Guideline on Quality Assessment for Gene-Editing Based Advanced Therapy Medicinal Products [for industry]

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MINISTRY OF FOOD AND DRUG SAFETY
National Institute
of Food and Drug Safety Evaluation

Cell and Gene Therapy Products Division
Biopharmaceuticals and Herbal Medicine Evaluation Department

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1. Introduction

As the potential of gene-editing tools, such as CRISPR/CAS9, has a huge impact on a wide range of areas, gene editing is currently utilized for drug development as a highly promising technology. This guideline applies to medicinal products whose therapeutic effects are achieved by gene-edited cells and those by gene-editing tools themselves or vectors expressing such tools, and intends to provide points to consider in the evaluation of these products. Such products are considered gene therapy products, and therefore they should comply with the requirements for gene therapy products prescribed in *Regulation on Review and Authorization of Biological Products* for marketing authorization.

As this guideline focuses on quality–related issues that warrant specific considerations by virtue of using procedures that involve gene-editing, general considerations on quality and nonclinical aspects of gene therapy products can be found in *Guide on Evaluation of Characterization, Manufacturing*, and *Quality Control of Gene Therapy,* and *Products Guideline for Nonclinical Assessment of Gene Therapy Products (Guide for Industry)*.

2. Scope

This guideline applies to products containing cells that are modified by gene-editing technology, or products expressing or containing gene-editing tools among the gene therapy products described in *Annex 3* of *Enforcement Rule of Medicinal Product Safety*.

3. Quality Assessment

For gene-edited products, *ex vivo* genetically edited cells (hereinafter referred to as gene-edited cells) can be used as active pharmaceutical ingredients (APIs), or gene-editing tools intended to induce *in vivo* gene-editing can be employed as APIs (hereinafter referred to as gene-editing products). Regardless of which category the product under investigation is classified into, detailed information on the tools adopted for gene-editing should be submitted as described in the following paragraphs.

3.1 General Information

- Types of Gene-Editing Tools

Description on the tools used for gene-editing should be given. For the 3rd generation gene-editing tool, information should be provided on the gRNA sequence, target site, the origin of enzyme species and its sequence (i.e., NCBI no.), and the recognition site and cutting site. If a gene-editing enzyme that is artificially altered is used, explanation should be given on the genetic alteration performed. It is recommended that an enzyme be used that can minimize the occurrence of non-targeted mutagenesis.

Description should be provided on whether the gene-editing tool is used in the form of an RNA and protein or in the form of an expression plasmid or viral vector, in addition to the information on how to deliver the tool into target cells.

- Strategies and Rationale for Using Gene-Editing

Information should be submitted including descriptions on the target site to be edited and the rationale for selecting the site, the method intended to be used for gene-editing (i.e., gene knockout or DNA replacement by homologous recombination using a repair template), and targeted gene expression profile by gene-editing, etc.

Diversity in human genome can lead to diverse genetic variations in the target site (alleles, SNPs, mutations, etc.). The types and frequencies of variations that are known to potentially exist in the target site should be described, and, if any, the methods to overcome the challenges posed by theses variations should be presented.

3.2 Manufacturing

The full range of manufacturing methods, raw material control, in-process control, and manufacturing change during development, etc. should be included.

- Manufacturing Methods

Regarding the entire range of processes used to manufacture the product, information on the reagents, solvents, and devices, etc. used for each unit process, description on the processes, and control items during the processes should be submitted in a tabular format together with a manufacturing process flow chart. Information on batch size and production scale should be given as well.

- Raw Material Control

A list should be submitted that describes the types of raw materials used for

manufacturing (starting material, cell culture medium, growth factor, reagent, column resin, etc.), their intended use, processes adopted, and quality control standards. Additional information may be necessary to ensure that the raw materials used are appropriate for manufacturing.

