Human Gene Therapy Products Incorporating Human Genome Editing

Guidance for Industry

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Table of Contents

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I. INTRODUCTION

In this guidance, we, FDA, are providing recommendations to sponsors developing human gene therapy[1] products incorporating genome editing (GE) of human somatic cells. Specifically, this guidance provides recommendations regarding information that should be provided in an Investigational New Drug (IND) application in order to assess the safety and quality of the investigational GE product, as required in Title 21 of the Code of Federal Regulations 312.23 (21 CFR 312.23). This includes information on product design, product manufacturing and testing, nonclinical safety assessment, and clinical trial design.

In general, FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

II. BACKGROUND

The level of interest in human GE as a scientific technology used in the treatment of human disease has increased substantially, and there has been rapid development of gene therapy products incorporating GE. While the potential of such products for the treatment of human

[1] Human gene therapy seeks to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use. FDA generally considers human gene therapy products to include all products that mediate their effects by transcription or translation of transferred genetic material, or by specifically altering host (human) genetic sequences. Some examples of gene therapy products include nucleic acids, genetically modified microorganisms (e.g., viruses, bacteria, fungi), engineered site-specific nucleases used for human genome editing, and ex vivo genetically modified human cells. Gene therapy products meet the definition of "biological product" in section 351(i) of the Public Health Service (PHS) Act (42 U.S.C. 262(i)) when such products are applicable to the prevention, treatment, or cure of a disease or condition of human beings. (See Federal Register Notice: Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products (58 FR 53248, October 14, 1993), https://www.fda.gov/media/76647/download).

disease is clear, the potential risks are not as well understood. To assist in the translation of these products from the bench to clinical trials, this guidance includes recommendations for how to assess the safety and quality of these products and address the potential risks of these products.

For the purpose of this guidance, human GE is a process by which DNA sequences are added, deleted, altered or replaced at specified location(s) in the genome of human somatic cells, ex vivo or in vivo, using nuclease-dependent or nuclease-independent GE technologies. Human gene therapy products incorporating GE are referred to as human GE products throughout this guidance.

FDA evaluates human GE products using a science-based approach weighing the benefits and risks of each product. The benefit-risk profile for each product depends on the proposed indication and patient population, the extent and duration of therapeutic benefit achieved, and the availability of alternative therapeutic options. Some of the specific risks associated with GE approaches include off-target editing, unintended consequences of on-target editing, and the unknown long term effects of on- and off-target editing.

Human GE is a rapidly evolving field and this guidance encompasses FDA's current thinking regarding the development of human GE products for clinical studies and licensure. As the field evolves, product design advances, and we gain information on the safety of human GE products, we may revise our recommendations to take into account such changes.

III. CONSIDERATIONS FOR PRODUCT DEVELOPMENT

A. General Considerations

A GE technology may be composed of a single or multiple GE component(s). For the purpose of this guidance, a GE component is considered any material that is essential for the intended genomic modification, including those that may not appear in the final drug product. GE components may include, but are not limited to, the editor, DNA targeting elements (i.e., elements used to dictate the target DNA sequence, such as guide RNA) and a donor DNA template (i.e., DNA sequence provided to repair the target sequence), if applicable. When developing a human GE product, we recommend that sponsors consider: 1) the method by which the DNA sequence change will be achieved; 2) the type of genomic modification needed for the desired therapeutic effect; and 3) the delivery method of the human GE components.

1. Genome Editing methods

GE can be achieved by either nuclease-dependent or nuclease-independent methods. Nuclease-dependent GE technologies introduce site-specific breaks in the DNA, which may result in modification of the DNA sequence at the editing site. Some examples of nuclease-dependent GE technologies include, but are not limited to, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), modified-homing endonucleases (meganucleases), and

clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) nucleases. Nuclease-independent GE technologies can change a DNA sequence without cleaving the DNA. Examples of nuclease-independent GE technologies include, but are not limited to, some forms of base editing and synthetic triplex-forming peptide nucleic acids. When choosing a specific GE technology, consideration should be given to the nature of the desired editing outcome (e.g., gene inactivation, restoration, or introduction), the ability to specifically target the desired DNA sequence, and the ability to optimize the GE components to improve safety, efficiency, specificity, or stability.

