidance, a synthetic generic glucagon, they are necessary. Referencing the recommended RLD has been outlined of the thinking here is that the API of the peptide can be demonstrated. It should be the same with analytical capabilities, but the immune intrinsic risk of impurity should be considered, and these should include the adaptive immune and I'll talk a little bit more about that in the next slide.

Exactly what adaptive? Immune. How does that play with the impurities? The innate immunogenicity risk really looks at the process where the impurities of the peptide product. This include contaminants in each of extract, both the innate immunity really is the first line

defense against any foreign invader. So as your body immune system, recognize any of these contaminants, you could have elevated immune response against these. The adaptive side looks like the peptide related impurities, the peptide related impurities are the deletion, insertion of the modified active peptide itself.

And these impurities. What you want to assess are the T cells. Or epitopes or the arm. And she binding region of these impurities. The presence of these. Any new impurities in the proportion of 0.1 to 0.5% is recommended to conduct this kind of assessments. These assessments. As the guidance stated, that has to be done in vitro or non-clinical assays this includes or here we just gonna abbreviate as in vitro in silico immunogenicity assessment.

Obviously. The adaptive immune system is being assessed. The T cell activation potential of the peptide impurities. So in silico studies looks at the binding. And then in vitro binding can can also be studied, studied adaptive immunity as well as other functional assays. That gives more complete picture of adaptive immunogenicity assessment. The innate immune activation can also be conducted on products that are not well controlled or have been in the immunogenicity risk. These include in vitro cell based assays or other kind of assays, which I'll talk about that in the next few slides.

So first, in silico adaptive immune systems, the assessment is looking at the sequence of the impurities. So if you have a sequence you can analyze using in silico methods. So in silico is a very it's a high throughput, it's fast and it gives you some sort of. It gives you a risk of the impurities. However, through our research we found that at risk of going through the in silico analysis can be overestimated? So in comparison to in vitro data, so that is why we typically recommend orthogonal analysis using a combination of in vitro assays to supplement or complement your in silico analysis.

And lately currently we have research project looking at using the the power of the in silico analysis approach to identify any kind of potential impurities or theoretical impurities that could increase the risk of adaptive immunity. And this is ready to using this to developed the in vitro adaptive assay working standard. And I can talk about that in the next slide a little more. So the current challenge with in silico is that the peptide products itself. Many of the products have unnatural and modified amino acids and ready, so these are done because you want to prolong the half life of the peptides. However, the in silico has a difficult time to predict or assess unnatural and modified amino acids. So that is something that is ongoing and need to be improved upon.

And another challenge is the validating the in silico model. I should probably aware there are multiple models out there, so it is. What sponsors typically submit are these data from the in silico model, but we don't really know what goes into the model. So one of the things that makes it difficult is we cannot judge the performance of in silico model and assessment. Another thing is. Because of proprietary information that's submitted, we don't get to see those. So another aspect is to use model master file apps to to be able to look into some of these proprietary information. So we encourage the submission of the MMF in this case for in silico modeling.

In vitro assay for adaptive immune assessment, there are many different types of in vitro assessments one can do on the peptide related impurities to T cell activation proliferation on to see the potential induction of adaptive immune response. This include MHC binding or MHC associated peptide proteomics or MAPS, T cell proliferation and response ending cells. So there are quite a number of these assays. So that makes one challenge is that because you have many different type of assay and these different assays can be run differently. There's not a standardized way or more passing, you know, best practices kind of protocol that one can use and then it's difficult to assess these impurities. I mean, sorry these assays altogether because we don't have a working standard. So without a working standard, it

becomes difficult to assess how well these assays work. And that is why we are currently funding a research project looking into using in silico modeling to generate some of these working standards. So perhaps we working standards can be coming in the next few years.

Another challenge with in vitro assay is understanding how. What's the relation of different amounts of impurities? Whether it has clinical effects and that is something that has to be assessed because currently we recommend 0.5%, but how relevant is that 0.5% and whether we can use in vitro assay to adequately assess that? That is a question that will remain to be assessed.

The innate immune response has a immune response. Assessing has one can use in vitro assays cell based assays. Cell and based on such as blue, raw blue or using PBMCs or whole blood. So these assays there are quite a number of them just like the adaptive, but it's more, I would say well developed because we have studied some of these assays in terms of a research as a part of research contract and we find that there are some parameters one can assess to make it more assertive for purpose. However, there's still remaining challenge. One of the which is the effect of the formulation buffer. The formulation buffer can have effect on a cell based assay or even PBMCs because it can affect the performance or or viability of the cells. So that is why formulation buffer is a challenge that one has to overcome come if your formulation has issues with this assay, another thing, just like adaptive assay is the innate assay also needs to be. Has needs to have a working standard, and that is something that we have working on to develop these working standard as well.

The immunogenicity assessment research is really looking into the, we're continuously having research effort into immunogenicity because our thinking and and can be evolved over time. Based on our research finding and also internal knowledge of case in point. If you recall, 2021, we had the first vasopressin injection approval and that approval is really associated with there's a change in thinking because traditionally we were thinking that before this we were thinking the RLD's risk safety risk plays a big part into the assessing the risk of the ANDAs, however, what we find is the ANDAs should have the same safety profile of the RLD. So in that respect, it's really the difference between the ANDA and the RLD that makes a very important difference.

So with this changing thinking, we begin to to approve from the vasopressin product in 2021 and then 2023. Actually, even between this we have parathyroid were approved. Based on the ANDA synthetic peptide guidance and then the only recently we approved the 1st liraglutide you heard GLP-1 product in December of last year. Until now we have 2 liraglutide approved, so we're looking forward to improving more of this GLP-1 products.

So the future direction is that I think we need to have continuous support for standard development for these in vitro assays and also we encourage collaboration with industry also also with academia in funding and publishing some of these best practices for the in vitro studies. One aspect that I want to bring attention to is something that we have already talked about because all my talk are based on synthetic peptide. But we have to recombinant that are coming in and we need to think about how we can evaluate the recombinant peptides. One challenge specific to recombinant peptide are host cell proteins or other kind of host cell contaminants. How do we assess the risk of these host cell proteins? Will be very important. I think research direction for us to consider another is the upcoming products that you heard earlier today from Rob. There's not only GLP-1 being approved, so one of the things that's challenging for oligo is the impurity characterization, especially when it comes to immunogenicity and other kind of toxicity effects. So we're looking forward to research collaborations or complex related to these areas.

And with that, I'll like to acknowledge everyone that I work with or as of today, OPQ and OPQR. Thank you.

MODERATOR: Thanks Eric for providing your introductory thoughts on. Our next speaker, and Likan Liang is a unit supervisor in Office of Pharmaceutical Quality Assessment 2 supervisory. He's a policy expert in the assessment of generic drug products and he's led a number of research teams. On a series of pre-development meeting requests and solutions. The feedback hopefully will die down. For what? We can get something. OK. All right. Thanks for the introduction.

Likan Liang: So in the interest of time, I'm going to skip some of the slides. Oligonucleotides are short strains of nucleic acids with therapeutic effect. They can be synthetic or semi-synthetic. You know. And the structures most of the cases, the structures of the nucleic acids have been modified. There are many ways to modify the nucleic acid structures, for example. So on this slide they can be modified at the 2' position, the 3' position or the on the basis. And oligonucleotides can be conjugated to, you know targeting ligands or other molecules at the 5' end, the 3' end or the 2' position, etc.

I'd like to draw your attention to the modification on the nucleotide linkage here. So on the slides is the so-called phosphorothioate modification of the of this linkage. In typically the in the naturally occurring linkage is the phosphodiester, which is non-chiral and after this PS modification. You have a chiral center and and in most cases the modification the PS modification, the process is leading to this modification. Non stereo specific. Therefore each of this stereocenters you have the R and S configurations as a mixture. So if you have multiple of such modifications, then you would have N 2 to N power of possible stereoisomers of in this linkage. But where N is the number of such modifications.

And here are some modifications on another type of oligo that is called phosphorodiamidate morpholino oligonucleotide, or the so-called PMO. In this case, the linkage can be, you know, modified as the phosphorodiamidate linkage. And in most cases, these modifications are also non stereo specific specific so you have the configuration R and S configuration and again you have the 2 to the power of N stereoisomers.

My apologies for the busy slide here. This table summarized the 22 oligonucleotides FDA has approved as of April 2025 and and I'd like to draw your attention specifically to the last two columns. Where you know you can see the number of PS and PDA modifications. And then on the last column you can see how many possible stereoisomers coming from those linkage modifications, and you can see in the diagram this can be from, you know, a few to thousands into millions and even to billions of stereoisomers.

So that presents a, you know, significant challenge for the generics. And you know most of the approved oligonucleotides are, you know, solution injectables. So as long as you have the same solution for injections and the same active ingredients and inactive ingredient in the same concentration as the RLD. The bioequivalence, self-evident and then to support the bioequivalence of pharmaceutical equivalents as well as well as the same safety profiles. Typically you can do a lot of comparisons comparing with the RLD.

This slide shows some of the areas that you can consider. For example, active ingredient sameness, mainly for the sequence and chemical structures that for example, the correctness of all these, you know modifications and the stereochemistry comes with it and also the composition including the isomeric composition. As well as potency and activity on that, when it is necessary and also. You know, comparative, physical chemical characterizations. For example, the higher order structures or aggregations in the drug products and other characterizations, especially when you have a complex formulation along with the oligonucleotide and then from safety and quality perspective, you need to compare the impurities with profiles. And levels with the RLD. And then you need to consider the risk in immunogenicity. And information and as well as other, for example of targeted effect.

So I'd like to go back to this slide that because we have so many different, you know, cases on the oligonucleotide, I mean the oligonucleotide. So we're using a term so-called composition. And then I will talk about in the sameness in this area later.

So one of the challenges, one of the key challenges for generic oligonucleotide is demonstrating and assessing the composition sameness. This is due to the high number of possible stereoisomers and the limited resolution power for any single method and compounded with interference from possible co-eluting impurities.

For oligonucleotide, with more number of stereoisomers such as the siRNAs in this table. It is possible to use one or more chemical methods to, you know, accurately quantify the levels and compare the levels of individual stereoisomers. And for those with very high number of stereoisomers more comprehensive approaches would be needed, and the agency has recommended the use of multiple orthogonal methods in these cases.

This approach could generate multiple, you know, profiles with detailed distribution information and combined with multiple other characterizations as a totality of evidence approach for sameness, you know, demonstration and assessment.

The sensitivity of the analytical method towards the isomeric composition changes needs to be demonstrated. One of the approaches may be to prepare and use multiple suitability test standards with intentional small perturbation to the composition. This may be achieved by slightly changing the R/S ratio at various combinations of such stereocenters. If the methods are sensitive to a variety of intentional small changes in the composition, it is more likely that these methods may be able to detect it as several differences between the test product and the RLD.

Also measuring the R/S ratio at individual stereocenters during the synthesis can help establish a baseline to ensure manufacturing consistency and for future potential future changes.

