Pharmaceutical research and evaluation technology for in vivo gene therapy products

Guiding Principles (Trial)

Drug Evaluation Center of the State Drug Administration

May 2022

Table of contents

I. Introduction

With the rapid development of biotechnologies such as gene delivery vectors and gene editing, The clinical application of gene therapy products continues to make new progress, providing solutions for difficult-to-treat diseases. New treatment options are provided. Gene therapy products generally use exogenous genes (or gene editing tools) into target cells or tissues to replace, compensate, block, Correcting, adding, or knocking out specific genes to exert a therapeutic effect. Import according to genes The human body has different methods. Gene therapy can be divided into in vivo gene introduction and There are two ways to introduce genes in vitro (ex vivo). In vivo gene therapy products will be Source genes (or gene editing tools) are directly introduced into human hair through appropriate vectors. exert a therapeutic effect, while in vitro gene therapy products generally combine exogenous genes outside the body (or gene editing tools) are introduced into cells to prepare genetically modified cells. or cell-derived products that are ultimately reinfused to exert therapeutic effects. due to internal and In vitro gene therapy products include product type, gene carrier type and design, carrier Targeted needs, management of starting raw materials, product purity, impurity levels There are certain differences in control, production mode and quality risks. Therefore, There are certain differences in R&D and technical requirements between the two types of products, and it is necessary to Row classification specifications.

In order to standardize and guide in vivo gene therapy products in accordance with drug development regulations Conduct research on laws and management practices with reference to the "Drug Administration Law of the People's Republic of China", The "Measures for the Administration of Drug Registration" and other relevant laws and regulations formulate this guiding principle. Book The guidelines are based solely on current knowledge and present scientific recommendations for pharmaceutical research on these products. Specific suggestions and general technical requirements, relevant technical requirements for specific varieties

Applicability should follow the principle of specific analysis of specific problems. During the product development process, the

Choose other more effective methods or technologies based on scientific considerations, but they must be consistent with the pharmaceutical

The laws of drug research and development and have sufficient and reasonable basis.

This guideline is mainly aimed at the pharmaceutical research system during the product application and marketing stage.

The pharmaceutical research in the clinical trial stage can be determined according to the research and development characteristics and research characteristics of each stage.

For research purposes, refer to this guiding principle to carry out research appropriate to the stage. due to birth

Material technology is developing rapidly, especially in aspects such as product design and quality research.

Pharmaceutical research on different types of products is different. With the development of technology and deepening of understanding,

With the accumulation of investment and experience, the relevant technical requirements in this guideline will also be gradually improved.

Revise and improve.

2. Scope of application

In this guideline, in vivo gene therapy products refer to products that enter the human body and

By modifying the genetic material of human cells in the body and expressing foreign genes,

Manipulate cell gene expression or regulate cell biological characteristics to achieve treatment

Medicines intended to treat diseases. Products typically consist of vectors containing engineered genetic constructs

It consists of a body or delivery system, and its active ingredients are usually DNA, RNA, genetically modified

Created viruses, etc., and some bacteria or fungi may also be developed as vectors for use in

due to treatment. Common mechanisms of action of products include transcription and/or translation of exogenous target genes.

Therefore, it regulates the expression level of target genes in cells and modulates the genetic material of target cells.

modification, etc., the mechanism of action of some products may require the use of protein ingredients

, such as various gene editing enzymes, to function.

Due to the complex and diverse composition of in vivo gene therapy products, there are relatively few

Components covered by proven technical guidelines (e.g. recombinant protein components),

This guideline will not be described in detail. Please refer to the corresponding technical requirements. Partial oncolysis

Viral products may also have similarities to gene therapy products in terms of design and mechanism of action.

Characteristics, please refer to this guideline appropriately. Taking into account the differences in production processes, this

The guidelines mainly cover products prepared based on biotechnology and may not be fully applicable.

For nucleic acid products produced through chemical synthesis processes, such as antisense oligonucleotides

products and their derivatives. For multi-component or complex products such as ribonucleoprotein

For white complexes, pharmaceutical research should be conducted on each component and combination product separately and comply with

meet the corresponding technical requirements. For example, enzyme-based gene editing technologies (e.g.

CRISPR-Cas, TALEN, ZFN, Meganuclease, etc.)

products, whose active components may be DNA, RNA (such as sgRNA) and/or protein

quality. According to the characteristics of the process, the pharmaceutical composition of chemically synthesized nucleic acid components (such as sgRNA)

When scientific research refers to this guiding principle, it is also necessary to refer to the chemical synthesis product phase.

relevant technical requirements. Pharmaceutical research on protein components should comprehensively refer to previously published

Pharmaceutical Research Essentials in the Released Guidelines for Recombinant Protein Biological Products

beg. In addition, some in vivo gene therapy products may require specific administration

device or used as a drug-device combination product, in a drug delivery device or drug-device combination product

For the device part, it is recommended to refer to the requirements of the relevant guidelines for medical devices.

3. General principles

Pharmaceutical research on in vivo gene therapy products should comply with the regulations of the People's Republic of China

Pharmacopoeia" (hereinafter referred to as "Chinese Pharmacopoeia"), especially "Human

"General Introduction to Gene Therapy Products". Gene therapy products for human use

Production should comply with the relevant requirements of the "Good Manufacturing Practice for Pharmaceutical Products" and its appendices.

Due to the special active ingredients and mechanism of action of in vivo gene therapy products, the product

Research and development, production, use and disposal should also comply with relevant biosafety regulations.

requirements.

1. General requirements for product design, R&D and production

Pharmaceutical research on in vivo gene therapy products needs to consider the special characteristics of each type of product

suitability and research phase. During the research and development process, we encourage

The research and development concept of "design" is used to explore the impact of production materials and production processes on product quality.

influence, establish the correlation between product quality attributes and clinical safety and effectiveness, and maintain

Continue to carry out process optimization and quality improvement, and establish a whole-process quality control system and

Full life cycle management concept.

In vivo gene therapy products have specific active ingredients and mechanisms of action, e.g.

Changes in the replication characteristics of viral vectors may cause non-specific infection of the virus

and diffusion. The genetic modification activity of some products can cause the genetic material of cells or biological

The physical properties are irreversibly changed. Therefore, product design,

The rationality of each link such as production and quality control, in the process of product development

Establish risk awareness and formulate corresponding risk control strategies based on risks. carrier

Selection and design require comprehensive consideration of vector type, replication characteristics, and genome integration.

Properties, on-target properties and off-target risks, scale-up, clinical indications, effects

mechanism, medication method and frequency of administration, immune risks, etc., the production process is strictly

Control the potential risk of contamination by exogenous agents and monitor homologous/non-homologous recombination of vectors

The potential risks of the group are established based on quality research and identification of critical quality attributes.

Good quality control strategy. The production process needs to ensure the safety of production personnel and Environmental safety control, especially for viral vector products. Production line cleaning and disinfection The poison must be fully verified, and cross-contamination and synchronization between different products must be strictly controlled. Residual contamination of a product between batches. Developed based on environmental and biosafety assessments Disposal procedures for materials, intermediates and products to avoid the release of active substances into the environment diffusion.

2. General R&D rules

Product development and production must follow the general rules of drug research and development, and gradually complete Improve and continuously optimize. Since the research purposes of products at each stage are different, different stages The R&D and production requirements also vary.

When applying for clinical trials, the pharmaceutical research on the product should be able to support clinical trials. development. This stage needs to be based on preliminary research and combined with the use of similar products. Identify and control factors related to product quality risks to minimize Low product safety risk for human use. For example, bacterial/viral species for production Preparation and testing of sub-batches and/or cell banks, as well as necessary stability evaluation and /or research; safety assessment and quality control of materials for production; clinical sample preparation Development and validation of preparation processes, preliminary establishment of intermediate control; representative samples quality research and establishment of early quality standards; development of quality control methods and Validation, including establishment and validation of safety testing methods, activity analysis methods Initial establishment; stability studies to support clinical trial development; container closure systems screening and suitability assessment, etc. Non-clinical studies evaluate products for use in humans important reference basis for safety, special attention needs to be paid to non-clinical research samples

Comparison or bridging analysis with clinical trial samples in terms of production process and quality analysis to support the safety assessment of samples for clinical trials.

During clinical trials, based on process development and understanding of product quality attributes, It is necessary to gradually clarify the product's production process, key process parameters, and Control projects and key quality attributes, etc., and establish stable production processes and perfect Quality control system. During the research and development period, the product production process may change as the process develops. development and optimization changes. Due to the innovative nature of this type of product, product awareness and Research accumulated during clinical trials, especially in early clinical trials, may Relatively limited and unable to fully understand the impact of changes on product quality, safety and effectiveness impact, change plans should be evaluated and implemented more carefully. Various change plans The implementation needs to be based on comparability studies suitable for the research and development stage. Reasonably evaluate the impact of changes on product quality characteristics, and may need to carry out Appropriate nonclinical or clinical trial bridging studies.

