Review

Gene Editing: The Regulatory Perspective

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Gene Editing: The Regulatory Perspective

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Abstract: Gene or genome editing (GE) revises, removes, or replaces a mutated gene at the DNA level; *it is a tool*. Gene therapy (GT) offsets mutations by introducing a "normal" version of the gene into the body while the diseased gene remains in the genome; *it is a medicine*. So far, no GE product has been approved, as opposed to 22 GT products that cost up to millions of dollars per dose. The FDA has recently added a guideline specific to gene editing that should be understood to enable faster development of GE products; at the same time, the FDA also needs to bring more clarification and make several amendments to this guideline to make it more rational.

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Introduction

Genetic mutations trigger human evolution and bring thousands of diseases. Until gene manipulation technologies arrived, treating the ailments of about a billion globally suffering from mutated, disease-causing genes was impossible. While the FDA has approved 22 GT products [1], and the EMA [2] has approved 13 products (labeled as Advanced Therapy Medicinal Products) (ATMPs), neither agency has approved any GE product. However, many are under development. [3] Thus, the developers should improve their understanding of the scientific, technical, and regulatory perspectives based on the lessons learned from the GT products to reduce the cost and time of bringing them to patients. This is the primary focus of this paper.

The first FDA-approved GT product Kymriah (tisagenlecleucel), an antigen receptor T cell that is chimeric (CAR-T) [4], costs about half a million dollars per dose; the most recent gene therapy product, Hemgenix, a hemophilia treatment, costs $3.5 million per dose. [5] This high cost comes from the amortization of development costs of about $1-5 billion per product [6] distributed over a smaller number of patients. However, there are many creative possibilities to reduce this high cost and the long time it takes to secure regulatory approval comprising outsourcing the work, partnering with academic institutions, using special IND approvals, and working closely with the regulatory agencies, questioning the listed testing requirements.

The *ex vivo* GE target cells include stem cells and immune cells that can be cultured and transplanted into the patient, such as SCID, ß-thalassemia, sickle cell anemia, and CAR-T therapy, including hematopoietic stem cells, T cells, and NK cells. These products fall under the category of GT, wherein modified cells are introduced, leaving the mutated genes in place. [7] The greater utility of GE comes from *in vivo* strategy that eliminates cell collection, isolation, expansion, editing, selection, and transplantation, making it more accessible and effective at targeting a single organ than the entire organism. [8] The GE is also ideally suited for precision or personalized therapies [9–11], primarily in cancer treatment. In 2021, the approval of 17 personalized (individualized, precision) medicines represented approximately 35 percent of all newly approved therapeutic molecular entities [12]. In the future, this category will likely include GE products as well. In addition, a better understanding of individual variability and using next-generation sequencing (NGS) technologies to discover new uncommon genetic illnesses have made individualized therapies more practical.

About 400 million people worldwide with 7,000 diseases caused by mutations in single genes [13] can benefit if GE products are made affordable. Gene editing needs are continuously expanding, such as the recent suggestions to monitor children for prospective genetic disorders and fix them well before they become evident.

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Regulators can do more to promote the standardization of off-target (and on-target) effect measurement, including implementing the proper methods, sample handling practices, quality control measures, data analysis, and clinical interpretation. For example, the EU guidelines for genetically modified cells' quality, non-clinical requirements, and clinical requirements do not specify methods for determining on- and off-target effects because they are still evolving. [14] The FDA's guidance is similar in not outlining how off-target effects discovered in pre-clinical studies should be monitored over an extended period. [15]

Since gene editing is a newer field, the regulatory control standards take a high risk-based approach, in the abundance of caution that has significantly hampered the entry of GE products. In certain situations, relying solely on product quality results will not be appropriate, requiring additional non-clinical and clinical data. In addition, release testing may be affected by the personalized nature of gene editing therapies, regulatory batch testing, and release requirements, which can consume a sizable portion of the batch.

Due to the significance of the in vivo cellular environment on gene editing efficiency, for instance, there are difficulties in predicting the clinical effects of gene editing treatments in humans using non-clinical efficacy models. A thorough investigation and attempts to develop such relevant non-clinical efficacy models should be discussed in any case and requested on a case-by-case basis in regulatory interactions, despite the idea that gene editing treatments may not need them or may not have any relevant non-clinical efficacy models.

Numerous unknowns exist regarding the handling and regulatory classification of gene editing products. Some gene editing products, for instance, are subject to GMO regulation and requirements in the EU, [16] where various competent authorities assess an ATMP GMO submission and a Clinical Trial Application (CTA) submission, resulting in timing and content inconsistencies. Several initiatives, such as standard application forms and good practice documents, are being made to clarify and harmonize the requirements. [17]

A Guideline on Assuring the Quality and Safety of Gene Therapy Products has been published by PMDA (not specific to gene editing). However, this raises scientific and policy concerns. Therefore, to maintain the orphan status of potentially curative ATMPs, significant benefit over an authorized ATMP must be demonstrated. Most ex vivo GE products will fulfill the ATMP definition as GTMPs or cell therapies. The US situation is more precise because the FDA classifies all gene editing products (both in vivo and ex vivo) as gene therapy products.