That stereoisomer composition, sameness, criterias are complex. And especially when the number of stereoisomers is high for example. What kind of orthogonal methods should be used to ensure the adequacy of composition? Sameness assessment is the individual method selected capable of dissecting the composition information or removing the interferences from the impurities? What would be the sameness, acceptance criteria and and how can the criteria help to ensure our the comparability of the generic in terms of the safety and efficacy?

Another challenging area for generic oligonucleotide is the impurity analysis. There are so many possible oligonucleotide related impurities and the resolution power of a single analytical method is very limited. This list co-elusion of impurity challenges. It is especially challenging for isobaric and isomeric impurities since they are difficult to be separated by chromatography or differentiated by, you know, conventional methods. This is also there's also a higher risk when the you know the synthetic approaches are different and your impurity profile may be different.

For comparative impurity profiling and analysis. Orthogonal method in complementary methods may be used and they can be coupled with, you know, high resolution mass spec or other more advanced technologies to address those co-eluting impurity challenges.

When compared your standards, or, you know, applicable regulatory guidance are not available. To support quality proposals such as impurity. Grouping approaches thresholds or limit one we consider conducting in depth RLD comparative studies and perform, you know,

a comprehensive risk assessments based on thorough product and manufacturing process understanding.

The challenges in generic oligonucleotide assessment also present themselves as opportunities for innovation. For example, one may develop, develop innovative and original methods to address the limitation of the current methods for oligonucleotide composition studies. And also. May explore computational approaches can help the sameness evaluation, especially when you have very complex you know profile like those millions of stereoisomers how how do you? Make sure they are comparable. Or investigate and develop novel strategies, strategies to address our complex sameness, quality and safety requirements.

With that, I'd like to acknowledge acknowledge the FDA colleagues working on addressing these challenges. And thank you for your attention.

Moderator: OK. Thanks Likan for providing the background information for the nucleotide class. So Next up we have our first public presenter, Manoj Kumar Pananchukunnath. He's the chief scientific officer at Biocon. He has a long years of expertise with many APIs and dosage forms with especially drug device combination products, injectable peptide fermentation and oligonucleotide products. And this morning, his presentation. Our title is challenges and immunogenicity risk assessment for complex active ingredients. Thanks.

Manoj Kumar Pananchukunnath: Thanks Cameron. OK. So the title slightly misleading because when I try to look at this topic I trying to comment it from an industry perspective. And we probably are poised with the unique experience of having to make these products across the globe and access being one of the, you know mandates the companies built upon. So getting these products across the globe presents us with the unique situation that the same data packages. Go across the globe, whether it's EMA, whether it's MHRA, whether it is TGA. Whether it's Health Canada, whether it's United States FDA and it's interesting to see how different review agencies come out with different outputs. And I think we might benefit from our experience of showing you some of those differences and may lead for food for thought to see that how? How can we bring together a little bit of harmonization in that area so that we don't do studies all over again and to the point we were talking about, I think Rob was mentioning in the morning, getting a product a year earlier to the market obviously helps with. Affordability and access and and that's probably where my discussion is gonna focus upon.

I'll go a little back into the basics and then a little go forward into what we are seeing when we present. These to different agencies. OK, standard legal disclaimer, I won't read out all the bullets, but it's gotta be there. It's gotta be there.

Now let me lay this topic out in terms of what I started with. We've we made these products both by recombinant routes. Fermentation has been a big history for us. We made these products by synthetic routes when we found that there wasn't pathways, you know, clear enough for us to do the investments on a recombinant based peptide pathway. And when we land up with these re engagements with agencies which start throwing up different challenges and. While the execution, the design and the purpose of the studies are one part of the challenges. The interpretation or the reaction to the results is another set of challenges which come and they're different when it comes to different agencies. And maybe that's probably what I'll attempt to do in the next few slides.

So going back a little. I think we all understand this. We we bundle together amino acids and then come up with a peptide. They're protected. They're deprotected. And there there's a complex chemistry going on when it's a synthetic process. And of course, in a recombinant process, it's the magic the organism does to produce the compound, and then we end up in

purification. We do end up with impurities and probably the larger gorilla in the room is impurities and impurities and impurity levels and sources of impurities, and the nature of impurity. And and if you have the impurity there, you tend to worry about these aspects of our immunogenicity as a subject. And one could talk about what if it's not there? And and that's probably one of the questions I'll put out there that you know what, if you had a pure product, an extremely pure product, then would you be still concerned about it when you have a brand which is already out there with extensive information on immunogenicity already? Proved.

So you end up in these two areas, the adaptive and innate. Don't think I need to get into too much detail on it, but it once related to the natural tendency of the drug product to evict immunogenic reactions. And the other is related to specific impurities, which are potentially possible to be present at levels which we feel are concerning.

Moving on just a little bit of you know light in terms of the definition of immunogenicity, which I don't want to read into, but the aspects about reference drugs I spoke about that there have extensive elaborate preclinical clinical studies and then we end up in the entire context of process related impurities which could be hosts which could be leachables extractables. This is where the entire analytical expertise in characterizing the product comes into play. And the peptide related structure could be aspects about incomplete reactions. Or, you know, you're ultimately putting a set of Lego breaks through in a sequence. And you put the wrong break in a place you get end up with an impurity.

So the question for us to dwell upon is what if we have a well defined high purity type product of a relatively small size as compared to a protein? What would be the appropriate way to deal with an immunogenic risk assessment? Considering the fact that you may be having an extremely pure small sized synthetic compound below 40 amino acids.

So why there are challenges? And we've been through this entire, you know, journey of trying to find a pathway for an rDNA recombinant based brand product which is out there. And then the follow on product, how do you get there? I mean, we've had the opportunity to try and discuss this with their various agencies because that's been our, you know, capability of building fermentation rDNA products in the past. But faced with unclear pathway, we went on to synthetic peptides. And then it's all about the manufacturing variability. You make formulations. Obviously there are patents out there, so we have to work around with patents to get freedom to operate, which means the agency's likely to see a slightly different composition or or different system than what the brand is, and it revokes more questions. And of course the most important thing is what is that immunogenicity study design? Which could take care of all scientific aspects about proving the outcome of what we're trying to achieve.

I'll go on to a little bit of comparisons and some of this maybe from guidances and most of it is from practical interactions with different agencies and for ease of comparison, I've just kind of bucketed it into the US FDA Canada bucket and and the EMA UK and and obviously, our India and other rest of the world countries so. Levels, as I see here, there are no explicit threshold in some of these countries. Levels have to be justified. There's no 0.5% here. There is a risk based approach where we're talking about in silico approaches, which is put, I think, an area of huge potential to eliminate a lot of the studies which have been happening in from in vitro, assay standpoint and the rest of the world also relies on a lot of PK data which the US waves it off versus the QQ guidances. What we've seen is the demonstration of comparable profile and immunity risk, while the requirement. Either ways is the same. The navigation of that pathway is a little more difficult in some cases versus the others.

On levels. Again, these are all publicly available information, but when you see there are thresholds which are far more defined in other parts of the world. There are expectations of

additional data controls, and and that's gonna be something I'll have a few examples to talk about that. By and large, you have an assay that the controls in that assay are subject to. Great. You know, interest and debate about what's, you know, demonstrating what from a control study perspective. And obviously, that's the entire crux of the assay you need to prove that the assay. Is specific and is is, you know, capable of giving you that assurance of what it's supposed to do.

So the question then here is, can we have an expectation to work upon harmonizing these assays and bring forth a certain level of standardization which helps the industry work on this faster and also provide results which are equally acceptable across different, you know, streams of regulatory engagement?

So going on to one of these examples where we're talking about the cytokine screening assay and the the designs, as you see are largely acceptable. The the construct of the assay in terms of when I say design it's it's the design of what you measure and how you measure and how do you acquire the assay and and the base of it. But when it when it comes to sensitivities is where you start seeing these differences start to keep in that largely, you know well. This data is about positive controls, clinical controls. Your PRR mechanism based innate immune response, modulating impurity requirements and spiking studies have generally been accepted all across, but we we still deal. Tend to have longer conversations and in some cases even ending up repeating the entire assay. To meet those additional expectations and it it's something which you know, which is an opportunity to look at in terms of which is the more robust and which is the more rigid control which allows us to do the studies in a way that the product is safe.

The T cell proliferation assay, for example. So again, largely our experience has been the designs are mostly acceptable. There aren't questions on the design, but obviously the controls become a very big, you know, chapter of discussion where it's acceptable in some cases not acceptable in other cases and. I mean, we all know if a controller is not acceptable, the assay has to be repeated and the repeat of that assay with the new controls is not a problem. It's time and money at the end of the day. And the question to see is if if there were standard, you know guidances about these kind of scenarios could look at these kind of controls then it would become much more easier. And I do see. That while we have this exchange and interaction with regulators. The. The contract research industry, which generally does these assays, is equally in those kind of conversations about what should be acceptable and what should not be acceptable in what scenario. So I do believe that's a region of huge opportunity for industry and agencies to work together and see what could, you know, help us get there with assurances of safety and also speed and time.

We're moving on to some more examples, so when I talk about your PRR mechanism based IR match, we have cytokines whose LQCs and HQCs are very well established in the assay. The controls used are, you know, well induced. They release cytokines above established LQCs both. EMEA and FDA also have noted that process related impurities from cell constructs are not a concern for synthetic APIs because they generally don't use any cell based mechanisms for synthesis. So the question on the design is why would we need those additional controls if the responses are established and you know acceptable?

Moving on to the innate study and again an example of how you know, different controls have been used. I'm not reading out all of them, but you do start to see that the IRMI controls do have now a requirement which in some cases is acceptable. Some case it's not acceptable or there are multiple justifications which are going back and forth on the usage and the suitability of these controls. So again, the question here is whether X lemma draw and P MW appear to be sufficient and accepted in many cases. Would we still want IR MI controls additional to these in study assays and if so, what could they be and how do we, you know, incorporate that into a guidance?

This is another interesting example. Unpublished data from our archives, where the requirement for a suitability control in EDU incorporation assay where the requirement kind of talks about using peptides which are similar length in structure but with minor modifications. So you have a 31 one residue substitution here, and the two substitutions here, and a completely different different amino acid sequence using a different peptide. And when you? Interestingly, look at the EDU assay comparison. The original controls KLH, Ceft and Herceptin all appear to have better responses than these slightly modified peptide controls, which are being looked at from incorporation in the assay. So it it does give you a sense of there is some amount of work here to be needed to understand that with better, sensitive, better responding controls, would you still need for the controls which don't appear? Have that advantage of being able to demonstrate the sensitivity of the assay.

So with those examples, let me get to certain you know, takeaways here we don't find universally acceptable protocols. There are uncertainties in study designs. The same study with two CROs. I've seen dramatically different designs coming in for the same purpose, which means there is ambiguity. Inconsistent data interpretation comes we do get hit with development delays. Obviously the costs of these assays are tremendously high. And obviously delays us from getting to the market in time. And in many cases it's not even clear whether immunogenicity is needed or needed. So it's like you go to the default position of. Let's go and do the study anyways, but whether it's needed or not, it's not clear.