2. Type and degree of genomic modification

The type of genomic modification needed for the desired therapeutic effect is another important consideration. Many GE approaches rely on intrinsic DNA damage repair pathways to perform genomic modification. Commonly utilized DNA damage repair pathways include, but are not limited to, homology directed repair (HDR) and non-homologous end-joining (NHEJ). HDR utilizes a homologous DNA sequence to repair the DNA break. NHEJ repairs the DNA break by rejoining two ends of cleaved DNA without a homologous repair template. Both HDR and NHEJ can be used to therapeutically modify the genome (Ref. 1). However, it is important to note that NHEJ is relatively independent of the cell cycle, while HDR is most active during S/G2 phase. It is also important to keep in mind that, although these processes can be accurate, they can also result in DNA insertions or deletions (indels) with possible unanticipated consequences. DNA cleavage events, which could be caused by multiplex on-target editing or a combination of on- and/or off-target effects, can also lead to chromosomal rearrangements, including translocations.

The degree of genome modification needed for the desired therapeutic effect (i.e., therapeutic editing threshold) may depend on the indication and the intended patient population. We recommend considering the therapeutic editing threshold (e.g., frequency of editing, number of cells edited) when developing a therapeutic product incorporating human GE. For some conditions, clinical data may be available to support a given therapeutic editing threshold. The potential efficacy of a human GE product will depend on its ability to achieve this therapeutic editing threshold. If clinical data supporting a therapeutic editing threshold are not available, we recommend sponsors provide a justification for the potential efficacy of the achievable editing threshold to initiate a clinical study.

3. Genome Editing Component Delivery Method

When determining the optimal delivery method of the GE components into cells, it is important for sponsors to consider the advantages and limitations of each potential method (e.g., the amount of nucleic acid the delivery vector can contain, efficiency and specificity of targeted delivery, and GE component persistence and stability). For example, with regard to persistence of the GE components, the

longer the GE component (e.g., the nuclease) is functionally active, the greater the risk of unintended genomic modifications, specifically off-target editing and chromosomal rearrangements. Therefore, to limit the degree of potential off-target editing, the duration of GE component persistence should be minimized to the time needed to perform the desired genomic modification, to the extent possible, based on individual product characteristics.

The optimal method for delivering the GE component(s) may depend on whether the product involves ex vivo or in vivo genomic modification. Ex vivo modifications are introduced into cells while the cells are outside the body. The modified cells are then administered to the patient. In vivo modifications result from administration of the GE components in their final formulation to the patient. Sponsors should consider whether ex vivo or in vivo genomic modification is best suited to their target indication and patient population.

For ex vivo genome modification, GE components are commonly delivered into cells via transfection, transduction, electroporation, or other mechanical methods. The GE components may be delivered as DNA, RNA, protein, or ribonucleoprotein complexes (RNPs). If HDR is the repair pathway being utilized, the donor DNA template can be supplied as a plasmid, or using a viral vector, such as recombinant adeno-associated virus (AAV). The chosen GE component delivery method may depend on the ability of the cell type of interest to be efficiently electroporated or transduced by a vector and maintain acceptable levels of viability following electroporation, transfection, or transduction, for example.

For in vivo genome modification, GE components may be delivered by viral vectors or nanoparticles. When choosing an in vivo delivery method, it is important to consider the ability of the delivery vector to target the cells/tissue of interest and minimize distribution to non-targeted tissue. Consideration should also be given to the ability to control expression of vector-delivered GE components (e.g., using tissue-specific promoters, small molecule inhibitors, etc.), if appropriate. Viral vectors may support sustained expression of GE component transgenes, and nanoparticles may allow the temporal delivery of GE components as DNA, RNA, or proteins. The potential for vector-mediated toxicity as well as pre-existing immunity to the GE components and vector should also be considered. The sponsor should select the appropriate delivery method based on the intended use.

