Human Gene Therapy Products Incorporating Human Genome Editing

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
March 2022

Table of Contents

Human Gene Therapy Products Incorporating Human Genome Editing

Draft Guidance for Industry

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

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, product testing, preclinical safety assessment, and clinical trial design.

The contents of this document do not have the force and effect of law and are not meant to bind the public in any way, unless specifically incorporated into a contract. This document is intended only to provide clarity to the public regarding existing requirements under the law. FDA guidance documents, including this guidance, should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA guidances means that something is suggested or recommended, but not required.

[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).

II. BACKGROUND

Over the past 10 years, 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 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- and off-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). These GE components may include the nuclease, 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 cleavage site. Some examples of nuclease-dependent GE technologies are 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, base editing and synthetic triplex-forming peptide nucleic acids. When choosing a specific GE technology, consideration should be given to the mechanism of action (MOA), the ability to specifically target the desired DNA sequence, and the ability to optimize the GE components to improve 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. Two commonly utilized DNA damage repair pathways are 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 unintended DNA insertions or deletions (indels) with possible unanticipated consequences.

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

3. Genome Editing Component Delivery Method

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

persistence of the GE components, the longer the persistence of certain GE components (e.g., the nuclease), 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.

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 in vivo or ex vivo genomic modification is best suited to their target indication and patient population.

For ex vivo genome modification, the cell type of interest may be amenable to electroporation or mechanical methods, in which case the GE components may be delivered as DNA, RNA, protein or ribonucleoprotein complexes (RNPs) for CRISPR/Cas9. If HDR is the repair pathway being used, the donor DNA template can be supplied as a plasmid, or using a viral vector, such as adeno-associated virus (AAV). The chosen method of delivery 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 or transduction.

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), if appropriate. Viral vectors may support sustained expression of GE component transgenes, and nanoparticles may allow the temporal delivery of GE components as messenger RNAs or proteins. The potential for vector-mediated toxicity as well as pre-existing immunity to the GE component 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

The general CMC considerations for product manufacturing, testing and release of human GE products are the same as those previously described (Ref. 2). 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.

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 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, GE component quality is considered critical for the manufacture of the final product because without these components, the resulting cell product would not have the same pharmacological activity.

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 sponsors provide lists of the reagents used during these processes and certificates of analysis. Descriptions of the following should 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 product 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.

This information is needed even if the GE component is manufactured by a contract manufacturer (Ref. 3) and may also be cross-referenced 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 (see 21 CFR 210.2(c); Ref. 5). However, for later Phase studies and for licensure, GE components must be manufactured according to CGMP standards (21 CFR Parts 210 and 211), with particular consideration for control of reagent quality, manufacturing process, and analytical methods.

We recommend each GE component be tested appropriately. In addition to evaluating the sterility, identity, purity and functionality of each component, as applicable, 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 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 components, as appropriate.

We also recommend GE components be assessed for stability. Outlines of stability study protocols and any available stability data should be provided in the IND. Stability studies should be conducted on all GE components (e.g., lyophilized and reconstituted materials, if applicable). Stability studies should include stability-indicating tests assessing critical product attributes, such as purity and functionality, 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 details on measures taken to ensure 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, potency/strength, 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 preclinical studies. For human GE products consisting of ex vivo-modified cells, this testing should include determination of GE efficiency (e.g., the degree of cleavage at the on-target site)

and specificity (e.g., the degree of cleavage 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 applicable), as well as any controls and, if applicable, reference materials used to ensure proper assay performance.

To help ensure product safety, the DP specifications should be developed based on the starting materials, manufacturing process, desired final product attributes and preclinical 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).

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 testing should include assays to evaluate the efficiency of incorporation of each GE component into the nanoparticles. Please also note that certain nanoparticles used for in vivo delivery of GE components may be considered a delivery device.

When establishing potency assays for in vivo human GE DPs, we recommend that assays be developed to measure the ability of the GE components to perform the desired molecular genetic and downstream biological modifications in the target cells or tissues. 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, descriptions of process controls and in-process testing should 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.

