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FDA CBER Guidance Webinar: Considerations for the Development of CAR T Cell Products

March 7, 2024

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DR. DENISE GAVIN: Good afternoon, everyone. Thank you for joining today's webinar. Today's event is hosted by the Office of Therapeutic Products, or OTP. We're in the Center for Biologics Evaluation and Research at the FDA (U.S. Food and Drug Administration). My name is Dr. Denise Gavin. I'm the director of the Office of Gene Therapy within OTP, and I'll be your moderator for today's event.

As you know, today's webinar is focused on the recently finalized FDA guidance document, *Considerations for the Development of CAR T Cell Products*. This guidance is intended to assist sponsors, industry members, and other stakeholders by providing you with practical, multidisciplinary information on how to develop safe and effective CAR T cell products. Based on the number of people registered and the questions submitted to today's events, it's clear there's great enthusiasm in this area of research, and we hope the information we share today will help expedite this area of product development.

Before we begin today's presentation, I want to share a few reminders with everyone about our event. Please note the webinar is being recorded. The recording and event materials will be posted on the FDA's website in the next few weeks. Closed captioning for this event is available directly in Zoom. We're not accepting live questions for today's event. However, we appreciate the many questions submitted during registration and plan to address a number of those questions during today's webinar. Lastly, please use the chat box if you are experiencing technical difficulties.

Now on to today's events.Before we begin, I'd like to introduce our panelists. Dr. Kim Schultz, director of the Division of Gene Therapy 2 within the Office of Gene Therapy; Dr. Allen Wensky, division director for Pharmacology/Toxicology 1 within the Office of Pharmacology/Toxicology; Dr. Yuxia Jia, medical officer in the Division of Oncology within the Office of Clinical Evaluation; and once we get to our Q&A session, we'll be joined by Dr. Xiaofei Wang, senior clinical pharmacology reviewer in the Division of Clinical Evaluation General Medicine within the Office of Clinical Evaluation.

I'd like to thank our panelists for their time today, and I'd also like to thank the working group for their efforts to finalize the guidance. Thank you all and welcome. Let's start this presentation.

We'll move into the presentation for today's events. Each panelist will review key highlights from the final guidance as it relates to their discipline.

I'll pass it to Kim to kick us off. Kim, whenever you're ready.

DR. KIMBERLY SCHULTZ: Thanks, Denise. We first posted the draft guidance on *Considerations for the Development of Chimeric Antigen Receptor T Cell Products* in March of 2022. For simplicity, we're going to refer to this as "the CAR T cell guidance" for the remainder of the webinar.

We received over 650 comments from approximately 85 stakeholders, and we really appreciate your thoughtful insights into where more clarity would be helpful. After consideration of each comment and with revisions as needed, the final guidance was then published at the beginning of 2024. Today, we'll be giving you an overview of these changes.

I'd like to take a minute to point out that it was our intention to focus on information that was directly applicable to CAR T cells. And as such, this is not all-encompassing, and it actually relies on a lot of information from a number of guidances for a complete picture of product development. We've referenced other guidances throughout the document, and we always recommend that you take advantage of the multitude of guidances that our office has developed, in addition to other FDA guidances and ICH guidelines.

Today, we will highlight information from the guidance, including the changes made to each section based on your comments, and then answer some questions that were submitted when you registered.

CAR T cells are a rapidly evolving class of gene therapies where T cells are genetically modified to express a CAR—a chimeric antigen receptor—to recognize a surface antigen. The field includes a diverse range of CAR designs, additional transgenes to modify cellular activity, manufacturing strategies, and target antigens. As the field has evolved, CARs have been explored on different cell types, and CAR T cells have been applied to a diverse range of indications, most recently to autoimmune diseases.

To be able to provide clear guidance, we have focused the scope of this guidance on ex vivo modified CAR T cells for oncology indications, both hematological malignancies and solid tumors. And this is based on our breadth of knowledge and experience to this date. Much of the information can be applied to CAR encoding cells and other genetically modified immune cells. And we will continue to engage with the field at conferences and through other outreach activities to support development as we and the field gains experience with CAR T cells and other clinical indications.