B. Chemistry, Manufacturing and Controls (CMC) Recommendations

This guidance is intended to address considerations specific to GE products and is not designed to be a stand-alone CMC guidance. The general CMC considerations for product manufacturing, testing and release of human GE products are the same as those previously described in FDA's guidance entitled "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications

(INDs): Guidance for Industry," January 2020 (Ref. 2) (hereinafter referred to as the "GT CMC Guidance"). Additional recommendations specific to human GE products regarding design, manufacture, and testing of the GE components, as well as the drug product (DP), are described below.

1. Genome Editing Component Design

Many platforms exist to design GE components, particularly the targeting elements. We recommend sponsors utilize design platforms that are most applicable to their genomic target and the type of intended genomic modification. A description of, and rationale for, the design and screening processes should be provided in the IND. The IND should also include the sequences of the GE components and/or expression constructs.

We recommend sponsors optimize the GE components to reduce the potential for off-target genome modification, to the extent possible. Optimization can be performed on the editor or the targeting elements, depending on the GE technology being utilized. GE components, such as guide RNA, can also be optimized to inhibit degradation. The optimization strategy should be described in detail in the IND.

2. Genome Editing Component Manufacture and Testing

GE components can be administered in vivo (e.g., using nanoparticles, plasmids, or viral vectors), or they can be used to modify cells ex vivo. When administered in vivo in the form of DNA, RNA and/or protein via nanoparticles, the GE components are considered the active pharmaceutical ingredients or drug substances. A GE component in its final formulation for in vivo administration is generally considered a DP. For example, when the GE components are expressed in vivo by directly administered plasmids or vectors, the plasmid or vector in its final formulation encoding the GE component is considered the DP. If used to modify cells ex vivo, the GE components are considered critical components for the manufacture of the final product because without these components, the resulting cell product would not have the same pharmacological activity.

The recommendations in section B.2 of this guidance apply to GE components used routinely in the manufacture of each DP lot, either as drug substances or critical components. If the GE component is used only once, for example in the manufacture of a master cell bank (MCB) that is then used in routine DP manufacture, the extent of information and release testing for the GE components may be reduced but should still be sufficient to support the quality of the GE component and safety of the starting material (e.g., MCB). In these cases, we recommend discussing the information available for the GE components and the GE component control strategy with FDA early in product development.

Detailed descriptions of how each GE component is manufactured, purified and tested must be provided in the IND (21 CFR 312.23(a)(7)). We recommend a description of the manufacturing process and any in-process controls for each GE component include a flow diagram(s) and a detailed narrative. We recommend that sponsors provide lists of the raw materials/reagents used during these processes and representative certificates of analysis. Summaries of the following should also be provided in the IND for each GE component manufacturing site:

- The quality control and quality assurance programs in place;
- Procedures in place to ensure component tracking and segregation;
- Procedures in place to prevent, detect and correct deficiencies in the manufacturing process; and
- Procedures for shipping of the GE component from the component manufacturing site to the final product manufacturing site.

The information regarding manufacturing and testing of the GE component is needed even if the GE component is manufactured and/or tested by a contract manufacturer (Ref. 3) and may be incorporated into the IND by cross-reference if it is present in an existing IND or Master File (Ref. 4). For most Phase 1 clinical investigations, sponsors should follow the recommendations in FDA's Guidance for Industry: CGMP for Phase 1 Investigational Drugs for the manufacture of these components (Ref. 5). However, for later Phase studies and for licensure, GE component manufacturing must comply with CGMP under section 501(a)(2)(B) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), with particular consideration for control of raw material/reagent quality, manufacturing process, and analytical methods.

We recommend each GE component be tested appropriately. GE components should be evaluated for sterility, identity, purity and activity, in a phase appropriate manner. Additional testing, such as that for process residuals, should be included, depending on the manufacturing process. Descriptions of the analytical procedures utilized for GE component testing, including the sensitivity (e.g., limit of detection/quantitation) and specificity of the procedures, should be included in the IND. Sponsors should also outline any in-process testing performed to ensure the quality of the GE components, as appropriate.

We recommend GE components be assessed for stability if being stored. Outlines of stability study protocols and any available stability data should be provided in the IND. Stability studies should be conducted on all applicable GE component presentations (e.g., lyophilized and reconstituted materials). Stability studies should include stability-indicating tests assessing critical product attributes, such as purity and activity, that may be affected during storage.

