UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES
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FDA CBER Webinar: Human Gene Therapy Products Incorporating Human Genome Editing

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DR. DENISE GAVIN: Good afternoon, everyone, and thank you for joining us for today's webinar. Today's event is hosted by the Office of Therapeutic Products, or OTP, within the Center for Biologics Evaluation and Research (CBER) at the U.S. Food and Drug Administration (FDA). 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 on developing human gene therapy products that incorporate human genome editing. This guidance is intended to provide sponsors, industry members, and other stakeholders with recommendations on what to include in an investigational new drug application, or IND, in order to assess the safety and quality of an investigational genome editing (GE) product. Additionally, it will provide tips on the design, manufacture, and testing on these products.

Based on the number of people registered and the questions submitted for today's event, it's clear there's great enthusiasm in this area of research. We hope that the information we share today will help expedite this area of product development.

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

And now, on to today's event. I'd like to take a moment to introduce today's panelists from OTP. Dr. Anna Kwilas is Chief of Gene Therapy Branch 4 in the Office of Gene Therapy Chemistry, Manufacturing, and Controls (CMC). Dr. Sandhya Sanduja is Chief of Pharmacology/Toxicology Branch 2 in the Office of Pharmacology/Toxicology. And Dr. Gavin Imperato is Chief of General Medicine Branch 4 in the Office of Clinical Evaluation, all in OTP. Thank you to our panelists for your time today. I'd also like to thank the working group for their effort to finalize the guidance document. Thank you all.

We'll now move to the presentation portion of today's event. Each panelist will review key topics from the final guidance. I'll pass it to Anna to kick us off.

DR. ANNA KWILAS: Thank you so much, Denise. As you all know, this guidance is broken down into five distinct sections: (1) background, (2) general considerations for product development, (3) CMC recommendations, (4) nonclinical recommendations, and (5) clinical recommendations. We'll briefly go through each portion of the guidance in this presentation.

For the purposes of this guidance, *human genome editing* (or *human GE*) was defined as a process by which DNA sequences are added, deleted, altered, or replaced at specified

locations in the genome of human somatic cells, either ex vivo or in vivo, using nuclease-dependent or nuclease-independent GE technologies. We tried to be as inclusive as possible in this definition so as not to limit this guidance to only currently available GE technologies, but also to technologies that may be developed in the future. Also, as with all gene therapies, FDA evaluates human GE products using a science-based approach that weighs the benefits and risks of each product, for each proposed indication. The benefit-risk profile for each product also depends on the indicated patient population, the extent and duration of therapeutic benefit achieved, and the availability of alternative therapeutic options.

There are many GE technologies currently available for therapeutic use. Sponsors should choose the technology and type of genome modification needed for the desired therapeutic effect. This includes whether double-strand DNA breaks are introduced, as well as whether homology directed repair or nonhomologous end-joining is employed. Sponsors should also consider the degree of genome modification needed for the desired therapeutic effect when designing their product. We termed this the *therapeutic editing threshold*. In certain instances, this can be determined using currently available clinical data from alternative therapies, such as stem cell transplants. However, often this needs to be interpreted through thorough nonclinical studies. Lastly, sponsors should select the most appropriate product design and delivery method, taking into account both efficiency and safety. This includes whether GE is performed in vivo or ex vivo and how the GE components are delivered.

Now for a few CMC recommendations. We'll start with the genome editing components. These components are considered the active pharmaceutical ingredients or drug substances when they are formulated into nanoparticles that are directly administered. However, they are considered critical components when they are used to perform routine genome editing in cells ex vivo. This is because even in the case of ex vivo products, without these components, the resulting cell-based gene therapy product would not have the same pharmacological activity. GE components can also be delivered in vivo via viral vectors (for example, an adeno-associated virus, or AAV). In this case, the vector itself is the drug substance and the drug product.

Whether the GE components are considered drug substances or critical components, the same general recommendations apply. Sponsors will need to provide detailed descriptions of how each GE component is manufactured and tested in their IND. It's important to note that, for Phase 1 clinical trials, sponsors should consult the FDA Phase 1 current good manufacturing practice (CGMP) guidance for an understanding of the level of CGMPs needed at this phase of development. For later-phase studies and for licensure, the expectation is that GE component manufacturing would comply with complete CGMPs.