The beginning of the guidance provides advice on general considerations when first designing your CAR T cell product related to a variety of aspects that may impact product safety, efficacy, and the regulatory framework. I'd like to point out two

areas that are further clarified in this final guidance. As CAR T cell design has evolved to target multiple antigens, we've seen recombination events during manufacturing, which reduce product consistency and resulted in reduced expression of the CARs. Therefore, recombination can impact product development. To avoid this, we recommend that you design your product in such a way to reduce recombination events, and recombination should be evaluated in the IND submission if it's a concern.

The final guidance also clarifies our expectations for monitoring previously administered CAR T cells. It is not yet clear how previous CAR T cells impact the safety and efficacy of the CAR T cell that you're developing. Therefore, we continue to request monitoring of previously administered CARs on autologous products through detection of common structural elements, although this monitoring does not necessarily impact patient eligibility.

Our encoding vectors can vary in type and design. However, in all cases, they are critical to CAR T cell quality and consistency. Therefore, a full drug substance section should be included in the IND. And the vector should be manufactured according to phase-specific expectations, including full CGMP manufacturing for late-stage clinical studies and licensure. Similarly, lot release expectations are phase-specific, with full safety testing and appropriate product characterization for early-phase studies. We recommend that lot release testing include a measure of vector concentration or strength such as titer for viral vectors, so that the amount of vector can be consistently applied in CAR T cell manufacturing. Biological activity may be demonstrated by transgene expression for early-phase studies, but transgene activity assays should be developed and incorporated for lot release as product development continues. And of course, assays should be validated for licensure. Stability studies are also important for your vector and should be initiated to support vector hold and storage times.

CAR T cells can be manufactured from a variety of cells. However, the CAR T cell guidance focuses on leukapheresis-derived materials, as this is the most common source currently. Details are given for allogeneic and autologous material in accordance with 21 CFR 1271. Consistent leukapheresis procedures are important to support consistent product manufacturing. Although you may use established procedures from the collection sites, we recommend that you ensure that the collection procedures are uniform across these sites and, importantly, that any additional manipulations such as washing or cryopreservation be described in the IND. Additionally, stability studies should support the time from collection to the start of manufacturing. And in particular, maintenance of the chain of identity should be described in the IND and validated for licensure.

The guidance also gets into recommendations for CAR T cell manufacturing and process control. Expectations for CAR T cell manufacturing align with the normal

product development expectations, including progressive characterization and control of your manufacturing process. Additionally, aseptic process simulation and validations are needed, as the final drug product cannot be sterilized.

Use of healthy donor materials is generally acceptable for autologous CAR T cell process development. However, we recommend that you demonstrate that the inherent patient characteristics are not going to impact the validity of those studies. To aid manufacturers, the CAR T cell guidance provides some examples related to comparability studies that may be needed if changes are made during product development or for multisite manufacturing.

Since the draft was written, OTP has released a new draft guidance that's all about comparability studies and managing manufacturing changes. We recommend that—in addition to referencing the information that's provided in the CAR T cell guidance—you refer to this comparability guidance for more information. This guidance does go into more detail related to the study design and the statistical analysis that you might consider when you're doing a comparability assessment.

As with all products, release testing of CAR T cells is needed to ensure product identity, quality, purity, and strength. These assays should be appropriately controlled to support product quality and interpretation of your clinical study. We recommend that you develop assays early, and that assay bridging should be performed if you change assays or methods during your development. The guidance also gives direct advice on some assays that are particularly pertinent to CAR T cells and special considerations if multiple transgene elements are expressed.

Based on your comments, we updated several portions of the CMC recommendations in the CAR T cell guidance. First, we recognize that our intent for patients who previously received CAR T cells was not clear. As I mentioned earlier, we revised the section to clarify that this is not necessarily eligibility testing, but it is important for characterization and interpretation of your clinical data.

We've also provided some recommendations for assay design when trying to detect previously administered CAR T cells. We have more clearly delineated recommendations for autologous and allogeneic products where separate advice was needed throughout the guidance. There's additional information for in-use stability testing related to both fresh and cryopreserved products. Overall revisions were made throughout the CMC section to point out phase-specific recommendations. For example, there's phase-specific approach in the analytical assay qualification expectations. Regarding analytical assays, we also give potency assay examples for different product designs and have added recommendations applicable to rapid CAR T cell manufacturing strategies, and also for multi-target CAR T cells, both of which have been of interest to the field recently.