3. Drug Product Manufacture and Testing

An IND should contain a detailed description of the DP manufacturing process, and any in-process controls. We recommend this description include a flow diagram(s) as well as a detailed narrative. We recommend lists of the reagents used during manufacture and certificates of analysis be provided. Please note that for DP intended to be sterile, but that cannot be terminally sterilized, sponsors should provide descriptions of the procedures in place to assure aseptic processing.

An IND should also contain a detailed description of the testing plan for the DP. To ensure that the DP meets acceptable limits for identity, strength,[2] quality and purity as defined in 21 CFR 312.23(a)(7)(iv), the DP testing plan should incorporate evaluations that address any safety concerns introduced due to the manufacturing process or identified during nonclinical studies. For human GE products consisting of ex vivo-modified cells, DP testing should include determination of GE efficiency (e.g., the degree of editing at the on-target site) and may include an assessment of specificity (e.g., the degree of editing at off-target sites). The DP should also be tested for sterility.

Sponsors should describe in detail the analytical procedures used for testing the DP. The descriptions should include the accuracy, precision, sensitivity, and specificity of the assay (as appropriate for the stage of development), as well as any controls and, if applicable, reference materials used to ensure proper assay performance.

To help assure product safety, the DP specifications should be developed based on the starting materials, manufacturing process, desired final product attributes and nonclinical studies. As discussed, the DP may consist of GE components intended for in vivo administration or may be composed of ex vivo -modified cells. In the following sections, we provide recommendations pertaining specifically to each of these human GE DP types:

i. In vivo-administered Human Genome Editing Drug Products

If the GE components will be expressed by a plasmid or viral vector that is administered to patients in vivo, the plasmid/vector in its final formulation is considered the DP and thus a complete description of plasmid/vector manufacturing and testing should be provided in the IND (Ref. 2).

[2] For purposes of this guidance, "strength" is the equivalent of "potency." As defined in 21 CFR 600.3(s), the word *potency* is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result. During the IND stage, sponsors must submit data to assure the identity, quality, purity and strength (21 CFR 312.23(a)(7)(i)) as well as stability (21 CFR 312.23(a)(7)(ii)) of products used during all phases of clinical study. Biological products regulated under section 351 of the PHS Act must meet prescribed requirements of safety, purity and potency for BLA approval (21 CFR 601.2).

If the GE components will be administered using nanoparticles, a detailed description of the nanoparticle formulation, a description of the manufacture of the nanoparticle components, as well as the DP, should be provided in the IND. A description of the tests performed on each nanoparticle component as well as on the DP should also be provided. Please note that release testing of the DP should include assays to evaluate the efficiency of incorporation of each GE component into the nanoparticles.

We recommend sponsors develop potency assays to measure multiple aspects of activity for in vivo human GE DPs. For early phase studies, potency assays evaluating the ability of the GE components to perform the desired genetic sequence modification may be adequate. However, for studies intended to provide primary evidence of effectiveness to support a marketing application, potency assays should include an assessment of the intended downstream biological modification (e.g., corrected cellular function). We recommend that, whenever possible, the potency assays be performed in the target cells or tissues (or a representative surrogate). We also recommend inclusion of such a potency assay in the DP stability studies. Additional information on the development of appropriate potency tests can be found in FDA's Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products (Ref. 6).

ii. Ex vivo-modified Human Genome Editing Drug Products

When describing the manufacturing processes for ex vivo-modified human GE DPs, sponsors should clearly indicate the timing of the GE step within the overall manufacturing process. Descriptions of process controls and in-process testing should also be included for critical steps that may have significant impact on the efficiency or specificity of editing (e.g., RNP formation step in the case of CRISPR-mediated editing). Acceptance criteria or limits should be provided and justified.

Release testing of ex vivo-modified human GE DPs should include evaluation of on-target editing efficiency and the total number (or frequency) of genome-edited cells. Additional characterization of the editing events occurring at the on-target site should also be performed. Assessments of off-target editing frequency, intrachromosomal and interchromosomal rearrangements, and residual GE components may also need to be included for release of the DP based on the outcomes of nonclinical studies. We also recommend that the number of edited cells or the frequency of GE be monitored during stability testing of ex vivo-modified human GE DP.