Thorough characterization of materials essential to manufacturing the product, such as cell bank, virus bank, etc., is required to demonstrate their appropriateness to be used for manufacturing, and subsequently ensure consistency in the manufacture of the product.

For products intended to induce *in vivo* gene-editing, depending on the form of the products, information should be provided on the cell banks used to manufacture gene-editing enzymes, or on the cell banks or virus seed banks used to manufacture vectors expressing gene-editing enzymes or gRNAs (plasmid, virus, etc.), etc. with regard to their establishment, validation, and storage.

For gene-edited cells, information on the cell banks and/or virus seed banks used to manufacture the cells and gene-editing tools is required, respectively. For example, if the product is based on gene-edited cells via plasmid transduction, then data on manufacturing methods, raw material control (manufacture of the cell bank, its suitability included), characterization, specifications and test methods, safety, etc. should be submitted for the cells and plasmids, respectively, as required for raw materials.

3.3 Characterization

As required for gene therapy products in general, in-depth characterization of the entire range of properties should be performed including the structure and biological characteristics of the products that involve gene-editing. As different components are used in line with how the products are constructed (i.e., gRNA+protein+vehicle, a vector expressing a gene-editing tool, gene-edited cells), each component should be taken into consideration to include test procedures suitable for characterization.

For products intended to induce *in vivo* gene-editing, the following key aspects of characterization testing should be addressed:

- Genetic sequence of the vector
- Primary, secondary, and high-order structures of the gene-editing enzyme, if used
- For plasmid vectors, purity, the ratio of plasmids of different forms (supercoiled, linear, open-circular, etc.), expression of the genetic insert

- For viral vectors, replication potential, the ratio of infectious viral particles to total viral particles, particle size of viral vectors and analysis of their aggregates, chromosomal integration tendency, expression of the genetic insert, release of viruses from infected cells
- Electric charge and particle-size distribution of the compound-based carrier used with the vector
- Estimated post-administration profile of the genetic material introduced into cells (target cell/tissue-specific delivery, delivery efficiency, copy number per cell, etc.)
- Analysis on on-target mutation
- Analysis on off-target mutation

For characterization of gene-edited cells, the following key aspects of characterization testing should be addressed:

- Characterization of the vector used for gene editing (refer to the tests listed above for the characterization of products intended for *in vivo* genome editing)
- Cell characterization (i.e., cell identity, survival rate, phenotype, cell function)
- Growth or differentiation capacity of gene-edited cells
- Infection efficiency
- Sequencing of the transgene
- Genetic stability during *ex vivo* culture or differentiation
- Vector copy number in the infected cells
- Chromosomal integration tendency of the vector DNA
- Release of viruses from the infected cells
- Restoration of replication competence of the non-replicating virus used
- *In vivo* persistence of the gene-editing tool
- Analysis on on-target mutation
- Analysis on off-target mutation

3.3.1 Analysis of On-Target Mutation

Analysis should be conducted to assess the profile of chromosomal rearrangements induced *in vivo* by the gene-editing tool at the target site in the cells, and their ratio to non-rearrangements.

Not only the intended mutations but also unintended ones should be investigated in the analysis of the gene editing profile created at the target site. The analysis should include various outcomes developed by non-homologous end joining (NHEJ) and variations occurring at the chromosomal level, such as translocations and inversions. For analysis of gene editing efficiency at the target site, analytical methods with a sufficient level of sensitivity, such as deep sequencing, etc., can be used.

3.3.2 Analysis of Off-Target Mutation

Since gene editing tools can induce off-target mutations, potentially leading to damaging consequences depending on the sites where the off-target effects have occurred, in-depth analysis of off-target mutations is necessary.