With this, I'll hand it off to Allen to talk about the preclinical recommendations.

DR. ALLEN WENSKY: Thanks, Kim. I will now go over our nonclinical recommendations for the development of novel CAR T cell products.

As many of you know from our regulations, nonclinical evaluations of CAR T cells are necessary to support a conclusion that it is reasonably safe to administer a product for a particular clinical investigation. That said, nonclinical testing of CAR T cells can be challenging due to the inherent biological complexity and variability of this product type and the limited availability of suitable animal models to test safety and activity. A case-by-case nonclinical testing strategy should be applied using in vitro, in silico, and in vivo testing strategies as appropriate, in conjunction with available nonclinical and clinical data from related products to support use of CAR T cells in a proposed clinical trial. This new guidance provides general considerations for nonclinical testing, which we have broken down into four subsections, which include (1) nonclinical considerations for the CAR construct, (2) nonclinical considerations for the cellular component of CAR T cells, (3) a discussion of the in vivo testing of novel CAR T cells, and finally (4) considerations for CAR T cells with additional modifications.

I will touch on several of the main takeaways from each of these subsections. First, the design of a novel CAR construct and the process by which the transgene is delivered to the T cells is critical in determining product safety and activity. To assess these constructs, nonclinical studies should be provided that evaluate the specificity of the CAR for the target.

In addition, the target expression profile should be evaluated. This will help address concerns about on-target and off-target activity, both desired and undesired.

In addition to the CAR construct, the cellular component of the CAR T cells used for vector transduction may affect the safety of the final CAR T cell product. Therefore, a thorough evaluation of risks—such as assessment of uncontrolled proliferation, clonality, or genetic abnormalities—may be important to inform a clinical trial and the safety of a particular donor cell source along with the associated manufacturing.

We recognize that in vivo testing of human CAR T cell products in animals may be limited in its ability to comprehensively assess safety, but the use of immunocompromised tumor models can be important for demonstrating the activity and ability of these cells to migrate to their target sites. In some cases, a species-specific surrogate is used for a more comprehensive look at safety.

Finally, the guidance discusses the growing use of CAR T cells with additional modifications such as suicide genes, gene-edited allogeneic CAR T cells, or the addition of T cell stimulatory elements. The guidance discusses the need for additional modification-specific testing strategies for safety and activity assessment as part of a complete nonclinical program.

Based on stakeholder comments and internal discussions, several changes to the draft guidance were made. I will highlight the most significant ones here. First, we more clearly defined and broadened the text describing the types of vectors that can be used to deliver genetic material encoding the CAR T cell. Examples of this include lentiviral vectors, transposons, mRNA, or other genetic material used for the same purpose. Cytokine release testing was added as an example of one of several types of assays that can be used to evaluate CAR T cell specificity for the target antigen or the lack of response to a suspected off-target antigen. We updated the section on studies conducted to address the potential for CAR T cells to undergo stimulation-independent growth to include both cytokine and antigen-independent uncontrolled growth. And finally, we included additional examples of novel components of CAR T cells—specifically detection selection markers and the inclusion of gene editing or gene silencing components to the CAR T cell final product.

I will now hand it over to Yuxia to discuss the clinical aspects of the new guidance. Yuxia?

DR. YUXIA JIA: Thank you, Allen. And thanks, Denise and Kim, for the introduction and the CMC and nonclinical information for this guidance. Now I will present the FDA clinical recommendations in the guidance for considerations for the development of CAR T cell products.

Selection of a study population should consider the anticipated risk and potential benefits for the study subjects to ensure that the overall study benefits outweigh the risk. CAR T cell therapies have been associated with considerable toxicities, notably cytokine release syndrome and neurological toxicities. In some cases, these toxicities can be life-threatening and fatal. Therefore, in defining the study population, we recommend that the sponsors consider these toxicities in the context of the potential benefit study stage of the disease and other available therapies.