Regarding GE component testing, these components should be evaluated for bioburden or sterility, identity, purity, and activity, as well as residuals based on their manufacturing process. If being stored, these components should also be assessed for stability. Outlines of your testing plan and your stability study protocols and any available engineering runs data or stability data should be provided in the IND. In the case of the stability studies, these should include stability-indicating assays and be conducted on all applicable component presentations (for example, lyophilized and reconstituted materials).

An IND should also contain a detailed description of the drug product (DP) manufacturing process. To recapitulate some earlier statements that I made, when GE components are administered via nanoparticle, the final formulated nanoparticle containing the components is the drug product, or DP. When GE components are expressed in vivo by a directly administered vector, the final formulated vector encoding the GE component is considered the DP. And when GE components are used to modify cells ex vivo, the ex vivo-modified cells in their final formulation are considered the DP. It's important to note here that, when describing the manufacturing process for ex vivo-modified GE products, sponsors should clearly indicate the timing of the genome editing within the overall manufacturing process.

As with all gene therapies, GE products should be evaluated for safety, identity, purity, and potency or strength, in a phase-appropriate manner. For early-phase studies, potency assays evaluating the presence of the desired genetic sequence modification, both genotypically and phenotypically, may be adequate. However, if intended to support a Biologics License Application (BLA), potency assays for BLA-enabling efficacy studies should include an assessment of the intended downstream biological modification or altered cellular function.

For in vivo GE products, the potency assay should also be performed in the target cells or tissues whenever possible. We also recommend inclusion of potency assays in the DP stability studies as early as possible. Ex vivo GE products should also be assessed for on-target editing efficiency and the total number (or frequency) of genome-edited cells, as well as off-target editing frequency, intrachromosomal and interchromosomal rearrangements, and residual GE components based on outcomes of nonclinical and process characterization studies, as well as a risk assessment.

If the ex vivo DP is an allogeneic gene therapy product, additional testing—such as testing for adventitious agents, alloreactive lymphocytes in the case of T cell or NK cell products, and abhorrent growth—may also be needed. Additional characterization of on-target editing events and product heterogeneity, in the case of multiplex editing, should also be performed. It should be noted that, if DP manufacture involves expansion or differentiation of a cell bank, it may be acceptable to perform some of this testing on that cell bank. However, this should be discussed ahead of time with FDA.

Regarding updates made to this guidance based on comments received, we clarified the definition of *GE component* and *therapeutic editing threshold* and further clarified when GE components are considered drug substances, drug products, and critical components. We also clarified that many of the recommendations provided in this guidance apply to GE components used in routine product manufacture and added recommendations for GE components used only once in product manufacture—for example, in the generation of a cell bank that is then expanded or differentiated to produce the DP. We clarified release testing expectations for ex vivo GE products and potency assay expectations for both ex vivo and in vivo GE products and clarified additional testing expectations for allogeneic ex vivo products.

I'll now turn it over to Sandhya to describe the nonclinical recommendations included in this guidance.

DR. SANDHYA SANDUJA: Thank you, Anna, for walking us through the CMC aspects in

the guidance. I will now cover the key points around nonclinical testing of human GE products to support investigational use in clinical trials. Before I do that, I would like to remind the audience that our 2013 guidance—which provides recommendations for nonclinical testing of human cell and gene therapies—generally applies to GE products as well.

So for GE products, what do we recommend? We recommend that pharmacology and toxicology data generated from proof-of-concept (POC) and safety studies should support the overall goal of establishing the rationale and safety of product administration in the intended clinical population. What does that mean? It means that we recommend that sponsors test the intended clinical product to demonstrate its activity and safety. If there are species-specific constraints between the human product and what could be tested in animals, whether these are models of disease or healthy animals, use of the surrogate product may be appropriate. And if that's the case, we request that sponsors provide their rationale for the use of the surrogate product, along with a discussion of how data generated using the surrogate product supports the activity and safety of the intended human GE product.

The nonclinical studies should also establish the feasibility and safety of the intended clinical route of administration. Proof-of-concept and safety data should identify pharmacologically active and safe dose level range, as well as the dosing regimen for the GE product. These studies should be appropriately designed to identify and characterize potential toxicities, which could be local or systemic; the timing of onset of these toxicities, whether acute or delayed, as well as their resolution; and the effect of dose level on these findings. This way, data from nonclinical studies should inform risk to the subjects, appropriate monitoring plan, and mitigation strategies.