Strategies and analytical methods employed to analyze off-target mutations should be described and justified. Strategies for analysis of off-target mutations should include analytical laboratory tests supported by *in silico* analysis based on sequence similarity. For laboratory analysis of off-target mutations, it is desirable to use a combination of various methods that are based on different principles, and also to include methods that will allow unbiased detection of genome-wide off-target effects. Table 1 shows some of the analytical methods currently available for off-target mutation detection. As analytical methods for the detection of off-target mutations are being actively developed, the most appropriate strategies and methods that reflect state of the art technologies should be used in reference to those presented in Table 1.

Given that cell-based analytical methods generate different results depending on the characteristics of the cells involved, it is recommended that various types of cells be used, such as cell lines with high delivery efficiency, target cells, and stem cells, etc.

In addition, the possibility of a gene editing tool producing different off-target mutations when applied to different individuals should be considered.

For each off-target mutation, biological risk assessment is required. If off-target mutations are observed in regions related to genes that control cell proliferation and differentiation, tumorigenesis, and/or tumor suppression, etc., the gene editing tool will not qualify for clinical application or use unless scientific justification is provided sufficiently.

Table 1. Types of Analytical Methods to Detect Off-Target Mutations and Examples

In silico prediction tools analyze similarities between the gRNA sequence and the reference DNA sequence based on their respective algorithms, thereby identifying highly likely off-target sites.

DNA-based analytical methods work by having the gDNA or gDNA with its shape changed (e.g., circularized gDNA library) digested by a nuclease, and then analyzing the genomic cuts at potential off-target sites. These methods can be used to identify off-target effects in the DNA obtained from individual patients.

Cell-based analytical methods have strength in that these approaches allow for detection of events occurring in the cells following the delivery of a nuclease therein, but the sensitivity of these methods may vary depending on the cell types, delivery methods used, etc.

Detection assays presented below are analytical methods free from bias incurred by sequence-based predictions.

	Analytical method	**Description**
DNA-based method	Digenome-seq	*In vitro* digestion of gDNA with a gene editing tool and fragmentation, followed by whole genome next generation sequencing to profile genome-wide off-target effects
	Circle-seq	*In vitro* digestion of circularized, fragmented gDNA and PCR amplification of linearized gDNA fragments, followed by next-generation sequencing
	SITE-seq	*In vitro* digestion of gDNA with a gene editing tool and biotin labelling and adapter ligation of cut sites in the gDNA, followed by next-generation sequencing of enriched and amplified fragments
Cell-based method	GUIDE-seq	Integration of double-stranded oligonucleotides into DSBs by NHEJ, followed by PCR amplification and next-generation sequencing of the tagged genomic sites
	LAM-HTGTS	A method to track translocated sequences resulting from joining between DSBs by gene editing

	BLESS	Direct *in situ* ligation of DSBs with biotin linkers in cells, which were fixed and digested with a gene editing tool, and removal of unlabeled gDNA fragments, followed by next-generation sequencing of enriched and PCR amplified fragments

3.3.3 Delivery Methods and Persistence

It is necessary to generate data that will confirm the suitability of the method used to deliver a gene editing tool into cells. Its suitability should be determined by assessment of target cell- or tissue-specific delivery, the number of cells where nuclease proteins were delivered against the total number of cells, and the copy number of vectors inserted per cell, etc.

Persistent expression of a gene editing tool may lead to an escalated risk of off-target mutations. Therefore, under such circumstances, scientific justification is required.

3.4 Specifications and Analytical Methods

For drug substances and drug products, analytical procedures and specifications should be established including those for identity, purity, potency, and safety (i.e., testing for sterility, endotoxin, replication competency). The analytical procedures should include those that are pharmaceutically required, and can be generated for individual drug products based on their characterization results.

For gene-edited cells, the ratio of gene-edited cells as intended to non-edited cells should be addressed in the analysis.

4. Additional Recommendations

When genetically modified organisms are used for domestic manufacturing, information needed for risk management of GMOs should be included in the manufacturing process section. The information required for risk management of GMOs is described in the Annex 14 of *Regulation on Review and Authorization of Biological Products*.

5. References

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Document History

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