In early-phase studies, sponsors should consider enrolling subjects with severe and advanced disease who have not had adequate response to available treatment, or patients who have no acceptable treatment options. In subjects who have early-stage disease and who have available therapies, the unknown benefits of first-in-human CAR T cell products may not justify the risk of the associated therapy.

For any study, the IND submission should provide a rationale and justification for the proposed study population. The informed-consent document must describe to the study subjects the reasonably foreseeable risk associated with the trial, as well as the alternative courses of treatment. These should be in accordance with Title 21 CFR 15.25.

For a tissue-agnostic approach, CAR T cells target a specific antigen expressed by the cancer cell, regardless of cancer type. Early-phase trials that include subjects

with different cancer types but share a common target antigen (e.g., tissue-agnostic approach) may face challenges in evaluating the efficacy and extent of toxicities.

The disparities in understanding the underlying comorbidities of the subjects, the impact of preexisting tumor burden on toxicities, and the differences in dose-response relationships may present challenges to the objectives of an early-phase study in evaluating the toxicities and dosing. We recommend that the IND submission include the rationale for the proposed study design and analysis.

For target identification, the anti-tumor effect of the CAR T cells depends on the binding of the CAR with the antigen expressed on the cancer cell. Therefore, it's essential to enroll patients whose tumors are known to express the antigen targeted by the CAR T cells.

Some CAR T cell products are developed specifically for pediatric conditions. Title 21 CFR Part 50 Subpart D provides the process for additional safeguards required for children in clinical investigations.

In terms of treatment plan, CAR T cell dose selection is complex, necessitating that several factors be considered, such as transduction efficiency and lot-to-lot variation. The totality of available data should be considered when proposing the study dose.

Clinical development of CAR T cells has often included dose escalation in half-log increments. The clinical protocol should provide specific criteria for dose escalation and de-escalation, and criteria for repeat dosing if proposed.

When there is no previous human experience with the proposed CAR T cells or related products, treating several subjects simultaneously may represent an unreasonable risk. To address this issue, consider staggered treatment to limit the number of subjects who might be exposed to the unanticipated risk within a cohort, followed by staggering between cohorts.

In some situations, manufacturing failure can happen, leading to unavailability of products for a given subject. A manufacturing delay or failure may prompt the investigators to use bridging therapy. However, such bridging therapy could confound the interpretation of treatment effects from the subsequent CAR T cell therapies because it may be difficult to ascertain whether any tumor response observed in these subjects is due to the bridging therapy, the CAR T cells, or both. To help understand the impact of any bridging therapy on the interpretation of the overall study results, we recommend that sponsors consider reassessing baseline disease before lymphodepletion and conducting separate pre-specified analysis for all subjects, for subjects who received bridging therapy, and for subjects who did not receive bridging therapy.

Clinical pharmacology considerations should include plans for pharmacokinetics, pharmacodynamics, and immunogenicity. For safety evaluation and monitoring, CAR T cell safety considerations should include the risk associated with not only the CAR T cell product itself but also the cell procurement and concomitant therapy. A clinical monitoring plan and toxicity grading criteria should be included in the protocol. Each clinical protocol should include a detailed definition of dose-limiting toxicities (that is, DLTs), study stopping rules, and justification for exemptions of any toxicities that will not be considered as DLTs.

CAR T cell persistence and long-term follow-up: We recommend that the clinical protocol describe the plans to determine the duration or persistence of the administered CAR T cells in 12 subjects. The duration of follow-up for subjects who have received CAR T cells depends on the underlying disease, persistence of the CAR T cell, and CAR vector. Subjects should have long-term follow-up after treatment with the CAR T cells containing an integrated transgene.

For allogeneic CAR T cells, there are considerations for CAR T cells derived from allogeneic sources, in addition to the clinical considerations already discussed. We recommend that the clinical protocol describe whether there is a plan for immunological matching of the donor and recipient and then clearly describe the methods for such matching. In addition, a major concern for recipients of allogeneic CAR T cell products is graft-versus-host disease, or GVHD. Clinical monitoring should include plans to collect information regarding the symptoms and signs of GVHD. A grading system to assess GVHD and a corresponding management algorithm should be included in the clinical protocol. DLT and study-stopping rules should incorporate GVHD as well.