When establishing potency tests for ex vivo -modified human GE DP, we recommend assays be developed that measure the properties of the cells and the intended downstream biological modifications resulting from GE. For example, we recommend that potency assays for a genome edited $CD34^+$ hematopoietic stem/progenitor cell product measure both the stem/progenitor cell activity and the biologically relevant outcome of the GE. For early phase studies, confirming the desired genetic sequence modification may be adequate to support potency of the DP. However, for studies intended to provide primary evidence of effectiveness to support a marketing application, potency assays should include an assessment of the intended downstream biological modification (e.g., corrected cellular function). We also recommend inclusion of such a potency assay in the DP stability studies. In some instances, surrogate potency tests may be acceptable; however, if proposing a surrogate potency assay, it is critical that the data provided supports a correlation between the output of the surrogate potency test and the biologically relevant outcome of the GE (Ref. 6).

Please note that if the ex vivo-modified human GE DP is an allogeneic human cell product, where a product lot is meant to treat multiple patients, additional DP testing and establishment of acceptance criteria may be appropriate. For example, additional adventitious agent testing, stringent acceptance criteria for the number of potentially alloreactive lymphocytes and absence of aberrant growth (i.e., if the DP is an allogeneic T cell product) should be included in lot release testing. Additional information on allogeneic products, including donor eligibility and testing recommendations for cell banks originating from allogeneic cells or tissues, are discussed in the GT CMC Guidance (Ref. 3)

Additional in-process, lot release, and characterization testing may be needed for more complex products (e.g., products incorporating multiple rounds of genome editing or the creation of multiple cell banks). Also, the timing and type of testing may depend on when the GE process is performed in manufacturing. For example, if a genome edited MCB is used to produce the DP without additional GE steps, some testing may be able to be performed on the MCB.

IV. CONSIDERATIONS FOR NONCLINICAL STUDIES

The overall objectives of a nonclinical program for a human GE product are generally the same as those described for gene therapy products in FDA's Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products (Ref. 7) ("Preclinical

Assessment Guidance"). These objectives include: 1) identification of a pharmacologically-active dose level range; 2) recommendations for an initial clinical dose level, dose -escalation scheme, and dosing regimen; 3) establishment of feasibility and reasonable safety of the proposed clinical route of administration (ROA); 4) support for the target patient population; and 5) identification of potential toxicities and physiologic parameters that help guide clinical monitoring and risk mitigation plans. More details for these general considerations in nonclinical studies are available in the above noted guidance (Ref. 7). The following general elements should be incorporated into the nonclinical development program for a human GE product:

- Nonclinical proof-of-concept (POC) studies should be conducted to establish feasibility and support the scientific rationale for administration of the investigational human GE product in a clinical trial.

    - The use of in vitro models (examples include, but are not limited to, cultured cells, tissues, explants, organoids, etc.) should be considered for evaluating the activity of a human GE product in the target cell type(s) for genomic modification.

    - The animal species and/or models selected for in vivo studies should demonstrate a biological response to the human GE product (see section IV.A of this guidance for further discussion). Given the differences in the genomic sequences between humans and animals, analysis of the biological activity may be done in a species-specific context (e.g., using a surrogate product), as appropriate.

- Nonclinical safety studies should be designed to identify potential risks associated with administration of the GE product. Toxicities may be related to the human GE product, delivery method, and/or modification of the genomic structure.

    - The safety assessment should include identification and characterization of on- and off-target editing, chromosomal abnormalities, and their biological consequences.

    - In vivo nonclinical safety studies for a human GE product (or surrogate product) should incorporate elements of the planned clinical trial (e.g., dose level range, ROA, delivery device, dosing schedule, study endpoints, concomitant therapies, etc.), to the extent feasible. Study designs should be sufficiently comprehensive to permit identification, characterization, and quantification of potential local and systemic toxicities, their onset (i.e., acute or delayed) and resolution, and the effect of dose level on these findings.