Gene editing products may also have difficulties maintaining orphan designation and proving significant benefits because of their long-lasting or potentially curative effects. In addition, clinical efficacy data to demonstrate superiority may not be attainable for scientific reasons, leaving clinical safety or even only non-clinical data as the primary evidence for a significant benefit claim.

CMC Considerations

By 2025, the FDA expects to approve 10 to 20 cell and gene therapy products annually based on an evaluation of the current pipelines and the clinical success rates of these products [18]. In anticipation, the FDA has been highly proactive in bringing regulatory guidance for Tissue and Advanced Therapies products by announcing an ambitious plan for 2022 that included the following guidance documents. [19]

- Human Gene Therapy for Neurodegenerative Diseases; Draft Guidance for Industry
- Considerations for the Development of Human Gene Therapy Products Incorporating Gene Editing; Draft Guidance for Industry (Issued March 2022)
- Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Therapies; Draft Guidance for Industry (Issued March 2022)
- Regulation of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) - Small Entity Compliance Guide; Guidance for Industry
- Voluntary Consensus Standards Recognition Program for Regenerative Medicine Therapies; Draft Guidance for Industry and Staff

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- Recommendations for Determining Eligibility of Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) ; Draft Guidance for Industry
- Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products; Draft Guidance for Industry
- Studying Multiple Versions of a Cellular or Gene Therapy Product in an Early-Phase Clinical; Trial Guidance for Industry"

Of significance is the guideline issued in March 2022, providing guidance giving recommendations on developing GE of human somatic cells, more particularly to assess the quality and safety of the investigational GE product following Title 21 of the Code of Federal Regulations 312.23, information that must be included in an IND application (21 CFR 312.23). This includes evaluating preclinical safety, designing clinical trials, manufacturing, testing, and designing products.

The FDA guidance on CAR-T products states, [20] "we recognize that the development, manufacture, testing, and clinical assessment of CAR-T cells is challenging. Therefore, careful design and appropriate testing of the CAR transgene2F and delivery vector are critical to product safety, specificity, and function." Though not present in the FDA guidance on GE products, the intent is assumed to be the same.

Despite the difference in the GE and GTs, the FDA applies the same Chemistry, Manufacturing, and Control (CMC) requirements to other GE products, both treated as new biological drugs. Thus, all requirements applied to new BLA approvals apply to GE, regardless of whether it is personalized medicine or a commercial product for distribution. [21]

However, the FDA promotes the need for increased mechanistic understanding, enhanced manufacturing capabilities, and new tools to fully achieve the potential of precision medicine and tailored therapies. The FDA is investigating new technologies (such as omics) to improve disease diagnosis, prognosis, and treatment advancements. FDA has also created precisionFDA, [22] a cloud-based community research and development platform that enables users worldwide to exchange GE data and tools for testing, piloting, and validating old and new bioinformatics methods for NGS processing.

Below are the FDA guidance and its analysis to optimize the workload to meet compliance.

Drug Substance

The GE guideline (in italics) describes how the drug substance (DS) is developed and qualified.

"Optimization of the GE components to reduce the potential for off-target gene modification to the extent possible. Optimization can be performed on the editor or the targeting elements, depending on the GE technology utilized. In addition, GE components, such as guide RNA, can be optimized to inhibit degradation. The optimization strategy should be described in detail in the IND."

This stage of development is responsible for most of the cost and time to qualify a product for testing. Off-target events are either related to the variability of the active components or innate responses. While several AI methodologies can ascertain the structural variability, the innate response will become more understandable once we have sufficient data. Of course, this would increase the risks and side effects of gene editing, but new technologies and further GE advancements might eventually get around these obstacles.

"GE components can be administered in vivo using nanoparticles, plasmids, or viral vectors, or they can modify cells ex vivo. The GE components are considered active pharmaceutical ingredients or drug substances when administered in vivo in DNA, RNA, and/or protein via nanoparticles.

In its final formulation for *in vivo* administration, GE components are generally considered a drug product (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. In addition, if used to modify cells *ex vivo*, GE component quality is deemed critical to manufacturing the final product because, without these components, the resulting cell product would not have the same pharmacological activity."

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"Detailed descriptions *of how each GE component is manufactured, purified, and tested must be provided in the IND: [23,24]*

- *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 the GE component from the component manufacturing site to the final product manufacturing site.*
- *The cGMP for Phase 1 Investigational drugs for manufacturing these components applies (FDA)."*

This designation and its requirements create significant ethical issues; administering a gene-altering therapy in healthy subjects should not be allowed. The same applies to Phase 2 studies. In addition, there is no guidance about personalized medicines that could not be tested in any other population except the patient for which it is created. Developers should prepare a robust safety profile from animal testing to convince the FDA that the testing should be conducted in patients, even at a small scale; studies with one or two subjects are familiar.