We really appreciated the feedback, comments, and suggestions from our stakeholders. Based on those comments and suggestions, we have incorporated clinical-related comments into the final guidance. Here, I'm going to highlight the major points.

In the final guidance, we clarified that if a test for the target antigen is not commercially available, then a companion diagnostic test may need to be developed to appropriately select subjects for the study. We also clarified that in some situations, it may be appropriate to initiate clinical studies of CAR T cell products in children if adequate justification is provided. We clarified language on dosing strategy based on body weight or body surface area and factors including age and disease. We have added toxicity grading with specific considerations for cytokine release syndrome (CRS) and for neurotoxicity. We have clarified that psychiatric toxicities may also be associated with CAR T cells and should be collected and managed appropriately. We have clarified language on the DLT definition. We recommend that a clinical protocol provide a plan for long-term follow-up in the

event that the sponsor decides to inactivate, transfer, or withdraw the IND before completion of the long-term follow-up.

Thank you very much.

Back to you, Denise.

DR. GAVIN: Thank you, Kim and Allen and Yuxia, for those highlights. It's really helpful.

We'll now move to the question portion of today's events. We'll try to address as many questions as we can, but please remember we're not able to discuss questions regarding specific investigational products or drug applications. We hope you can stay on with us for the entire time, but I also would like to reiterate that the event is being recorded, so you can visit the full discussion after it's posted on our website.

Let's begin with our first question. Kim, could you address this one, please?

Can you explain the advantages of reporting vector copy number per CAR-positive cell, instead of per total cells?

DR. SCHULTZ: Thanks. We get this question quite a bit. In recent years, the FDA has updated its recommendation for monitoring the average number of vector integrations in your drug product, which we refer to as vector copy number, or VCN. Insertional mutagenesis is a risk with integrating vectors that would theoretically rise as the number of integrated vector copies increases. Thus, vector copy number is an important safety test as well as a measure of manufacturing consistency.

We recommend that, as part of CMC development, you optimize the amount of vector used to reach your target CAR expression but minimize vector copy number. Previously, we accepted vector copy number measurements either as a function of total cells or as that of CAR-positive cells. Although both require calculations from multiple assays and neither is a perfect measure of vector integration, representation of vector copy number per CAR-positive cell is more representative because it removes CAR-negative cells from the equation, which, in most cases, don't have vector integration.

When expressing vector copy number per total cells, the same vector copy number value may be obtained for lots with different transduction frequencies, but the implications may be very different. For example, a VCN of three based on total cells for a product containing 60% CAR-positive cells equates to an average of five VCN per CAR-positive cell. But if, in another lot with three vector copy numbers per total cells, there was only 10% CAR-positive cells, this would equate to 30 copies per CAR-positive cell. You can see that there's a big difference depending on how you do your calculation.

Therefore, we believe that reporting vector copy number per CAR positive cell on the final certificate of analysis provides a more useful assessment of patient risk and allows for a better comparison of product variability across lots. In your IND, you may justify an appropriate acceptance criterion for VCN per CAR-positive cell based on your development experience and refine that criterion as your study continues.

Back to you, Denise.

DR. GAVIN: Thank you, Kim.

Allen, what are essential nonclinical studies for the IND application when submitting for a new CAR and new target?

DR. WENSKY: Thanks, Denise. This is a very broad question, but it highlights one of the goals of our new CAR T cell guidance in helping to facilitate the development of a successful nonclinical program and to encourage early communication with our stakeholders for their specific novel CAR T cell product.

Not surprisingly, the time duration and scope of nonclinical studies needed to begin a clinical trial with a novel CAR T cell product depends on many factors. The nature of the product (including previous clinical and nonclinical experience), the disease it's targeting, and the additional components or modifications of the CAR T cell (such as the inclusion of gene editing) are all part of the process in determining the types of studies that should be considered as part of the IND application. In general, the nonclinical assessment is typically focused on characterizing the activity of the product, target specificity, and overall safety profile of the CAR T cell product of interest.

Following the guidance, following referenced documents in the guidance, and communicating early with the FDA are key to a successful application.

Back to you, Denise.

DR. GAVIN: Thanks, Allen. That's very helpful.

Yuxia, what should be considered when developing CAR T cell products for pediatric conditions?