Testing of ex vivo-modified human GE DPs should include evaluation of the following:

- On-target editing efficiency, including characterization of the editing events occurring at the on-target site;
- Off-target editing frequency;
- Chromosomal rearrangements;
- Residual GE components; and
- Total number of genome-edited cells.

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 functional outcomes of the genomic 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 functional outcome of the GE. In some instances, surrogate potency tests may be acceptable; however, it is critical that the data provided supports a correlation between the output of the surrogate potency test and the functional 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 testing and establishment of acceptance criteria may be appropriate. For example, in addition to meeting the donor eligibility screening and testing criteria outlined in 21 CFR Part 1271, Subpart C, additional donor screening and testing may be warranted. More extensive analysis of the GE events occurring at both on- and off-target

sites, additional adventitious agent testing, establishment of stringent acceptance criteria for the number of alloreactive lymphocytes and absence of aberrant growth (i.e., if the DP is an allogeneic T cell product) may also be warranted.

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).

IV. CONSIDERATIONS FOR PRECLINICAL STUDIES

The overall objectives of a preclinical program for an investigational 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 biologically active dose range; 2) recommendations for an initial clinical dose level, dose-escalation schedule, 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 for a particular investigational product. More details for these general considerations in preclinical studies are available in the above noted guidance (Ref. 7). The following general elements should be incorporated into the preclinical development program for an investigational GE product:

- Preclinical in vitro and in vivo proof-of-concept (POC) studies are recommended 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 should be considered for evaluating the activity of a 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 investigational GE product or species-specific surrogate 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 and applied to the clinical product, as appropriate.

- We recommend preclinical safety studies be designed to identify potential risks associated with administration of the GE product. Potential toxicities may be related to the delivery modality for the GE components, expression of the GE components, modification of the genomic structure, and/or expression of the gene product.

- The safety assessment should include identification and characterization of off-target activity, chromosomal rearrangements, and their biological consequences, as feasible.

- In vivo preclinical safety studies for an investigational GE product should incorporate elements of the planned clinical trial (e.g., dose range, ROA, delivery device, dosing schedule, evaluation endpoints), 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 potential resolution, and the effect of dose level on these findings.

- We recommend biodistribution studies be conducted to characterize the distribution, persistence, and clearance of the GE product, as well as any expressed GE components in vivo. Evaluation of the biodistribution profile of the edited genetic sequence and persistence of the gene product may provide additional information on the extent of editing activity in target and non-target tissues.

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

A. Product Evaluated in Preclinical Studies

- The investigational human GE product should be evaluated in the definitive POC and safety studies, when 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) for 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 biological relevance of the surrogate compared to the investigational human GE product.

- For ex vivo-modified GE products, the clinical cell source should be used for the definitive preclinical studies. If an alternative cell source is used in any studies, scientific justification should be provided for the cell source selected.

- Each GE product lot evaluated in the preclinical studies should be characterized according to appropriate specifications, consistent with the stage of product development. This information will be critical to establish comparability of the product used in preclinical studies to the clinical product, if necessary.

B. Assessment of Activity

We recommend preclinical in vitro and in vivo POC studies assess 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;
- Durability of the genomic modification and resulting biological response; and
- Effects of genetic variation on editing activity across the target population.

C. Assessment of Safety

We recommend preclinical studies be conducted to identify and characterize the risk of GE at on- and off-target loci and include the following:

- Identification of off-target editing activity, including the type, frequency, and location of all off-target editing events.
    - The use of multiple orthogonal methods (e.g., in silico, biochemical, cellular-based assays) that include an unbiased genome-wide analysis is recommended for identification of potential off-target sites. When possible, the analysis should be performed using the target human cell type(s) from multiple donors.
    - Verification of bona fide off-target sites should be conducted using methods with adequate sensitivity to detect low frequency events. The analysis should be performed using the target human cell type(s) from multiple donors.
    - 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 rearrangements, large insertions or deletions, integration of exogenous DNA, and potential oncogenicity or insertional mutagenesis. For ex vivo-modified cells, this may include assessment for clonal expansion and/or unregulated proliferation.