The guidance provides recommendations for assessment of activity. In the specific context of genome editing, sponsors should evaluate whether a GE product is working as it is expected to, which means testing if editing at the desired locus is achieved and translates to the desired functional outcome. This can be demonstrated using appropriately designed in vitro and/or in vivo studies using animal models, as feasible. The in vitro models—which may include cultured cells, tissues, explants, and organoids—should be representative of the target cell types. For in vivo studies, the animal species or model that is selected to look at activity should be biologically relevant to assess activity of the human GE product.

It is critical to determine the level of editing that is needed to achieve a therapeutic effect. Therefore, the expectation from POC studies is to identify those dose levels that would lead to a clinical activity. For instance, in an ex vivo setting, what is the minimum number of corrected cells that would need to be infused into patients to see a therapeutic effect? Similarly, in an in vivo setting, what dose levels of the product are expected to achieve desired editing in target tissues to see a clinical outcome?

The guidance also provides recommendations for assessment of safety in the specific context of genome editing. Sponsors should conduct appropriately designed in vitro and in vivo studies to evaluate safety, which involves identification and characterization of on- and off-target editing events. This should be done using multiple methods. In silico

prediction tools are usually a good start, and they should be followed by biochemical and cellular assays to identify and validate on- and off-target events. We recommend using genome-wide and targeted approaches. Using more than one method is recommended to reduce bias. This may also be helpful in reducing false positives and false negatives.

For these studies, appropriate controls should be included to trust the quality of assays and allow data interpretation. For instance, for ex vivo GE products, this should be evaluated using the intended clinical cell type. For in vivo GE products, sponsors should evaluate cell types that can be edited based on the in vivo biodistribution data. Biodistribution data can be collected in stand-alone studies or along with POC or safety studies.

Once the type, location, and frequency of genomic alterations have been identified, it is critical to assess their impact on physiology of cells. By *physiology*, I mean how these edits are impacting cellular function, their survival, their differentiation capacity, and their proliferation capacity. Other factors that would tie into safety assessment overall would include assessment of immunogenicity of the product (which could be due to the vector or to the encoded transgenes), risk associated with the delivery device, safety of the clinical procedure, and any concomitant therapies.

For in vivo safety studies, animal species and models that are used should be biologically relevant to assess product activity, either using the clinical or surrogate product. We do not default to inclusion of any specific species or use of more than one species. The plans for safety studies should be justified by the sponsor as it fits to their product. Depending on the product and the impact on genomic integrity, the risk of tumorigenicity may have to be characterized. Similarly, if biodistribution data are indicative of gonadal distribution and editing in gonadal cell types is seen, additional evaluations for germline transmission and the risk of development and reproductive toxicity would need to be considered.

Finally, I would also like to highlight the key revisions that were made to the nonclinical section of the draft guidance. These changes were made to address the public comments and clarify our expectations with additional examples. Throughout the guidance, the term *preclinical* was changed to *nonclinical* to align with the Food and Drug Omnibus Reform Act (FDORA) of 2022 regulation that defines *nonclinical* to include all in silico, in vitro, and in vivo testing of products that happens before or during clinical trials. We also included examples of in vitro models and possible biological consequences of on- and off-target editing on cell physiology. We also clarified what cells could be used to characterize on- and off- target sites, and we changed *chromosomal rearrangements* to *chromosomal abnormalities* to be inclusive of all changes at the chromosomal level.

With that, I would like to take the opportunity to acknowledge and encourage the constant efforts in the field that are going into product optimization, as well as characterization of off-target effects to enhance safety of human GE products. I will now let my colleague Gavin Imperato go over the clinical aspects covered in the guidance document. Thank you.

DR. GAVIN IMPERATO: Thank you very much, Sandhya, and good afternoon, everyone. I will first discuss some general considerations for clinical development, then discuss some particular components of clinical development programs for GE products, and then close with a summary of the clinical comments that were incorporated into the final guidance.

The first consideration is that clinical development programs should address both the risks associated with the gene therapy product itself and the additional risks associated with the genome editing, as these may be discrete. And they would include off-target editing and unintended consequences of on-target editing, which may be unknown at the time of product administration. We advise that trial design should include an appropriately defined patient population, an efficient and safe approach to product administration (including data-based dosing, dose schedule, and treatment plan), adequate safety monitoring, and appropriate safety and efficacy endpoints. I'll discuss a few of these components in more detail.