DR. JIA: Thank you for the question. Sponsors who have plans to develop CAR T cell products to treat pediatric conditions should consider how to incorporate additional safeguards for pediatric subjects into their clinical investigations and into their overall development program. Clinical development programs for pediatric indications usually obtain initial safety and tolerability data in adults before they begin to study those products in children. We recognize that in some situations, it may be appropriate to initiate clinical studies of those cell products in children. As mentioned earlier, that should be in accordance with Title 21 CFR Part 50 Subpart

D, because that provides the process for additional safeguards required for children in the clinical investigations.

Back to you, Denise.

DR. GAVIN: Thank you, Yuxia. I have a question for Xiaofei, if you are here:

What are FDA's recommendations for a dose-finding study for CAR T cells?

DR. XIAOFEI WANG: Thanks, Denise. That's a good question. Dosing is an important component in CAR T cell therapy, and we recommend that sponsors conduct dose-finding studies during the clinical development of these products.

The initial starting dose in a first-in-human study can be based on preclinical and clinical information from other similar CAR T cell products. Due to concerns of species-specific immune response, there are some limitations of preclinical testing of CAR T cells. Therefore, clinical experiences of the same or similar CAR T products are very important to facilitate dosing in first-in-human studies.

We generally recommend that half the lab increase for a dose-escalation scheme. The dose-finding study should include sufficient sampling time points to characterize the kinetic profiles of CAR T cells. Based on our available experiences with autologous CAR T cells for the treatment of hematological malignancies, the expansion after repeat dosing may be substantially lower than the expansion after the first dose. Therefore, we generally do not recommend the intra-subject dose escalation for autologous CAR T cells indicated for hematological malignancies.

Additionally, the general principles of dose optimization may also apply to CAR T cell product development. Due to the product complexity, we recommend that sponsors communicate with FDA early and often to obtain input on the dose optimization of CAR T cell products.

Thank you.

DR. GAVIN: Thank you, Xiaofei.

Kim, do you have any recommendations for how sponsors could control the impact of donor variability on drug product variability in late-phase CAR T cell development?

DR. SCHULTZ: Of course. The FDA recognizes that patient-to-patient variability in starting material attributes is a large contributor to lot-to-lot variability—particularly for autologous CAR T cell drug products. With any drug manufacturing process, you should appropriately characterize your manufacturing during development and implement appropriate process parameters and controls to support a consistent manufacturing process. Additionally, you may consider how initial manufacturing steps could be controlled; these might include incoming material

testing or cell selection or other processes to support a greater understanding of your process capabilities.

And I do have to say that we have seen a number of developers achieve robust and consistent manufacturing as evidenced through their split apheresis studies. However, even with a consistent manufacturing process, there is going to be an expected level of lot-to-lot variation in your final drug product attributes.

For autologous products, we encourage that your clinical studies include a sufficient number of product lots and patients, so that the attributes that you experience during your clinical study are representative of the totality of the experience that's expected for your intended indication. That way, you can gain the CMC experience to inform what an acceptable level of variability is for your commercial product. For allogeneic cells, the incoming material is generally more uniform, as it comes from healthy donors who, for instance, have not been subjected to chemotherapy. It can also be more controlled with incoming material testing acceptance criteria.

But we still recommend that you should use CAR T cell lots that are made from a range of donors as part of your clinical study, so that you can define your manufacturing space and understand the clinical impact of attribute variability.

Back to you, Denise, for the next question.

DR. GAVIN: Thank you, Kim.

Allen, could you address whether safety assessment in an immunocompromised, tumor-bearing animal model is required?

DR. WENSKY: Thanks, Denise. Due to the xeno-response to the human CAR T cell product in animals, severely immunocompromised animals bearing target tumors are typically used to provide proof-of-concept data. However, limited safety information can be obtained using these models due to lack of a functioning immune system in the recipient animals. In addition, in many cases, the species of animal being tested does not express the targeting antigen, or the clinical product is not cross-reactive in the selected species.