"GE components must be produced following cGMP standards for the later Phase studies and licensure, with special attention paid to reagent quality control, the manufacturing process, and analytical procedures."

Compliance with the cGMP standards ensures that the safety and efficacy of a commercial product remain the same when multiple batches are produced. However, when personalized medicine is produced, it starts with a new material that cannot be standardized. Therefore, the reagent quality and analytical methods should be validated, and the latter is only suitable if validation is impossible.

"Each GE component is tested; in addition to evaluating each component's sterility, identity, purity, and functionality, additional testing, such as that for process residuals, should be included, depending on the manufacturing process."

Functionality testing can only be established in animal species; these do not always reflect human responses. Process residuals are also challenging to establish regarding their safety.

"Descriptions of the analytical procedures utilized for GE component testing, including the sensitivity and specificity of the procedures, should be included in the IND. Developers should also outline any in-process testing performed to ensure the quality of the components, as appropriate."

A description does not necessarily mean validation; some test methods are selected only because they are suitable and not validated. Likewise, it is not sure what "ensure the quality of the components" means during manufacturing.

"GE components are 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."

Stability testing is required when a product is commercially distributed with a listed shelf-life; only stability data during the testing period is required for the IND purpose. Products manufactured for immediate administration should only be required to demonstrate meeting the release specification during administration. Developers can also choose the option of storage requirements known to provide a stable environment, such as -70C; when Pfizer's Covid-19 vaccine was approved, it was supposed to be stored at a very low temperature to avoid conducting complete stability testing; it was later changed (FDA). Some gene and cell therapy products must be held at -70 to -190C, at which temperature and stability testing are optional for IND purposes.

Drug Product

"An IND should contain a detailed description of the DP manufacturing process and any in-process controls. It is recommended that this description include a flow diagram(s) and a detailed narrative.

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In addition, it is recommended that lists of the reagents used during manufacture and certificates of analysis be provided. The DP cannot be terminally sterilized; developers should provide details on measures taken to ensure aseptic processing."

Clinical INDs are widely used to customize gene therapy products in hospitals and academic units; IND aims to ensure that the product has reasonable promise to demonstrate the expected outcome, is safe, and that human testing is not abused. While commercial INDs include all information listed above, given that these studies are conducted in a few qualified patients, basic safety should suffice to secure IND approvals. In addition, developers may establish academic institutes [25] and hospital relationships to take a faster route to IND approvals.

"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, 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 the 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."

Much of the requirements to address safety issues are not likely to be available at the time of filing the IND; safety concerns due to the manufacturing process will still be unknown, and the same holds for the requirement of the efficiency of GE products. Sterility is, of course, required.

"Details of the analytical procedures used for testing the DP. The descriptions should include the assay's accuracy, precision, sensitivity, and specificity (as applicable), as well as any controls and, if applicable, reference materials used to ensure proper assay performance."

Test methods required to release a product must be validated. Still, for some tests specific to GE, only suitability can be demonstrated, mainly if it is used with a reference material or the reference product if it is a copy of an approved product.

"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."

The DP specifications are established for biological products based on the product character; there are no targeted final product attributes to be assessed; starting material specification will come from the supplier, and it should be acceptable to the FDA, provided the material claims cGMP-grade.

With these technologies' ongoing improvements, ZFNs, TALENs, and CRISPR-Cas9 have already begun using them in human clinical studies. However, while ex vivo GE results in highly effective cell therapies, correcting genetic diseases with ex vivo GE could be more practical. Higher side effects like off target editing, inefficiency, and the induction of negative immune reactions come at a cost.

"Suppose the GE components are expressed by a plasmid or viral vector administered to patients in vivo. In that case, the plasmid/vector in its final formulation is considered the DP. Thus, a complete description of plasmid/vector manufacturing and testing should be provided in the IND."

This is required.

"Suppose the GE components are administered using nanoparticles. In that case, a detailed description of the nanoparticle formulation, the manufacture of the nanoparticle components, and the DP should be provided in the IND. In addition, a description of the tests performed on each nanoparticle component and the DP should also be provided. Please note that testing should include assays to evaluate the efficiency of incorporating each GE component into the nanoparticles. Please also note that specific nanoparticles used for in vivo delivery of GE components may be considered a delivery device."

Needs clarification of what is considered a delivery device since the currently approved nanoparticles for Covid-19 are not treated as a device.

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"When establishing potency assays for in vivo human GE DPs, it is recommended 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. Including such a potency assay in the DP stability studies is also recommended."

The ability of GE components to perform is only determined after initial testing.

"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 significantly impact the efficiency or specificity of editing (e.g., RNP formation step in the case of CRISPR-mediated editing). In addition, acceptance criteria or limits should be provided and justified."