The first component I'd like to address is study population. The major take-home point here is that, in its first concept, the study population is to the sponsor's discretion. We do advise sponsors to thoroughly consider the risk-benefit profile of the proposed treatment when defining their patient population. Essentially, an appropriate study population will ensure maximum benefit while minimizing the potential risk to study subjects. It should be based on the product mechanism of action, the study rationale, and balancing, again, the potential risks of the product.

We acknowledge that genome editing products may have significant risks and an uncertain potential for benefit, so first-in-human trials involving these products generally should enroll only subjects for whom no other treatment options are available or justified. And we acknowledge that that may not be true across the board. Subjects with severe or advanced disease may be more willing to accept the potential risks of an investigational human GE product. However, these subjects may be predisposed to experiencing more adverse events or may be receiving concomitant treatments, which could make safety or effectiveness data difficult to interpret. And in some instances, subjects with less advanced or more moderate disease may actually be more appropriate for inclusion in first-in-human clinical studies.

The next consideration is for dose and dose schedules. Clearly, adopting safe and effective product delivery methods is important for minimizing any potential adverse events related to product delivery to target tissues, recognizing that the targets may be quite different depending on the mechanism of action of the genome editing product and its intended clinical use. Both the delivery and the proposed dose schedule should be supported by comprehensive nonclinical data and, where available, also guided by previous clinical experience, if that's applicable, from similar products, including cell and gene therapy products that may or may not have been genome edited.

In terms of the treatment plan, we recommend that any risk or risks anticipated in association with the genome editing product be mitigated by staggered enrollment, with a specified time interval between administration to sequential subjects within and between cohorts. The staggering interval should be of sufficient duration to detect acute and subacute adverse events prior to treatment of additional subjects at the same dose, or prior to increasing the dose of subjects who are treated subsequently. The staggering interval should also take into account the expected duration of activity of the genome editing product. Selection of a study cohort size will depend on the size of the proposed patient population and the amount of acceptable risk in that study population for the genome editing product. In addition, there are several other considerations—such as assessments of

tolerability, feasibility, and pharmacologic activity—that may ultimately influence the cohort size.

I'll next discuss monitoring and follow-up for product-related adverse events, as this is critical for genome editing products. A thorough safety monitoring strategy, with a well-defined toxicity grading system, and a toxicity management plan is crucial for clinical trials that evaluate human genome editing products. Specific consideration should be given by sponsors for adequate monitoring of any off-target editing and adequate assessment of the outcomes of off-target editing, as well as unintended consequences of on-target editing that are anticipated from nonclinical studies. Additional monitoring should also capture adverse events that are related to aberrant cellular and chromosomal changes, immunogenicity, and tumorigenicity. The product-related adverse event monitoring plan and toxicity grading and management strategy should be well described in the clinical protocol.

Long-term follow-up is a critical component of safety monitoring for genome editing products. Prior to enrolling subjects in a clinical study that evaluates a human GE product, they should be asked to provide voluntary, informed consent to long term follow-up, or LTFU. The long-term safety and therapeutic effects of intended on-target editing, as well as off-target editing and unintended editing at the on-target loci, may be unknown at the time of GE product administration. So we recommend, in accordance with our established guidance for long term follow-up, that sponsors conduct LTFU for up to 15 years after product administration, and we advise sponsors to consult with our LTFU guidance for additional details on LTFU. We also recommend that a plan be provided for follow-up, including funding, in the event that the sponsor ceases to operate or decides to inactivate, transfer, or withdraw the IND before the completion of the long term follow-up, to ensure subject safety.

We recommend that study endpoints be based on the proposed indication. For efficacy studies, the primary endpoint should reflect a clinically meaningful effect of the genome editing product on how patients feel, function, or survive. The experience gained from early-phase studies can be helpful to guide the selection of a primary endpoint for late-phase studies.

Accelerated approval is an expedited approval pathway whose goal is to ensure that effective products for rare and serious conditions are delivered to the market in an expedited fashion. It may be an appropriate pathway for approval of a human genome editing product intended to treat such a condition where there's a lack of available alternative treatments. Under accelerated approval, a surrogate endpoint or marker (for example, a laboratory measurement) that is reasonably likely to predict clinical benefit will need to be selected. Alternatively, sponsors may choose to use an intermediate clinical endpoint that is reasonably likely to predict an effect on irreversible morbidity or mortality or other clinical benefit for accelerated approval.