- Assessment of biodistribution should be conducted to characterize the distribution, persistence, and clearance of the GE product, any expressed GE components in vivo, editing activity in target and non-target tissues, and the potential for inadvertent germline modification. These evaluations may be conducted independently or in conjunction with POC and/or safety studies.

Specific recommendations for the assessment of activity and safety of a human GE product are as follows:

A. Product Evaluated in Nonclinical Studies

- The intended clinical GE product should be evaluated in the definitive POC and safety studies, as feasible.

- Due to differences in the genomic sequences between animals and humans, POC and/or safety studies may warrant the use of a surrogate GE product (e.g., substitution of the human elements including GE components, promoter(s), and transgene(s) with the respective species-specific elements in the GE product) in situations where administration of the investigational human GE product would not be informative. We recommend sponsors provide scientific justification for the administration of a surrogate GE product, and establish the biological relevance of the surrogate compared to the human GE product.[3]

- For ex vivo-modified human GE products, the clinical cell source/type should be used for the definitive nonclinical studies. Scientific justification should be provided if an alternative cell source/type is used in any studies.

- Each human GE product lot evaluated in the nonclinical studies should be characterized according to appropriate specifications, consistent with the stage of product development. Retention of adequate samples from each nonclinical lot is recommended in case future reanalysis is warranted.

B. Assessment of Activity

Pharmacology studies conducted to assess the activity profile of a human GE product can address important considerations such as the following:

- Specificity and efficiency of editing in target and non-target cells;

- Functionality of the corrected or expressed gene product (e.g., protein, RNA), if applicable;

- Editing efficiency required to achieve the desired biological activity or therapeutic effect; and

- Durability of the genomic modification and resulting biological response

[3] The nonclinical program for any investigational product should be individualized with respect to scope, complexity, and overall design. We support the principles of the "3Rs," to reduce, refine, and replace animal use in testing when feasible. Proposals, with justification for any potential alternative approaches (e.g., in vitro or in silico testing), should be submitted during early communication meetings with FDA. We will consider if such an alternative method could be used in place of an animal test method.

C. Assessment of Safety

Comprehensive safety studies should be conducted to characterize the risks of a human GE product. These studies can include the following:

- Identification of on- and off-target editing events, including the type, frequency, and location.
    - Multiple methods (e.g., in silico, biochemical, cellular-based assays) that include a genome-wide analysis are recommended to reduce bias in identification of potential off-target sites. When possible, the analysis should be performed with the relevant human cell type(s) obtained from multiple donors. For in vivo GE products, the analysis should also include the major cell types in which editing events are detected.
    - Verification of off-target sites should be conducted using methods with adequate sensitivity to detect low frequency events. For ex vivo GE products, the final clinical product obtained from multiple donors should be evaluated. For in vivo GE products, the analysis should also include the major cell types in which editing events are detected.
    - Appropriate controls should be included to confirm the quality of the assay and to assure interpretability of the results and its suitability for the intended use.
- Assessment of genomic integrity, including chromosomal abnormalities, insertions or deletions, and potential oncogenicity or insertional mutagenesis. This may include assessment for clonal expansion and/or unregulated proliferation of edited cells.
- Evaluation of the biological consequences associated with on- and off-target editing, including, but not limited to, viability and function of the edited cells (e.g., differentiation capacity of progenitor cells).
- Assessment of immunogenicity of the GE components and expressed transgene(s).

V. CONSIDERATIONS FOR CLINICAL STUDIES

We recommend that clinical development programs of human GE products address both the risks associated with the gene therapy product itself as well as the additional risks associated with the GE, including off-target editing and unintended consequences of on-target editing, which may be unknown at the time of product administration. Clinical 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. Additionally, long term follow-up is recommended for clinical trial subjects receiving human GE products for evaluation of clinical safety. In general, the overall study design, assessment of adverse events (AEs), and subject follow-up plans should be well described in the IND. The overall considerations for clinical trial design for GE products are similar to those outlined for other cellular and gene therapy products (Ref. 8) and are briefly described in section V.A-F of this guidance.