During the marketing application stage, the commercial production process is finalized after process development. Yiying can continuously and stably produce products that meet the target quality, pharmaceutical research Data should support the safety, effectiveness, and quality control of the product. At the same time, formulate Work plan for continuous verification and optimization of production process after launch to better guarantee product quality.

4. Risk Assessment and Control

In vivo gene therapy products have different characteristics and production processes. Products exist in terms of production materials, production processes, quality control, stability, etc. The risks vary greatly. R&D can combine the characteristics of products and processes, refer to

ICH Q8 and Q9 quality risk management concepts, scientific use of risk assessment tools,

Based on the principle of specific analysis of specific varieties, various risk factors in production are analyzed.

Identification and assessment, and formulating a plan based on the risk assessment results that is suitable for the product development stage

appropriate risk control strategies. In addition, early product knowledge or past use of similar products

The safety data used can also provide important reference for risk assessment.

Common risks include (but are not limited to) the following:

1. Production materials

(1) Contamination of intracellular/exogenous factors, cell characteristics and genetics used in production cells

Stability, cell gene modification, cell tumorigenicity, tumor promotion and tumorigenicity

Risks etc.

(2) Toxicity, immunogenicity and introduction of exogenous factors of raw materials used for production

Risk; excipients, especially new excipients and compound excipients, are safe for human use

properties, immunogenicity, compatibility and stability of excipients; physical and chemical properties of production consumables

Quality stability, sealing performance and compatibility, etc.

2. Active ingredients

(1) Historical passage and transformation of viral vector species, vector types and

Transformation method, replication characteristics of the vector, genome integration ability and integration tendency,

Viral genome stability, infection and expression properties, homologous and nonhomologous recombination

risks, immunogenicity, pathogenicity, etc.

(2) The correctness and stability of the nucleic acid sequence and the regulatory characteristics of the regulatory elements,

Tumorigenicity, targeting specificity, and tissue expression properties of genes and regulatory elements, above

Target/off-target risks, genome integration characteristics and integration mutations, tumorigenic risks, etc.

(3) Plasmid loss, resistance changes, or biological changes in bacterial or fungal vectors

and changes in genetic characteristics.

(4) Target specificity and editing efficiency of gene editing tools, as well as editing

Effect of tool-related impurities or degradation products on specificity.

3. Production technology

(1) Production technology and process control, process changes and process deviations, process

risk of contamination and confusion.

(2) The interaction between product production, production environment, personnel, etc.

4. Product quality

(1) Whether the quality attribute characterization or quality standards are comprehensive and sufficient?

Whether the quality risk of conducting research without effective methods is controllable.

(2) Whether the analytical method can meet the needs of quality control.

(3) Quality monitoring during the production process, such as genetic recombination and

Control of mutation risks, etc. Aggregation, aggregation, and

Inactivation, degradation, microbial contamination, confusion, etc.

5. Container sealing system

Compatibility-related risks (such as adsorption of products, leakage of packaging materials, etc.)

and risks related to sealing performance.

Develop reasonable quality risks based on comprehensive risk identification and assessment results

Control Strategy. Control strategies should be developed with the aim of reducing product risks, e.g.

For example, quality control should be carried out based on the risks of raw materials, and the production process should be fully inspected.

Characterization and validation studies, comprehensive verification of quality control methods, and establishment of

The quality control system for the whole process from raw materials, production process to release inspection is based on

Quality research establishes reasonable quality standards and determines product quality based on stability research.

Storage, transportation and use conditions should be selected based on compatibility and sealing studies.

inner packaging materials, etc. The revision of risk control strategies should be carried out throughout the life of the product.

life cycle, with the accumulation of production experience and understanding of product quality attributes,

Revised and improved.

5. General considerations for product design

Product design should be based on patients' clinical needs and fully consider product expectations

Various factors such as mechanism of action, process performance, safety risks, effectiveness, etc.

It is also necessary to consider the safety and convenience of the product during storage, transportation and clinical administration.

Benefits. Due to the factors that need to be considered in the design of different types of products, there may be greater

Differences, this section mainly focuses on the current main types of in vivo gene therapy products

(Viral vector products, nucleic acid products, bacterial vector products) proposed products

The general factors that need to be considered in product design are provided for reference. The design of specific products still needs to be

Take comprehensive consideration into account with product features. Because different types of products differ in functionality or

There may be certain similarities in the mechanism of action. Therefore, when designing the product,

It may be necessary to cross-reference considerations for other types of products. With gene therapy

With the development of the field and the enrichment of knowledge, the factors to be considered in product design will also continue to

complement and improve.

1. Viral vector products

Viral vector products in this guideline refer to products that use viral vectors to

Gene therapy products that transfer target genes from foreign sources into the human body. Among them, the virus carries

Viruses refer to viral particles modified to mediate the transfer and/or expression of foreign genes,

Common ones include adeno-associated virus vectors, adenovirus vectors, herpes simplex virus vectors, etc.

According to whether the nucleic acid carried by the vector is integrated into the genome of the target cell, it can be divided into integrated

and non-integrating viral vectors; according to the replication characteristics of the vector, they can be divided into non-integrated

type, conditionally replicating and replication-competent viral vectors. Viral vectors and foreign genes

The selection and design should be based on the carrier characteristics, mechanism of action, population antibody levels,

Clinical indications, route of administration, and frequency of administration (i.e., potential need for retreatment)

Wait for analysis and research to determine.

1.1 Target genes and regulatory elements

The target gene refers to the gene in the product that mainly plays a therapeutic or regulatory role

Or nucleic acid sequence, which can exist in the form of DNA or RNA, such as functional proteins

White coding sequences, nucleic acid transcription sequences with targeting effects, etc. target gene

The selection and design of products need to consider the pathogenesis of the disease, the mechanism of action of the product, human

Sequence differences between species, as well as the immunogenicity and functional activity of gene expression products

and security etc. From the natural sequence through codon optimization, gene mutation, recombination,

The target gene transformed by modifications such as insertion, deletion and/or rearrangement must have sufficient

The basis for the transformation, and confirm the rationality of the sequence design through in vitro and/or in vivo studies

sex. Targeted binding through platform calculation or design based on sequence rules

Nucleic acid sequences, such as sgRNA, siRNA, etc., need to be evaluated for the rationality of the design.

And during the development process, the target specificity and on-target/off-target risk of the sequence are evaluated.

Evaluate and validate.

The selection and design of target gene expression regulatory elements need to be based on the target cells

The type, regulatory requirements for gene expression and regulatory characteristics of the elements are determined and evaluated.

The expression level, persistence and specificity of the target gene by the regulatory elements

(if applicable) and other aspects of the control are in line with expectations, pay attention to the abnormality of the control components

Anticipated control risks, such as: remote control functions of components, multiple identical components

The risk of gene knockout caused by the group, the insertion of regulatory elements on the cellular genome genes

Start, enhance, terminate, insulation adjustment effects, etc. When designing, it is necessary to control

Assess the tumorigenic/tumorigenic properties of components and avoid using components with potential tumorigenic/tumorigenic properties

Risk components, if used, should be modified to remove their tumorigenic properties

/Tumour-promoting properties. If it contains a protein that controls gene expression in a transient or tissue-specific manner

For control components, the corresponding control characteristics of the components need to be confirmed.

1.2 General principles for viral vector selection and design

Viral vectors are generally selected and designed to effectively deliver and express the target gene.

Therefore, the purpose is to reduce the pathogenicity of the vector and reduce the risk of recombination and mutation of the vector.

Common vector modification methods include deletion, recombination and/or replacement of viral genes, etc.

When selecting and designing viral vectors, the following aspects generally need to be considered (but are not limited to)

the elements of:

(1) Study the basic infection rate of wild strains or vector viruses in the population and

Neutralizing antibody titer levels to assess the body's immune response to viral vectors in vivo

distribution, transduction efficiency and therapeutic efficacy.

(2) Delete genes or components related to safety risks as much as possible, for example

Pathogenic/tumorigenic genes, otherwise, legacy genes/components need to be assessed for product safety

sexual influence.

(3) Minimize non-essential elements of the viral vector or the packaging base of the virus

Due to splitting, the probability of recombination and reverse mutation of the viral vector is reduced (such as

replication-deficient vectors).

(4) Minimize the interaction between viral vectors and human susceptible viruses or endogenous viruses

Homologous sequences reduce the risk of recombination to produce new infectious viruses or replicating viruses.

risk.