There are practical challenges when we talk about impurities to be used in the adaptive studies. They have to be extremely pure standards. And producing the peptide itself is a challenge. And then producing an impurity insufficient quantities to be used in the assays and other practical challenge. And of course, the excipient matrixes tend to produce challenges which are all you know. These are science based challenges. But the question is how much of it is dictated by need and how much of it is dictated by lack of, you know, we don't know which is the best way to do and maybe. Research projects to identify the best ones the best approaches. Would immensely help you know the subject.

So just to take a high level summary of some of these harmonization opportunities. If if our impurity profiles are exactly the same and and believe me with the orthogonal level of testing we do today, at least in peptides, I understand oligos have a even higher level of complexity. But if if the impurity profiles can be proved to be similar, then do we really need an adaptive study? And if you really need an adaptive study, do we need it at end of shelf life? We could test that specification and control that specification and not wait till end of shelf life. If the manufacturing process is not the same as a drug substance drug as RLD and Nate could suffice, and that again comes back to the earlier question about purity and comparative purity of the product.

I spoke about the peptide suitability control so I won't repeat that. I spoke about cytokine standards as an example, so I wouldn't repeat that 10 times to this question 5, 6 and 7 this this is an interesting journey of if you have a synthetic peptide versus a synthetic peptide in the RLD, then you're basically dealing with a synthetic process and if that profile is demonstrated to be exactly the same analytically. Do we really need to get into an immunogenicity risk assessment because the? Comparison itself is a risk assessment.

We have situations of this which is an evolving area and we would love to see this develop more about if we had to go back and see if an rDNA recombinant peptide substitutes for an R DNA recombinant RLD and what would be the requirements there and we've seen. That technology has moved so far along that our downstream processing can help us control these R DNA product profiles. To something which is even better than the RLD. You know, lesser and lower level of impurities is something which we have consistently achieved. But the pathway today is not there. So that's something to look at because from an access and a

cost perspective, any days in an rDNA process is much more efficient in terms of productivity and capability to produce larger quantities. And finally, when you look at a synthetic API based on rDNA, which is the current approach which has been adopted across synthetic processes, again are capable of yielding lower impurity profiles than RLD designated impurities and also lesser impurities. And if that's the case, can that be the starting point of deciding how immunogenicity risk assessments need to happen? With with basically seeing what do you have in comparison to the RLD with the high level of the sensitivity and a high level of orthogonal complexity.

So in conclusion, there are major scientific evidence is available to harmonize protocols for synthetic peptides. It just needs the work of collaborating and getting it together. Emphasis on in silico models touched upon earlier. I think that's an excellent area to go which removes a lot of the work actual in the lab before you actually come to the experiments, and we need more specific guidances for immunogenicity risk assessment, more harmonized guidances to move forward.

I learn by acknowledging my team's inputs in getting this analysis together. And names are out there. Experts in each of the field have contributed to putting this to heartful. Thank you to them.

Moderator: Thank you, Manoj. Next up, we have Andrew Graves, who's director of immunogenicity assessment at Teva Pharmaceutical Industries. Andrew leads a group of immunologists supporting complex generic drug candidate programs spanning non-clinical in vivo studies. Human clinical studies and in vivo immunogenicity prediction studies. And he has a specialization in the development and validation of complex immune immunoassays. His presentation title this morning is research initiatives for harmonization of immunogenicity risk assessments for generic peptides. Thanks Andrew.

Andrew Graves: Thank you, Cameron Yan and the organizers for inviting me to speak with you today. It's good to see the progress that we're making and. Still, some room to go. Quick legal disclaimer that Teva wants me to remind you that I speak for myself, not necessarily for Teva, but they really hope you like what I say.

A couple quick things. We'll provide some background and then some of my thoughts on standardization, harmonization as well as expansion. In terms of background, Eric already touched on this in his presentation, but the watershed guidance for industry that the FDA published in May of 2021 started to enable the ability for us to put forward generic peptides through the ANDA pathway. At the time of publication. This guidance was limited to synthetic peptides going through the ANDA pathway and the further research would aid both industry and the agency by establishing and harmonizing some best practice approaches leading to what I believe would be higher quality submissions as well as easier and speedier review process for the agency.

We believe that there's also an opportunity to kind of expand this guidance and the lessons we've learned from it, beyond its original scope. So adaptive immunogenicity risk is typically summarized using combination of in silico and in vivo or in vitro approaches and well in silico provides rapid and high throughput option for computational risk assessment. Current platforms are somewhat limited in its accurate prediction due to the presence of both non natural amino acids and some products as well as many of the impurities we need to test.

In vitro adaptive immunogenicity risk can be assessed using multiple platforms, including HLA binding assays, MAPS, PBMC, cytokine release assays, DC-T coculture assays. And although all these assays are readily accessible and commonly used, the predictive value of these assays in terms of clinical outcomes is not thoroughly been established yet? So even

though we may look at something and say, ah, there may be a risk that clinical outcome is it meaningful. We don't know. And there's no way of knowing currently.

Subtle differences in the assays may lead to difficulty in interpretation in comparison between the assays or studies, which I would imagine makes particularly hard from a review standpoint to understand how one submission compares to another submission. There's also, as mentioned, the innate immunogenicity risk assessment generally using a reporter cell line or cytokine release assays. The goal is to examine compare products for the presence of potential innate immune response, modulating impurities or IRMIs, which in turn could trigger loss of immune tolerance and as with adaptive immunogenicity, the link to the clinical outcome has not been fully established. And differences in the readout, the statistical approaches and the controls that are used. Can lead to difficulty in data interpretation comparison.

Now these problems have existed since the May 2021 guidance was first published. There has been a lot of progress in trying to get to a point where this is harmonized or standardized amongst the field, but as I mentioned at my outset, my thesis is there's room to go.

So talking about standardization and harmonization. Although that progress is being made. For standards with both in vitro adaptive immunogenicity platforms as well as innate mutations platforms, I believe that there is an opportunity here for some additional research. And let me explain why.

So for the adaptive immunogenicity standards, they're in development after a contract was awarded to EpiVax and CUBRC to develop specifically positive and negative controls. To use for adaptive immunogenicity assays for a generic peptide drugs, which again I think this is a phenomenal step forward. Additionally, there are innate immunogenicity standards being developed in assistance from FDA, NIH, and NIST. They're undergoing some testing prior to deployment. But parallel to this. You know, we know that establishing these control standards is an excellent first step. Evidence in the field suggests a lot of research may be needed. However, before we fully deploy these controls.

And what do I mean by that? Well, a joint task force that was established by HESI and AAPS was set up to establish control standards for CD4 T cell assays for in vitro platforms. Their scope was confined to large molecule biologics particularly. For innovators, so unfortunately the peptides are out of scope. For what they're looking at, but in their first phase, they had approximately 11 labs participating and the initial set of data was presented at the 2024 Immunogenicity and Bioassay Summit downtown DC. Some controls that are known to be clinically immunogenic were immunogenic in all the platforms that were tested. On some controls known to be clinically immunogenic. Not immunogenic in all platforms? Does this mean that some platforms are limited in their predictive ability, or is it possible that different platforms are providing slightly different readout?

An example of this may be that maybe you have a product that you're testing in. Vitro adaptive immunogenicity assay that in one type of assay causes a response in one type of assay, it doesn't. You would assume without any further information. Well, one assay is clearly superior to the other. But on the flip side, maybe what's going on is that one assay is telling you if that antibody specifically is immunogenic, whereas in the other assay format, it's able to form a complex with its target and the immune complex is what's actually immunogenic. And both sets of information are true and accurate. But because you don't know the limitations of each platform, you make a very knee jerk reaction in that assessment.

So that's just something to think about in terms of why I would be concerned for the deployment of these standards. So where is the research opportunity here? I think that the

HESI AAPS consortium. Because of its limited scope is. Unfortunately not as applicable to what we're mostly interested in for generic peptides. However, I do think that there is a need to ensure and understand what these different platforms may say and look at generic sponsors to assist in this broad utility of these control standards being made in. Development both. The adaptive as well as the innate immunogenicity standards that are being prepared.

So we propose, or I should say I propose FDA should consider partnering with industry sponsors and CRO through CRCG or USP or AAPS or APS to establish a consortium for part of the roll out of these controls to ensure that they're able to be utilized across multiple formats. The consortium could also research other aspects of immunogenicity risk assessments, for example, expectations for fit-for-purpose assay validations, or applying different statistical approaches to a standardized data set to understand its impact on data interpretation. To me, the ideal outcome is for one or more white papers outlining best practices and expectations for immunogenicity risk assessments, supporting ANDA applications for generic peptides.

So that's one research opportunity. Another that I'd like to mention is on the theme of expansion. So as Manoj had mentioned, we'd spent a lot of time and effort in developing these assays. A lot of time in effort into coming up with something that would be worthy of approval, but now how can we leverage these assays for other opportunities? So the guidance again published in 2021 was limited in its scope. To only generics of synthetic origins. And there may be a number of regulatory concerns, especially at the time that was published, that would prevent generic peptides of recombinant origin from being considered through the 505(j) pathway.

Must the generic product be manufactured from the same host? Meaning, if the innovator made it in E. coli, does the generic manufacturer also have to make it in E. coli or should they make it in CHO? Oh, should they make it? Yeast. And does that influence how we interpret the immunogenicity risk? What are the specification limits of host cell proteins in the final product and how do you assess the immunogenicity of host cell proteins present in the final product?

Despite these questions, expanding the 505(j) pathway to accommodate recombinant generic peptides should be a benefit that expands availability of generic products to the American public. In the maturation of in vitro in silico immunogenicity tools may allow for consideration of recombinant generic peptides in the near future, with some additional research, I think that this is important. I'm pretty sure that other colleagues in industry will probably agree that there are opportunities out there where recombinant APIs actually provide a cleaner profile of impurities compared to some of the generic APIs that we have readily accessed to.

So what can we do on that front? Well, we actually have some data of our own that I can share. This was originally presented at WRIB earlier this year, but we took a look and thought, OK, well, when it comes to assessing the immunogenicity impact or potential from HCPs, what do our current assays tell us? And we focused a little bit more on the innate immunogenicity assays. Our general feeling is that between the in silico tools that are currently available in the adaptive immune. Tools that are currently available that many of those risks for adaptive immunogenicity can be readily assessed, but to my knowledge, there hasn't been as much published about what would we be able to do to assess innate immunogenicity risk for HCP. So this is our setup that we use. Looking at innate immunogenicity.

Very briefly, we looked at 5 donors. We saw the PBMCs from these healthy donors. We assess them for viability. We arrest those PBMCs and then treat the PBMCs with HCP in this

set of experiments. We used either commercially available HCP from E. coli or commercially available. Excuse me. HCP from CHO in the absence or presence of drug and we after treating harvest the samples where we look at the. Content of pro-inflammatory cytokines present in the cell supernatant. Or looking at the cell viability and phenotyping of the cell pellet.