A. Study Population

Selecting the appropriate study population ensures maximum benefit, while minimizing the potential risk to subjects. We recommend the choice of study population be well supported based on the product MOA and study rationale, along with balancing the potential risks of the product. Human GE products may have significant risks and an uncertain potential for benefits. Therefore, first-in-human trials involving such products generally should enroll only subjects for whom no other treatment options are available or justified. Factors to consider in determining the study population include:

- The MOA of the product in the context of a specific disease;
- The anticipated duration and magnitude of therapeutic benefit;
- The availability, safety, tolerability, and effectiveness of alternative therapeutic options for the patient population;
- 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 AEs or be receiving concomitant treatments, which could make the safety or effectiveness data difficult to interpret. Therefore, in some instances, subjects with less advanced or more moderate disease may be appropriate for inclusion in first-in-human clinical studies.

B. Dose and Dose Schedules

Adopting safe and effective product delivery methods is important for minimizing any potential AEs related to product delivery to target tissues. Both the delivery and the proposed dose schedules should be supported by comprehensive nonclinical data and, where available, guided by previous clinical experience from similar products, including cellular or gene therapy products that may or may not have been genome edited. Additional aspects of dose and regimen for clinical trials evaluating human GE products are similar to those for other cellular and gene therapy products and can be found in section IV.D of FDA's Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products; Guidance for Industry (Ref. 8).

C. Treatment Plan

We recommend that any risk(s) anticipated in association with the GE product be mitigated by staggered subject enrollment, with a specified time interval between product administration to sequential subjects within and between cohorts. For first-in-human studies, the staggering interval should be of sufficient duration to detect acute and subacute AEs prior to treating additional subjects at the same dose, or prior to increasing the dose in subjects treated subsequently. The staggering interval should also take into account the expected duration of activity of the human GE product.

Selection of study cohort size depends on the size of the proposed patient population and the amount of acceptable risk in that study population for the GE product. In addition, other considerations, such as assessments of tolerability, feasibility, and pharmacologic activity may influence choice of cohort size. Additional cohort size considerations are outlined in section IV.E.2 of FDA's Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products; Guidance for Industry (Ref. 8).

D. Monitoring and Follow-Up

1. Assessment of Product-Related Adverse Events

A thorough safety monitoring strategy, with a well-defined toxicity grading system, and a toxicity management plan is crucial for clinical trials evaluating human GE products. Specific consideration should be given for adequate monitoring of any off-target editing and adequate assessment of the outcomes of off-target editing and unintended consequences of on-target editing as anticipated from nonclinical studies. Additional monitoring should capture AEs 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 described in the clinical protocol.

Applicable reporting requirements outlined in 21 CFR 312.32 for adverse experiences associated with the use of the human GE product must be followed. Additional information concerning good clinical practice can be found in FDA's E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1); Guidance for Industry (Ref. 9).

2. Long Term Follow-Up

Prior to enrolling subjects in a clinical study evaluating a human GE product, they should be asked to provide voluntary, informed consent to long term follow-up (LTFU). As discussed, 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. Therefore, we recommend that sponsors conduct LTFU for up to 15 years after product administration, as outlined in FDA's Long Term Follow-Up After Administration

of Human Gene Therapy; Guidance for Industry (Ref. 10). FDA also recommends that a plan be provided for follow-up, including funding, in the event the sponsor ceases to operate or decides to inactivate, transfer, or withdraw the IND before completion of the long term follow up.

E. Study Endpoints

We recommend that study endpoints be based on the proposed indication. For efficacy studies, the primary endpoint should also reflect a clinically meaningful effect of the GE product on how patients feel, function, or survive. The experience gained from early-phase clinical studies can help guide the selection of a primary endpoint for late-phase studies. Further information may be obtained from FDA's Guidance for Industry: Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products (Ref. 11). Additionally, accelerated approval may be an appropriate pathway for approval of a human GE product intended to treat a serious or life-threatening disease or condition, where there is a lack of available alternative treatments. Under accelerated approval, a surrogate endpoint or marker (e.g., a laboratory measurement) that is reasonably likely to predict clinical benefit will need to be selected; alternatively, an intermediate clinical endpoint that that is reasonably likely to predict an effect on irreversible morbidity or mortality or other clinical benefit will need to be selected.[4] Approval under this pathway may be subject to a requirement to conduct an appropriate postapproval study or studies to verify and described the predicted effect.[5] FDA is supportive of the use of accelerated approval for GE products and encourages sponsors to discuss the potential eligibility of a GE product for such program, including the proposed surrogate endpoints or intermediate clinical endpoints, early in development of the clinical trial. FDA's Guidance for Industry: Expedited Programs for Serious Conditions – Drugs and Biologics (Ref. 12).