(5) The infection specificity/tropism and gene transduction efficiency of viral vectors have important effects on the production

The safety and effectiveness of the product are affected, and the tissue or cell tropism of the viral vector

Should be consistent with the indications.

(6) Study the stability of viral vector gene sequences and evaluate the risk of sequence mutations

Potential safety risks and effects on effectiveness.

(7) Study the effects of modification of viral vectors on their immunogenicity, pathogenicity and other

influence on biological properties and assess whether susceptibility to relevant antiviral therapies has developed

biological changes and whether the viral vector may be reproductively toxic.

1.3 Special considerations for viral vector selection and design

1.3.1 Integration features

Integrating viral vectors may have longer life span than non-integrating viral vectors

effective gene expression activity in vivo, but the integration process may affect the genes of human cells.

Impact due to group integrity or expression properties. Therefore, according to product design needs

Please make a choice. For integrated carriers, currently known technologies should be used wherever possible

Conduct safety screening and/or design modifications on the carrier using surgical methods to reduce the risk of insertion

Risk, and on this basis, analyze the integration mode and integration position of the vector in the genome

Distribution trend of points, and evaluate whether their insertion leads to gene mutation or gene loss in cells.
survival/activation, or the risk of cells becoming cancerous.

For non-integrating viral vectors, theoretically it leads to changes in the cellular genome
The risk of insertional mutations is relatively small, but needs to be adequately studied, evaluated and
/or confirm the non-integrating characteristics of the vector. For example, for AAV (Adeno-
Associated viruses) and other vectors that are generally considered to have non-integrating characteristics are still in use
Integration of vectors into the genome has been reported under certain circumstances, so studies are needed to confirm
control risk. In addition, due to the non-integrating nature of the vector, the target gene is free in cells.
Outside the cellular genome, the expression timing of the target gene is easily affected by the stability of the vector gene.
effects, and may lead to dilution and loss of the target gene due to target cell division. because
Therefore, it is necessary to fully consider the mechanism of action of the carrier, integration risks, and target base.
Select and design viral vectors based on expression timeliness, etc.

1.3.2 Replication Features

Non-replicating viral vectors theoretically cause viral spread or infection in the body
The risk of loss of control is relatively small, but it still requires reasonable and reliable detection methods.
Confirm the non-replicative characteristics of viral vectors and consider the vector's intended use
Select and design viral vectors based on factors such as the impact of diameter and tropism on efficacy. No
Design of replicating viral vectors and selection of viral packaging systems for production should be adequate
Consider the generation of replicative forms through homologous or nonhomologous recombination during production and use
the possibility of viruses, and establish control strategies for the potential risks of replicating viruses
strategy, e.g., replicating samples or final products at appropriate stages in the production process
Detection of viruses, etc.

For replicating or conditionally replicating viral vectors, their replication characteristics may

Causes non-specific infection, immune response, cell lysis and other effects, which need to be combined with the carrier

Choose carefully based on physical properties, structural design, safety and expected mechanism of action. this

When selecting and designing class carriers, the following aspects should be considered (but not limited to):

(1) Analyze the in vivo replication of viral vectors based on the mechanism of action and indications

Necessity of ability.

(2) The carrier should avoid containing any known risk of human tumorigenicity

components.

(3) If the viral vector has been modified, the viral load after modification needs to be evaluated.

changes in the body's pathogenicity, infectious activity, etc., and control related safety

sexual risk factors.

(4) The tissue/cell infection and/or replication specificity of the viral vector should be consistent with

Compatible with clinical treatment mechanisms. If necessary, consider designing to increase the carrier's in vivo

Deactivation or clearance mechanism.

1.3.3 Targeting characteristics

The efficacy and safety of viral vector products are related to the infection characteristics of viral vectors.

heterosexuality/tropism and/or expression specificity of the gene of interest are closely related, therefore, the vector

The selection and design of the carrier need to consider the tissue/cell tropism of the carrier, combined with the receptor distribution characteristics.

points, as well as factors such as cell specificity in the regulation of target gene expression. Different administration

Pathways also have an important impact on the target distribution of viral vectors. The development process should be based on

Selection and design of vectors, in vitro biological property studies, and non-clinical studies,

The targeting specificity/tropism of the vector and the quality of the target gene in clinical trials and other aspects

Expression activity and expression regulation (if applicable) are confirmed.

2. Nucleic acid products

Nucleic acid products in this guideline refer to nucleic acids with specific functions

After active ingredients enter human cells through physical or chemical mediation, they

Products that are transcribed, sheared, translated, or directly act in cells. Products are often

The common active ingredients are RNA or DNA. Depending on the mechanism of action, nucleic acids

Products such as mRNA, shRNA (Short hairpin RNA), and miRNA

(Micro RNA), plasmid DNA, minicircle DNA

Functions can also be exerted by single active ingredients such as RNA, DNA and proteins.

Two or more components combine to form a functional system, such as

CRISPR-Cas system. Nucleic acid products often require the help of

Certain physical transduction devices (such as electrotransfection) or chemical delivery auxiliary media (such as

Liposome complexes, cationic polymers, cationic peptides, etc.) to improve nucleic acid

Transfection efficiency, some peptides, antibodies, ligands, etc. with nucleic acid transport function

It can also assist in the transport of nucleic acids.

The structure of nucleic acid products, the design of target genes and related regulatory elements should be

It has reasonable scientific basis and fully considers the characteristics and characteristics of various types of nucleic acid active ingredients.

mechanism of action so that it conforms to its intended function. When designing nucleic acid products, the purpose of

For the design of genes and regulatory elements, please refer to "1.1 Target Gene of Viral Vector Products"

and control elements". In addition, depending on the characteristics of the product, there may be

The following factors need to be considered (including but not limited to):

(1) For the use of shRNA, miRNA, CRISPR-Cas, zinc finger nuclei

Targeted binding to target genes such as ZFN, TALEN or Meganuclease
sequence or gene expression product sequence to function, the product's
Target specificity and safety risks caused by off-target, reduce the relationship between the target gene sequence and
Homology of non-target sequences, optimizing related nuclease structures, improving binding or editing
editing specificity.

(2) For CRISPR-Cas, ZFN, TALEN or Meganuclease
Products that work by editing the genome within cells should be considered
Gene error repair and genome structure caused by genomic gaps or double-strand breaks
Risk of rearrangement. At the same time, the duration of action of the editing enzyme and the editing
Effects of enzyme residues on genome stability and cytotoxicity.

(3) For products with genome integration properties such as transposons, consideration should be given to
Integration site specificity of the product or distribution trend of integration sites, gene integration
Stability, as well as insertional mutations and tumorigenic risks caused by integration, etc., reasonable design
Calculate vector sequence, ratio of transposase/transposable DNA, number of transposable genes, transposase
The duration of enzyme expression and expression regulatory elements, etc.

(4) For mRNA-like protein coding sequences, the 5'-cap or like
The type and design of the analog structure, the sequence and length of the poly A tail and its length distribution,
Translational regulatory elements, types of nucleoside modifications, sequence self-replication ability, delivery system
system, etc., on the immunogenicity, expression activity and vector stability of the product.

(5) Are the nucleic acid type and structure consistent with its expression duration in vivo?
Adaptation, self-amplifying function of nucleic acids and gene persistence
The necessity and safety risks of the product, its genome integration function and its effect on target cells

Functional necessity of genetic modification of the genome.

(6) Stability, genotoxicity and immunity of the product's nucleic acid structure and sequence

Original nature.

(7) Oncogenic genes should be avoided in nucleic acid sequences. If possible, should

Remove non-essential elements and screening genes (such as antibiotic resistance genes) where possible.

(8) Chemical stability, human safety, and transfer of chemical delivery auxiliary media

staining efficiency, as well as compatibility between delivery media and delivery complexes. If possible

If possible, the design and screening of delivery auxiliary media may be considered to enhance the product's in vivo

Targeting/tropism, or reduced immunogenicity.

3. Bacterial vector products

Bacterial vector products in this guideline refer to products that have been genetically modified

As a vector, bacteria used to express target proteins or specific nucleic acid sequences in the human body

Microorganisms, such as Salmonella, Listeria, Escherichia coli and other bacteria modified

The product. The construction of bacterial vectors is generally based on the structure and biology of wild strains

Characteristics, by transferring plasmids, episomal vectors, or

The genome of the strain is modified and completed. The modification of bacterial vectors should be based on reasonable

scientific basis and product mechanism of action to reduce or remove the pathogenicity of microorganisms,

The goal is to achieve or enhance the therapeutic function of the carrier. The design and construction of carriers should be

Note the genetic characteristics, biological characteristics, pathogenicity and purpose of the modified bacterial vector

Gene expression activity, in vivo distribution characteristics of the vector, and impact on normal human flora

impact, environmental safety, horizontal gene transfer, and challenges to conventional treatments

changes in sensitivity, etc. Modification of bacterial vectors should be avoided b -Inside

Amide antibiotic resistance genes. The design of foreign target genes can refer to viruses

General requirements in product "1.1 Target genes and regulatory elements".