However, these studies do provide valuable information on the activity of the product, demonstrating the ability of the product to migrate to the site of a tumor and to kill the tumor target. Some safety information, such as aberrant proliferation, or off-tumor targeting, may be assessed when appropriate. But these data are typically just one component of the safety assessment for a novel CAR T cell product. Additional sources of data to support an IND application can include immunocompromised, tumor-bearing animal model data from previously published studies using a similar CAR T cell product, nonclinical data using a surrogate product, or cross-referenced data from studies using the same CAR T cell construct or product.

Back to you, Denise.

DR. GAVIN: Thanks, Allen.

Yuxia, would you discuss how retreatment with CAR T cell products will be addressed from a safety perspective?

DR. JIA: Yes. Thank you, Denise, for the question. As we know, CAR T cells can persist in the subject for a long time and have an extended duration of activity. Additional lymphodepletion in the context of repeat CAR T cell dosing might lead to severe and prolonged cytopenias and pose life-threatening risks to study subjects. Therefore, most CAR T cell trials use a single administration or a onetime dosing regimen. Repeat dosing, if proposed, should be based on a preliminary understanding of the CAR T product's potential benefit and toxicity. At this time, proposals will be reviewed and evaluated on a case-by-case basis.

Thank you for the question, and back to you, Denise.

DR. GAVIN: Thank you. That was very helpful. Xiaofei, we have a big question for you. It's two parts, and I'll go ahead with both questions, and then you can provide your answer.

Part 1: What are FDA's recommendations for a clinical pharmacology package to support a BLA, or Biologics License Application? And Part 2: Would the agency like to review the translational clinical pharmacology analysis plan, which specifies PK, PD, and response analyses up front during the clinical development process?

DR. WANG: Thank you, Denise. Thank you for the questions. Generally, for BLA submission, we recommend that the applicant provide information to support the dose selection from a kinetic or cellular kinetic assessment, pharmacodynamic assessment, and dose exposure response relationship evaluation. For the CAR T cell products that are made using retroviral vectors, such as lentiviral vector or retroviral vector, we also ask sponsors to include replication-competent testing to address potential safety concerns.

For the second question, the answer is yes: The corrective analysis to identify potential factors that may impact CAR T cell PK, PD, safety, and efficacy can provide important information in the development of CAR T cell products. Although it is not required, we do encourage the sponsors to submit a general translation clinical pharmacology analysis plan to FDA for review during the clinical development for CAR T cell products.

Thank you.

DR. GAVIN: Thank you. That was very helpful. Kim, this is your last question:

What are FDA's recommendations for allogeneic CAR T cell lot release testing?

DR. SCHULTZ: Lot release tests should monitor the critical quality attributes for your specific product to ensure the product's safety, identity, purity, and potency. A lot of your release testing strategy will depend on your product design. In general, recommendations for allogeneic CAR T cell lot release testing are similar to those for autologous CAR T cells, in that the general critical quality attributes are similar between autologous and allogeneic products. For allogeneic CAR T cells, there are additional safety tests for release, including the number of residual T cells expressing conventional alpha/beta T cell receptors, cytokine-independent growth, and additional adventitious agent testing, similar to testing for a cell bank, including testing of HHV-6, -7, and -8. This is in addition to the donor screening and testing that's required under 21 CFR 1271.

Residual alpha/beta TCR-positive T cells pose a risk for graft-versus-host disease. Therefore, the threshold for the allowable residual alpha/beta TCR-positive T cells is determined on an indication-specific basis with our clinical review teams. However, we often recommend that, for first-in-human studies, you limit allogeneic products to 100,000 alpha/beta TCR-positive T cells per kilogram patient weight for populations that are not at significant risk for graft-versus-host disease and 70,000 for populations that are at risk for graft-versus-host disease. That's 70,000 per kilogram.

As expectations for safety testing are based on a multifactorial risk assessment—including product design, donor matching with the recipient, disease indication, and some other aspects as well—we recommend that you discuss the safety testing panel with the FDA during your pre-IND meeting. You may also be able to interact with the FDA throughout development in order to justify changes in these allowable limits as you gain additional experience with your product in clinical studies.

Lastly, expectations for testing related to genome editing would be specific to the product. I recommend that you refer to the recently published guidance for human gene therapies incorporating genome editing in the webinar that we just held on February 29.

Thanks for the question, and back to you, Denise.