- Evaluation of the biological consequences associated with on- and off-target editing, as feasible.

- Immunogenicity of the GE components and gene product expressed.

- Characterization of the kinetic profile of GE components expression and editing activity.

- Assessment of viability and any selective survival advantage of the edited cells.

- Preservation of cell functionality following GE (e.g., differentiation capacity for progenitor cells).

- Evaluation of the potential for inadvertent germline modification.

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 unintended consequences of on- and off-target editing, which may be unknown at the time of product administration. Clinical trial design should include appropriate patient selection, an efficient and safe approach to product administration (including data-based dosing, dose schedule, and treatment plan), adequate safety monitoring, and an appropriate choice of 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 be designed to enroll only subjects for whom no other treatment options are available or acceptable. Factors to consider in determining the study population include:

- The MOA of the product in the context of a specific disease;

- The anticipated duration of therapeutic benefit;

- The availability and effectiveness of alternative therapeutic options for the patient population;

- Subjects with severe or advanced disease may be more willing to accept the 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 well established, 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 preclinical 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. The staggering interval should be of sufficient duration to monitor for 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 unintended consequences of on- and off-target editing. Additional monitoring should capture AEs related to aberrant cellular proliferation, immunogenicity, and tumorigenicity. Such AEs should be anticipated from pre-clinical studies, if possible, and toxicity grading and management strategy should be outlined 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 should be asked to provide voluntary, informed consent to long term follow-up (LTFU). As discussed, the long term effects of intended, as well as unintended, editing at on- and off-target loci may be unknown at the time of GE product administration. Therefore, we recommend that sponsors conduct LTFU at least 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).

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. 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).

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 pediatric subjects. If enrollment of pediatric subjects is justified, 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.

VI. COMMUNICATION WITH FDA

We recommend sponsors of human GE products communicate with the Office of Tissues and Advanced Therapies (OTAT) 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. 12), and INitial Targeted Engagement for Regulatory Advice on CBER producTs (INTERACT) meetings, which can be used earlier in development to discuss issues such as preclinical development or manufacturing, so that sponsors can obtain non-binding regulatory advice.[2]

[2] For additional information about INTERACT meetings, please see https://www.fda.gov/vaccines-blood-biologics/industry-biologics/interact-meetings.

VII. REFERENCES

1. Cox D.B., et al. Therapeutic genome editing: prospects and challenges. Nature Medicine, 2015. 21(2):121-131.

2. Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs); Guidance for Industry, January 2020, https://www.fda.gov/media/113760/download.

3. Contract Manufacturing Arrangements for Drugs: Quality Agreements; Guidance for Industry, November 2016, https://www.fda.gov/media/86193/download.

4. Drug Master Files: Draft Guidance for Industry, October 2019, https://www.fda.gov/media/131861/download.*

5. Guidance for Industry: CGMP for Phase 1 Investigational Drugs, July 2008, https://www.fda.gov/media/70975/download.

6. Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products, January 2011, https://www.fda.gov/media/79856/download.

7. Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products, November 2013, https://www.fda.gov/media/87564/download.

8. Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products; Guidance for Industry, June 2015, https://www.fda.gov/media/106369/download.

9. E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1); Guidance for Industry, May 2018, https://www.fda.gov/media/93884/download.

10. Long Term Follow-Up After Administration of Human Gene Therapy Products; Guidance for Industry, January 2020, https://www.fda.gov/media/113768/download.

11. Guidance for Industry: Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products, May 1998, https://www.fda.gov/media/71655/download.

12. 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.

672 # APPENDIX

673 # Abbreviations and Acronyms

674

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
OTAT	Office of Tissues and Advanced Therapies
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

675