6. Production materials

Production materials in this guideline refer to materials used in the production process

All biological and chemical materials, including starting raw materials (e.g. cell matrices,

Bacteria, virus strains, plasmids, etc.), materials used or added during the production process (such as culture

Culture medium and its additives, tool enzymes, purification fillers, buffers, etc.), excipients,

And production consumables (such as culture bags, storage bags, pipetting lines, filter membranes, etc.)

wait. The materials used in production are closely related to the quality, safety and effectiveness of the product.

Materials that are suitable for product characteristics and have stable supply should be selected through risk assessment. specification

Establish a quality management system for production materials, including risk assessment, supplier review

audit, risk and quality control to ensure product quality and reduce the risk of production materials

related quality risks.

1. Starting raw materials

Different types of in vivo gene therapy products may have different starting raw materials for production.

There are certain differences. Common starting raw materials include plasmid DNA, bacterial seed batches, virus

Toxin seed batches, toxin-producing cell lines/banks, etc. Starting raw materials should have clear origins and

Complete traceability information should generally be constructed in accordance with the requirements of the "Chinese Pharmacopoeia"

manage. Quality control of starting raw materials should be based on their type, characteristics, history and

Source information, preparation process, and product type, production process, and route of administration

Risks in other aspects are determined, and quality control should be commensurate with the risks.

1.1 Plasmid DNA

Plasmid DNA for in vivo gene therapy product production, transient transfection for

Production of viral vectors, in vitro transcription for RNA nucleic acid product production, or transfection

Transfection/transformation for the construction of bacterial seeds, viral seeds, and cell lines. Various plasmids

The structure, gene and element design of DNA should comply with the general principles and expectations mentioned above

purpose, the source and sequence of the original plasmid used for construction should be clear, and the construction process

The construction results of each step should be confirmed. Plasmid sequences, especially as end products

The sequence of active ingredients of the product should not contain genes or elements with potential tumor risk.

If necessary, such genes/elements should be replaced or modified. to avoid

Antibiotic abuse and the risk of allergies caused by antibiotic residues, it is recommended to avoid using them

b -Lactam antibiotic resistance genes are used as plasmid screening genes.

Disposable removal of bacterial seed batches, viral seed batches, or cell line construction processes

In addition to the plasmids used, the starting material plasmid DNA should be based on bacterial seed batch lines.

system, prepared using proven fermentation and purification processes, the scale of plasmid production should be

Subsequent production steps are scaled accordingly. General package of quality standards for plasmid DNA

Including plasmid identification, content, purity, confirmation of complete plasmid or important gene sequence,

Supercoil ratio, sterility, bacterial endotoxins, antibiotic residues (if applicable), -

General physical and chemical properties (such as pH value, etc.), process-related impurities (such as host bacterial DNA

Residues, host bacterial RNA residues, host bacterial protein residues, etc.), etc., specifically

The project should be determined based on the preparation process and usage analysis of the plasmid. Each batch of plasmid passes

Release testing can only be used for product production. The storage stability of the plasmid should be able to support subsequent

Production of continued production steps.

Bacterial seeds, virus seeds or single-use during the construction of cell lines

Plasmids do not need to be prepared through bacterial seed batch systems, but the plasmid sequence should be

Confirm while avoiding the risk of contamination.

When transiently transfecting plasmids used for viral vector packaging, the aforementioned viral vectors should be considered.

General requirements for body design. In order to reduce the recombinant production during virus production and use,

There is a risk of producing replicable or pseudo-wild-type viruses. It is recommended to give priority to verified tools.

There is a virus packaging system with a higher security level, and the necessary components for virus packaging are appropriately

Split into different plasmids and try to remove non-essential viral genes to reduce quality

Homology between plasmids, packaging cells and wild virus sequences. example

For example, when constructing the AAV packaging system, the structural genes of AAV are separated into different

in the transcription unit of the plasmid and reduce the Rep/Cap gene sequence and ITR as much as possible

Similarity between sequences, etc.

1.2 Bacterial seed batch

Bacterial seed batches can be used for nucleic acid gene therapy products (such as plasmid DNA,

minicircle DNA, mRNA, etc.) may also be cultured as bacteria

Vector gene therapy products. The source of the bacterial strain should be clear, and the bacterial seed batch should be

The preparation process should be clear and complete, and the use of human or animal sources should be avoided as much as possible during the preparation process.

Source raw materials and confirm the monoclonality of seed batches.

The preparation and testing of bacterial seed batches should comply with the general chapter "Biology" of the "Chinese Pharmacopoeia"

"Management and Quality Control of Bacteria and Viruses Used for Product Production and Testing" and "Gene Therapy System for Human Use"

According to the requirements of "General Introduction to Products", the testing of seed batches generally includes colony morphology, identification,

Staining microscopy, growth characteristics, biochemical reaction characteristics, viability, antibiotic resistance,

Electron microscopy, plasmid sequencing, restriction enzyme mapping, expression of transgenes and/or

Activity analysis (if applicable), etc., the bacterial seed batch should be free of other bacteria, fungi,

Bacteriophages and other contamination. Genetically modified bacterial vectors should pay attention to the modified bacteria's

Phenotype and genotype, changes in the genome or important regions of the genome (e.g., introduced therapeutic

therapeutic genes and regulatory elements, as well as a region of at least 0.5 kb flanking the gene of interest)

Perform sequencing to confirm the insertion site of the modified gene, gene copy number, etc.

Analysis, if necessary, the expression level and functional activity of the target gene should be detected. for

The attenuated bacterial vectors should be identified for their attenuated properties and stability, and

Detect changes in their antibiotic susceptibility. Bacterial seed batches for plasmid and other production or

For bacterial vector seed batches containing plasmids and episomes, the plasmids or episomes contained must be

Add the sequence for confirmation.

Consider bacterial growth characteristics, genomic integration, or modified genes during passaging.

factor, expression and activity of target gene, plasmid sequence, plasmid copy number and plasmid

Changes in the loss ratio need to be carried out using conditions that simulate or represent the actual production process.

Passage stability research, the genetic and phenotypic characteristics of the seed batch should be able to meet production needs

beg.

The storage stability of bacterial seed batches should be sufficient to meet production needs.

1.3 Production/Packaging of Cell Banks

Production/packaging cell banks are used for viral packaging and production and can impact viral vectors

quality and yield, there may be risks of introducing exogenous factors, cell culture residues

The retained cellular proteins, DNA and other impurities have certain immunogenicity, and some fine particles

Cell lysis products or DNA may also pose a certain risk of tumorigenicity. in addition,

The types of production/packaging cells that may be suitable for in vivo gene therapy product development are relatively few.

Many, such as passage cell lines/lines and insect cell lines with or without tumorigenic properties

Etc., different cells may present different types of risks. For the above reasons, it should be

Prioritize cell substrates with clear origins, clear culture history, and no virus contamination.

The quality is used for virus packaging and production. If using cell matrices containing endogenous viruses,

The necessity and safety of its use should be evaluated, e.g., human infection with endogenous viruses

activity, immunogenicity, process residue levels, viral gene expression activity, etc., must

When necessary, a validated virus removal/inactivation process unit should be added to the process and

Virus residue and activity are tested at appropriate stages. For tumorigenic or

Cells with unknown neoplastic risk, especially novel cell matrices and newly established cell lines/lines,

It is necessary to refer to the relevant requirements of the Chinese Pharmacopoeia to evaluate the corresponding risks of producing/packaging cells.

risks and carry out corresponding research when necessary. Due to tumorigenesis or tumorigenesis of tumor cell lines

The risk is relatively high, so it is recommended to choose with caution. If cells with tumorigenic properties are used,

Impurity removal performance needs to be combined with clinical risks and benefits, route of administration and production process

(such as residues of viable cells, residues of tumorigenic gene fragments, etc.) to evaluate its use

Necessity, rationality and safety, analyze whether cells carry tumorigenic risks

dangerous genes or other factors, and their residual levels and gene fragment sizes should be addressed if necessary.

Small to control. The use of tumorigenic cells is generally not recommended. In addition, it is necessary

Intact cells, especially tumorigenic ones, through manufacturing process controls and/or product release

Cell residual levels are controlled.

In order to ensure the stability of product quality, production/packaging cells need to be built into a library

Management, preparation and testing of cell banks should comply with the general chapter "Biology" of the "Chinese Pharmacopoeia"

"Preparation and quality control of animal cell matrix for product production and testing" related requirements.