CQAs are not known for new biological products as they are tested in the form presented; the quality attributes are then classified as critical once efficacy and safety are established. Therefore, there will be other options than defining these attributes at the pre-IND stage.

"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*
- *The total number of genome-edited cells.*
- *The number of edited cells or the frequency of GE be monitored during stability testing of ex vivo-modified human GE DP."*

Most of these testing protocols are available from qualified CDMOs that can provide the service efficiently.

"When establishing potency tests for ex vivo-modified human GE DP, the assays be developed to measure the properties of the cells and the intended functional outcomes of the genomic modifications resulting from GE. For example, potency assays for a genome-edited CD34+ hematopoietic stem/progenitor cell product are recommended to 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 support a correlation between the output of the surrogate potency test and the functional outcome of the GE."

The relationship between surrogate potency and functional outcome is established once the clinical testing is completed. However, even then, the potency outcome rather than the relationship will require unnecessary extensive testing and exposure to patients.

"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, additional donor screening and testing may be warranted to meet the donor eligibility screening and testing criteria."

Additional requirements are not necessary, as already described for GT products. Also, each lot of the product is derived from different sources, making it impossible to establish acceptance criteria for the allogenic supply.

"More extensive analysis of the GE events occurring at both on- and off-target sites, additional adventitious agent testing, the 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."

Listing these requirements as "may be warranted" leaves much uncertain; additional testing is also not defined; is it over and above what is required for GT products or other biological products? The testing should be relevant and only needed since, at this stage, acceptance criteria are not established, and it is unclear what "stringent;" means; these should be suitable and relevant.

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"Additional in-process, lot release, and characterization testing may be needed for more complex products (e.g., products incorporating multiple rounds of gene editing or creating multiple cell banks)."

Additional "may be required" needs clarification; only necessary testing should be required.

Safety and Toxicity

Off-target GE refers to nonspecific and unintended genetic modifications that can arise based on whether the repair of the DNA takes place (nonhomologous end joining (NHEJ) or homologous recombination (HDR) responsible for site-specific modifications [26] ; the latter is only active in dividing cells; thus, not applicable to the liver, neuron, muscle, eye, and blood stem cells. (Figure 2) However, because HDR genes are found in all cells, they can be activated by giving the cells certain medications. The low rate of HDR in most cells is one factor that raises the possibility that genes will be disrupted rather than fixed in the first clinical application of CRISPR. Assume these complexes fail to bind at the target, frequently resulting from homologous sequences or mismatch tolerance. Then they will cleave off-target DSB and result in non-specific genetic alterations [27], resulting in off-target effects like unintended point mutation [28] deletions [29] , insertions, inversions, and translocations.

Although viral vectors, unlike their non-viral counterparts, are extremely efficient at inducing transgene expression in cellular targets, their clinical utility is hindered by significant immunogenicity and toxicity. Covalent modification of proteins contained in the virus coat with PEG [30] been shown to improve both the physicochemical and biological properties of several recombinant viruses used for gene transfer, including adeno-associated virus (AAV) [31] pseudo-typed lentivirus [32], retrovirus [33,34] , and baculovirus. [35]

The Cas9 enzyme, intended to cut a specific DNA sequence, may also cause cuts in other genome regions, increasing the danger of mutations that increase cancer risk. The safety risk associated with CRISPR has received the most attention. However, CRISPR can be made more specific or tweaked to reduce off-target effects or increase the enzyme's capacity to exchange single DNA bases.

Another issue arises with the current use of CRISPR as *in vivo* tool by introducing the Cas9 DNA into cells through a viral vector; even after Cas9 has made the desired cuts, cells will keep bringing it out for years, raising immune response to the enzyme, even if it is made highly specific. However, the risk is reduced if nonviral methods, such as lipid nanoparticles, are used, a rising technology.

Other difficulties include patients needing repeated treatments and the possibility that any gene-edited cells may eventually perish, depending on the condition. Less effectively than a single vector, two distinct viruses are frequently used to boost the maximum amount of DNA a viral vector can carry.

The safety and bioethical issues raised by somatic gene therapy using CRISPR/Cas9 are frequently like those raised when recombinant DNA technology and human gene therapy first became available [36]. The non-clinical challenge in safety and toxicity is thoroughly identifying off-target toxicities after gene editing. The effectiveness of the gene editing tool, its delivery method, the DNA target, the cell type and stage of differentiation, the chromatin structure, the length of the nuclease exposure, and the administration method (in vivo vs. ex vivo), among other factors, all affect off-target effects. In addition, editing errors can result in chromosomal translocations, insertions, deletions, and single nucleotide point mutations, all of which can be pathogenic to varying degrees.

Even when gene editing is successful, it can result in single nucleotide mistakes, added extra DNA, or "scarring" of the genome. [37]

In addition to the DNA repair, mainly through error-prone nonhomologous end joining (NHEJ), CRISPR-CAS9 has resulted in deletions and genetic changes close to the cut site, raising the possibility of pathogenic effects that are close to the target. These can involve significant deletions or rearrangements that span thousands of base pairs.