So just a quick background by looking at flow, we're able to look at a wide variety of different cell types. So we have our T cells. Our B cells are monocytes. Dendritic cells are all covered in our flow cytometry view and we're able to not only assess how many are present, but also the viability of those cells at the time. We can also see here that clearly the treatment is having some level of effect. This is a look at just the viability gate pre and post treatment here across these five donors and you can see that just simply treating with HCP, we see that there's quite a big difference in the viability profile. That's good thing for us to know and obviously we have to keep that in mind when we're assessing what is the innate immunogenicity risk.

So in our first set of experiments, we looked at the presence of E. coli HCP that we purposely put in to a drug that I'll refer to as drug A. Drug A is relatively common peptide, but it's not known to have any influence on the immune system. So here you can see we look at two different cytokines. We have a very low response to the drug alone. We insert HCP at various concentrations and we see this nice. Titratable effect, where we see that there's more cytokine being elicited with more HCP being present. This carried through for a number of different cytokines and we have our LPS only control our HCP only control. They obviously responded very highly as we'd expect. So to us, we said, oh, this is wonderful news. It looks like we are able to detect if E. coli HCP were present per innate immunogenicity standpoint.

What else can we do? Well, we wanted to look and see what was the impact on other types of drugs. So we looked at another peptide, this peptide is known to have an anti-inflammatory component to it. And So what would the outcome of that be? Well, probably unsurprisingly, when we put the E. coli HCP in, we have again our LPS and our HCP control. Very high response. Our drug alone, very low response and then. The only time that we saw anything significant was at the one nanogram per mill level of HCP that you can see right here with cytokine 1. Only one cytokine, not multiple cytokines and only at the highest concentration.

Now I want to clarify. This is the exact same experimental setup. It's the exact same platform. This difference is clearly being influenced by the presence of the drug. That's the only variable here. So to us, this again demonstrates that one of the concerns when we're looking at these types of assays is that we can have great looking assay that maybe its sensitivity can be hindered depending on the drug that you're looking at.

We also looked at this in terms of the cell viability to see if we could detect anything going on. So at the exact same concentration of E. coli HCP, we noticed that in the presence of drug A, which has an inert immunogenicity effect, it doesn't prevent any immune response from happening that we saw a dramatic loss of viability at higher levels of HCP. Whereas in the presence of drug B, that very suppressive drug. But I mentioned previously, we saw that the viability of the cells was retained. So this is again demonstrating to me that this is truly a drug effect that is impacting your readout. It's not necessarily that your readout is insufficient.

We also looked then to see, well, what about CHO? Chinese hamster ovary is another expression system that people will sometimes use. So again, we ordered in commercially available HCP and we put it in the presence of drug A. We know that drug B is a little bit immunosuppressive, but in drug A we see here again, LPS works very well, but even at relatively high levels going up to. 0.2 micrograms per mil. We're not really. Seeing much in

terms of different levels of cytokine, nothing statistically significant anyways. And so it makes sense on the surface that a eukaryotic expression organism would allow for a lower immunogenicity profile from an innate standpoint. It may still carry different risks from the adaptive standpoint, but to me, this again kind of proves what the hypothesis is on paper. We would expect that the CHO should be less immunogenic in here. We can demonstrate that that is truly the case.

So what does this all mean? We believe that adaptive immunogenicity in recombinant generic peptides can be adequately assessed using in silico and in vitro assays. Our early experiments suggest that innate immunogenicity platforms such as our PBMC cytokine release assay that we've demonstrated here can provide adequate assessment of the innate immunogenicity risk of HCP present in generic drug products. In particular, we've demonstrated that the assay could detect. Immunogenicity potential in response to low levels of E. coli HCP. As expected, sensitivity, the HCP was impacted by the nature of the drug as well as the type of HCP that was present. Suppressive drugs hinder the sensitivity of the assay while also increasing cell tolerance. Higher levels of HCP and given it to mammalian origins. CHO HCP triggers undetectable levels of immune response compared to similar concentrations of E. coli HCP.

So where is a research opportunity here? Additional work and controls will be required for a thorough evaluation of innate immunogenicity platforms and their suitability for supporting 505(j) submissions for recombinant peptides, we must establish the expectations for industry to leverage in silico and in vitro platforms for supporting recombinant submissions, including appropriate controls. The levels of sensitivity that are required in statistical approaches that are acceptable. There's an opportunity to leverage learnings from parallel research going on for BSUFA 3. That's aimed for enabling quicker approval biosimilars through supportive in silico, in vitro assessments as well.

The ideal outcome again to me is guidance or White Paper on employing in vitro immunogenicity assays for 505(j) submissions for recombinant peptides and/or potentially other classes of generic drugs.

So to conclude. Substantial and combined research by FDA and industry has led to a continued maturation of both in silico and in vitro platforms are now used to support 505(j) submissions for synthetic generic peptides, and despite this, maturation of these platforms, many types of platforms and many different approaches do make it difficult to assess the validity of an assay or compare results across assays or sponsors. Now, I believe is the time for the FDA to support these research initiatives to standardize, harmonize and expand. And the utility of these assays, creating an environment where industry has confidence in their submissions will simultaneously allowing for a clear and more thorough and more standardized approach to submission evaluation for agency reviewers with that. Wanna just quick thanks to my team for helping me put together all this data and thank you all for your attention.

Moderator: Thanks Andrew. So Next up, we'll move to the topic of oligonucleotides. And we have Sudhir Agrawal who's the president of RNA Sciences and also an affiliate professor at University of Massachusetts. Sudhir's research interests have encompassed the discovery and development of RNA therapeutics, including antisense and immunotherapy and he specializes in the chemical biology of nucleotides to advance RNA therapeutics. So his presentation titled this morning is immunogenicity of oligonucleotides. Thanks.

Sudhir Agrawal: Thank you, Cameron. Good morning, everyone. Thanks for the invitation. I'm really glad to have this opportunity to share our experience with immunogenicity. These are my disclosures.

So All in all, drugs are designed to bind to mRNA. It could be coding RNA or could be non coding RNA. But these class of compounds could also be presented itself as pathogen associated molecule patterns and could bind to pattern recognition receptors. And that's the topic for immunogenicity of oligonucleotides. So there is a desired effect and there is a undesired effect. Upon binding to the RNA, it could engage multiple mechanism. It all depends on the sequence of the compound we are using and also the modifications we are using. So in simple way antisense can bind to the target and recruit endogenous RNA to cleave the target or it can based on the modification just bind to the target and modulate splicing or RNA processing as well. So there are multiple mechanisms, but it all depends on. The sequence. Modification.

So I'm gonna divide my talk into 3 portions. One will be on the chemistry and because chemistry brought all these issues we're talking about. And then come back to innate immunity and pattern recognition receptors. And then really, what can we learn from it and to see in going forward, can we improve these class of drugs so we can create better? Drugs.

So really going back to very early days of antisense, this is mid 80s, unmodified DNA were showing promise as antisense compounds but the idea was to make these class of compounds as drugs. Many modifications were explored but ultimately oxygen replacement with sulfur led to drug like properties and. I listed here three. One is a phosphorothioate DNA. Other ones are modified. RNA. We quickly learn that the modified DNA leads to RNase H engagement, because that will lead to a catalytic effect. And so this was pursued as antisense, but the other modifications were on hold because they were less potent as antisense. But I'll come back to that as well.

So, first generation antisense compounds were based on phosphorothioate chemistry. DNA looked very promising. What was studied widely in the field? This is what we can call plus RNase H mediated degradation. Focus was on antisense for antiviral and anti cancer but as these studies progressed we started to see some unintended unexpected observations of. For immune activations and other characteristics and so development of these class compounds were discontinued. But I wanna share with you what we saw as we progress this compound.

So first time when these compounds were administered, I'm gonna take experience with this 25 mer phosphorothioate DNA, which was as a complementary to HIV as an anti HIV agent. Intravenous bolus injection led to hemodynamic changes and complement activation. So this was a very confusing period in the space because the idea of complement activation is it because of sequence. Is it because of the chemistry? Is it because of any contaminant? But then it's clear that we this leads to complement activation but also affects the clotting factor. So it's not one factor, maybe a charge related, but it took us some time to understand elucidate. That this is real effect and a sequence independent. But also a length dependent effect. In addition to that, we also saw first time that these class of sequences or compounds could lead to thrombocytopenia. So it was dose dependent and also duration dependent. So if the infusion was slowed down, then the effects were mitigated. So ultimately we established that it is plasma concentration related. It is also sequence independent. But length dependent as well. But it can be avoided by slow infusion. So if the infusion rate is slower than these can be mitigated and that's where I think the the guidance from the regulator thought is at that time. Was that no compound? We should be given by intravenous bolus injection. So again, this can be mitigated.

The other observation we made when we went into clinical studies with a similar compound again in a very controlled environment with a slow infusion to avoid complement activation that the HIV infected patients who we were employing these for clinical studies showed. Immune reactions, so that was injection site reactions. Local lymph node reactions and flu like symptoms and. That was more with subcutaneous administration than intravenous

administration. But more importantly, rather than suppressing the HIV, what we saw after the analysis of the HIV levels that the levels had increased rather than suppressed. But that was transient. But the trial was discontinued. But this was an observation that these class of compounds could lead to. Immune activation in humans. But also that leads to a systemic effect and can lead to a retrovirus upregulation. And so that is again sequence dependent and highlighted in the sequence in Orange CG and I'll come back to that, that why that became important in the development of the field.

So one question, as was highlighted earlier by that, stereochemistry does play a role. So these compounds where had the 2^22-25 isomers and so we went back to the bench and asked the question is it stereochemistry? So we made R isomer. But from the immunology point of view, R isomer was less stable. So it had less immunogenic reaction but was not useful as a drug, but S isomer was more. Active as immunostimulator but was more stable, so the property did not correlate for us to pursue a stereo pure compound. So that's why everyone is still using stereo mixtures.

So by that time, we knew that the phosphorothioate DNA is causing all these issues. But then we collected the data for RNA. Interestingly, even though Sulphur is present in RNA, the complement activation and clotting factor were less so. It's not sulfur of the DNA, but when it is in RNA, it has a different effect. Immuno immune responses were also different in case of modified RNA, especially 2'-O-propyl. So the idea was. These class of compounds have a desirable properties, but they're also undesirable properties. And how can we milk them together? And that's where the chemistries came together, which is called gapmer chemistry. We introduced this in mid 90s and then a splice modulator which is completely RNA. So in antisense all the class of drugs which have been approved or are in development are employing these type of chemistries and going forward.

So this has enabled approval of candidates. So taking back what we license learn from this one was in mid 90s that antisense has the required property and it does exist. But then we are dealing with polyanionic related effects and we are related to cytokine induction. So we didn't know at that time that pattern recognition receptors were not even discovered at that time. So we knew this is happening, but then. There was a enough evidence that cytokine induction could be playing the role for anti viral. An anti cancer activity. So if those things were done in. Knockout mice for immune system then activity was lost. So it was not Antisense it was immune mediated.