Testing of cell banks generally includes: cell identification, cell number, viability, genes

Type and phenotype (if applicable), growth characteristics (if applicable), exogenous factors, etc., for

For cells with unknown tumorigenic properties, it is recommended to perform tumorigenicity testing. Exogenous factor detection

Testing generally includes sterility, mycoplasma, spiroplasma (insect cells, or production process

Plant-derived ingredients), exogenous viral factors, etc. are used in it. of foreign viral agents

The test can be carried out in accordance with the requirements of the pharmacopoeia and combined with the characteristics of cell modification after risk assessment.

definition, generally should include non-specific viruses, retroviruses, cell species-specific

viruses, as well as potential risks that may be introduced during cell strain or cell bank construction and culture.

in exogenous viral agents. Cell culture history or if bovine blood was used during the culture process

Raw materials of animal origin such as serum, porcine trypsin, etc. should be tested for bovine origin, porcine origin, etc.

Detection of viruses related to corresponding animal species.

Based on production needs, if the cell matrix is genetically modified, such as tissue

For adult expression of viral packaging proteins or replication cofactors, genetic modification should be considered

The necessity and applicability of the modification method, the modification process should not add exogenous factors

The risk of introduction should be minimized or minimized when selecting modified genes.

The risk of process restructuring. For example, non-replicating or conditionally replicating types such as adenovirus

Viral vectors should be used in cell lines that do not contain homologous sequences or have few homologous sequences.

In viral vector production, it reduces the risk of recombination during the production/packaging process. For the classics

Stable passage cell lines/lines established through genetic modification are also used in characterization studies of cell banks.

Respond to the results of genetic modification, such as gene sequence, modification site, copy number, expression

Confirm the level is reached.

In order to confirm that production/packaging cells within a limited number of generations can produce products with stable quality

A specific viral vector needs to be carried out in detail under conditions that simulate or represent the actual production process.

Study on the stability of cell passage and determine the production limit passage of the cell bank based on the research results.

Second-rate. Cells within a limited passage number should not affect the genetic and phenotypic characteristics of the packaged virus.

Can support virus production. For new cell matrices or newly established cell lines/lines, it is necessary to

Pay attention to the changes in tumorigenicity during its passage, and if necessary, conduct further research on its tumorigenicity.

Conduct research; genetically modified stable passage cell lines/lines need to pay attention to the passage process

Whether the sequence and copy number of the viral packaging gene are stable, and whether the viral packaging product is

Whether the quantity is uniform and meets quality requirements.

1.4 Virus seed batch

Production viruses may include viral vector seeds and/or packaging viruses,

Its source, culture history, and construction process should be clear and complete, and the virus characteristics must meet

meet production needs, and the safety risks should be controllable after assessment. For educational background

The history is unclear, there is a risk of contamination by other non-target viruses, or the strain is monoclonal.

Virus seeds cannot be guaranteed, so it is recommended to choose with caution. If you need to use it, you can build

The process uses multiple rounds of plaque purification, limiting dilution purification, or DNA/RNA

Rescue and other methods to ensure the purity and monoclonality of the strain.

Virus vector seeds and packaging viruses that can be used for library construction should be

Inventory management to reduce batch-to-batch variation in products. Quality control of virus seed lots should be

Comply with the requirements of "General Introduction to Gene Therapy Products for Human Use" of the "Chinese Pharmacopoeia", and the test items

The target should be specifically determined based on the characteristics of the seed batch, culture history, library construction process, etc.

Generally include identification (genomic and immunoserological characteristics), viral titer, purpose

Transcription/expression of gene sequence (if applicable), expression product of target gene sequence

Biological activity (if applicable), viral vector contamination by exogenous factors (such as bacteria, viruses

bacteria, mycoplasma, exogenous viruses, etc.), replicating viruses (the vector is non-replicating or

Conditional copy type) etc. In addition, it is necessary to pay attention to the historical passage and construction process of seed batches.

Specific exogenous factors that may be introduced during the process, such as those that may be introduced by insect packaging cells

Species-specific exogenous factors such as Spiroplasma and Rhabdovirus. viral vector genes

The group sequence should be consistent with the theoretical sequence. If there is any difference, the source and origin of the mutation need to be analyzed.

The stability of the viral genome and the impact of mutations on product quality, safety and effectiveness

sexual influence.

Passage stability studies of virus seed batches should represent or simulate actual production

production process conditions, paying attention to the genetic stability of the virus seed batch and the quality of the target gene

expression characteristics, replication characteristics and product quality changes, etc., based on the stability of passage

The research results provide a reasonable basis for formulating the production limit passage times for virus seed batches. Develop virus seeds

The storage stability of batches should be studied, and the storage of virus seed batches should be able to meet production needs.

2. Other production materials

Other production materials refer to other materials used in production other than starting raw materials.

Raw materials used (such as tool enzymes, antibiotics, culture media, detergents, purification reagents

agents, etc.), excipients and consumables, etc.

The raw materials used in the production process should comply with the general chapter "Production and Production" of the "Chinese Pharmacopoeia"

"Relevant requirements for quality control of raw materials and auxiliary materials used in the production of physical products", the quality of raw materials

The quality should be consistent with its intended use. It is recommended that key raw materials be given priority by drug supervision.

Regulatory agency approved products or pharmaceutical grade raw materials. The selection of raw materials should be based on

After adequate evaluation, its source, components, functions, quality, stage of use, use

The quantity and other conditions should be clear, try not to use non-essential raw materials, and reduce the cost of raw materials.

Risk of carryover and introduction of exogenous agents. Comprehensive review of risks related to raw material production

Risk factors and raw material manufacturers' control of corresponding risks, based on risk assessment

Establish reasonable internal control standards. If possible, avoid using serum, porcine pancreatic eggs

Raw materials of animal or human origin such as white enzyme should be replaced with serum with clear ingredients as much as possible.

Substitute with other products or reconstituted products. If it is deemed necessary to use it after research, necessary measures should be taken

risk control measures (e.g. c ray irradiation treatment, etc.), and for the species of raw materials

Establish a complete quality control system regarding sources, production areas, production processes, quality standards, etc.

system to assess its TSE/BSE safety risks. Spongiform encephalopathy epidemic is strictly prohibited

Raw materials prepared from susceptible animals originating from epidemic areas shall not be used without passing safety tests.

Tested serum/plasma. Assess the safety of production reagents and avoid the use of beta-enzymes

Amide antibiotics, streptomycin, and other toxic and harmful reagents such as ethidium bromide.

If toxic or hazardous raw materials are used in production, downstream purification should be proven

The process can remove it well or provide usage warnings.

For relevant requirements on excipients, please see "1.2.2 Excipients" under "7. Production Process"

part.

Consumables and containers used in the production process, such as disposable reaction bags, pipetting

Pipelines, liquid storage bags, filter membranes, etc. should have stable physical and chemical properties and consume less

The material should have good compatibility with solutions in direct contact, production intermediates, etc.

Analyze consumables and contents based on factors such as their material, stage of use, supplier research, etc.

Evaluate or study the compatibility of the device.

7. Production process

1. Production process development

The production process generally refers to the cultivation/fermentation and purification of cells or bacterial microorganisms.

The entire process from chemicalization to final product packaging and storage. Due to different product types, different

There may be big differences in the production processes of products, such as plasmids and viral vectors.

Products, the production process may involve induced expression, plasmid transfection, virus infection

For other operations, some nucleic acid products such as RNA may also use non-cell systems.

An in vitro transcription process is used for production.

The development of production processes should be based on an understanding of the quality profile of the target product, resulting in

The correlation between production process and product quality, through process research, gradually

Improve the process and complete the development process from laboratory to commercial scale production.

Finally clarify the process steps and key process parameters. If a reduced model is used for process

research, the representativeness of the scaled-down model should be confirmed to support its findings.

Can fully represent the actual production process. If feasible, it is recommended to use a closed type

The production process reduces environmental exposure and storage during the production process. Produced

If there is temporary storage of intermediate products in the process, the temporary storage conditions and temporary storage time limit should be studied.

research and verification. Based on risk analysis, establish a full-process control strategy and rationally design

Control in the production process, especially the contamination and contamination of external factors during the production process

Quality control of key intermediates. During the life cycle, the production process should follow the

With the advancement of process technology and in-depth understanding of products, continuous optimization is carried out in response to process changes.

Conduct corresponding comparability studies to ensure product quality.

1.1 Dope solution process development

1.1.1 Fermentation/culture process

Production processes starting with bacterial seed batch fermentation or production/packaging cell culture

The fermentation/culture process can directly affect the quality of the product. Based on product quality

The understanding of properties requires understanding of fermentation/culture conditions, such as the scale and model of fermentation/culture.