In vivo, *in vitro*, and *in silico* studies are sensitive and objective techniques to comprehend on and off-target. There are two types of these: biased approaches, which use information about the gene editing product to evaluate on/off-target effects, and independent or "unbiased" approaches, which are unconcerned with the gene-editing product and thoroughly examine the DNA (or other

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molecular targets). GUIDE-seq and CIRCLE-seq have distinguished themselves as complementary and sensitive methods for defining engineered nuclease activity among these unbiased techniques. [38,39]

Preclinical Studies

There is significant room for advancement in non-clinical approaches to reduce risks related to use in patients, such as by modeling the best gene editor and its delivery method and maximizing a gene editor's active time.

Animal testing of gene editing products is a hotly contested issue. [40]. Nevertheless, animal modeling has been helpful in several situations. With the aid of CRISPR, numerous animal models with mutations that closely resemble the range of mutations found in people with Duchenne muscular dystrophy (DMD) can be created quickly. These models offer a testing ground for sequence-specific therapies like CRISPR, which seek to reframe or skip DMD mutations to restore functional dystrophin expression. [41]

Animal models are crucial for validating delivery systems inside living things. In addition, these models serve as a testing ground for novel therapeutics and a method for adverse events, such as toxicity and immunogenicity, to be detected. Regulatory authorities treat nearly all genome-editing therapeutics being advanced to the United States and the European Union clinic as needing target-indication-specific in-animal efficacy and safety studies. The SCGE program aims to develop in vivo reporter systems that broadly apply to various delivery and editing techniques, regardless of the target cell, tissue type, or disease that must be treated. These reporters should be able to identify and measure GE in the targeted tissue and editing occurrences brought on by non-specific delivery to other tissues all over the body.

Since mice are an ideal tool for the preliminary testing of new delivery formulations due to their small size, low cost, and well-established utility, large animals are required for preclinical determination of safety, efficiency, dosing, and reagent distribution. As an alternative, models are being developed to assess new delivery formulations' efficiency, specificity, and safety in wild-type and reporter-animal models, such as mouse reporter systems. Additionally, large animals like pigs and non-human primates can now be genetically modified accurately and efficiently thanks to engineered nucleases.

New non-invasive techniques are required to measure the effects of editing in addition to creating new model organisms, such as in vivo cell tracking using cutting-edge imaging techniques like total-body positron emission tomography (PET), magnetic particle imaging (MPI) and chemical exchange saturation transfer magnetic resonance imaging (CEST MRI). [42]

"The overall objectives of a preclinical program for human GE products are generally the same as those described for gene therapy products.[43] These objectives include:

Identification of a biologically active dose range;."

Dose ranges are established in typical phase 2 studies; given that the GE products can only be tested in rare patients, conducting a full dose-response analysis is impossible. However, developers should be able to determine a safe range of doses to start the trials with gradual increases if necessary.

"Recommendations for an initial clinical dose level, dose-escalation schedule, and dosing regimen."

These recommendations can only be estimates and need not be based on experimental evidence in humans; appropriate animal studies should allow sufficient data to make these projections.

"establishment of feasibility and reasonable safety of the proposed clinical route of administration (ROA)."

There are limited routes of administration, and it is not feasible to try out several routes; based on the types of delivery systems, such as viral or LNP, the routes of administration and formulations are well-defined.

"support for the target patient population; and"

The target population is easily defined.

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"Identify potential toxicities and physiologic parameters that help guide clinical monitoring for a particular investigational product."

Toxicities of GE products cannot be identified based on the product design; the side effects of off-limit editing are the primary risks that can only assess in initial small-scale studies. Recent data on the possible immunogenicity and other side effects of formulation components such as polyethylene glycol should be addressed based on the frequency of observed effects; this is particularly important since the tested population will be much smaller than the patient populations that demonstrate these side effects.

"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 administering the investigational human GE product in a clinical trial."

Animal testing models where genetic modifications are made offer a better solution; while these provide a scientific rationale, the lack of correlation between animal data and anticipated human response remains a barrier; good scientific presentation to support the rationale should be provided.

"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."

In several instances, such models may not be available, and the guidance tells this by stating that it "should be considered," not that it must be done.

"The animal species and models selected for in vivo studies should demonstrate a biological response to the investigational GE product or species-specific surrogate product. 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."

The FDA is admitting that animal models may not be relevant, and these studies will be redundant. The FDA should provide more details about evaluating a "specific-specific" surrogate product.

The preclinical safety studies are designed to identify potential risks associated with administering the GE product. For example, 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 gene product expression.

All of these concerns are addressed earlier; the conclusion is that the developer should provide supportive data to ensure safety; newer technology, AI-based modeling, and the NGS engagement can be helpful.

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

Off-target projections are difficult to make and cannot be extrapolated from animal data; the developer should request IND approvals to determine these in a smaller population.

"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, and evaluation endpoints) to the extent feasible. In addition, 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."

