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FDA CBER OTP Town Hall: Gene Therapy Chemistry, Manufacturing, and Controls

April 25, 2023

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DR. NIRJAL BHATTARAI: Good morning, everyone. Thank you all for joining us for today's town hall. Today's event is hosted by the Office of Therapeutic Products [OTP], formerly known as the Office of Tissues and Advanced Therapies, within the Center for Biologics Evaluation Research [CBER] at the Food and Drug Administration [FDA]. My name is Nirjal Bhattarai, and I'm the chief of the Tumor Vaccines and Biotechnology Branch in the Office of Cellular Therapy and Human Tissue within the Office of Therapeutic Products, and I'll be your moderator for today's event.

So, our topic for today's town hall is gene therapy chemistry, manufacturing, and controls, or CMC for short. Some of you may have joined us from previous town halls, including our September 2022 town hall, which also focused on gene therapy CMC. For those of you who have joined us before, welcome back. For those of you who are joining us for the first time, welcome and thanks for attending today's event. Next slide, please.

So, before we begin, I want to share some background about OTP's town hall series. OTP launched its first town hall series in 2022 to engage with product development stakeholders and researchers to discuss topics related to OTP-regulated products. These town halls have a question-and-answer [Q&A] format with the goal of providing regulatory information to stakeholders to help advance drug development. All of the recordings from previous town halls are on FDA.gov, and we encourage you watch them for additional information. Next slide, please.

Please note that this town hall is being recorded. The recording and the event materials will be posted on the FDA's website in the next few weeks. We will also provide a transcript. Closed captioning for this event is directly available in Zoom. This event is a question-and-answer format. If you have a question, please type your question directly in the Q&A box in Zoom. The Q&A box can be found at the bottom of your screen in Zoom. We appreciate the questions submitted in advance and look forward to seeing your questions today. We will do our best to address as many as we can today, but please note that the FDA is not able to comment on or answer questions regarding specific investigational products or drug applications. We will also not address any questions that we consider out of scope for the event. Lastly, please use the chat box if you are experiencing any technical difficulties. And let's get on to today's event. Next slide, please.

As many of you know, the FDA requires sponsors to provide information about CMC as part of an investigational new drug [IND] or biologics license application [BLA] for gene therapies and other products. The CMC information should describe the sponsor's commitment to perform manufacturing and testing to ensure product safety, identity,

quality, purity, and strength, including potency. This is why we're here today: to help answer your questions about gene therapy CMC.

I'd like to take a moment to introduce today's panelists: from the Office of Therapeutic Products, Office of Gene Therapy CMC, Dr. Andrew Harmon, who is a team lead in Gene Therapy Branch 1 within the Division of Gene Therapy 1; Dr. Anurag Sharma, who is acting team lead in Gene Therapy Branch 2 within the Division of Gene Therapy 1; and Dr. Graeme Price, who is a team lead in Gene Therapy Branch 4, Division of Gene Therapy 2. And all of our panelists today are from the Office of Gene Therapy CMC. And I also want to thank our panelists for your time today. Next slide, please.

So, we will now move on to the Q&A portion of today's town hall. We'll begin by answering questions submitted during the registration process, followed by responding to your questions submitted during today's event. As a reminder, you can submit your questions to our panelists in the Zoom Q&A box at any time during the event, which can be found at the bottom of your screen in Zoom. We'll try to address as many questions as we can. Plus, please remember, we are not able to discuss anything specific about investigative products or drug applications. We will not be also able to discuss any questions related to our draft guidance documents under public comment period or under revision or final guidance document publication. We hope you can stay with us for the entire time but would like to reiterate that the town hall is being recorded, and you can visit the full discussion after it is posted on our website.

With that, I think we can start with our first question. Next slide, please.

All right, so the first question we're going to discuss today is:

What is the difference between characterization testing and release testing?

And Dr. Graeme Price will respond to this question.

DR. GRAEME PRICE: OK, good morning, and thank you for the question. So, the key difference between characterization and release testing is [the purpose]. The purpose of characterization testing is to gain information on the product, whereas release testing is to demonstrate that the product is of an acceptable quality for use. The release test results should be reported on the product certificate of analysis, but characterization test results need not be formally reported. However, both release and characterization test results should be recorded and submitted to an IND or BLA at relevant places in Module 3.

Now, we strongly encourage characterization testing of gene therapy products. Information gained from characterization testing is valuable for multiple purposes, including for identifying critical quality attributes; guiding analytical assay development, particularly in the case of potency testing; and for establishing product comparability if the manufacturing process changes.

Appropriate types of characterization tests vary from product to product. The examples are extended assessments of cell surface phenotypic markers, such as those associated with immune cell activation, differentiation of exhaustion in the case of ex vivo modified cells, and characterization of non-vector DNA impurities inside capsids by next-generation sequencing, vector genome size analysis and mass spectrometry to detect capsid amino acid modifications for AAV [adeno-associated virus] vector products. So back to you now, Nirjal.

DR. BHATTARAI: Thank you, Graeme, for that response. Next slide, please. So the next question is:

At what stage of product development do release assays need to be qualified?

And Andy will respond to this question.

DR. ANDREW HARMON: Hi. Well, as something to strive for, I'm going to start by saying that many experienced gene therapy manufacturers actually qualify their assays at the very start of clinical studies. And that type of focus on the analytical development really aligns with our general advice to begin with the end in mind. While qualification from the jump is the best-case scenario, I can also say that, in general, you will have to have noncompendial assays qualified before initiating any studies that are designed to obtain primary efficacy data for a license. You may notice that we use that specific phrase, "studies designed to obtain primary efficacy data," and that's because, in the rare disease space, a pivotal study can sometimes occur before a conventional phase 3 study would.

I also want to highlight some other key situations that could affect the timing of assay qualification. The first is, if you're preparing to perform analytical comparability in order to implement a manufacturing change before a pivotal study, in this situation, it's our recommendation to qualify noncompendial assays before you start your comparability study so that you can enhance the precision and the quality of the data obtained from that comparability study. The second situation has to do with strength-determining assays, specifically for AAV vector-based gene therapy products, which are often a qPCR [quantitative polymerase chain reaction] or ddPCR [droplet digital PCR] assay. OTP has seen just how critical the strength assay is for accurate dosing and safety of AAV products. And because of this, we expect this assay to be qualified prior to initiating any clinical study. This is also unique in that sponsors need to fully validate that AAV strength-determining assay prior to a pivotal study, and this is much different from other assays, where we expect validation to occur prior to a BLA submission. Nirjal, back to you.

DR. BHATTARAI: Thank you, Andy, for that response. So next slide, please. So our next question is:

What is the difference between assay qualification and assay validation?

And this will be responded to by Anurag.

DR. ANURAG SHARMA: Hello, good morning. So, the objective of both assay validation and qualification is to demonstrate that the assay is suitable for its intended purpose. For qualification, the assay performance is tested for the parameters such as accuracy, precision, specificity, sensitivity, linearity, and range for its intended application. In general, predetermined assay performance acceptance criteria are not required for qualification, but the qualification data can help identify the variability in assay attributes to reoptimize the assay to achieve acceptable performance. The assay qualification data also form the basis for developing a scientifically sound validation plan and establishing predetermined acceptance criteria to demonstrate that the assay does what it is intended to do on a routine basis.