Formula, culture medium and added ingredients, culture temperature, pH value, osmotic pressure, stirring speed

degree, pCO2, dissolved oxygen, culture time, inoculation conditions, transfection/infection conditions,

Conduct sufficient research on harvesting time, etc., and formulate conditions and parameters suitable for production.

For example, the development of viral vector packaging processes should consider improving the packaging efficiency of vectors

and packaging accuracy, reducing empty carriers, incorrectly packaged carriers, inactive carriers,

Formation of impurities related to products such as free nucleic acids; plasmid DNA, minicircle DNA, etc.

For nucleic acid products, the sequence correctness, structural integrity, and recombination efficiency of the nucleic acid should be considered.

rate (such as microcircle DNA, etc.) and conformation, etc. During the fermentation/culture process, avoid

Introduce unnecessary process-related impurities, eliminate impurities and/or exogenous factors in appropriate steps

sub for testing. For the packaging production of viral vectors, it is generally recommended that unprocessed

The culture harvest fluid is tested for contamination by exogenous factors. in virus seed or harvest

Testing of fluids for exogenous agents is interfered with because product viruses cannot be adequately neutralized.

In this case, control cells can be set up in production and used for exogenous

Factor check. For non-replicating or conditionally replicating viral vectors, the appropriate

Sensitive methods are used to monitor replication-competent viruses during the process stages.

1.1.2 In vitro transcription process

Nucleic acid products such as mRNA prepared by in vitro transcription can be

Research on raw materials, transcription templates and transcription conditions to control the quality of transcription products.

Important raw materials for transcription, such as nucleotides, modified nucleotides, 5'-caps, or similar

Substances, tool enzymes (such as transcriptase, etc.) should be subject to appropriate quality control, which can be

Pay attention to its purity and impurities, and the transcriptase also needs to pay attention to its fidelity, etc. Transcription template

The preparation should ensure the sequence accuracy and purity of the template and reduce impurity residues. body

External transcription conditions should be fully studied to improve the accuracy and uniformity of the transcribed sequence.

Uniformity and integrity, reducing side reaction products such as incomplete RNA, double-stranded RNA,

Formation of truncated RNA, long RNA, etc.

1.1.3 Purification process

Purification processes should be based on product type, upstream processes, and potential impurities

And it is determined that it should be able to stably remove it without affecting the integrity and activity of the product.

or reduce process and product related impurities. If a helper virus is used during the culture phase,

Packaging with viruses, or with other potential virus contamination risks, should be based on the target

Differences in physical and chemical properties between the product and non-target viruses, which increase during the purification process

Necessary viral removal/inactivation process steps such as detergent inactivation, low pH inactivation

Or process units such as virus removal filtration to control the safety risks of non-target virus residues

risk. During the purification process, contamination by exogenous factors and product risks can be considered at critical steps.

Quality is monitored.

1.2 Preparation process development

1.2.1 Preparation prescription

The selection of dosage form should consider the stability of product storage and transportation, clinical use

Many factors such as convenience and safety can achieve clinical treatment purposes.

Under the premise, try to design and select dosage forms that are easy to store, transport and use. system

The design of the dosage form needs to be compatible with the dosage form and should be able to effectively maintain the functional activity of the product.

properties and stability to meet clinical drug needs. Due to poor stability, some products

It needs to be stored under low temperature, freezing or freeze-drying conditions. Preparation prescriptions are usually

When it is necessary to add cryoprotectants, freeze-drying protectants, active protectants, etc. with special

Excipient ingredients with specific functions. On the premise of ensuring product activity and stability,

The design and screening of prescriptions should try to select products with simple structures, clear components, and controllable quality.

Prescription excipients with less safety risk. The selection of excipients should be based on sufficient basis and their functions should be

It should be clear that the dosage should be reasonable and supported by corresponding research data. try to avoid

Select excipients with high toxicity and high safety risks.

1.2.2 Excipients

Excipients refer to auxiliary materials used in product formulations, such as stabilizers, buffers

systems, etc., their selection, dosage and quality standards should be determined based on the formulation development of the preparation.

Certainly. The quality of excipients should meet their intended functions and comply with the general principles of the Chinese Pharmacopoeia

Relevant requirements for "quality control of raw materials and excipients used in the production of biological products", priority

Choose excipients that meet pharmaceutical standards. If using multiple sources (e.g. animals, plants

excipients from synthetic sources) or from multiple suppliers, especially complex ones such as liposomes

For excipients, corresponding product characteristics should be developed based on the source and risk of excipient change.

Qualification and comparability studies to demonstrate products produced using excipients from different sources

Have equivalence.

If the prescription contains drugs that are intended for first use in the human body or during the intended route of administration,

New excipients used for the first time should be systematically based on the risk factors related to excipient production.

Evaluate the safety of excipients and formulate corresponding quality standards. In the absence of human safety

If supported by clinical research data, it is necessary to refer to the "Nonclinical Safety of New Pharmaceutical Excipients"

"Guiding Principles for Sexual Assessment".

Nucleic acid products often require the use of certain chemical delivery materials/media to

Promote or improve the transfection efficiency of nucleic acids, such as nanoparticles, liposomes, cationic

Ionic polymers, etc. Since auxiliary delivery materials/medium are different from conventional excipients, in

Protection, transfection and/or intracellular release during in vivo delivery of nucleic acid products

and other auxiliary effects, it can be considered as a functional excipient. Selection of delivery material/medium

The selection needs to have a reasonable basis. The material/medium itself should generally be considered.

Quality production process, quality control, human safety, material/media stability

etc. For the delivery system composed of delivery material/medium and nucleic acid, the system also needs to be considered.

Nucleic acid protection, delivery efficiency, intracellular nucleic acid release function, and delivery system

stability, as well as process stability and quality changes of the delivery system. for

For multi-component delivery systems, quality control and safety should be carried out for each component of the system.

evaluation, and at the same time conduct an evaluation of the interactions between system components and the stability of the system.

Conduct research to determine whether it functions as intended.

1.2.3 Preparation production process

Preparation production technology needs to be studied in combination with product characteristics, preparation formulas, dosage forms, etc.

Determine, the scale should match the production scale of the original solution, and try to avoid

Mixed batch operations. If mixing is indeed necessary, the mixing process should be fully verified.

Each mixed batch must be produced according to the prescribed process and meet the proposed standards. Developed

During the process, you may need to pay attention to the preparation method of the prescription, process operation time, and filling accuracy.

Control of accuracy and sterile conditions, etc. Special dosage forms such as freeze-drying should also deal with the freezing of preparations.

Dry curves were studied.

For nucleic acid products such as mRNA, the complex/

The encapsulation process may require attention to temperature, feed ratio, solution concentration, stirring rate,

The buffer system, mixing flow rate and mixing sequence have an important impact on the quality of the delivery system.

The impact of process parameters, pay attention to the compounding/encapsulation process and purification steps on the compounding/encapsulation

rate, particle morphology, particle aggregation/dispersion, nucleic acid leakage, impurity residues, nucleic acid

The influence of integrity and stability, etc., and reasonable setting of control items in the process.

1.3 Optimization and changes during process development

The production process may change as the process is developed, e.g. production site

changes, changes in equipment, replacement of raw materials, optimization of processes, changes in scale

Expansion, adjustment of quality control strategies, etc. The implementation of changes should be based on sufficient feasibility

Comparative research. Comparability studies can refer to the general principles of ICH Q5E, based on changes in

More risk assessment to establish comparable study programs or bridging programs. During risk assessment,

The research and development stage of the product, the type of change, the process involved in the change, etc. should be considered.

impact on product quality. In the early clinical trial stage, because the production process has not yet been optimized

Final determination, batch number is small, process changes can be based on limited batch study number

Comparable studies should be conducted, but attention should be paid to safety, purity, impurities, structure,

Changes in relevant quality attributes such as content; with the accumulation of batch data and product

With a deeper understanding of process and quality, the implementation of changes should be based on a more comprehensive and strict

Comparability studies. Depending on the type of change, pharmaceutical comparisons may include process

and in-process control, release testing, expanded quality research, stability, etc.

aspect. The batches for comparative studies should be based on the type of change, importance of quality attributes

variability and variability, data analysis methods, and development stages. It is generally believed that wind

Changes with higher risk/impact need to be addressed in process control, characterization studies, release testing and

Conduct more comprehensive statistical analysis with more batch data in terms of stability and other aspects. in production

When product quality attributes are not completely comparable, the quality observed in the study should be

Differences are evaluated. When existing knowledge or platform experience cannot predict quality attributes

The impact of differences on product safety and/or effectiveness, or anticipated changes in product quality

When product safety and/or effectiveness may be adversely affected, consideration should be given to

One step to add non-clinical and/or human bridging study data.