Approval under this pathway may be subject to a requirement to conduct an appropriate postapproval study or studies to verify the predicted effect. We're quite supportive of the use of accelerated approval for genome editing products, and we encourage sponsors to

discuss the use of accelerated approval with us early in their clinical development programs.

I'll next discuss considerations for research involving children. We acknowledge that, for many genome editing products, there may be direct applicability to an exclusive pediatric population. We do recommend that, when possible, clinical studies should enroll individuals who can understand and consent to the study procedures and risks. For clinical investigations that involve children, the critical statutory requirement here is prospect of direct benefit, which requires, among other things, that the risks of the investigational product are justified by the anticipated direct clinical benefit to the children. And this can be demonstrated based on both clinical and nonclinical data.

Where it's possible, we do recommend that studies enroll at least an initial cohort of adult subjects to obtain preliminary safety and efficacy data. If the enrollment of children is justified based on the benefit-risk assessment, then an effort should be made to enroll adolescents prior to the enrollment of younger children and infants. And this, again, will be specific to the particular indication and target population.

This slide summarizes the clinical-related comments that were addressed in the final guidance. We added that there should be adequate clinical assessment of the outcomes of potential off-target editing and unintended consequences of on-target editing. We also clarified language on monitoring the length and magnitude of therapeutic benefit and staggering intervals. We added monitoring of adverse events anticipated from nonclinical studies, clarified that subjects should be asked to provide voluntary informed consent to long term follow-up, clarified when enrollment of pediatric subjects is justified, and added language on accelerated approval of products when indicated.

Thank you very much for your attention. I will now turn it back to Denise to moderate the question-and-answer session.

DR. GAVIN: Thank you, Anna, Sandhya, and Gavin, for those highlights from the guidance. We'll move on to the question-and-answer portion of today's event.

We will answer questions submitted during the registration process. We'll try to answer as many questions as we can, but remember: We're not able to discuss any questions related to specific investigational products or drug applications. We hope that you'll stay with us for this entire time, but we'd also 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 go to the first question. Anna, this question is for you.

What are FDA's recommendations for guide RNA (gRNA) purity analysis?

DR. KWILAS: Thanks so much, Denise. This is a question that we get quite often, so I'm hoping that I can provide some additional feedback regarding gRNA purity analysis. So gRNA purity can be assessed in multiple different ways. For high-performance liquid chromatography (HPLC) and mass spectrometry purity analyses, as anyone who's submitted an application to FDA knows, we recommend the purity of gRNA full-length product to be greater than or equal to 80% and that you identify any impurities that are

present at greater than or equal to 1%. However, we do acknowledge that the acceptance criterion can be dependent on the sensitivity of the method that's used to determine purity.

There are multiple different HPLC and mass spectrometry methods that can be used. Therefore, if you're unable to obtain gRNA purity of greater than or equal to 80% due to this method sensitivity, we ask that you provide further justification for the proposed acceptance criterion. In your justification, we'd like you to include data on the impurities present and a risk assessment on how these impurities may affect the safety of your product as it pertains to off-target editing.

It's also important to note that we do not consider the direct sequencing assay as a strict identity assay. The sequencing assay should not only be used to confirm the correct full-length gRNA sequence, but it should also be used as an orthogonal purity assay to identify and quantify the presence of sequence variants.

Because sequence-related impurities can affect the safety and efficacy of the product, and to ensure that manufacturing yields product with a consistent impurity profile, characterizing the identities of these impurities in the gRNA is very crucial, which is why we recommend that the orthogonal assays are used. Therefore, we also recommend using a sensitive, high-throughput sequencing method, such as next-generation sequencing (NGS), which is more sensitive and has a better capacity for quantifying sequence variants as well.

We also got a lot of questions about read depth. For this type of analysis, we recommend a read depth of 30X, which we believe would be sufficient in this application. Regarding impurity of gRNAs, I also would be remiss if I didn't mention that residual solvent and elemental impurities should also be assessed based on your guide manufacturing process. Thanks, Denise.

DR. GAVIN: Thanks, Anna. Sandhya, this next question is for you.

How should nonclinical studies inform clinical dose levels?