The assay validation is more comprehensive in nature and in addition to parameters that I stated earlier. The assay performance is tested for robustness to determine variability under different operational conditions, such as hold times, temperature, seeding density, region concentration, etc. And, as pointed out by my colleague Andy in the previous question, you are required to qualify all noncompendial analytical assays on lot release before initiating clinical studies that are intended to provide primary evidence of effectiveness to support a marketing application. And this assay will need to be validated prior to submission of a license application.

For additional details, please refer to ICH [International Conference on Harmonization] Q2 guidance on validation of analytical procedures. Thank you. Back to you, Nirjal.

DR. BHATTARAI: Thank you, Anurag. That was really helpful. All right, so next slide, please. So, next question:

What are the FDA's expectations for potency testing at phase 1 or 2 versus phase 3 studies?

And Graeme will respond to this.

DR. PRICE: Thank you. So, potency testing and potency assay development may be challenging. The purpose of potency testing is to ensure [that] a product is able to perform its intended function when administered to patients. Because products differ widely, there's no one-size-fits-all potency assay. Potency tests have to be specifically designed for each product and should reflect the product's proposed mode of action. For example, in the case of a gene-modified T-cell product, it may be reasonable to assess secretion of a particular cytokine following stimulation with cells expressing the intended target antigen or measuring the ability of modified T-cells to kill target cells in a cytotoxicity assay. For a directly administered vector product such as AAV to treat a metabolic disorder, measurement of enzyme activity in transduced cells may be appropriate.

Now, a key consideration is a distinction between measuring gene expression and showing product activity. At early stages of product development, it may be sufficient to demonstrate which transgene is expressed. However, potency testing that demonstrates

product function should be in place and qualified prior to the initiation of clinical studies intended to provide evidence of product efficacy in support of licensure.

In some cases, a single test may not be sufficient to show product function. So, potency may best be determined by a panel of tests assessing different aspects of the product that contribute to its function. A potency test must be fully validated prior to submission of a biologics license application. Potency testing should also be performed as part of a product stability assessment program.

Now, a good potency assay should reflect the product's mode of action and be reproducible, accurate, robust, and stability indicating. Potency assays should also be quantitative and allow lot-to-lot comparison of a product.

We recommend a stepwise approach to potency assay development, where information gained from investigating biological activity of a product for characterization studies is used to guide potency assay design and optimization. It may be advantageous to assess multiple potency assays simultaneously during product development, discarding those that are not fit for purpose and carrying suitable assays forward for qualification and eventual validation.

Of note, the potency test must be fully validated prior to submission of a BLA, and I'll emphasize that. Potency testing should be performed as part of a product stability assessment program.

Now, for additional information, you can refer to the 2011 FDA guidance titled "Potency Tests for Cellular and Gene Therapy Products" and the 2020 CMC "Information for Human Gene Therapy IND Applications" guidance, which we generally refer to as the gene therapy CMC guidance. These and other useful guidance documents are available in the Cellular & Gene Therapy Guidances section of the FDA website [https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/cellular-gene-therapy-guidances] and really are a great resource for sponsors. We encourage you to look at them. So, back to you, Nirjal.

DR. BHATTARAI: Thank you, Graeme. That was really good. So, next slide, please. This question is for Andy:

A variety of methods exist to determine the percentage of full, partial, and empty capsids in AAV final drug product, and each method may lead to different results. Does the FDA have a cut-off for the percentage of full capsids required for AAV products?

DR. HARMON: So, this is a very important topic, and there's actually a lot of exciting work happening in the field on characterization of capsid content and considerations of what actually constitutes a full, partial, or empty capsid. What I can say is that the FDA is not prescriptive on the particular analytical method you choose to assess full capsids for product release. We also cannot give you a "magic number"—a percentage of full capsids that would be, quote-unquote, "acceptable," and that's for a number of reasons.

The first is that different analytical methods are going to give you varying results, even if you are testing the exact same sample. Additionally, different products and indications have different tolerances for empty capsids based on the proposed dose, indication, and route of administration.

All of that being said, if you intend to release an AAV vector product where the vast majority of the product is empty capsid impurity, then you're likely going to see a lot of questions from the FDA about why you believe these levels of impurities are justified for your specific product and clinical scenario. And that's regardless of the assay you choose to use to assess the percentage of full capsids.

Ultimately, it's very important that, as a sponsor, you have a robust downstream manufacturing process in place that is going to result in a product with high purity and that you obtain data as early in product development as feasible with your chosen capsid assay or assays. And this data is going to be important for monitoring both your process and setting initial acceptance criteria for that percent full attribute. Nirjal, back to you.

DR. BHATTARAI: All right, thank you, Andy. That was good. So, next slide, please. The next question is:

What is the FDA's expectation on the use of viral vector reference materials?

And this will be responded to by Anurag.

DR. SHARMA: Thanks, Nirjal. So, we encourage sponsors to use viral reference materials to calibrate their internal reference standards and then use the internal standards as controls for routine use in their analytical assays and for validation of assays, which may include particle count, infectious units, capsid content, vector copy number, potency, etc. So, well-characterized reference materials can serve as our fixed reference point and can help to improve the precision of your analytical assays.

We believe that the use of viral vector reference materials would lead to production of more consistent, safer, and higher-quality vector products. The use of viral reference materials would allow comparison of different clinical and nonclinical studies across the field, which may also assist in the development of regulatory policy regarding the use of various gene therapy viral vectors.

A few viral reference materials are already available, such as AAV serotype 2 and AAV serotype 8. A lentiviral reference material is being developed and is almost ready to be available to users. Similarly, efforts are ongoing to replenish the now-exhausted AAV serotype 5 reference material.

Developing these reference materials is not CBER's primary responsibility, but we support the development of these reference materials. And organizations such as the Standards Coordinating Body [SCB], NIST [National Institute of Standards and Technology],

ISBioTech, and others are primarily involved in developing and maintaining these reference materials.

We also encourage industry to lead or contribute to developing such consensus reference materials, which will be greatly beneficial to the field of gene therapy. And, if needed, we will be interested in supporting such efforts by providing our input. Thank you. Back to you, Nirjal.

DR. BHATTARAI: Thank you, Anurag. Next slide, please. So, the next question is:

Can we make changes to the manufacturing process during our pivotal clinical trial?

And Graeme will respond to this.

DR. PRICE: Manufacturing changes during late-phase clinical studies are one of the major concerns that we have in the lead-up to BLA submission and during BLA review. While we understand the need to introduce manufacturing changes to facilitate scale-up and scale-out in preparation for commercial manufacturing, our experience is that this can lead to challenges down the road. So clinical assessment of product safety and efficacy, including establishing product label claims, is made much more complicated when changes are introduced during pivotal studies. And this can potentially reduce the number of product lots and hence the number of treated patients included in the clinical efficacy assessment dataset.