Due to the current limited knowledge and application experience of gene therapy products, one

It is generally not recommended to modify the production process during or after confirmatory clinical trials.

Make significant changes. Changes at this stage may increase the complexity of comparability studies.

Uncertainty about the nature and results may even affect the acceptability of clinical trial data.

During the product development process, attention should be paid to retaining staged or representative process samples.

Work in this way to facilitate later retrospective analysis or comparability research.

2. Confirmation and verification of production process

In the process of process development, it is necessary to carry out research according to different stage research purposes.

Production process validation or validation studies appropriate to the stage. Clinical trial application stage

section, the production process of samples for clinical research should be confirmed, and the production process should be done well.

Risk control in the production process, such as contamination by external factors, cross-contamination, product

Confusion etc. After the commercial production process is determined, representative

Commercial production processes undergo standardized process verification. The content of the validation study should be based on

It is determined based on the production process and risk analysis of the product, generally including cell culture, transformation

All process steps including induction/transfection, in vitro transcription, purification, preparation, etc. validation study

The number of batches and the complexity and variability of the process, as well as the amount of preliminary process research

Adequacy, platform experience, etc., generally no less than three batches, if there are other special

situation, it is recommended to communicate with regulatory agencies in advance. Validation studies should focus on

The controllability of each step of the process, the quality of intermediate products and the stability of process performance,

Conduct a deviation investigation for deviations that occur during the verification process, and based on the investigation results

Develop a corrective plan. Additionally, validation studies may include (but are not limited to)

Sterility verification of production processes, filter sterilization verification, and service life of fillers and membrane packages

Life study, transportation verification, cleaning verification, facility and equipment verification, etc., the production process

If there is temporary storage of intermediate products during the process, the temporary storage conditions and conditions of the intermediate products should also be addressed.

Time limit for research. The results of the validation study should demonstrate the stability and control of the process.

The rationality of the setting of control items in the system and process. After listing, it should be commercialized

Continuous validation studies are carried out during the production process.

For gene therapy products such as viral vectors, if there is virus removal in the process

/Inactivation unit, you can refer to ICH Q5A appropriately for process virus removal/inactivation

The effect is verified, and safety assessment is carried out based on the results of the verification study. Verification research

Studies should demonstrate that the process effectively removes or inactivates packaging used in the production process

Use non-target viruses such as viruses and endogenous viruses. At the same time, the target virus vector

Properties such as activity and structure are not affected unintendedly.

8. Quality research and quality standards

1. Quality Research

Quality research runs throughout the product life cycle. With the deepening of knowledge and analysis,

The development of analysis technology is constantly supplemented and improved. According to the stage of development or research purpose Different, quality research can select representative batches produced by the corresponding process (if not Clinical study batch, pilot process batch, clinical sample batch or commercial process validation batches, etc.) and samples of appropriate production steps (e.g. starting raw materials, production Intermediates, raw solutions, preparations, etc.) are studied. Market application stage, quality research Studies should generally include at least representative clinical sample batches and commercial process validation batch. If there are differences in quality characteristics between the original solution and the preparation, samples should be taken separately. Perform analysis. For nucleic acid complexes formed in combination with chemical delivery materials/media, Nucleic acids, complex components, and complete complexes should be studied separately.

Confirm the product's critical quality attributes through comprehensive quality studies quality attributes (CQA). Quality research generally chooses advanced, mature and flexible sensitivity to meet analytical needs. Due to possible limitations in the analysis method itself, limitations, we can consider using different methods with complementary principles to conduct research at the same time. specific Research projects should be determined based on the type of product, mechanism of action and production process. Seen quality research projects include (but are not limited to): identification, structural analysis, biological Chemical activity, purity, impurities, content, transfection/infection efficiency, general physical and chemical properties wait.

1.1 Identification and structural analysis

For viral vector products, they can be divided into genome, structural protein and complete The vectors are identified and structurally studied at different levels including whole virus particles. Genome Sequencing, restriction endonuclease digestion patterns, and PCR amplification specificity can be used to Fragment and other methods are used to analyze the virus genome, target genes and related regulatory sequences.

OK to confirm. If base or sequence mutations are observed during research, the cause of the mutation should be

analysis, combined with the impact of mutation sites on gene function and disease

Genetic stability characteristics of viral vectors to evaluate the impact of mutations on product safety and effectiveness

Impact. Structural protein and virion levels, by isolation of capsid proteins

Identification, immunoblotting, serotyping of viral particles, microscopic structure, particles

Size distribution, refractive index and other methods are used to evaluate the expression of structural proteins and the organization of virus particles.

Install to confirm. For some viral vectors with complex structures or limited analysis methods,

Consider combining the phenotype and activity of the viral vector, as well as the target cells infected by the viral vector.

Post-cellular gene sequence analysis, etc., to comprehensively conduct virus identification and structural analysis.

For nucleic acid products, the correctness of the nucleic acid sequence and the completeness of the structure are required.

Confirm integrity. If nucleic acids exist in single/double strands, linear, circular, supercoiled, etc.

For multiple topoisomers, each component should be identified and proportionally analyzed. partial core

The functional activity of acid products may be related to the secondary or higher-order structure of the nucleic acid, such as

hairpin structure, etc., it is recommended to study such structures. For mRNA etc.

The structure and modifications, such as nucleotide modification, capping modification, PolyA tail, etc., should be

Identify and confirm respectively.

For delivery complexes formed by combining nucleic acids with chemical delivery materials/media,

Suggestions on the structure of nucleic acid molecules, delivery complexes, and nucleic acids and delivery complexes

Study the interactions between objects. For example, studies of complexes may include e.g.

Isoelectric point, structural form, particle size and distribution, surface charge, complex component ratio

Examples: Nucleic acid complexing/encapsulation efficiency, particle aggregation, nucleic acid release, specific environment

Stability, etc. In addition, the delivery materials/media used such as lipids should also be identified.

Identification and content studies.

For bacterial carrier products, it is recommended to check the staining characteristics and microscopic appearance of the strains.

To study phenotypic and biochemical characteristics such as bacterial morphology, colony morphology, and culture characteristics, using

Sequencing, PCR, restriction enzyme analysis and other methods are used to analyze the strain's genome and/or negative

Plasmid and episome sequences, especially characteristic sequences and engineered sequences

Confirm the plasmid size, copy number, plasmid loss rate, and foreign target gene

Detect mutations, etc.

1.2 Biological activity

Biological activity is an important indicator reflecting product quality and clinical effectiveness.

At the marketing stage, it is necessary to establish biological mechanisms that are the same as or related to the product's in vivo action mechanism.

Activity analysis methods are used to study the functional activity of products. If there are multiple types of products available

The mechanism of action of energy should be studied separately. According to the activity and

Relevant to the product's mechanism of action and determine one or more appropriate activity detection methods

method as a quality control item. If there are steps or functions between different functional activities

The relevance/continuity should be reflected in the method as a whole as much as possible, such as product

transfection/infection activity and gene replacement, compensation, blocking,

Correlation of correction effects. For products containing multiple active ingredients, separate

Establish methods to study the activity of each ingredient, taking into account the relationship between active ingredients

Possible interactions such as interference and synergy. The methodological system should be simulated as much as possible

In vivo action conditions of the product, select cell types that are the same as or related to the in vivo process

type, analyze the product's transfection/infection efficiency, gene expression/inhibition level, expression product

activity of the drug, and other factors related to the mechanism of action of the carrier or delivery system.

For some products with selective delivery characteristics, the tissue/cell of the product should be

tropism, infection specificity, or selectivity of gene expression. active

Analytical methods should consider establishing appropriate activity controls.

1.3 Purity, Impurities and Contaminants

1.3.1 Product-related impurities

Product-related impurities are unintended,

Products in non-functional form. Potential product-related impurities in viral vector products 1

Generally include incompletely packaged viruses (such as empty shell virus particles, non-enveloped virus particles

etc.), incorrectly packaged virus particles, hybrid virus particles, no

Active virus particles, virus particle aggregates, free virus genomes, etc.; nucleic acids

Common product-related impurities in similar products such as enzyme digestion and recombination related sequences and codes

Wrong sequences, incomplete sequences, degraded fragments, structural anomalies and incorrectly modified sequences

columns, as well as related impurities generated during the liposome assembly process; bacterial carrier products

Product-related impurities of the product may be non-monoclonal strains, plasmids, modified genes

Lost/rearranged etc.

In order to control product quality, it is recommended to separate and

identification, assess its safety risks, and consider the control of impurity residues based on the assessment results.

control strategy. In impurity analysis, the target product and product-related impurities can be analyzed

Due to differences in chemical properties, it is necessary to choose an appropriate method to separate each component.

If necessary, it may be necessary to test the components of the product using testing methods that combine multiple principles.