DR. SANDUJA: Thanks, Denise. This, too, is a frequently asked question, and I will try to answer it with respect to different considerations that go into determining a clinical dose level. We recommend that sponsors propose clinical dose levels for GE products based on the activity and safety data from supporting nonclinical studies. As I mentioned earlier during the talk, it is critical to determine the degree of editing that is needed for the desired therapeutic effect—what we call in the guidance *therapeutic editing threshold*. For instance, in an ex vivo setting, what is the minimum number of corrected cells that would need to be infused into patients to see a therapeutic effect? Similarly, for an in vivo setting, this would mean dose levels of the GE product that, when administered in vivo, are expected to achieve the desired percentage of editing in target tissues or organs to see a clinical benefit.

Another thing to note here is that, when extrapolating from animals to humans, the anatomical differences, as well as the route of administration, should be taken into account. We request that sponsors provide their rationale for their method of dose level extrapolation and justify that with supporting data. A human-equivalent dose level should

therefore be calculated based on minimally effective and safe dose information from nonclinical studies or clinical experience, as applicable.

I would like to note here that determination of a therapeutic threshold may depend on the indication, as well as the intended patient population. And for some conditions, clinical data may be available to support a given therapeutic editing threshold. Thanks. Back to you, Denise.

DR. GAVIN: Okay, this next question is for Gavin.

Can accelerated approval be used for genome editing products?

DR. IMPERATO: Thanks, Denise. Yes, absolutely. Accelerated approval can indeed be used for genome editing products. Accelerated approval is a powerful tool, and we strongly encourage sponsors who are developing genome editing products to consider it. The basic concept of accelerated approval is to allow for earlier approval of products that are intended to treat serious conditions, and it's based on the use of a surrogate or intermediate clinical endpoint.

The critical piece of guidance that I'd like to give to sponsors regarding the use of accelerated approval is that it can be quite challenging to develop a surrogate or intermediate clinical endpoint for a given clinical development program. And so, we really encourage sponsors to think critically about the particular surrogate or intermediate clinical endpoint that they plan to use, early in their development programs, and to engage with us to receive feedback regarding the type and quantity of data that may be required to use those particular endpoints in a given clinical development program.

We acknowledge that genome editing products have tremendous potential for the treatment of rare and serious conditions. The Center for Biologics Evaluation and Research is committed to serving patients with rare and serious diseases, and we fully intend to use the accelerated approval pathway as a means of delivering those products to patients, particularly when there's unmet need. Thank you.

DR. GAVIN: Thank you, Gavin. Anna, this next question is for you.

Are potency tests required for each drug substance component (mRNA, gRNA, etc.) of an in vivo genome editing product?

DR. KWILAS: Thanks so much, Denise. Potency assay testing and development is always a big CMC topic of discussion. In this case, the answer is pretty simple: No, potency testing is not required for drug substances. However, we do recommend that you assess the activity of the material to an adequate extent before using it to manufacture the drug product. And this is to ensure the appropriate drug product functionality. This could include assessing protein expression of an editor, for example, if it's provided via mRNA, or it could include cleavage or targeting activity using a surrogate DNA template for a gRNA. These are just considered part of the drug substance lot release testing—again, just to qualify them for use in the drug product manufacturing process. But strict potency testing of these materials is not necessary. Thanks.

DR. GAVIN: Thank you, Anna. The next question is for Sandhya.

How should the risk of tumorigenicity be assessed for GE products?

DR. SANDUJA: Thanks, Denise. I think it really depends on what the product is and what data are indicative of risk for potential tumorigenicity or oncogenicity. So these studies should be considered and addressed depending on the actual product and the genomic alterations (including sites of on-target and off-target edits) and the genomic integrity of the edited cells, which can be indicative of such risk. Therefore, we recommend that sponsors follow a stepwise, science-based, and data-driven approach to this.

While evaluating such risk for tumorigenicity or oncogenicity, they should follow a comprehensive weight-of-evidence approach based on totality of data, which could include data showing changes in gene expression, impact on cell viability, proliferation, persistence of the product or the editing components, any changes seen in organ weights or sizes, or presence of any abnormal lesions. For such evaluation, if in vitro approaches are used, data should be provided to demonstrate that a particular in vitro assay can adequately assess the risk of tumor formation. Similarly, if in vivo studies are conducted to evaluate the potential risk of tumor formation, the duration of these studies and selection of animal species or model—as well as animal numbers—should be appropriate to allow evaluation of the risk. Back to you, Denise. Thank you.

DR. GAVIN: Thank you, Sandhya. The next question is for Gavin.

What are FDA's recommendations for the patient population for a first-in-human study?