Similarly, commercial specifications are based on lots shown to be safe and effective in clinical studies. So, there's a risk that specifications established using a different process may not fully reflect the commercial process, and this may lead to a higher number of other specification lots. So for these reasons, we strongly recommend that you use your intended commercial manufacturing process to make product for pivotal clinical studies and do not make changes to that process once these studies are underway.

Now, one of our major BLA review criteria is that the future commercial product is comparable to the product used to demonstrate safety and efficacy in clinical studies supported with a BLA. If manufacturing or analytical method changes are introduced during these studies, you will need to show comparability between lots manufactured using the pre-change and post-change processes. The burden of establishing comparability increases with the phase of product in clinical development. So, if changes are made during later phases, then comparability studies will need to be more robust.

The goal of a comparability assessment is to show that the product quality attributes are highly similar between the two processes and have no adverse impact on product safety or efficacy. A comparability assessment should use appropriate analytical methods, qualified where appropriate, to examine multiple quality attributes and have predefined acceptance criteria that reflect an acceptable level of variability.

I'll emphasize that simply showing that the new process can generate that meet established lot release acceptance criteria is insufficient to demonstrate comparability. A good comparability assessment will look at multiple product attributes, and a thorough understanding of product quality attributes gained through characterization studies can make the design of comparability studies and setting of comparability acceptance criteria much easier. When designing comparability studies, we generally recommend the use of statistical methods to assess equivalence, such as the two one-sided t-test method.

Now, ideally, comparability assessments would be performed in head-to-head studies directly comparing products resulting from the two processes. A good example of this is using split apheresis runs for ex vivo modified cell products. Here the same cellular starting material would be split into two portions, with the old and new manufacturing processes performed on each portion in parallel. Now, this approach is particularly useful for controlling variability in the starting material but is not going to be applicable in all product classes.

If you can't demonstrate comparability, then additional clinical studies may be required. So, therefore, we recommend that you consult with your review team before implementing manufacturing changes and when designing comparability studies.

Ultimately, making manufacturing changes during pivotal studies is something we strongly recommend you avoid. Using the lot commercial manufacturing process in pivotal studies is much less complicated, reduces risks to commercial development, and saves you time and resources in the long run. Back to you, Nirjal.

DR. BHATTARAI: Thank you, Graeme, for that response. Next slide, please. So, our next question is:

What are the CMC requirements to start a phase 3 clinical trial?

And this will be responded to by Andy.

DR. HARMON: Thanks, Nirjal. So here, again, we have to be careful of relying on the conventional phase 1, 2, and 3 designations. While many gene therapies do follow this development path, for certain rare disease indications, the main efficacy study may occur earlier than the conventional phase 3 study. Also, this is actually a huge question, so for this town hall, let's focus on some of the major CMC considerations for getting ready for a pivotal or efficacy study.

Firstly, you're going to want to give some serious thought about whether your manufacturing process, manufacturing facility, and analytical testing are the same or fully representative of what will be proposed for commercialization. As my colleague Dr. Price just discussed in the previous question, we have seen the potential for significant issues regarding product comparability and significant issues regarding pooling of clinical data when manufacturing changes are made in the middle of a pivotal study. So it's always our

advice to think with the end in mind and deal with manufacturing changes and comparability exercises before starting your pivotal study.

Another big area of focus before the pivotal [study] is your approach to release testing of your drug substance and drug product. At this point in product development, you need to have your potency assay or potency assays in place for product release. This was discussed in more detail in an earlier question. But your potency assays should reflect your product's mechanism of action and be appropriately quantitative. Prior to a pivotal study is when you'll be qualifying all of your noncompendial release assays, including your potency assay. And remember, there's also that special situation if you are a sponsor of an AAV product, as prior to your pivotal study is when you'll be fully validating your AAV strength-determining assay.

You should also be using your manufacturing data at this point to really tighten your release acceptance criteria. And this should be done prior to your pivotal study, because you want a very consistent product to be used to obtain your efficacy data.

One of the other things we want sponsors, especially of in vivo gene therapy products, to be aware of is that the expectation for a commercial product is that dosing will be based on labeled nominal strength. Your pivotal study should reflect commercial expectations, and for CMC, that means you need to start releasing product lots based on a strength target and an acceptable strength range of your drug product.

As always, the 2020 gene therapy CMC guidance is really the first place to start when assessing the state of your CMC program. This guidance also makes reference to other specific guidances related to topics like validation of analytical methods. So I would recommend taking another look at the CMC guidance at each stage of your product's development. Back to you, Nirjal.

DR. BHATTARAI: Thank you, Andy, for that really comprehensive response. So next slide, please.

What is the FDA's expectation for viral clearance studies for gene therapy products?

And this will be responded to by Anurag.

DR. SHARMA: Thanks. So we recommend that you include viral clearance steps in your manufacturing process when possible. And the inclusion of viral clearance also depends on the type of product. For example, it may not be feasible to add viral clearance steps in the manufacturing of cell-based products or even certain viral vectors. However, viral clearance may be crucial for AAV-based products, especially those utilizing helper viruses, such as herpes virus, adenovirus, or baculovirus, and for insect cell lines as producer cells may harbor a retrovirus.

So, you cannot completely guard against contamination either against unintentional introduction of adventitious viruses or with other viral products manufactured in the same multi-product facility. If your process has built-in robust virus clearance steps, then it gives us additional assurance regarding product safety. And this may help you by not having to reject the lots or even mostly avoid consequences, such as manufacturing shutdowns or adverse events in patients. Based on our experience, designing robust viral clearance steps in your manufacturing process will help you to avoid these potential issues.

In addition to viral clearance, your virus safety assurance strategy should also include measures to prevent introduction of contaminating viruses and testing of materials at various process stages, such as cells and viral banks, bulk harvest, and final product. In general, we recommend you include orthogonal steps that depend on multiple mechanisms to remove viruses. Having robust virus clearance steps, such as detergent treatment, chromatography, nanofiltration, etc., in the manufacturing process and adequate testing of the potential viruses will allow initiation of phase 1 clinical trials. You should have validated viral clearance studies by the BLA submission demonstrating several logs of viral clearance using relevant model viruses.

We don't have defined expectations on the levels of viral log reduction that should be achieved, but you should provide justification for the levels that you are able to achieve and that they are adequate to support product safety. Please also be aware that whenever you make significant changes to your manufacturing process, you should consider and evaluate the effect of that change on viral clearance.

For additional information on this topic, please refer to ICH Guideline Q5A on viral safety evaluation. Thank you. Back to you, Nirjal.

DR. BHATTARAI: Thank you, Anurag. Next slide, please. All right, so the next question is:

What information should I submit if I want to change an analytical method?

And this will be responded to by Graeme.

DR. PRICE: OK, so in general, analytical method changes should be submitted as an amendment under the IND or a prior approval supplement to the BLA. The type of information that you should submit should include a detailed description of the new method highlighting differences from the previous method, the rationale for the change, a risk assessment of the potential impact of the method change on product quality, a package providing assay qualification or validation data as appropriate for the phase of product development, the proposed acceptance criterion and a justification for this acceptance criterion, and results of a bridging study to show equivalent or better assay performance from the existing method.