F. Special Considerations for Research Involving Children

When possible, clinical studies should enroll individuals who can understand and consent to the study procedures and risks. For clinical investigations involving children, associated with greater than minimal risk, a reviewing Institutional Review Board must find, among other things, that these risks are justified by the anticipated direct clinical benefit to the children (21 CFR 50.52). Such prospect of direct benefit should be evidence-based (e.g., from adult humans or appropriate animal models). Therefore, it is important to enroll at least an initial cohort of adult subjects, whenever feasible, to obtain preliminary data on safety and feasibility, bioactivity, and preliminary efficacy to support enrollment of children. If enrollment of children is justified based on the benefit-risk assessment, then an effort should be made to enroll adolescents prior to enrollment of younger children and infants, as appropriate for the specific disease of interest. See the draft guidance entitled "Ethical Considerations for Clinical Investigations of Medical Products Involving Children; Draft Guidance for Industry, Sponsors, and IRBs,"

[4] See FD&C Act section 506(c)(1)(A).
[5] See FD&C Act section 506(c)(2)(A).

September 2022 (Ref. 13) for additional recommendations on including pediatric subjects in cell and gene therapy trials.

VI. COMMUNICATION WITH FDA

We recommend sponsors of human GE products communicate with the Office of Therapeutic Products (OTP) in the Center for Biologics Evaluation and Research (CBER) early in product development, before submission of an IND, to discuss the product-specific considerations for transitioning these products to the clinical phase of product development. There are different meeting types that can be used for such discussions, depending on the stage of product development and the issues to be considered. These include pre-IND meetings prior to submission of the IND (Ref. 14), and INitial Targeted Engagement for Regulatory Advice on CBER producTs (INTERACT) meetings, which can be used earlier in development to discuss issues such as nonclinical development or manufacturing, so that sponsors can obtain non-binding regulatory advice.[6]

[6] For additional information about INTERACT meetings with OTP, please see https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/otp-interact-meeting.

VII. REFERENCES

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12. Guidance for Industry: Expedited Programs for Serious Conditions – Drugs and Biologics, May 2014, https://www.fda.gov/media/86377/download.

13. Ethical Considerations for Clinical Investigations of Medical Products Involving Children; Draft Guidance for Industry, Sponsors, and IRBs, September 2022, https://www.fda.gov/media/161740/download.*

14. Draft Guidance for Industry: Formal Meetings Between the FDA and Sponsors or Applicants, December 2017. https://www.fda.gov/media/109951/download.*

* When finalized, this guidance will represent FDA's current thinking on this topic.

APPENDIX
Abbreviations and Acronyms

Term	Description
AAV	Adeno-Associated Virus
AE	Adverse Event
Cas	CRISPR-associated
CBER	Center for Biologics Evaluation and Research
CGMP	Current Good Manufacturing Practice
CMC	Chemistry, Manufacturing and Controls
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeat
DNA	Deoxyribonucleic Acid
DP	Drug Product
FDA	Food and Drug Administration
GE	Genome Editing
HDR	Homology Directed Repair
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IND	Investigational New Drug
Indels	Insertions or Deletions
INTERACT	INitial Targeted Engagement for Regulatory Advice on CBER producTs
LTFU	Long Term Follow-Up
MOA	Mechanism of Action
NHEJ	Non-Homologous End-Joining
OTP	Office of Therapeutic Products
PHS	Public Health Service
POC	Proof-of-Concept
RNA	Ribonucleic Acid
RNP	Ribonucleoprotein Complex
ROA	Route of Administration
TALEN	Transcription Activator-Like Effector Nuclease
ZFN	Zinc Finger Nuclease