So this is a a data from mid 90s we were working and I showed you the sequence we were seeing all these immune activation and complement activation. Without knowing what it is, we felt maybe sequence length dependent, so we truncated from one side or we truncated from the other side. As you can see, when we truncated from the five prime end, we mitigate the proliferation in the cells, but not from the three prime. So naively we at that time we were thinking it's sequence length dependent, but then it became clear then when we cut down the sequence and we maybe remove the CG, then we mitigate it, but then also the gapmer chemistry which is on their right hand side of their. Slide when we convert that sequence into gapmer then we mitigate that, that's what. So hypothesis at that time was maybe gapmer chemistry mitigates the immune system, but it's not. It's the CG modification which leads to the and then we sort of hypothesized and published that this may be binding to some proteins which is later on innate immune receptors.

So. This summarizes it. That antisense designed to bind to mRNA, but it's now interacting with certain proteins which is complement in clotting cascade, but also interacting with pattern recognition receptors and that could lead to mechanism of action but also safety issues. So with that. Brings back to immunogenicity because now with the pattern recognition receptor discovery, it became clear what we were seeing was. Because of this receptor.

So these receptors are present in all of us. There are nucleic acid base receptor in addition to small molecules and peptide base receptors. So most of the nucleic acid receptors are intracellular. They are in cytosolic compartment or endosomal compartments and that there are variety of them which will recognize sequence, pattern and type of nucleic acid. And so I've highlighted some of them. And they are expressed differently in species, so rodents will be different. Primate and humans are quite similar. They will recognize patterns of nucleic acid, single stranded, double stranded and there are some preferences of nucleotide structures. So for each receptor there is a requirement. So for TLR like receptor 9 it will be bacterial DNA which is unmethylated. But TLR 7-8 and 9 will be for RNA. Different type of structures, and similarly, RIG-I and inflammasome. So each one of them will be expressed in different cell type and leads to a different cytokine profile.

So what we have done was to really do a extensive SAR studies and I've highlighted that any place changes in the DNA could lead to changes in the cytokine profile. So it's very active profile. Any changes will lead to cytokine induction. Most importantly, the ends are very important. So if you block the five prime end of the nucleic acid, you mitigate the immune activation. If you provide 2-5 prime ends, you can increase the immune activation so the the ends are very important.

So this is a good example, I'll take it from one of the studies. So the center sequence which is highlighted is apparent sequence. But if you take the same sequence and insert only one RNA unit which is highlighted in orange, the cytokine profile induce varies dramatically. It could completely mitigate it. To completely change the cytokine profile and IL-6 and IL-10 ratio. So that tells you that the sequence is important, but the modifications are also important.

So this is another example of various type of DNA. This is all included in that publication. Details of the sequence. Most of these class of compounds will have a bell shaped dose response in PBMCs so that becomes important at what dose to test for looking for immunogenicity but not one cytokine depending on the structure cytokines will vary which were induced and which ones are not induced so it. Needs to be looked at as a panel of cytokine.

Similarly for RNA based compounds for TLR seven and eight that these compounds have certain compounds will have interferon. Some will not have interferon will have IL-6 or TNF or IL-10, and so that needs to be looked at.

So in summary, we have a sequence and chemistry and modification dependent desired effect which will be hybridization dependent leading to modulation of the protein expression which will be drug like property. But then we have a sequence independent hybridization independent effect and I talked to you about today. One is interaction with the pattern recognition receptors leads to. Cytokine induction and the other one will be. Polyanionic related effects, which will lead to complement cascade or polyanionic effect. But these are dose dependent. So as long as the dose is maintained there are no effects. See in human studies. But then there are some what we can call DAMPs, damage associated module patterns which will be due to chronic dosing and accumulation.

So there are well established assays used for these based on the reporters alliance or rodents or in primates or in human PBMCs I. Think one thing we have noticed that for these ones we need to use fresh PBMCs because nucleic acids are not taken up in the frozen PBMCs and the results could be very different. So always in based on gender. Or patient's profile. Or donor's profile could vary completely. The cytokine profile.

So that brings me to third topic. So we know that these class of compounds, depending on the sequence and modification have these issues. How can we fix them going forward so that way we can create better drugs because if they are interacting with these proteins and receptors means they are being bound to it, so they are being hijacked, the active compounds is being hijacked. How can we fix that?

So one of the effort we are pursuing is really to say, OK, if we have a antisense or a functional domain of nucleic acid that's in blue color. Can we change the shape of it by extending it? And so the shapes you see is like a circle or transient cyclic structure. And so in this case we are changing the shape, maintaining the same sequence and chemistry. We're changing the shape, it will change the protein binding, but upon entry into the cell, it will find the. Target and only then it opens up and in that case the compound then becomes much more specific. For this matter, I just talked to. About the role of five Prime Minister, if you block the five Prime Min. Ister on the right hand side is the CPG oligo TLR 9 icon is in this case you can see three prime, three prime linkage. So five prime is accessible with 2C activation. The bottom sequence five prime in this block. So we don't see immune activation with this sequence. So at least we mitigated this effect. But when we test this for its potency in cell culture system now based on the potent sequence to modified sequences, we are seeing about 24 improvement in potency. So this is sort of a guiding us that by certain chemical engineering we can mitigate some of these undesirable effects and then move forward in creating better candidates.

So in summary, I think chemistry has provided drug like properties to oligonucleotides but also. Has brought in some complexity, including the stereochemistry and and some of the safety signals we see, but the gapmer and the modified RNA, are now widely used. Immunogenicity is sequence dependent and modification dependent. Certainly formulation dependent as well on the the future is chemical engineering or following your tides using the same building blocks which have been used widely in the and the oligonucleotide field by chemical engaging. Hopefully we will be able to mitigate some of these risks and create better drugs, and with that, I'd like to acknowledge. Contribution of my colleagues over last 30 years. From early days in mid 80s to now and then more recently with RNA sciences and collaboration with alloy. And we know this in the audience was contributing to one. Thank you very much.

Moderator: OK. Thanks Sudhir. Last but not least, presenter in this session for our panel discussion is Raman Bahal. He's associate professor at University of Connecticut. His lab is developing new therapeutic modalities for targeting genomic DNA and RNA interface of nucleic chemistry and its delivery strategies. His presentation title this morning is immunological challenges associated with nucleic acid based therapeutics. Thanks, Raman.

Raman Bahal: All right, great. Thank you, everyone. First of all, good morning. It's my actually first time in the FDA and it's pleasure to be here. So really we have a great talk. So I'm going to kind of draw more into this like where I can present you some more case studies like what are the kind of immunological challenges nucleic acid based have been facing so far. So during Sudhir's presentation, I'm going to. Start with actually nucleic acid based drugs instead of oligonucleotide based because I want to kind of bring attention like lot of this kind of correlation between immunology. And nucleic acid actually started before the advent of these kind of antisense ways technologies.

So in general, the nucleic acid based drugs we can classify actually into 3, three types, one where we can come up with a knockdown, a messenger RNA by using technology like ASO and siRNA as explained so well and the 2nd is like some of the nucleic acids that can actually target the proteins directly and inhibit its action. And one of the example is an aptomers and there is a kind of FDA approved aptomer call Macugen, which is kind of approved. For a lot of for therapeutic purposes, and the third is like which is more recent, like

how we can increase overexpression on some proteins. And here we are the example of mRNA coming to a picture and one is like very recent example how CRISPR or chemically modified CRISPR have been used for a lot of clinical applications.

So if we look into kind of literature, there are a lot of nucleic acid based products actually being tried in a clinical trials, but there are only few that haven't actually got approval and these are kind of range from a number of diseases ranging from genetic diseases to. Cancer to infectious diseases, cardiovascular and ophthalmic diseases.

So there are kind of a lot of challenges associated with kind of nucleic acid based drug products. And one of the major challenges that kind of the research having focused on is the immunological responses. So where did this start? So this started actually long time back about like 5-5 decades historically. So the first there was a discovery of interferon production. But naturally, in a in a physiological system there are DNA and RNA sensor present as a defense mechanism. So one is called DNA sensor, another are RNA sensor and in early 1960s it had been found that the DNA sensors actually kind of activate the interferon gamma production and similarly along the period of time it had been known that there is a specialized group of receptors which are called Toll like receptor which are present more in the endosome and there are about 11 members of this family.

So the DNA sensor actually activates. The Toll like receptor 9. And whereas the Toll like receptor 3, 7 and 8 are activated by RNA and when it's come to the RNA there are more complexity involved. Whether it is a single strand RNA or double strand RNA, it can activate a different kind of immune response and as we kind of progress in number of years we kind of found out it’s not only the Toll like receptor, there are different aspects of the immune system which actually kind of activated. By these nucleic acid base systems. So in the in the summary, what we found so far that Toll like receptor 3 are activated by double strand RNA Toll like receptor 7. That is kind of active 7 and 8. It is activated by a single strand RNA but rest. Like Receptor 9 is activated by a single strand DNA.

So here just to clarify, all these are actually used for more defense system when the when the host. Infected with it, viral or bacterial RNA. These defense plays an important role in a protective measurement and over the year with the with the kind of increase in kind of knowledge about immunology, it’s been known that other than Toll like receptors, there are different epithelial cell and fibroblast present on the mucosal surface they. Can also activate the innate immune response systems. For example, there are retinoic acid induced system which actually activate the immune response. In response to the RNA and there are a lot of cyclic GMP and AMP level which are actually activated by the DNA levels.

So. So these are kind of natural sensing of the cytosolic immune system comes when it's come to the natural backbone associated with the DNA and RNA and later on as Sudhir explained really well like how the oligonucleotide chemistries actually kind of come into a role and we got an idea? That siRNA. Then activate the Toll like receptor 3, whereas the phosphorothioate ASOs it can activate at Toll like receptor 9 are considering the time frame. I'm not gonna go into details of the biology, but they are kind of from here. I’m gonna a detour and trying to Find give you some key studies that how oligonucleotide chemistries their designs have been kind of play a role. Like what kind of immune system it can activate?

This is a kind of very kind of a very comprehensive study being done as like CG dinucleotide it activate the Toll like receptors. So there is a quite a bit study done in terms of the chemical engineering, like where the CG dinucleotide should be present in a nucleotide sequence in some case in the case study have been done where there are two CG dinucleotides. Are set apart with some kind of **** pyrimidine sequences. The there are some T sequences which are kind of truncated on the three dash end and the T dash and the T nucleic bases which are added on the five dash end. And as mentioned like the five dash end is very important as

we have a more T. Sequences on the CG dinucleotide. It actually increase immune response and similarly if we substitute some of the T with the adenine, it actually decreases immune response.

So overall, what actually been found? Out like in a oligonucleotide, when there is a CG dinucleotide present on the five dash end and there is an adjacent group are present. It activate immune response. So. So this is kind of pretty comprehensive study, which could be even kind of used till now like when we are designing some kind of oligonucleotide product, what kind of nucleic nucleotides we can place in the sequence that can actually mitigate the immune response.