Introduction of new methods late in product development can be problematic. As we noted in earlier answers, commercial specifications are set based on batch analysis data from products shown to be safe and effective in clinical studies supporting the BLA. If test methods or specifications change during these studies, it can complicate both assessment of the clinical data and specification-setting and may result in some lots being excluded from these analyses. If methods change late in product development, it may be [possible] to test retained samples of pre-change lots using the new method. However, this may not be feasible in some cases, and we would recommend that you contact your review team for advice prior to implementing any testing or manufacturing change late in product development. Back to you, Nirjal.

DR. BHATTARAI: Thank you, Graeme. Next slide, please.

So, the next question is:

When preparing a BLA submission, can CMC information be incorporated into the BLA by reference to a master file?

And Andy will respond to this question.

DR. HARMON: So, for any question about master files, one of the best resources out there is the combined CBER and CDER [Center for Drug Evaluation and Research] guidance that was published or revised in November of 2019. This guidance specifically discusses the FDA's approach to the use of master files in BLA applications under the Public Health Service Act, and that would include BLAs for cell and gene therapies. Specifically, the guidance states that the FDA generally does not permit applicants to include information about drug substance manufacturing or drug product manufacturing by reference to a master file.

So, let me give you a few examples. If you are a sponsor developing an AAV product and you use a CMO [contract manufacturing organization] for drug substance and drug product manufacture and reference that CMO's master file in your IND, you would not be able to use that master file reference in your BLA. You would need to provide full details of drug substance and drug product manufacture in Module 3 of the BLA. And the same goes for information and data for critical manufacturing components. As another example, let's say you are developing a CAR T-cell product and using a lentivirus vector to modify the T-cells. If you use a CMO to generate your lentivirus and your CMO has a master file for lentivirus manufacture, then at the IND stage, you are good to go for referencing that master file in order to cover vector-specific CMC information. However, at the BLA stage, you would not be able to reference that master file for lentivirus manufacturing. Your BLA would need complete manufacturing information about the vector, and this information would be placed in a separate drug substance section of the BLA, even though the lentivirus vector is a critical manufacturing component and technically not a drug substance.

So, the take-home message is really that at the BLA stage, we generally expect all manufacturing information to be submitted directly to the BLA. As a sponsor, it is important for you to be aware of the difference in how master files can be used at the IND stage of product development versus the commercial stage and plan accordingly. Back to you, Nirjal.

DR. BHATTARAI: Thank you, Andy. That was really helpful information. Next slide, please. So, the next question is:

What is the FDA's expectation for host-cell DNA limits for AAV-based products?

And Anurag will respond to this.

DR. SHARMA: So, our standard recommendation for products made in continuously dividing cells that are tumorigenic or tumor-derived is that you limit the amount of residual host-cell DNA to less than 10 nanograms per dose and the DNA size to below 200 base pairs. However, we understand that it can be challenging to meet these limits for products such as AAV, as that AAV capsid can package host-cell DNA in a nonspecific manner, which is then resistant to nuclease treatment.

So, if you are unable to meet these limits, then please justify your proposed limits with manufacturing data that apply to your process. Additionally, you should provide a comprehensive risk assessment of the oncogenic potential of your product that takes into consideration the highest dose that will be administered, levels and size of residual DNA in the product lots, the patient population, target tissue, and route of administration. You should also discuss process optimization plans to reduce the level of residual host-cell DNA in the commercial product to the extent possible.

In addition to host-cell DNA, you should also control the level of relevant transforming sequences in your product with acceptance criteria that limit patient exposure. For example, products made in 293T cells should be tested for adenovirus E1 and SV40 large T-antigen sequences. Your tests should be appropriately controlled and of sufficient sensitivity and specificity to determine the level of T sequences in your product. Thank you. Back to you, Nirjal.

DR. BHATTARAI: Thank you, Anurag, for that response. Next slide, please. So, the next question is:

For cell-based gene therapy products where vector transgene expression is tested as part of drug product release, is it also necessary to test transgene expression on vector release for first-in-human trials?

And Graeme will respond to this question.

DR. PRICE: So this question really concerns the requirements for testing vectors used to make ex vivo modified cellular products. Here, measurement of transgene expression is needed as part of the vector lot release testing program to ensure that the vector is functionally active before making DP (drug product) loss. Even when a cellular drug product will be tested subsequently, testing the vector is critical, because the cellular starting material can be very limited in supply or irreplaceable, especially in the case of autologous products. Release testing of a vector reduces the risk to the eventual cellular product.

As we outlined earlier, measurement of transgene expression is sufficient to ensure vector activity for first-in-human and early-stage clinical studies. An assay to assess biological function of the transgene encoded by the vector must be in place prior to initiation of studies intended to demonstrate efficacy in support of licensure. The vector activity assay must be validated prior to a BLA submission.

Know that in most cases, it would be acceptable to test vector activity using a similar assay as that used to test cellular drug product potency for lot release. And this can really help streamline and make your life easier if you can use the same assay for both purposes. So back to you now, Nirjal.

DR. BHATTARAI: Thank you, Graeme. Next slide, please. And just a quick reminder to folks: Please type in your questions in the Q&A box, and we'll respond to that very shortly in our live Q&A session. So, the next question is:

To what extent can knowledge from a similar product be leveraged when the products differ only in terms of the transgene?

And Andy will respond to this.

DR. HARMON: Thanks, Nirjal. So, to give you one example, we definitely see a lot of sponsors in the AAV product space having a goal of setting up a manufacturing process for a specific serotype of AAV so that they can just swap out different transgenes to treat different indications. So, in this example, what are some of the ways that you can leverage previous manufacturing knowledge in a new IND submission? Well, one thing you may be able to do is use delivery device compatibility data from, say, AAV product A to support the IND for AAV product B. But you have to keep in mind that this will only work if both products are the same AAV capsid, the same formulation, and at the same concentration. The proposed administration procedures or in-use hold times at the clinic for the products also have to be similar, so this can be tricky.

Another possibility is using previously obtained data to inform the setting of preliminary acceptance criteria for release testing. If your products always use the same manufacturing process and the same AAV capsid, you may have a good idea of what can be achieved for certain attributes, such as capsid titer, process-related impurities, or product-related

impurities. And therefore, you can set preliminary quantitative acceptance criteria right from the start.

There are also some opportunities to utilize data from similar products for qualification of certain noncompendial assays, such as, for example, capsid ELISAs [enzyme-linked immunosorbent assays] or, as another example, host-cell DNA tests. However, the big caveat is that certain assays will always need to be qualified in a product-specific manner, and these include strength assays, such as vector genome titer, and potency assays.

Also, keep in mind that at the BLA stage, your analytical validations and process validations are generally going to be product specific. So while previously obtained data with a similar product could speed up your path into the clinic, it may not be as helpful for commercialization. Nirjal, back to you.

DR. BHATTARAI: Thank you, Andy, for that really helpful information. Next slide, please.