These requirements are based on "to the extent possible," "sufficiently comprehensive," and "to the extent feasible." the developers should take note of it and submit to the FDA reasons for not submitting all the data requested above.

"Biodistribution studies are conducted to characterize the GE product's distribution, persistence, clearance, and any expressed GE components in vivo. Evaluation of the biodistribution profile of the

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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."

There needs to be more clarification on how these testing should be conducted and whether animal data would suffice. This information will become available once the limited clinical testing is completed.

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

The investigational human GE product should be evaluated in the definitive POC and safety studies when feasible."

It may not be feasible for most GE products; this should be pointed out.

"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., the 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. Therefore, it is recommended that developers provide scientific justification for the administration of a surrogate GE product and establish the biological relevance of the surrogate compared to the investigational human GE product."

The FDA has yet to clarify these approaches that may not be practical, such as testing a surrogate product. Therefore, developers should seek clarification in the earliest meetings with the FDA.

"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."

Using clinical cell sources is advised to avoid additional justifications.

"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 establishing the comparability of the product used in preclinical studies to the clinical product, if necessary."

Lots produced during preclinical stages should be at scale to avoid later justifications and validation; these are small-scale productions, so they should not bring a more significant cost burden.

"The preclinical in vitro and in vivo POC studies assess the following:

- *Specificity and efficiency of editing in target and non-target cells;*
- *The 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;*
- *The durability of the genomic modification and resulting biological response; and*
- *Effects of genetic variation on editing activity across the target population."*

This testing should be outsourced to a qualified CDMO with qualified or validated test methods.

"It is recommended that preclinical studies be conducted to identify and characterize the risk of GE at on- and off-target loci and include the following:

- *Identify off-target editing activity, including all off-target editing events' type, frequency, and location.*
- *Multiple orthogonal methods (e.g., in silico, biochemical, cellular-based assays) that include an unbiased genome-wide analysis are recommended for identifying potential off-target sites. In addition, the analysis should be performed using multiple donors' target human cell type(s) when possible.*
- *Verifying bona fide off-target sites should be conducted using methods with adequate sensitivity to detect low-frequency events. In addition, the analysis should be performed using multiple donors' target human cell type(s).*
- *Appropriate controls should be included to confirm the assay's quality and assure the results' interpretability and 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*

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ex vivo-modified cells, this may include assessment for clonal expansion and/or unregulated proliferation.

- *Evaluating 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."*

The extensive testing details provided by the FDA should be discussed before developing and testing a new product; many of these requirements will likely be waived in a comprehensive evaluation plan. Most of this work should be outsourced to a qualified CDMO.

Clinical Studies

Safety concerns for clinical testing include the effects of gene editing and the method used to deliver the gene-editing tool. [44] The clinical development programs for human GE products address the inherent risks of gene therapy products and additional risks related to GE, such as unintended effects of on- and off-target editing that may not be known during product administration.

The clinical trial design incorporates appropriate patient selection, a productive and secure method of product administration (including data-based dosing, a dosing schedule, and a treatment plan), adequate safety monitoring, and a sensible selection of endpoints. Long-term follow-up is necessary for clinical trial participants receiving human GE products to assess clinical safety. As a result, the IND should thoroughly describe the overall study design, adverse events (AEs) evaluation, and subject follow-up plans. The general factors to be considered when designing clinical trials for GE products are the same as those mentioned for other cellular and gene therapy products. [45]

Choosing the proper study population ensures maximum benefits while reducing potential risks to subjects. Based on the product MOA, the study's rationale, and weighing the product's potential risks, the study population's selection is solidly justified. Human GE products may carry substantial risks and uncertain benefits. As a result, only patients who have exhausted all other treatment options should be included in first-in-human trials for such products.

"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 receiving concomitant treatments, making the safety or effectiveness data difficult to interpret. Therefore, in some instances, subjects with less advanced or more moderate diseases may be appropriate for inclusion in first-in-human clinical studies*." [46]

The risk of adverse events (AEs) associated with product delivery to target tissues must be reduced using established, secure, and efficient product delivery methods. When available, previous clinical experience from similar products, including cellular or gene therapy products that may or may not have undergone genome editing, should also be used to guide the delivery and the proposed dose schedules. These products may or may not have undergone GE. [47]

The GE product's potential risk(s) are reduced by staggered subject enrollment, with a set amount of time elapsed between product administration and successive subjects within and between cohorts. Before administering the same dose to additional subjects or raising the dose already administered, the staggering interval should be long enough to monitor for acute and subacute adverse events (AEs). The staggering interval should also consider how long a human GE product is anticipated to be active.