This is a kind of very interesting study with siRNA and I I believe like here we are, the stereo isomer actually play a very important role this in this study. The sequence and the target independent angiogenesis suppression happen with the siRNA TLR receptors. So here it has been shown like this siRNA actually can target nonspecifically to the TLR receptors and activate the immune response. So this is not kind of more kind of Watson-Crick based pairing or any Hoogsteen pairing involved in binding to the sequence, this could be more because of what kind of stereoisomer chemical modification happened? In particular, siRNA that can activate some of these immune response.

So when a lot of that information there is a quite a bit of actually matter have been used, that can actually be employed to decrease the immune response. For example, there is a kind of advent of pseudouridine having actually placed on mRNA that can decrease immune response. The presence of five dash cap on mRNA can decrease the immune activity and they were there kind of two kind of modifications being done when it's come to oligonucleotides 1. Can we do chemical modification on a specific nucleotide that can decrease immune response? For example, there are some sugar modifications have been done which can for. Methoxyethyl. And locked nucleic acid base polymers. But there are. Kind of. New chemistries are actually being involved where rather than targeting the nucleic base, there is an alternate backbone. Chemistry can be included and as Likan kinda mentioned, the morpholino or one of the examples. So where the backbone chemistry have been kind of changed and there are other example like peptide nucleic acid are actually kind of being kind of tested to decrease immune response or some of these oligonucleotide based drugs.

This isn't kind of another data publish like we\'re this as we talk about like the CG dinucleotide it activate the Toll like receptor by inducing the chemical modifications in some of these CG dinucleotide in particular it can drastically decrease the TLR 9 kind of activity. So this example. Kind of clearly shows like how the chemical modifications on the per nucleotide unit basis are overall. In the design of the nucleic acid can decrease the immune stimulatory activity, but now there is a kind of another challenge when it’s come to the delivery. In the previous studies, when it’s come to a oligonucleotide based drug, very high doses of oligonucleotide based drug product have. Been used but there is another innovative idea. Can you deliver some of these oligonucleotide to the target site of action? But not really a high dose where the delivery actually plays an important role. Control. So delivery though it having kind of really a very focus on on numerous oligonucleotide based products, but it also brings in another challenge, but it’s come to immunological responses, for example when it’s come to the delivery, the prior methods were based on only using the antisense ASO based products.

Then there is a kind of like. Lipid nanoparticle based systems have been used and there is a kind of excellent example of Onpattro that actually got FDA approval. For delivery of siRNA based technology. But there are other methods can also import like called bioconjugates where you can come up with this. Some very biocompatible ligand to conjugate directly to

some of these oligonucleotides and to deliver to the side affection. And there are kind of lot of usage on the adeno associated viral based vectors too.

So when it’s come to the lipid nanoparticle, it is a kind of excellent way in terms of nucleic acid delivery. But at the same time, there are some concern that LNP based delivery system can also activate the immune response. For example, it have been known that LNP it. Contains a lot of polyethylene glycol and PEG is kind of exhibit which can activate either IgE or complement or autoimmune based diseases. Based activation system, so you can believe like if we have some. Oligonucleotide which is encapsulated in LNP. Now we have to deal with the immune response that is associated with the oligonucleotide as well as the immune response that is associated with some of the excipients which are associated with the LNP too.

There isn't another alternative that have been actually employed by a lot of nucleic acid base companies, and one example is a bioconjugates. So there here are a few example of the bioconjugates using cholesterol or antibody mediated system and more recently like there are galactose which are more carbohydrate ligands and the carbohydrates one of the most ubiquitous receptor which are found in the physiological system. So these are more target delivery, there are less of target effects, but still it can target immune response depending upon what kind of oligonucleotide we are trying to conjugate. Some of these delivery system.

So overall, what we have learned so far, there is a consideration. And when we go from kind of one species to another and we the evolution happened. There is a different concentration about the immune system in correlation with the nucleic acid or oligonucleotides. So from bacteria to mammals, there is always an kind of activation nucleic acid system which is kind of more protective based mechanism. But as Sudhir mentioned like when we go from primates and humans the immune systems are more intact and will give us exact idea like how some. Of these oligonucleotide based drug products are going to. Respond to the immune system.

So what are the methods? Early on, we could actually imply to detect some of these nucleic acid mediated immune activation. There are some innate immune system activation. We know that there are Toll like receptor and there's some families which are actively involved and there is a complement activation. There is an immune stimulatory effect, definitely one of the assays like we could use some of the PBMC. And not the thought and not the frozen one. The fresh PBMCs itself. To find out how the immune system it is kind of respond to immune system. Another common assay that we actually use for any chemistry or design associated with the nucleic acid base product is the cytokine array analysis where we treat the PBMC cell for some of these oligonucleotides or oligonucleotide based conjugate delivery system and look into array of cytokines and see if. There is a kind of anomaly in the cytokine production. There are some standard assays, like in terms of activation of immune cells we could use and there are detection of whether it’s an early on response or later on a response. We there are a lot of kind of assays in a time dependent manner could be assessed like it is shorter in the longer time frame to differentiate between innate as well as adaptive immunity.

So overall, what my overall kind of the take home lesson is like the immune system generally so far outcomes when it’s come to a oligonucleotide or a oligonucleotide based drug products. So there is a still whether a kind of quite a bit of data is available in on particular class of nucleotides. So there's a kind of long way to learn, like what kind of nucleotides are gonna activate, which kind of immune response system? And when it’s come to the delivery system, there is another challenge that is associated with some of these oligonucleotide based drug products. That actually need to be evaluated. There is a kind of always a challenge when it’s come from transitioning from rodent study to the humans, but still.

Depending upon if they were kind of new drug delivery system have been evaluated. Still, few lessons can be drawn from some of those pre clinical studies.

With this, I would like to thank the organizers for giving me an opportunity and I would like to thank UConn School of Pharmacy for supporting my research. Thank you.

Moderator: Thank you, Raman. So that brings us to the end of our formal presentations. So I'd just like to thank all of our presenters that made this presentations this morning. So let's just give them a round of applause before we move on to the panel. Item.

Panel Discussion
So in the interest of time, we're not going to pause for a break at this point in the schedule. We're going to go directly into the panel discussion. So. Before we get started, I just want to remind our audience members that if you do, if you would like to make a public comment, please queue up at the microphones and then we will have you make that public comment before we start the panel discussion.

So. Before that happens, I would like to. Introduce the additional members of our panel that will be joining us for the discussion. So on the FDA side, studying at the far left, we have Jae Hee Lee. He's a staff fellow in chemical engineer from the Office of Research and Standards in in OGD, and his expertise is in the development and application of advanced methods in large language models, AI or machine learning, data analytics, and in silico immunogenicity assessment.

Next, we have Mohan Rajamanickam. Mohan is senior research scientist. He's in the office of. Pharmaceutical quality research is also part of OPQ. He has extensive experience with product quality and immunogenicity assessments of therapeutic proteins, complex peptides and oligonucleotide drugs, and also specialist experience in the development and validation of bioassays. From monitoring the control of process and product related impurities in the peptide products.

Next we have Paresma or Pinky Patel. She's division director of DPQA 3, the Office of Product Quality Assessment 3. In this role, Pinky leads assessment groups responsible for the evaluation of chemistry, manufacturing and controls. Thank you. Information with a focus on drug substance quality throughout the clinical development and submission of marketing applications.

And finally, we have Kui Yang. She's senior research scientist from also in the office of Product Quality Research, and she's joining us online from St Louis. Her research centers on the characterization of complex active ingredients and impurities, with a specialization in mass spectrometry of complex products and a current focus on synthetic oligonucleotides.

OK. So I guess. We don't have anyone in the audience who would like to make any comment at this time. So we'll guess we can get started with the discussion. And before I get started, I would like to invite also Eric and Likan to the podium. To join the panel please.

So. I would like to take the opportunity to thank all our speakers again for the very informative and thoughtful presentations, which lay a great ground to start this panel discussion. So the first half of our panel discussion, we will primarily still focus on generic products. I think as Rob mentioned in the next 10 years, what we have two primary goals. One is to do the things we are not able to do right now. 2nd is to do the things we make progress that we can do. But do it better. In a more efficient way? So with that, the first discussion point, which was already mentioned in several talks and wanted to see you know, elaborate further in terms of where you think we could? Start at a specific point is to enable. The recombinant peptide. The generic recombinant origin peptide. What's your

understanding in terms of the progress we have made through the synthetic generic peptide where we can leverage and what needs to be further developed to get to that point?

Manoj Kumar Pananchukunnath: Thank you. I think it's a very interesting subject. It's close to at least my heart, because we spent about three years trying to develop a recombinant peptide through. E. coli fermentation routes and only to realize that there's no pathway to move forward from an agency perspective. And then we went all the way to the start line and started working on synthetic peptides. Now I think the two outstanding issues majorly which I sees. One is the understanding around immunogenicity for recombinant RLD versus a recombinant. Follow on generic peptide and the 2nd is the aspect work. Clinical efficacy studies and the way the guidance today hits you is it's. You doing almost everything. The brand has to do to get it qualified. And if we can come out with that pathway, I think chemistry is advanced to a point. I mentioned also we see we can produce pure peptides today because of chromatographic systems have evolved to a level of efficiency and sophistication that if you want to work on it. We can remove any impurity and in the synthetic route what happens is this adds to the cost. There's a ton of solvents which gets used and stuff upstream, downstream, everything put together. But in recombinant, the upstream is very cheap. It's just food, stuff and water and some micro organisms which are gonna work on it for a few weeks or a few days. So from a cost perspective, the capability perspective if you are to say that produce a pure product, we can look at J pathway in. Some form. I think that's where we should be headed in the next, you know, several months, weeks, years, whatever time it takes. But the number of peptides which are appearing on the horizon. Are all going to present us with the same problem over and over again. And as that access question comes in, I think this is a pressing question for us to resolve.

Moderator: Yeah. Thank you.

Andrew Graves: Again from my side. I my interpretation is at the time that the guidance was initially put out in 2021. There were some concerns about what can we really know in terms of clinical risk without running a clinical study. And my impression is that there was relatively widespread belief that. We can probably take some rest and look at what's really going on from an adaptive standpoint with synthetics. But recombinant API is a whole another ball game. And through all the efforts that we've had in that time, I think now there's a lot better understanding of what assays are available and what the assays are capable of telling us. That now the question would be, are we at a place where the existing portfolio of assays, when we look at maybe how we look at biosimilars in terms of looking at those HCP values, can we take all these learnings and mold them into something that would allow? Recombinant API through 505(j). Now I'm only speaking as somebody who's primarily interested in immunogenicity, but I would argue that we're either there or we're very close to being there. My question for the agency would be if you disagree, what's the lingering concerns that we're not thinking of that do need to be addressed before that could be considered? And I mentioned this in light of, I believe there's a product that had gone through 505(b)(2), that's a recombinant that was recently maybe approved for a therapeutic equivalents. I may be mistaken, but I want to say it may have been a teriparatide product. And. My point of view is if we're getting to a point where we can allow through 505(b)(2), what's the hang up for a 505(j)? That's the genuine concern and curiosity from an industry perspective. And again, from my perspective, very willing to help map this out, but we just don't know what the hang up would be. Thank you.