Can process characterization and/or validation be executed at smaller scale, and how many PPQ [process performance qualification] lots are required for process validation?

Anurag?

DR. SHARMA: Yes. So, the validation of commercial manufacturing process should be supported by data from commercial-scale vectors. The use of scaled-down models is not appropriate for process performance qualification. Scale-down models can be used at the process design and characterization status to determine process variability. However, you should demonstrate the validity of scaling down. The scaled-down version should represent the intended commercial manufacturing process as closely as possible.

Regarding the number of PPQ lots required for process validation, again, there is no one-size-fits-all number. You should have a statistically appropriate number of vectors based on process variability to demonstrate that the process is reproducible and will consistently deliver quality products.

In general, if you have greater understanding and knowledge of the product, that will better inform the number of PPQ lots required. The required number of PPQ lots may also differ based on the scale and frequency of the product manufacture. Thank you. Back you to you, Nirjal.

DR. BHATTARAI: All right, thanks, Anurag. So we'll spend a few more minutes to answer two more questions, and then we'll switch to a live Q&A. So the next question, please. The question is:

Can the FDA please provide recommendations for the use of raw materials that are required for manufacturing but are not available as a GMP [good manufacturing practice]-grade or a compendial reagent?

And this will be responded to by Graeme.

DR. PRICE: OK, this is a good question, but I'll start with clarification. While many times we see reagents and raw materials referred to as "GMP-grade," this is really a form of shorthand. While suppliers may use various trade names and terminology for a material, the FDA doesn't recognize or define a GMP grade for reagents. A more correct terminology would be, "The reagent is manufactured under a quality system compliant with current good manufacturing practice, or CGMP, requirements, as detailed in 21 CFR 210-211."

Now, key concerns that we have with reagents and raw materials are that they are what they say they are and that they're free of contaminants that could be carried forward in the manufacturing process and pose a risk to patients. Many suppliers manufacture more than one material, and it's vital that there's no cross-contamination between them. A good example of this would be plasmids used for vector manufacture. If the same bioreactors were used for bacterial fermentation or the same chromatography columns are used during production and purification of different plasmids, then there's the possibility of cross-contamination of each plasmid lot of previously manufactured plasmids. And this can have a direct effect on vector purity and pose a significant risk to patients, who could receive a mixture of the correct and contaminated vector manufactured using the plasmid starting material.

In terms of microbiological contaminants, major concerns include adventitious viruses, which can get into reagents or raw materials in many nonobvious ways. So for example, recombinant cytokines may be produced in E. [Escherichia] coli, which would reduce for risk of viral contamination, but then purified using affinity columns containing murine antibodies from ascites that's contaminated with murine mass viruses, which would then contaminate cytokine preparation and be amplified during culture of a cellular product, which, again, would pose a risk to the patient. So such concerns with reagent quality are a common hold issue in the IND stage and can apply to materials using product manufacture but contain components of biological origins, such as antibodies, cytokines, sero-recombinant proteins, and enzymes.

We recommend the use of the highest-quality reagents available at all stages of product manufacture. Some ways to achieve this are to use reagents described in master files wherever possible. Although the FDA cannot tell you whether or not an appropriate master file is available, we recommend that you contact manufacturers to inquire whether such a master file is available for a different reagent and, if so, provide a letter of authorization to cross-reference that master file. And this may involve considering multiple alternative manufacturers of reagents.

In some cases, such as for human serum albumin and certain cytokines or monoclonal antibodies, an FDA-approved product may be commercially available and should be considered for use. If a suitable quality reagent is not available, then you should provide information regarding source of the material, how it was manufactured, and how it was tested and found suitable for use in your manufacturing process. In some cases, you may be

required to test each material to ensure that it is free from adventitious viruses prior to releasing it for manufacturing use.

An important point to consider is that the CGMP regulations require each lot of incoming components, in this case including reagents, used in manufacturing be quarantined until tested for conformity with written specifications for purity, strength, and quality or are assessed for alternative procedures before being either released or rejected, as described in 21 CFR 211 Subpart E. While phase 1 trials are exempted from the 211 CGMP regulations to allow greater flexibility, manufacturing must still be well-documented and well-controlled.

Ultimately, when sourcing reagents, it is your responsibility as a sponsor to establish procedures confirming that each reagent is of suitable quality for use in your manufacturing process and provide this information to the FDA to review. Back to you, Nirjal.

DR. BHATTARAI: Thank you, Graeme, for this comprehensive response. And so, maybe one last question before we move to the next session, and maybe I'll ask Graeme to respond to this last question, too.

How focused should pre-IND questions be, and are there some questions that are too expansive?

DR. PRICE: OK, so this is a great question. So, pre-IND meetings are intended for the FDA to provide feedback on the design of preclinical studies, the initial clinical protocol, and product manufacturing, and quality controls needed before initiation of human studies. This feedback should then be used to guide what information is needed to be included in the IND submission. To facilitate effective review and discussion, we typically recommend the questions are limited to a maximum of 12 in total across all review disciplines. So that's pharmacology, toxicology, clinical, and CMC.

Now, from the CMC standpoint, we often see multipart questions along the lines of "Are our drug substances and drug products, raw materials, manufacturing process and process controls, release testing, specifications, and stability protocols acceptable?" While these are reasonable questions, when combined, they're a lot to deal with in the context of a single pre-IND meeting, particularly when there are multiple other multipart questions that need to be addressed for both CMC and other disciplines. So, we recommend that before preparing a pre-IND package, sponsors familiarize themselves with relevant guidance documents that may provide answers to many commonly asked questions. This can allow the number of questions asked in the pre-IND package to be pared down and focused on specific areas where additional guidance is really required.

An additional factor to be considered is that the high interest in the cell and gene therapy field has led to very heavy and sustained workloads for reviewers. This has resulted in many pre-IND meetings requested as teleconferences being converted to written response only. We understand that this is suboptimal in some ways, as it doesn't allow for

interactive discussions, but I would emphasize how helpful it is to have very clear and defined questions, so that the reviewers can provide the most accurate and useful advice in the context of a written response. Thank you. Back to you, Nirjal.

DR. BHATTARAI: All right, well, thanks, Graeme. That was really helpful.

All right, folks, so thank you to all who submitted the questions during the registration process. And now we'll spend the remainder of today's time answering your live questions, and I ask all my panelists to join me in responding to some of the live questions and answers.

All right, so let's start with the first question that we just got today:

If a gene therapy assay has been qualified during the early phase of clinical development, such as phase 1/2, can the qualification be used as a validation for late stage?

And Anurag, this is for you.

DR. SHARMA: Yeah, thanks for the question. So, as I mentioned earlier, also, during the submitted questions session, for the qualification, one of the purposes of the assay qualification is that it forms a basis of the assay validation later in program development. For example, what would be the appropriate acceptance criteria for the validation exercise for that particular [critical quality] attribute [CQA]? And during the product development, you gain more experience regarding that particular CQA, and that information can be used to further optimize the acceptance criteria and better inform the acceptable limits for the validation exercise later, during the later stages. And if needed, the assay can be further re-optimized if you think that the acceptance limits need to be narrowed down further. But if all is good, then you can move directly to the validation.