DR. IMPERATO: Thanks, Denise. So in the first iteration, the choice of a patient population for a given clinical development program is to the sponsor's discretion, and it will undoubtedly reflect the known mechanism of action of the product and its intended use in a clinical disease setting. That said, in the context of regulatory review, we often provide input to sponsors to help them think through and make modifications to patient populations to optimize benefit-risk.

And that's really the critical take-home point that I'd like to leave with sponsors: that it's really important—particularly for genome editing products, where there are many unknowns—that the benefit-risk profile of the patient population is optimized to the fullest extent possible. In certain circumstances, this will involve treating an initial patient population that does not have any approved therapies for that particular indication. It may also involve the treatment of patients who have more severe disease. But we also acknowledge, given how unique genome editing products are and what their proposed clinical use ultimately is, that there can be significant variability. So these general rules may not ultimately apply.

This is, again, another plug for sponsors to engage with us early in their development programs. Even at the INTERACT stage, we often find it valuable to provide some preliminary feedback as sponsors are thinking through how they may ultimately conduct a clinical trial. And the clinical teams are pleased to provide that feedback regarding clinical study design early in product development. Thank you.

DR. GAVIN: Thank you, Gavin. The next question is for Anna.

Is G-band karyotyping sufficient for the assurance of chromosomal stability for a genome-edited gene therapy product?

DR. KWILAS: Thanks, Denise. This is also a very common question that we get from sponsors. While G-band karyotyping is a very common assay and it's really been around for a very long time, it's also not very sensitive. So FDA recommends that sponsors utilize very sensitive assays to confirm chromosomal stability following genome editing. And this is to help ensure product safety.

Currently, there are multiple alternatives to G-band karyotyping—many recently developed, but then also ones that have been around for a while. These alternatives are often not only more sensitive but also less labor intensive, less variable, and potentially higher throughput. That allows for the testing of more samples and, therefore, a greater safety assurance. FDA recommends that sponsors consider all of the techniques that are available and choose the technique that's most applicable to their product that offers a high level of safety assurance.

Although it's a little off-topic, I'd also like to bring in the use of whole genome sequencing for establishing chromosomal stability and genomic stability in general. For certain products (for example, products that are derived from induced pluripotent stem cells, or iPSCs), FDA highly recommends the use of whole genome sequencing to establish genetic consistency throughout the manufacturing process. In this case, whole genome sequencing can be performed on starting cell banks, as well as genome editing cell banks, and other intermediate steps in the process. And again, this is just another method of assuring both genetic stability and chromosomal stability, specifically using both short-range and long-range sequencing methods.

And, as I mentioned earlier, we do get a lot of questions about read depth. So for something like this, where you're looking at clonal populations but also populations that might have divergent sequences, we do recommend a 50X to a 60X coverage in these cases. I'll turn it back over to you, Denise.

DR. GAVIN: Thank you, Anna. The next question is for Sandhya.

Does the Agency have recommendations on how to approach the nonclinical safety assessment of genome editing activity observed in reproductive organs?

DR. SANDUJA: Thanks, Denise. This, too, is one of the frequently asked questions, given the development of GE products using lipid nanoparticles delivery. And my answer is going to apply to products in general that incorporate GE. So for nonclinical safety assessments of genome editing activity observed in reproductive organs, we recommend that sponsors take a stepwise, science-based, and data-driven approach to the need to evaluate potential for germline transmission, as well as the risk for development and reproductive toxicity. As I mentioned earlier during my talk, the risk would actually depend on the product as well as the likelihood of exposure to editing components in the reproductive organs and specific risk to the target patient population.

So, specifically, the determination of whether to conduct additional nonclinical safety evaluation of genome editing in gonadal tissue should be based on the biodistribution profile of the human GE product. If the product distributes to gonadal tissue and editing is observed, then sponsors should proceed with a more comprehensive assessment of the risk and provide their scientific justification for what their approach is going to be. For example, depending on the type, frequency, and location of those genome edits that are seem in gonadal tissue, in some cases, this can involve more targeted studies to determine the specific cell types that are being edited, where editing is observed, and how these edits are really impacting viability and/or function of these cell types. And scenarios like this would require a more comprehensive assessment of potential for germline transmission, as well as the risk for development and reproductive toxicity.

I want to mention that these therapies are highly novel and that the potential effects of gene editing in gonadal tissue are not really well understood, and that's why having early discussions regarding the risk of development and reproductive toxicity can be very helpful and may be included in a pre-IND meeting package. Back to you, Denise. Thank you.