Moderator: Thank you. And switch to our FDA panelists to continue.

Mohan Rajamanickam: So thank you, Manoj, and thank you, Andrew for bringing this up. And I'll just first address the question that you brought up, which is an interesting question. If we have everything that we want to approve A505(b)(2) and have therapeutic equivalents, what's hang up in the 505(j) space, the hang up is technical. So before you have the

approval of the 505(b)(2), which may involve clinical studies and we do not have the. Opportunity of such clinical studies in the 505. J space. But I would also like to point out that right from Rob. Eric. And Jan are all in the are all on the same page and we understand the importance of recombinant peptides and we are, although we are on the opposite sides of the table, we are looking at the same thing with the same context. So I would like to bring that up first and then the assays and the predictive nature of the assays is what is missing currently and with the impurities that we have with the recombinant peptides. We all agree and understand that we have an increased risk because of these impurities that could act as adjuvants and can modify immunogenicity. And so if we can have opportunities or methods that have been perfected for predicting that we will be in that space, what we want to know from the industry is like what Manoj pointed out. There is a lot of savings in the upstream and how much of that are you willing to invest on the methods that can be used to derisk in terms of host cell impurities and what is the analytical development that'll help us. Do this. That's what we're interested in and what kind of research is needed to understand the host cell impurities. Or associated variants that could be produced in the recombinant products. That's what we are interested in and I would pass it on to other colleagues at FDA.

Eric Pang: Yeah. So I would like to echo what everyone has said so far. And also to Andrew's point, I think we're, you know, very close to it, I think. You know, like the study that you presented that's, you know, something that are very interesting. I think additional research in that area will be helpful to help us understand, you know, the current battery of assays. Is that sufficient for evaluating the risk and potentially you know the additional risk of the host cell protein? I think that's what is the most important part about the recombinant peptide. So yeah, I think. In terms of pathways, you know we are open to recombinant peptides in the J space, but you know, as you understand the issue is really how do you demonstrate? These host cell remnants not going to be impacting the efficacy and safety, that's all.

Moderator: Thank you. And through I think through the talks and the discussion just occur, it sounds like much progress has already been made in the assay development, but there could be still some specific technical gaps in assay development such as assay control, specificity controls for individual assays and also. The suitability controls. So for those. Very specific technical considerations. What would be your common suggestions? In terms of how to zoom in and focus that specific point, be able to expand then to the whole space.

Manoj Kumar Pananchukunnath: So If I got it right, your question is on what do you think we can do to better the methods and assays and how do we apply them. See, right now I think most of the interactions we have are reactive. We submit something. Somebody has five questions on it. We will go back and work on it and a lot of the time companies don't just do this in House. So then you have a third player in the room which is the CRO. And I think a focused approach to seeing what could be the battery of assays and what could be the design of those assays as a topic itself as opposed to somebody submitting an assay. Anybody reading it will have 5 comments, and those comments may get incorporated. They may carry over to the next product. They may not get carry over to the next product. So how do we break the cycle of engagement, which is not productive? It's not moving us through improvement. It's only just getting 1 by 1 assays through the door. So maybe that would be my suggestion if we could set up forums or workshops specifically aimed at where we can, we can participate to address the larger question of what are. Is the ideal design as per knowledge today. What is the best conduct of the assay and how do we work towards it?

Andrew Graves: And I think that's the point I was trying to drive with my presentation is that we have these interactions. But right now they're mostly timed with review cycles and they're very individualized. Meaning, one sponsor has one review and then another sponsor has another review. And the information trickles out, but it trickles out very slowly and. Can be somewhat inconsistent, and so if there were a white paper. For and, I look at the guidance

that that formulated around anti drug antibodies is a nice example of where we came from and how we got to a place that now it's not that there are never any questions or concerns about how do I run an ADA assay. For this particular product I'm working on, but there's a lot fewer questions because there's a lot more consistency between industry and the reviewers. On what are the expectations for these assays? How do they need to run? And so using that as a template to me, I feel like the the biggest watershed was having white paper out there that, that encompassed all of these perspectives and identified. And it doesn't mean that all the gaps have to be closed right away. But at least if there's an acknowledgement of this is a gap that we don't know the answer to right now. That can also help in this forum to target for the research that we need to do to close those gaps. And my only concern is that because we are seeing now some products getting approved that there may be a complacency of sorts between the FDA and between industry saying oh, we know what we need to do now. But we're finding out that that's not always true because we. Send something in for review that we think, hey, we have everything that we need. We did this very similarly to previous products and now a new issue is a race. Right. So again, it would be helpful I think, for that transparency to be there for these are the level of expectations for what we know and these are the things that we don't know. We haven't come to a consensus on yet.

Moderator: Thank you. And anyone from the FDA side would like to comment, but then the same time, I also want to from the FDA side, if you could also comment on the assays part of one approach in silico immunogenicity, what do we need to do to enable the implementation of that, so Mohan, go ahead first

Mohan Rajamanickam: So thank you, Manoj, and thank you, Andrew for bringing up and it was eye opening to have comparison between other jurisdictions and US FDA and having tick Marks and into into those. It was a opening for us, so we really appreciate the thought that you have put into it and bringing up the differences. So I think there is a lot of confusion between the types of controls that we use for both of the innate immunogenicity and have an assay control and I don't think there is any difference of opinion with any jurisdictions in terms of assay control. The only difference comes in terms of suitability or in terms of a sensitivity level of calibrator control that we have differences in. So whenever we have a look at the RCA results and we have this common confusion of whether the test material is not immunogenic or the assay is not set up right. And so we need a control that is so sensitive to the type of test material that you. Evaluating to make sure. That the assay was done right. And the test material is not immunogenic. And that's the confusion that we constantly. Struggle with and I think it's the same struggle that the industry as well. I absolutely agree about the reactive approach that you have said. We can strive to be consistent and from your tick marks we were not able to differentiate if there is inconsistencies, but at least I think we were consistent in saying the same thing for all the assays. We can try to be consistent, but unless we know what exactly it is, I don't think we'll be able to put forward white papers or guidances, and that's what the research effort is now to develop these controls. And then like what? Andrew said after developing these controls, if we can consistently, consistently develop methods that could be implemented at different places to understand variability of the assays and differences in in terms of. Results. Then we can have an approach where everybody will agree on a control and then. That could be harmonization and that should be harmonization. I completely agree on that and this is the status quo in now. We have every new field or a modality not only related to this pathway by pathway in multiple pathways in multiple modalities. When it is new, it's always common to ask the people in the industry who. Are the experts to bring in approaches and then with the approaches to see if it is acceptable or not? And that's where we where that's how we better in and we have evolved a little bit. And that's how we are only gonna rotate. We will evolve with all your input. That's the state of signs and the state of our pathway right now and not necessarily be having information and not disseminating it are not being transparent. That's not the case. I just wanted to point that out. Thank you.

Jae Lee: So one thing about harmonization, I think Andrew also brought up, you know, maybe having the consortium when it comes to like the standards. But the other way is, you know, having this consortium also working on this White Paper idea, I think that'll be a good development as to. In terms of the in silico immunogenicity assessment tool as Doctor Pang mentioned earlier that there is a lack of access to information about the model itself for FDA to assess, especially when it comes to proprietary information, so. We have implemented a new initiative called model master file in which you can the sponsors can support or CRO's can submit detailed in silico model description, especially when it comes to proprietary information as well as the evidence of credibility. To that, that can be demonstrated using risk informed framework and an FDA will review the model independently from of a specific application and then that can promote the model reuse and also transparency and also regulatory clarity. So the goal is to use this platform. Form or a regulatory mechanism to be able to for FDA to be able to review those models that can be used for different applications for the specific scope of use. And the question of interest.

Moderator: Thank you. Is there anything to add to the in silico from the panel? If not, we'll switch gear to discuss the generic oligonucleotide drug products. This is a newly emerged treatment area compared to other drugs and obviously we're facing various technical and regulatory challenges. So the first part, we will primarily focus on discussing the API characterization. To support API sameness. What are the current technical limitations, challenges then, given those limitations, how we can? Try our best to still move things forward. What would be the strategies there? So for that I'm well first invite our online panelist, Dr. Kui Yang to make a comment.

Kui Yang: Everyone greeting. Can you hear me?

Moderator: Yes.

Kui Yang: OK, great.

Moderator: Thank goodness.

Kui Yang: So for the for the synthetic oligonucleotide API sameness as Likan presented, the major challenge is for the stereochemistry characterization, because majority of the approved oligos has modified. Internucleotide linkages and that brings in the chiral center. And therefore the. Depends on the number of the those linkages having chiral center. The number of the stereoisomers could be really big. I think it's fair to split those approved. Products into two groups. The first group is those products has relatively small number of those linkages, such as siRNA. With up to four PS linkages. Each single strand, and that brings in up to 16 stereoisomers for separation of those numbers. Of stereoisomers the current analytical capability, the current analytical technology already show have the capability for their separation. However, even in this case, challenges remain. For example, one of the challenges is. You need to demonstrate your method is fit for purpose, for example. Need to demonstrate your method is sensitive. Is precise, accurate and robust in terms to characterize the stereoisomeric composition, and to do that one approach you can attempt is design your suitability control samples. Like for example you can. Intentionally change one of the linkages. There are three configuration ratio, for example by changing the activator using different type of activator as used in your in your test product and or spiking a different activator into the user activator to change the ratio of the two activators, that will also. Operating control the R/S ratio to your fullest sequences. So those control standards can be well designed to, to be used, to demonstrate your method is fit for purpose and your method have the resolving power to show the changes in. The isomeric composition, if there is a change. If there is a difference between your product versus RLD, so the. Demonstrating the method is fit for purpose, that is. Challenging you, you need to well design and. To show the method as sensitive to. To demonstrate the difference, if there is a difference between

test product and RLD. Another challenge. It could be the way you set the acceptance criteria for the sameness in stereochemistry for example you now you have your method established and your method is validated and demonstrated for the purpose and now you measure. Your test product and measure RLD and how do you set? To the acceptance criteria to show there's a the sameness during the HSC assessment. Those can involve analyzing a number of RLD to generate the acceptable range. And also. Could involve using suitable statistical analysis. Because now you're not. Deal with just a few of percentages. That can be a bunch of a percentage and you should. Assess them and to give you the sameness assessment as a totality. Way. And another group is more, more, more challenging. That is the like those antisense single stranded antisense. Oligonucleotide product have a bigger number. Like over for example over 10 those modified linkages that can produce the huge number of so for those. Current or even near future analytical capability may not able to. To perform the separation of all of those stereoisomers. For so for that case. Then there's some technologies, or the OR some approaches can be used. For example, you can break down the full length into shorter fragments. Then you look at those shorter fragment piece by piece and then put them together to categorize the stereochemistry for the for the for the full length or. Other technologies that is, or something separation. To LC and MS. for example. Like ion mobility technologies, way that add additional separation. By utilizing the size or shape of those isomers in the gas phase so that can also facilitate some separation that when able to achieve. Yeah, by LC and MS. So yeah, that's it.