But, if your qualification was robust enough and you set the acceptance criteria prior to the qualification and your qualification exercise meets those acceptance criteria, in that case, validation might not be needed, or maybe the verification might be sufficient. But what we commonly see is that the robustness validation for most of the assay is missing by the time you approach the BLA. So usually, additional validation exercises might be needed.

So, ultimately, you know, we need assurance that your assay is in control and there's less variability and it does what it is supposed to do on a regular basis. Thank you.

DR. BHATTARAI: Thank you, Anurag, for that response.

So, our next question is:

How much drug product stability data are needed for a phase 1 trial? Are drug substance stability data also needed for phase 1?

And I'll ask Andy to respond to this question.

DR. HARMON: So, this is a good question, and I want to get the take-home message out there up front, and that's that some stability data are required at all stages of product development. So, as you submit that big IND application, we're going to be looking at stability to support your entry of the product into the clinic. And so, I'll start with phase 1, because that's what the question focused on. We will be looking for some preliminary stability data. Now, it might not be that you have all of your CGMP lots released and already on a formal stability protocol. It may be that your preliminary stability data are process engineering lots, process development lots, or sometimes your proposed phase 1 clinical lot [and you're] beginning to obtain preliminary data on what the stability of that product looks like.

In one of the questions we answered in the previous set of pre-submitted questions, we also talked about leveraging some data from similar products. And here's one other, I think, situation that I didn't mention where that could be helpful. So just as one example, if you have the same AAV capsid and same formulation buffer at the same storage conditions, then you might also be able to submit some data there. But, we're going to be looking for data even at phase 1 to support how you intend to store your product.

So, the question also brings up specifically drug substance stability data. So within the products that we review, there's often a case where, at the drug substance stage, before final formulation and vialing, this drug substance is frozen and held. And in this case, we also need to see preliminary stability data supporting that long-term storage of the drug substance. And those same things apply. You might have data being generated from engineering lots. You know, we're starting with these assays for stability, but we're intending to add other assays later. So drug substance stability is going to be part of that.

There's the other side of that coin, where some sponsors very quickly and directly forward-process drug substance into drug product. And if that's the case, if you're not freezing it for long-term storage, that's more of a process validation issue, and you wouldn't necessarily be putting drug substance that isn't frozen for long-term storage on a stability protocol.

There are a few other minor points I want to make. As product development goes on, you're going to be really figuring out what assays are going to be part of your stability program. But, for gene therapy and cell therapy, you know, you can look at the guidances and look at the information in the field and already get a sense of some assays that you know are going to be generally stability-indicating, so things like, for an AAV, the potency of the vector, the expression of the transgene. For a CAR T-cell, it's going to be critical that you start getting data about viability of those cells if you're freezing them. So, we don't expect you to have all the assays locked down, but we expect you to start generating data to help you in product development.

One of the other key issues is that you should not forget about in-use stability. And, part of in-use stability is delivery device compatibility. So when I talk about "in-use," I'm talking thawing the product at the clinic and that time window when the pharmacist is going to be

thawing it; it's going to be transported to the patient's bedside; if it's being administered in a syringe, it's going to be manipulated or an IV bag is going to be set. You need to submit preliminary data to support that in-use stability window and show that contact with that delivery device does not damage or decrease the quality of the product.

So, just very briefly, we usually have the same advice for kind of developing a delivery device compatibility study. For things like a CAR T-cell, like I said, you're going be looking at viability of the cells after exposure to the delivery device. For a vector product, you're going to be looking at a measure of physical titer to see if the vector's sticking to the tubing and a measure of activity to see, after exposure of the delivery device, if the vector is somehow inactivated. So a lot of info there, but basically, we're expecting some stability data, even at phase 1, and then that bar is going to increase as you move on in product development. Nirjal, back to you.

DR. BHATTARAI: Well, thank you, Andy, for that comprehensive response. So, let's move on to our next question. So, then, our next question is:

Could a manufacturing change that's shifted the phenotype of cells to an earlier senescent state, such that, for example, more of the final drug product has a higher ratio of central memory T-cells compared to the initial clinical product, constitute a new version of the drug product, therefore requiring a secondary IND?

So this will be for Graeme.

DR. PRICE: This is a good question, and I'll answer it in the context that the same vector and basic manufacturing strategy are used for the two versions. So, in most cases, a new, less differentiated version of the product would be a new product, requiring a new IND, as the cellular composition would have changed relative to the original version. This can potentially change the potency and safety of the product. This would affect multiple aspects of the clinical study, including establishing an appropriate dose, which may be lower than that from the previous version of the product, because the cells are just younger and more active. So, you should provide preclinical data, allowing assessment of the impact of the change in product safety and activity relative to the original version. And this information may be useful for setting a starting dose.

In some cases, such as when the new product is intended to treat the same indication as the original product and will be used under the same clinical protocol or a similar clinical protocol, it maybe be appropriate to submit the IND for the new product as a secondary IND, as outlined in the FDA guidance titled "Studying Multiple Versions of a Cellular or Gene Therapy Product in an Early-Phase Clinical Trial." If the two products are manufactured using the same vector and share CMC information, then this information can be cross-referenced from the primary IND, which will certainly make life easier for both the sponsors and for the reviewers. So back to you, Nirjal.

DR. BHATTARAI: Thank you, Graeme. So, let's move on to our next question. Our next question is:

In the IND and BLA filings, is it necessary to list and provide the schema for the complete cloning history of each plasmid used for the therapeutic vector production, given that the cloning history can be long and complex and sometimes not fully known? For a complex sequence, would a detailed description of each genetic element be sufficient?

And I'll ask Anurag to respond to this question.

DR. SHARMA: Yeah. Thanks for this question. So, in general, it may be sufficient. We ask for all these details so that we can determine what is the source of all these elements and the sequence that you are using in your plasmid, if there are any safety concerns. But it may not be necessary to provide us each step-by-step detail of how you generated these plasmids. But, as you mentioned, then, if you have detailed sequence analysis, you can provide that information. You can provide us the full sequencing report and provide any available information about where all these sequences are coming from. So, that might be sufficient. And if you see any discrepancy from the expected sequence, you provide that description and that rationale and any unintentional consequences for that discrepancy, and we also recommend that you perform some sort of risk assessment for all those discrepancies and lack of availability of these details. So, the short answer is that this might be sufficient. Thank you. Back to you, Nirjal.

DR. BHATTARIA: Thank you, Anurag. All right, so let's move on to our next question, and the next question is:

What are the expectations regarding AAV dosing based on nominal titer versus lot-specific measured titer, as measured at batch release? Do the expectations change as clinical development progresses?

And I'll ask Andy to respond.

DR. HARMON: So, this is a really important question for the AAV field. It's a question that I could probably discuss for a few hours. So we're going to try to get over a few key points in this town hall format.