The proposed patient population size and its acceptable risk level for the GE product are considered when choosing the study cohort size. Other factors, including assessments of

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pharmacologic activity, tolerability, and feasibility, may also influence the choice of cohort size. Clinical trials evaluating human GE products must have a thorough safety monitoring strategy, including a clearly defined toxicity grading system and a toxicity management plan. It is essential to pay close attention to proper off-target editing monitoring and assessment of the results of unintended off-target and on-target editing consequences. In addition, the AEs connected to tumorigenicity, immunogenicity, and aberrant cellular proliferation should be monitored further. Pre-clinical research should be used to anticipate such adverse events, and the clinical protocol should include information on toxicity grading and management techniques.

"The necessary reporting procedures for adverse effects of using the human GE product must be followed.".

Before enrollment, subjects should be asked to consent voluntarily and knowingly to ongoing monitoring (LTFU). The long-term effects of deliberate and unintentional editing at on- and off-target loci may not have been known when GE products were administered, as was previously mentioned. Developers are advised to perform LTFU at least 15 years after the administration of the product." (long term follow up)

The 15-year follow-up of patients may not be feasible; the filing should state it.

Fast-to-Market Strategies

The gene editing technology development to test its applications is now one of the most straightforward processes, with all components required off the shelf available. The supplies are available with cGMP certification, qualifying their use to test in humans. Most companies develop their technology or outsource; today, the demand for CDMOs is high, but many new entries are anticipated to overcome this constriction soon. But the current trend shows that the percentage of cell and gene therapy companies who outsource CGT manufacturing is expected to drop from 44% to 22% over the next five years, especially as their products move closer to commercialization (9) mainly to retain control, speed up development, maintain confidentiality, and expertise needed. Several companies have built manufacturing facilities to support clinical and commercial manufacturing. Still, even modest facilities require significant capital investments in the tens of millions of dollars and significant burn rates. In most cases, a CDMO can speed up the development cycle without waiting for a facility to be built and qualified; it applies particularly to startup companies.

The process of designing and testing GE products is well-defined, and technology is widely available. [48] The good news is that CDMOs can now provide clinical-grade materials and full cGMP supply, a newer trend in the field of GE. Table 1 lists a few prospective CDMOs that can help startups and ready-to-market projects. Notably, several educational institutions also offer these services, [49] besides commercial suppliers. (Table 1)

Table 1. A shortlist of suppliers of GE components and products (no conflict of interest).

Resource	Link
Applied Stem Cells	https://www.appliedstemcell.com/
Genscript	https://www.genscript.com/
Synthego	https://www.synthego.com/
TriLink Biotechnologies	https://www.trilinkbiotech.com/
ThermoFisher Scientific	https://www.thermofisher.com/
Biotechne	https://www.bio-techne.com/
Wuxi Biologics	https://www.wuxibiologics.com/
Lonza	https://bioscience.lonza.com/
Avid Bioservices	https://avidbio.com/
Takara	https://www.takarabio.com/
Aldevron	https://www.aldevron.com/
AXOLabs	https://www.axolabs.com/
Cornell University	https://research.weill.cornell.edu/

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PPD	https://www.ppd.com/
CRISPE Therapeutics	https://crisprtx.com/
Fujifilm Diosynth	https://fujifilmdiosynth.com/
Sarpeta Therapeutics	https://www.sarepta.com/
Charles River	https://www.criver.com/
Addgene	https://www.addgene.org/crispr/
Labmate	https://www.labmate-online.com
Synbio Technologies	https://synbio-tech.com/

The FDA and EMA provide details about the regulatory filings and details of biological products, including gene therapy products; these reports should be the starting step to understanding the scope of studies expected by the agencies to reduce the time to approval. For example, a recently approved gene therapy product ZYNTEGLO took ten years from filing to approval. [50]A word of caution for developers is to know that the regulatory guidelines provided by the FDA or EMA are neither binding on them nor the developer. Following the path of similar approved products can often lead to a longer path; the developers must question every requirement before and during the development process.

The fast development of CRISPR tools involves a systematic approach:

To generate a complete knock-in design, edit up to 30 bases in any human gene using CRISPR-Cas9 or TALEN technology, design all necessary oligos for precise SNP or amino acid changes, and design the required reagents to add a GFP or RFP tag to a target gene without the need for cloning are now available as off-the-shelf items.

The design of the gRNA is essential to the CRISPR-Cas9 system's editing effectiveness, and several proprietary designs are offered for maximum editing effectiveness without sacrificing specificity. For instance, transfection of the Cas9 protein and gRNA bypass transcription and translation to increase editing efficiency, allowing CRISPR plasmids to remain in the cell for longer than 72 hours and potentially contributing to off-target events. In addition, within 24 hours, the Cas9 Protein v2 is removed from the cell, reducing the possibility of off-target cleavage events.

Quality control is required to improve delivery circumstances, increase editing effectiveness, and establish hit selection criteria. Additionally, these controls aid in developing assays with improved signal-to-noise ratios, resulting in greater assurance in the hits identified by the screens.

Nontargeting gRNA sequences that don't recognize any sequences in the human genome serve as negative controls. There are various package sizes for the negative controls. When developing assays and running your screens, negative controls are used to check for non-specific cellular effects on the plate.