Moderator: Yeah. Thank you for that very informative comment. And if any of the FDA side think you have anything?

Paresma Patel: Yeah. I mean, I think Kui kind of touched on the most important parts of the API sameness piece. And I think in my mind at least personally, I think the oligonucleotide space is particularly challenging for generic drug development, a little bit more so than the peptides slightly less. I think mature right and I think the other piece that's difficult here is right. These are just inherently heterogeneous molecules because of the manufacturing process. On top of that, you also have structurally complex complexity depending on the type of oligonucleotide you have. So you have aptamers that are 3D and then you have these other you know and how they even act are different. The mechanism of action it's like. So in reality I feel like there are really three classes of drugs, but we just lump them in as all. Oligonucleotides. But I think the biggest challenge here is likely also the analytical capabilities and where we probably need a lot of research in thinking about how do we deal with all of the impurities, right? All of the known impurities, the additions, deletions, but then the other known impurities and what level is going to be acceptable for a generic right? And this is where the new drug development has. This advantage of having the clinical studies right? And I think this is an area which also right we really need harmonization. This is the area we need global harmonization and I understand the challenge because I think now that EMA at least has some sort of draft guidance out there, industry has a little bit more direction. And I think internally we you know, we have a lot of conversations about. This and generic drug development is relatively new in this space now. So I think, but I do think this is an area where there could be a lot of research and thinking about how to approach. This in the methods that would be appropriate for approaching it too, and then that would tie in very clearly with control strategies.

Moderator: Yeah. Thank you for those comments. So I really personally, I really appreciate the talks from Sudhir and Raman like working us through the history of oligo and it sounds like actually from chemistry standpoint lots has been done in the past 20-25 years. So what we have known about the chemistry I would be curious to know from your perspective how those known chemistry can guide us to develop those. Targeted methods to really understand the alternative and to deal with the current technical limitations.

Sudhir Agrawal: I think the issues which are brought by Kui and Pinky up there real, these are complex molecules and. Stereochemistry does play a role as we know it. In addition to sequence, and I think it analytical tools are available to help. But they're not there to analyze how many isomers are present. And so I think those tools needs to be developed, but I think one can also learn from approved drugs because there are different batches made at different times and so how they are consistent from each other. Are the same methodologies used and that could create a consistency and create an experience that by different batches using chemistry conditions purifications? How those batches are qualified and that could be guidance for the generic people that that methodology can be seen as. I think you know just simple step of sulfurizing reagent change could change the manufacturing process. Purification can change the outcome as well. So all of those have to be standardized.

Raman Bahal: Great, I do agree. They're oligonucleotide drug products based overall, like if you're looking to the basic science behind it, it is complex and but it is evolving at the same time. So so let me break down into into two parts. One is why it is complex. One of the reason is when you go from one sequence to another one, there was a different purine to pyrimidine ratio. So which is it's difficult to draw a lesson from here and apply to other one. So and at the same time the another is the length, the length of the nucleic acid. Some people say like if you use 17 monomer you can apply a sameness 24. That's not true actually, because if you have something more 24 which are more CG dinucleotide that can form secondary structure so so I believe like yes there is a room. What what we could do is like take one class of nucleic acids and try to map out what are the lessons we learn from it. And then go from there step by step. But definitely there is more work need to be done to even draw the map for that one class of drugs based too.

Manoj Kumar Pananchukunnath: Can I just add on to that please?

Moderator: Yeah.

Manoj Kumar Pananchukunnath: So sitting here listening to the conversation, I'm going almost 1 1/2 decades back to go back soon and lo and. Those were equally complex. Those days, methods were not known. I think a bunch of universities were done a lot of stuff to create knowledge base and stuff and we are sitting probably in that space where either the methods are not suitable, they're not capable, or we have to bring in new technologies. And as I speak, when we talk to companies which make analytical instruments, they're focusing this as a research area that you know, how do we increase the sensitivity of the mass spec? How do you increase the separation of the column chemistry? Stuff like that. But I just want to point out one big, big issue which I think is going to pop out is the way we evolve ourselves is today's sameness is three by three. And I thought you also mentioned about multiplicity of. RLD lots. I think one of the products in my past experience, we use 90 lots to arrive at the specification ranges for. Acceptance because of just because of the heterogeneity and the wide variability in the brand itself. And you wanna capture that accurately so that you get a range for your product. Oligo some of the products are $125,000 a vial. Some of them are $200,000 a vial and imagine us doing a three lot pH measurement to compare. Well, the pH of the RLD is similar to our product. $600,000 for pH value osmolality, so the standard protocol we have will force us both agency and the industry to work on a three by three or maybe even more comparison wherein the concerns lie on the more serious tests, the isomer contents, the, the, the fragmentation and and how how the structure is built up? So I think there is a need for us to evolve guidances which kind of keeps some of these. Nice to have tests. It really doesn't matter if pH is 3.1 versus 3.7. We we can argue for a long day about the significance of that difference, but I'm more worried about the amount of samples we would procure from the market and use for studies, because every vial is 1 product of the market for rare diseases and already low availability of. Products in the market and it's unaffordable. So food for thought. I mean my request is

because if you think about evolving Guidances separately for oligonucleotide. It'll help all of us. Be practical and scientific at the same time.

Likan Liang: Here is a verbatim transcription of the text with grammar corrections and repetitions removed, staying as close to the original content as possible:

So I want to address some of the past data questions. For those with lower numbers, that's probably more straightforward and we can just think about it as part of the active ingredient. So if you are able to analyze individual serums, then set a specification, just like part of the API. That's easy.

It will be more challenging when you have a lot of modern serums and you cannot find a single serum on the curve. For example, if you have 2 million or a million and you have a sharp peak, how can you tell what the sameness is?

But if you spread it out into really broad peaks of curves and multiple curves because you have impurities there, and if you think about what level each dye has and the total 100% divided by whatever - for example, the siRNA 16 oligomers, so 100 / 16 is about 6 point something, so each one is 6 point something there.

Is there any influence from your method on the accuracy of that peak? And if you're talking about huge numbers and each one is tiny, there are a lot of challenges for the method itself. On top of that, for those broad peaks, what is the acceptance criteria when you cannot look at the individual anymore?

For example, if you use PCA to compare the shape of the curve to try to set up some acceptance criteria, it actually depends on how well your method is dissecting this information. If you have a really narrow peak for a million versus a huge broad peak, you probably have different kinds of accuracy in terms of controlling the sameness. Even though you can use some kind of statistical methods for the PCA - okay, that's statistically similar enough from a statistical perspective. But if your method is not able to spread out those dyes, a sharp peak being the same doesn't mean much, right?

Moderator: Thank you. In the interest of time, the last topic - but not the least - knowing how immunogenicity in general is a challenging technical question, and knowing the heterogeneity associated with oligo products, I would like to get comments on how we can initiate some work in this space. Knowing the technical limitations and challenges, such as impurities cannot be characterized to the similar level we can do for peptide products, how do we get to the point where we can really start to involve more clinical relevance considerations into our development and assessment?

Raman Bahal: I think one question to address, for example in siRNA space, there is less task to humor versus single-stranded. In peptides, it's also very important in the oligonucleotide space too. We do come across those challenges when we have like N-1 or N-2. How to characterize those things in quality control space? At the same time, if we could use some appropriate controls which in fact has N-1 or N-2 kind of oligonucleotides, and we spike them in some of those immune-related experiments, we can actually get some kind of acceptable limit too.

There are some standard assays that could be used, which are more PBMC-based or flow cytometry-based, that can set up the bar for what percentage is going to be more acceptable in those kinds of criteria.

Sudhir Agrawal: I think when we look at it, the discussion is mostly on stereochemistry, but also when you manufacture these compounds, you have a parent peak which is required as

a drug, but then you have failed peaks. You go through a purification which separates out based on the charges, and that will lead to the parent product. But there's always what we call N-1, which are failed sequences, and the goal is to remove that during purification, but always there is 5 to 10% left, which is a heterogeneous product.

So I think one way to resolve this would be to fix that this should be the minimum amount present of the apparent peak or parent product, and that could be a percentage of 5 to 10% or whatever it is of the hetero mixture. And then looking at it as a total mixture, because now the parent peak is well established as a drug and its stereochemistry, but the uncertainty is the hetero peak. Can that be purified and analyzed for immune reaction separately and along with the parent peak or as a separate peak?

But then what assays are supposed to be used? We have used essentially human PBMCs, but there are caveats of gender response, age response, race response, and the donor's background. Those need to be looked at, and it's not one-sided. A panel of cytokines flow is very informative and gives a lot more data because then you can look at multiple cell populations in one assay, but those need to be standardized.

Mohanraj Manangeeswaran: Thank you. I agree with both of those comments. Heterogeneity is key, both in terms of stereoisomers and in terms of sequences or failed sequences, remnants, whatever. If we do not have methods currently to completely characterize the product and compare it to the reference product, that residual uncertainty is going to jump onto the safety of other areas including immunogenicity, so that's key.

In terms of immunogenicity risk, I think we are far behind compared to peptides. What was explained in terms of using PBMCs to look at the totality of whatever is in your product and compare it to the reference to see if it elicits any kind of activation or secretion of cytokines, it's possible to understand the innate immunogenicity risk.

As mentioned, we will have issues with the controls. We may need specific controls because we have developed them for peptides and have used innate immune response assays in that space. We may have to have controls specific to the oligonucleotide products like an N-1 or a kind of polynucleotide that's modified, which could be used as our control or suitability control for the assays. We may be closer to developing an innate immune response assay that's specific for oligonucleotides.

But when we come to adaptive immunogenicity risk, even in terms of NDA products where we do clinical studies, we do ADAs and we have an anti-drug antibody response, and we may even have an anti-drug antibody that may have relevance in terms of increasing or decreasing PK, but we do not really understand the mechanism by which oligonucleotides elicit an anti-drug antibody response. So it's going to be a bigger challenge in terms of using assays to monitor adaptive immune response.

I think we understand the cost of a vial of oligonucleotide and we also understand that many times it's used in rare diseases, and the amount of available drug in the market should not be taken away for something that does not have a real clinical risk. So we are looking into the context, and together as a community, we are looking into what is the clinical relevance of immunogenicity and how that can be addressed in terms of the adaptive immune space. We are still evolving and hope we will have techniques to look at that risk, but we are not there yet in terms of adaptive immune response.

Moderator: Okay, thanks. I guess we're out of time now. I would just like to thank all of our panelists and presenters that have contributed to this engaging discussion. Certainly, we ought to take a lot of this information home and see where we move forward. So thank you everyone. Let's give a round of applause to everyone.

Thank you. So I think we're going to pause for lunch now. I believe we're coming back at the same time, 1:00.