Firstly, I just want to briefly explain that some folks might have more familiarity with what we describe as dosing based on measured titer. And that's when, at drug product release, you determine the strength of the vector, and then that specific strength is labeled on the vials in the drug product lot. And, then you would be doing a dose calculation measurement at the clinic to determine, say, the dose administered.

A different approach to this is what we call dosing based on nominal titer and release based on nominal strength. So, in this case, you're going to have very strict acceptance criteria, where you're going to say the target strength in vg per mL [viral genomes per milliliter] of

this drug product lot is X. And then along with that acceptance criteria, you're going to have an acceptable strength range, and that could be "X plus a certain percentage or minus a certain percentage will meet the specifications for release."

After that lot is released, the vials will all be labeled with the same nominal strength. And so, that makes the calculations at the clinical sites much easier. You're not doing a complex calculation. Either there'll be a very simple dose calculation or sometimes a dosing table.

One example that is very overly simplified is, the middle of the night, your kid is sick with a fever. You go to grab some NSAIDs [nonsteroidal anti-inflammatory drugs]. You're not going to be doing math based on the weight of your child or the specific strength of that drug. You're going to see that it's labeled, "This is the strength," and on the dosing table, if your kid is this heavy, you're going to give them that much drug. So, that's the approach to dosing based on nominal titer.

To the question, because your pivotal study –- yeah, the approach both to release of drug product and to the dosing of your drug product -- has to reflect commercial expectations. So because of this, you should expect to make that transition to dosing your AAV based on nominal titer prior to initiation of any study designed to obtain primary efficacy data. That being said, it's our recommendation to get experience with this dosing approach as early in development as feasible. But we know and expect that generally, for entering the clinic in phase 1, folks are going to be dosing based on measured titer, because that's kind of the simpler approach, the approach that folks are more used to, and doesn't rely on that final kind of qualification and lockdown of manufacturing and of your analytics for determining the product strength. And our advice of doing this as early in development as possible is also very relevant to this AAV rare-disease space that we're operating in. Your pivotal study -- and we've made this theme a few times now -- may be earlier than a conventional phase 3, so you might not have that extended product development time to get familiar with dosing based on nominal titer. You might need to be prepared to get familiar with this approach earlier than expected. Nirjal, back to you.

DR. BHATTARAI: Thank you, Andy, for that response to a very important question. Let's move to our next question:

How does one establish and support the expiry date of a master cell bank or a master virus bank?

And I'll ask Anurag to respond.

DR. SHARMA: Hi, yeah. So for master cell bank stability, we recommend that you monitor viability or stability over time after cryopreservation. So that gives you a good idea of that master cell bank as –- you know, is stable over time, and you can check its performance, of its ability to yield the product. And we also recommend that you perform, a one-time, end-of-production study, in which you culture your cell for a similar number of

passages as expected in the regular production of your product and test for any development of regeneration of adventitious viruses or genetic stability. So that's a recommendation for a master cell bank.

Similarly, for a master viral bank, we do not have a definite recommendation, but this is something for your own good. So, both the master cell bank and viral bank are very critical components which you'll be using for a long period of time throughout the life cycle of your product. So it makes sense that you want to take care of these to monitor stability, how long they are good for. So, for a master viral bank, also, you can develop a protocol and test in a timely manner for critical quality attributes, such as infectivity and transgene expression, as well as the stability and whether the identity is minted or not.

So, on somewhat similar lines, [the testing is like] what you would do for a drug product -- and Andy very well described in much detail some of the stability recommendations. But, for a master viral bank, the requirements are not that stringent, but this is something for your own benefit, so it's good for your program in the long term. Thank you. Back to you, Nirjal.

DR. BHATTARAI: Thank you, Anurag. All right, so let's move on to our next question:

What is the FDA's expectation for encapsulated host cell DNA and residual host-cell protein for AAV-based products?

And I'll ask Andy to respond.

DR. HARMON: So I think that my colleague Dr. Sharma's answer during the pre-submitted questions really set up a very nice discussion of parts of this already. So, for residual host cell DNA, folks who also submit drugs to CDER, you're well aware of that WHO [World Health Organization] standard that's looking at a specific number of nanograms per dose. The difference for AAV-based products -- and my colleague mentioned this -- is that AAV will encapsidate these DNA residuals. So, the enzymatic destruction of them in the drug substance isn't really possible, because they're protected by that capsid. So that's something that we're aware of, and my colleague talked about how, if you are not able to meet those WHO standards, then you should be submitting data and a justification for what you think your acceptance criteria are.

So I want to say that -- at phase 1 in particular -- for certain residuals, it might be appropriate, on the COA [clinical outcome assessment] to report results, but you should also think about all the process development data, engineering data, and, in some cases, that similar manufacturing process -- similar capsid data that you can leverage for setting a preliminary expectation. So, a phase 1 best-case scenario: You should be striving for preliminary acceptance criteria based on the data you have. And then our expectation for both the host cell DNA and the residual protein is that, as product development keeps going and you get more and more experience with your clinical manufacturing process, you

should be able to refine and tighten those acceptance criteria. So basically, it's a data-driven approach for setting those things.

And I think one other point that I wanted to make is that this is going to be based on your specific data and your specific product, so what you are able to achieve, what is shown to be safe in the clinic -- and so, really, it's one of those other situations where we can't give you a specific cutoff for those things, but we would look forward to seeing the data for your particular manufacturing situation. Thanks. I'll give it back to Nirjal.

DR. BHATTERAI: Thanks, Andy. All right, so our next question is:

Will the agency consider a waiver for replication-competent retrovirus, or RCR, testing for ex vivo modified cell therapy with sufficient data package demonstrating RCR-negative results with statistical analysis and justification?

And I'll ask Graeme for a response.

DR. PRICE: OK, so I think I have to preface this answer by saying both yes and no. So in terms of vector testing, no, we wouldn't consider a waiver under our current guidance. So RCR testing has to be performed on both end-of-production cells and vector supernatant using a sensitive, culture-based assay, right? And that's a requirement for the vector. For the cellular drug product testing, which is normally done using PCR-based methods to test something like VSV G [vesicular stomatitis virus G] envelope or retroviral envelope and gene -- and if you have accumulated sufficient manufacturing and clinical experience to demonstrate that your transduced cell product is consistently RCR negative, you can provide this information as an IND amendment to support reduction or elimination of the testing of the ex vivo modified cells for RCR.

So, in this submission, we recommend that you would include a discussion of the safety features in vector design that reduce the likelihood of generating RCR and a description of the vector testing methods that you're currently using and the experience you have in manufacturing RCR-free cellular products. So this information can be provided to support removal of RCR testing for lot release of ex vivo transduced cells. And we have a guidance on this. It's titled "Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication-Competent Retrovirus During Product Manufacture and Patient Follow-Up" and contains really much more detailed answers to this question. It's really very helpful, and I suggest you refer to that guidance. Thank you.

DR. BHATTARAI: Thank you, Graeme. All right, so our next question is:

What is the FDA's expectation for drug substance and drug product acceptance criteria setting for first-in-human trials? Would it be acceptable to use or report results for some of the test items, other than safety and potency-related items?