Validated gRNA sequences showing high editing efficiencies in various cell types—up to 90% in some cell types—are considered positive controls. The conditions that provide the greatest editing efficiency in cell models are identified using these controls during the assay development process. Then, when you run your screens, they can act as on-plate positive controls.

Some Key CRISPR-Cas9 system resources are listed in Table 1. Additional resources include:

- CasOFFinder is used to predict off-target sites and off-target editing. [51]
- Ready-made Cas9 vectors:
- Alt-R CRISPR-Cas9 from Integrated DNA Technologies [52]
- GeneArt CRISPR Nuclease Vector Kit [53]
- LentiCRISPR vector V2 52961 [54]
- Resources for obtaining ZFNs and TALENS for DNA targets include commercial vendors: [55]
- Zinc Finger Tools [56] is a public website that lets users look for potential nuclease target sites in a DNA sequence of interest. In addition, this website provides researchers with a database of described zinc finger domains and a reverse engineering feature that forecasts the binding locations of recognized zinc finger proteins.
- Zinc Finger Consortium. Context-dependent Assembly (CoDA), one of the publicly accessible techniques for designing zinc finger domains, [57] Oligomerized Pool Engineering (OPEN), and Modular Assembly [58].

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- TALENs. [59]
- Cellectis Bioresearch and Life Technologies; https://cellecta.com/
- Modular assembly Voytas Kit, [60,61] the Zhang kit. [62] These kits produce TALE libraries and arrays using a variety of techniques.
- Library of prefabricated TALE arrays; FLASH [63]
- Library of TALEN plasmids for over 18,000 human protein-coding genes. [64]
- Mammalian expression vectors to produce TALENS in just two days methodology. [65]

Cost Containment

While the suggestions made above to reduce the regulatory cost seem practical, there is no assurance that the regulatory agencies will soon be willing to switch their thinking from traditional to creative. As a result, the cost of these products shall remain unaffordable unless covered under insurance plans. Even when the insurance plan picks up most of the cost, the out-of-pocket costs remain formidable. Declaring GE tools suitable for inclusion under the Pasteur Act is one solution to this problem. [66] The developers are paid a one-time reimbursement by the government or a joint fund developed for the purpose; this will allow all patients to receive the treatment free of charge. This proposition applies best to gene therapies where the number of patients is much smaller and, in some cases, predictable [67]. These numbers can range from a dozen to a thousand. [68] This will also allow the developer to estimate their lifetime cost burden.

The approach to bringing "biosimilar" gene therapy products when the GE products are approved, "biosimilar" will not be applicable; even if it did, the 12-year delay of exclusivity would be an impediment. But if it is filed as a new biologic, the exclusivity period will not apply. GE products can capitalize on the concessions given to similar new biologics (approved under 351a).

The FDA's instructions for COVID-19 products are essential: "To the extent that it is both legally and scientifically possible, the development of the COVID-19 vaccine may be expedited using the knowledge obtained from related products made with the same well-characterized platform technology. Similar to how some manufacturing and control processes may draw on the vaccine platform with the right justification, reducing the need for data specific to a given product in some cases." (FDA). Developers can build a case of safety and efficacy using an approved product or a product for which public domain data are available to secure testing concessions.

Another important consideration is the compliance with intellectual property that keeps entry of similar drugs coming to patients; in the case of gene therapy, an intellectual property, of all patents issued to protect gene therapy, only 27% remain valid as of the end of 2022. [69]

Material costs are often the major hurdle in developing new products, but a revolution regarding GE products has already come to fruition. Off-the-shelf GE kits perform perfectly well and provide a widely available starting point. [70–73]

Generally, a kit costing less than $200 can perform the complete gene editing of a bacteria, including all chemicals, bacteria, media, and glassware. Comparing these costs to create a GE product ready for non-clinical testing is inappropriate, but these costs should be at least a few thousand dollars. The safety and efficacy testing costs would be higher, but a total of $100K to reach nonclinical testing is possible. The cGMP-compliant chemicals and supplies will add a few thousand more, allowing the developers to develop many test products to select the most suitable human use.

Conclusions

GE will change the map of human destiny if it becomes accessible to billions who need it most. Unlike a biological drug, it has no pharmacology or contact toxicity pattern. The RNA-based CRISPR tool is well-characterized and specific to its target. Unlike biological drugs, there is little variability from batch to batch. It is almost like chemical drugs since the structures of its components are well-defined. The likely caution is the possibility of off-target genome editing, and the tests created to measure this have proven unreliable. [74] Additionally, as these tools can only be tested in patients, there is a need for regulatory agencies to allow faster testing once GMP compliance is in order. Given

the available GMP-grade materials, all off-the-shelf, this should lead to the development of many products without spending billions of dollars.

The future of GE tools will depend on bringing more rationality at the regulatory level and more creativity at the development stage to avoid facing the price structure that is now holding back gene therapies.

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