DR. GAVIN: Thank you, Sandhya. That was very helpful. The next question is for Gavin.

Is it possible to enroll pediatric subjects in a first-in-human study?

DR. IMPERATO: Thanks, Denise. Yeah, it certainly is possible. We acknowledge that, for genome editing products, it's likely that the products will be associated with greater-than-minimal risk. As such, the critical regulatory statute that will apply is prospect of direct benefit. That will essentially mean that the risks of the product are justified by the anticipated direct clinical benefit to the children.

So, as I mentioned previously, we do advise that, where possible, clinical studies enroll individuals who can understand and consent to study procedures and risks. Simultaneously, we do acknowledge that, for genome editing products, the therapeutic rationale for the use of the product clinically may be such that its intended population is pediatric and studies in adults or adolescents may not be feasible. In that case, the clinical evaluation will rely on data that are generated from nonclinical studies. And so we strongly encourage sponsors to think through these considerations as they plan their clinical development programs and to interact with us early to ensure that if, ultimately, they are planning to conduct a first-in-human study in a bona fide pediatric population, they have adequate evidence based on nonclinical studies to support that first-in-human pediatric investigation. Thanks. Back to you, Denise.

DR. GAVIN: Thanks, Gavin. The next question is for Anna.

What additional lot release or characterization testing should be considered when using donor templates or performing multiplex genome editing?

DR. KWILAS: Thanks, Denise. So if the method of genome editing introduces double-strand breaks and you're using donor templates to make the intended genomic modifications, we recommend characterizing template insertion at the intended as well as potentially unintended sites—for example, off-target editing sites, if any have been

identified. We also want there to be evaluation of any insertion at other on-target sites that are still unintended—for example, in the case of multiplex genome editing.

Additionally, when you're performing multiplex genome editing, it is important to characterize the frequency of cells with different combinations of edits. This information is important to understand the composition of your product, as well as to set thresholds for the frequency of cells believed to be actual therapeutic active ingredients. Thanks, Denise.

DR. GAVIN: Thank you. That kind of goes into the next question, which is for Sandhya.

What considerations need to be accounted for when considering how genetic diversity might affect on-target and off-target editing? Does the Agency recommend: (1) specific public genome databases for identifying population-specific variants, (2) an acceptable allelic frequency cutoff, and (3) conducting such analyses before, during, or after clinical studies have been completed?

DR. SANDUJA: Thanks for that question, Denise. Since there are several parts to this question, I will try to answer them in the same order.

So we do not require or endorse use of any specific public genome databases for identifying population-specific variants. However, what we do recommend is that sponsors use an appropriate database that is relevant to the patient population proposed under the clinical study to capture genetic heterogeneity.

We recommend that you select a reasonable variant allele frequency and provide appropriate justification for your selection.

And finally, for the third part, we recommend that you perform these analyses and appropriately design confirmatory tests to measure editing potential at target sites. These analyses should be part of your nonclinical testing strategies that are performed prior to initiation of clinical studies. Back to you, Denise. Thank you.

DR. GAVIN: Thank you. Thank you all for your help answering all of these questions.

I'd like to thank the audience for joining us today for this event. And I'd like to thank the panelists and all of those who helped prepare today's webinar.

As a reminder, the recording of today's event will be posted on FDA.gov in the coming weeks, as well as the slides. For more information, you can visit the FDA website to read the FDA guidance document on developing human gene therapy products that incorporate human genome editing.

Thank you, everyone, for submitting questions in advance of today's event. We had hundreds of questions, and thousands of people registered for the event. So thank you all for your enthusiasm on this topic. While we received far more questions than we were able to address today, it's clear there's lots of enthusiasm in this area, and we hope we can continue this dialogue.

I encourage everyone to visit the OTP virtual town hall recordings, of which there are many over the last year or two, and hope that you get more information on cell and gene

therapies. I also want to put in a shameless plug for another webinar we're hosting on March 7 to discuss a recently finalized guidance, *Considerations for the Development of CAR T Cell Products*. Registration for this event is free, it's open to the public, and we hope we see you there.

You can find more information about other FDA-hosted events at our OTP meetings and workshop page, and we'll drop a link for that for you in the chat box. You can also follow us on X; our handle is @FDACBER. And you can sign up for our listserv. A colleague of mine will post the links in the chat for you.

Thank you again for joining us, and please enjoy the rest of your day. Thank you to everyone. Good afternoon.