And I'll ask Anurag for a response.

DR. SHARMA: Yeah, thanks for the question. So, we do understand that during the early stages, you have very limited manufacturing experience and understanding of your product attributes, so it may be challenging to set acceptance criteria. And you may also have some concerns that if you set acceptance criteria, then it may lead to the rejection of the lots, which, of course, is not desired; no one likes that.

But we do recommend that you set numerical acceptance criteria for these test parameters and especially those related to safety and dose-related assays. And this can be based on some of your early development lots, pilot lots, engineering lots, stocks lots – so, based on whatever product knowledge is available at that time, you can set acceptance limits.

And at this date, these limits can be sufficiently wide so that you are also happy that you don't have to reject lots, and we also have some assurance. But, as you move ahead, you gain more experience. Then we would expect that you keep narrowing or tightening these acceptance criteria with the gain in knowledge. Thank you.

DR. BHATTARAI: All right, thank you, Anurag, for that response. All right, so our next question is:

For a phase 3 study, if there are two final products that are injected together (for example, two AAV vectors), do you expect one potency assay with both products or each of them separately?

And, this is for Andy.

DR HARMON: So, I think this is a great question. It's also a very complex question. I like that my colleague Dr. Price already discussed some of the basic recommendations for potency. There's no expectation that you need to develop a single "golden" potency assay. We see a lot of sponsors that have products with complex mechanisms of action, and they need a panel of sometimes multiple potency assays to really show appropriate control and assurance of potency of their product. So that's the quick answer for that part of it. You might need multiple potency assays, depending on how this product with multiple vectors works.

And, this can also be very complicated, if those two distinct drug products work in harmony together to exert an effect. So let's say, best-case scenario, you have one single potency assay. You're putting those vectors into a dish. They're working together. You see that readout for potency, and you have a nice quantitative result. The complexity is, what happens if you don't get that result? There's a potential that one drug product, for whatever reason, when released, lost its potency or isn't working, and you wouldn't know which one it is in maybe this situation. So this is a really perfect scenario where our advice would be to, in addition to really doing a lot of product development work and analytical development work on the assay that will work best, really take time and effort to develop appropriate reference standards and reference material. If you have a reference material for

Vector A that you know is solid, you can swap that in, combine it with the to-be-released Vector B, and you can really determine whether Vector B is working or not.

So I don't have a very specific answer for this, besides that your potency assay approach will reflect what works best for your product. It could be one perfect assay. That's kind of the dream. It could be multiple assays that have to account for multiple parts of the mechanism of action or multiple drug products that are combined when administered. Nirjal, I'll send it back to you.

DR. BHATTARAI: Thank you, Andy. Let's keep on moving and get a few more questions answered. All right, so the next question is:

Could you give us a high-level expectation for identifying stability-indicating assays for cell and gene therapy products?

Graeme?

DR. PRICE: Well, this is a huge topic, and I think the answer is, yes, I can give you a high-level expectation, but more detailed requirements and expectations will be very dependent on the product class. So, one thing I'd emphasize is that accelerated and stress stability studies are very useful for identifying stability-indicating parameters, and I think these are extremely valuable and should be exploited.

So what stability-indicating parameters do we see for cell and gene therapy product? Well, as I just said, they vary very widely from product to product, so it's difficult to give a cross-cutting answer. But, typical parameters that you could assess would include things that affect the function of the product: vector titer, potency, viability in the case of cellular products, degradation products, any pH change in media that the product is suspended in during long-term storage.

I'll emphasize that, really, it's not just drug product that needs to be tested; it's drug substance as well, because any stability changes to, for example, a vector could have downstream effects on the product made with that vector. So other than that, I think I'd refer to the recommendations present in ICH Q1A(R2), which will give some more useful guidance in terms of the type of things that should be assessed in stability studies. Back to you, Nirjal.

DR. BHATTARAI: All right, so we have a few more minutes, so let's try to get at least two more questions answered. All right, so our next question is:

For nonviral in vivo therapies, do you recommend sterility or bioburden release testing for the RNA drug substance?

And I'll ask Anurag for a response.

DR. SHARMA: Yeah, thanks. So, in general, sterility testing for drug substances is not required, and bioburden is acceptable, especially if there is a terminal sterilization step in the drug product manufacturing and that drug product is subsequently tested for sterility, which is a requirement. So, that might be acceptable. But, in certain cases, if your product is an LNP [lipid nanoparticle], it needs to be complexed with messenger RNA. There's no terminal sterilization step in the messenger RNA manufacturing at the drug substance stage, then you are mixing it with LNP and testing for sterility. In that case, we may prefer that you test the messenger RNA drug substance also for sterility, because if you complex it with LNP and if there is any contamination, then it is getting diluted, and the chance of detecting the contamination in the drug product is lessened.

So it depends on the situation, but in general, testing for bioburden at the drug substance state is generally acceptable. Thank you, Nirjal.

DR. BHATTARAI: All right. Thank you, Anurag, for that response. And maybe one last question for today's town hall, and the question is:

What is the recommended number of lots for doing a comparability study to support a manufacturing change prior to a pivotal trial? Also, do all the lots need to be manufactured at clinical scale or under CGMP conditions?"

And I'll ask Graeme for a response.

DR. PRICE: So, I think that the key part here is the "prior to a pivotal trial" clause. So the number of lots needed for comparability studies will be dependent on the phase of product development. So, the later you get in product development, particularly as you approach and enter a pivotal trial, you will need more information to demonstrate comparability, and the barriers become greater at this point. So, the number of lots should be justified based on the statistical assessment of your existing data, relative to the amount of information that you already have. So at late stage, we'd expect a fairly robust statistical justification for the number of lots that you'd use.

In terms of whether the lots need to be manufactured at clinical scale or under CGMP conditions, I would say, yes, they do. And the reason why is because the comparability study is to demonstrate the product is highly similar between the old version and the new version, and that's going to be what goes into patients. So, you'll want to be comparing the actual product manufactured at clinical scale or commercial scale that you'll be administering to patients, right? That's going to be important for demonstrating that the material used in the pivotal clinical studies is equivalent to the ultimately used commercial material, right? And the ultimately used commercial material will be manufactured at full scale.

Okay. So, I think that's about the best answer that I can give based on the limited scope of the question. Back to you, Nirjal.

DR. BHATTARAI: Thank you, Graeme, for that response. All right, folks, we've come to the end of today's town hall. So, thank you all for attending today's OTP Town Hall. I'd also like to extend a big thank-you to our panelists for their time and all the good responses. I hope that those were very helpful to you guys.

As a reminder, a recording of today's town hall will be posted on FDA.gov in the coming weeks. For more information, you can visit the FDA website to read the FDA guidance document about gene therapy CMC and find other OTP and gene therapy resources.

We plan to host our next town hall meeting on June 8, and the topic will be cell therapy CMC. This will also include tissue-engineered medical products regulated in OTP. The event is free, open to the public, and registration is currently open on FDA.gov.

With that, I will end today's town hall. Thank you, again, for joining, and have a great day, everyone. Thank you.