DR. GAVIN: Thanks, Kim. Allen, how is nonclinical data used to determine the first-in-human dose?

DR. WENSKY: That wasn't my question. I think that's for clinical.

DR. GAVIN: That's the question I have. Do you have another question?

DR. WENSKY: My question was: "Is there a minimal duration for the cytokine-independent growth assay?" We can start with that. And I know that this question will be addressed overall for nonclinical/clinical, I think, by the clinical group.

Is there a minimal duration for the cytokine-independent growth assay?

There are no pre-specified requirements for the design of cytokine-independent growth assays. The sponsor should design the assay such that the potential for uncontrolled growth in the absence of cytokines can be reasonably assessed. This includes the use of multiple donors and appropriate controls.

This may include untransduced donor cells, CAR T cell donors incubated with stimulatory cytokines, or unstimulated donor cells.

Back to you, Denise, and sorry for the confusion on my end.

DR. GAVIN: That's OK. This is a good question anyway, and I think that it is the next question. Right? Yes, here we go.

What factors should be considered when selecting study doses for CAR T cell products from a clinical perspective?

DR. YUXIA: Thank you, Denise. Allen is right that dose or dosing regimen is a very common question that comes up not only for clinical but also for nonclinical and clin pharm, and so we share the insights and use data and information from all the disciplines and information. I'm going to just address from a clinical perspective here, and there are several factors to be considered when selecting CAR T product to study doses.

The first one: I would say that the transduction efficiency can differ from lot to lot. That's very different from other therapeutic products, and those variations can result because of the percentage of the transduced cells. To mitigate this variability in dosing, we recommend that the CAR T dose selection be based on the number of viable transduced CAR T cells in the product, instead of the total cell number.

Now the second: I would say, if available, previous clinical experience with similar CAR T cell products, even if for a different indication, would be very helpful. Luckily, we already have several products that are approved for the hematological malignancies; in some circumstances, those can help to justify the clinical doses. However, we do recommend that sponsors exercise that approach with caution, because such an approach can sometimes be challenging. The in vivo behavior of CAR T cells may be different, depending on the disease, depending on the antigen load, and depending on the study population and the CAR construct.

The third component or factor: I would say the choice of a preconditioning lymphodepletion regimen may influence CAR T cell in vivo proliferation and should be considered when selecting CAR T cell doses. It's quite a complex process.

I will pause here. Thank you. Back to you, Denise.

DR. GAVIN: Thank you. That's very informative. It looks like we have time for one more question, and this is for Kim. This comes up a lot, so we thought we would throw this one in there:

What are the expectations for visual inspection of CAR T cell drug product?

DR. SCHULTZ: Thanks, Denise. CAR T cells are typically administered intravenously and, like all parental products, are subject to 100% visual inspection per USP. Therefore, each drug product lot should be inspected against a black-and-white background prior to release, so that defects and particulates will be visible. Containers with visible particulates should not be released.

We recommend that you properly qualify your manufacturing process, product contact materials, and final containers to reduce the risk of particulates in your final drug product. Inspection of the final containers at multiple points prior to filling has been adopted by some manufacturers as a mitigation strategy.

Lastly, I'd like to point out that visual inspection is separate from appearance testing. Appearance testing is a lot release test that looks for conformance to the expected product profile. Visual inspection specifically looks for particulates and defects that may increase patient risk.

Back to you, Denise.

DR. GAVIN: Thank you. Thank you, everyone, for joining. I'd like to thank you all for joining today's event. I'd like to extend a thank you to our panelists, to the working group who put this all together, and to the backstage team. Thank you very much.

As a reminder, a recording of today's webinar will be posted on FDA.gov in the coming weeks. For more information, you can visit the FDA website to read the full guidance. I'd also like to note that we held a similar webinar, as Kim mentioned earlier, regarding the genome editing guidance. You can also find that at FDA.gov if you're interested in learning more. To find more information about other FDA OTP-hosted events, visit our OTP Meeting and Web Workshops page.

Additionally, I wanted to remind everyone that FDA is also accepting comments on a draft guidance on the topic of potency assurance for cell and gene therapy products. That will be open until March 27.

Thank you all again for joining. I hope you found this webinar helpful. Have a great day. Thank you very much.