Isolation and characterization studies. Test results can be in the form of absolute purity and/or relative purity.

Formula expression, such as anion exchange HPLC purity, UV spectrophotometry purity,

Gel electrophoresis purity, SEC-MALS, ratio of viral vector infectious particles, etc.

Products may produce a variety of unexpected variants during production and storage.

Variants discovered by testing should be identified and analyzed, referring to the principles of ICH Q6B

concept, based on the differences in functional activity and safety between the variant and the target product,

Consider controlling variants as product-related substances or product-related impurities.

For non-replicating or conditionally replicating viral vectors, the most appropriate

samples, using sensitive analytical methods to detect reproducible

or wild-type virus. According to the type of replicable virus, residual risk, process

Set reasonable residue standard limits for controllability, clinical dosage, etc., such as adenosis

For virus-related gene therapy products, it is generally recommended to use replication-competent adenovirus

(Replication-Competent Adenovirus, RCA) controlled at 1 RCA/3

Within ×1010 VP (Viral partials). Lentiviruses and retroviruses

Products with greater safety risks should not detect replicable viruses.

1.3.2 Process-related impurities

Process-related impurities are mainly introduced by the production process, such as host cell proteins

White, host cell DNA, host cell RNA, packaging plasmid, packaging virus

Toxins, production reagents (such as culture media, DNA templates, tool enzymes, purification reagents

and fillers, etc.), as well as leaching of equipment and consumables such as production pipelines, packaging, containers

substances, etc., the impurity removal performance of the production process and the residual level of impurities should be evaluated.

Research and evaluate the safety risks of impurity residues and, if necessary, potentially safe

Impurity residues with sexual risks are included in product quality standards for control.

If packaging viruses are used during the production process, the residues of the packaging viruses should be

retention levels, infectious activity, replication capacity and/or expression activity, and evaluate

Evaluate its residual safety and formulate corresponding control strategies based on the evaluation results. Production

If you use tumor cell lines (such as HeLa cells), tumorigenic cell lines, or carry

Cells with oncogenic genes and virus-derived sequences (such as HEK 293T cells),

While ensuring that no intact viable cells remain, it is necessary to control the amount and residual amount of DNA.

Fragment size is controlled and standard limits are reasonably drawn up. If possible, it is recommended that

The amount of residual DNA is controlled within 10ng/dose, and the size of the residual DNA fragments is

Control it below 200bp. For products with known potential safety risks

Risk specific transformation sequences, such as the Adv E1A sequence carried by HEK 293T cells

sequence, SV40 large T antigen sequence, HPV carried by Hela cells (Human

Papilloma virus) E6/E7 genes, etc., should be controlled separately. for

Viral vectors such as AAV that easily package non-carrier DNA into viral particles are

Relevant foreign DNA should be considered when selecting packaging cells, helper viruses, and packaging plasmids

Potential risk of packaging into viral particles.

For new or complex delivery systems, preparation of polymeric excipients such as liposomes

Process-related impurities, as well as impurities arising from polymer degradation, should also be included in the impurities list.

Qualitative considerations.

1.3.3 Pollutants

Contaminants generally refer to microorganisms or related components introduced during the production process.

Such as bacteria, fungi, mycoplasma, exogenous viruses, bacterial endotoxins, etc., need to be

Its pollution risks shall be studied and controlled.

1.4 Content

Viral vector products can be measured by factors such as total particle number, genome copy number,
Structural protein content, infectious titer or infectious particle number and other tests to determine the virus
content. In order to effectively control product immunogenicity while ensuring product efficacy
risk, the specifications or dosage of viral vector products are recommended to be based on the corresponding volume of virus
Expressed as total particle number or genome copy number, while controlling the infectivity titer as
and the ratio of infectious particles to ensure the number of active virus particles. Nucleic acid products
The nucleic acid content can be determined by detecting DNA/RNA concentration, copy number, etc., and also
The content of special excipients (if applicable) in each component of the delivery system needs to be studied.
The content of bacterial carrier products can be expressed by the number of viable bacteria or the number of colonies. Content testing should be
Whenever possible, use standards or controls for calibration.

1.5 Analysis of other characteristics

Other characteristic analyzes may include appearance, clarity, and content of important excipients.
Amount, visible foreign matter, insoluble particles, pH value, osmotic pressure, loading volume, etc. for
Integrated virus and nucleic acid products respond to the integration site and integration stability of the product
Study the sex and site distribution trends, and analyze the mutagenicity or tumorigenicity caused by the insertion
risk. For non-integrating viral vectors, it is recommended to evaluate the non-integrating properties of the vector.
OK to confirm.

1.6 Considerations related to gene editing technology

At present, people from all walks of life are interested in CRISPR-Cas, TALEN, ZFN, Meganuclease
The risk perception of enzyme-based gene editing technologies is relatively limited, and editing enzymes
There may be certain differences in editing or cutting effects in different cells, and the current

Research methods still have certain limitations in analyzing editing or cutting effects. because

Therefore, for CRISPR-Cas, TALEN, ZFN, Meganuclease, etc.

For gene therapy products designed as editing tools, it is recommended to use multiple methods to edit

System risks are comprehensively analyzed and assessed. For example, the editing system itself has

Certain limitations, sequence targeting specificity, analysis of off-target sites, editing enzymes

fidelity and editing efficiency, production and quality control of editing systems

Production, screening of the advantages of editing technology on cell tumor promotion/tumor formation, and editing system components

Immunogenicity, non-specific insertion of editing tool-related sequences, multi-target editing

Genome rearrangements, genome mutations, etc. During the development process, research should be combined with

Development and methodological improvements will improve the quality control strategy of the editing system.

CRISPR-Cas, TALEN, ZFN, Meganuclease and other enzyme-based

The analysis of off-target risks and off-target sites of gene editing tools requires a combination of multiple methods

Make a comprehensive judgment based on the above information. Due to different sequence design rules and algorithms, different

Candidate target binding sequences such as sgRNA recommended by the same sequence design software or platform

There may be large differences. It is recommended to comprehensively compare the design and potential of multiple platforms.

Analysis of off-target sites screens candidate sequences. When analyzing off-target sites, use biological

Bioinformatics tools, sequence homology alignment, off-target system scoring tools and other methods

While theoretically screening potential off-target sites, you can refer to the treatment plan.

Conduct in vitro cell simulation experiments, through karyotype analysis, genome fragmentation detection, deep

Methods such as sequencing are used to analyze potential off-target sites and genome rearrangements.

analyze. When simulating experiments, it is necessary to simulate the functioning of the editing system in the body as much as possible

temperature, ion concentration, pH value and other conditions, consider targeted binding such as sgRNA

Sequence heterogeneity, isomers of editing enzymes, and concentrations of each component of the editing system.

off-target conditions under the worst conditions. For the detected off-target sites, the

Risk analysis can be conducted on site location, gene function, etc., and if necessary, can be combined with animals or

Comprehensive judgment based on human trial data.

2. Quality standards

As an important part of product quality control, quality standards are based on product

Standards used to control product quality determined by product quality research, generally consisting of inspection items

It consists of objectives, analytical methods and standard limits. Quality standards generally include stock solutions (e.g.

(if any), semi-finished products (if any) and preparation quality standards. Due to differences in technology,

Some products may not have a clear production stage of raw liquid, and the standard should deal with raw liquid, semi-

The finished product and formulation stages are clearly defined. Some items in the quality standards include:

Unable to test in preparations or bulk solutions, or use samples from other intermediate stages

When testing is more conducive to controlling product quality, you can consider passing the inspection

Test appropriate intermediate products to control product quality.

2.1 Inspection items

The inspection items in quality standards are usually determined based on quality research.

Quality attributes that have an important impact on product safety and effectiveness, generally including identification,

General inspection items, physical and chemical properties, purity, impurities, content, biological activity, external

Source factors, etc. Specific inspection items should be based on product type, production process, quality

Research, stability and risk assessment etc. determined. Liposomes, nanoparticles and other special

Appropriate quality control items should be selected based on specific characteristics of the dosage form, such as particle size

and distribution, refractive index, encapsulation efficiency, surface charge, etc. For non-replicated types or strips

Replicating viral vectors require control of replicating or wild-type viruses. system

If the medicine uses a special container or a combination of medicine and equipment, it must also be based on the function of the device.

Add specific checks. For other inspection items, please refer to "Chinese Pharmacopoeia" "Human Basics"

Due to the requirements of "General Introduction to Therapeutic Products", some inspection items may not be repeated in the original solution and preparation.

Retest, but the impact of the preparation process on the corresponding quality attributes should be considered. For research

Inspection items that are considered important by the research and are not included in the quality standards should have sufficient reasons.

or supported by validating research data.

2.2 Standard limits

In quality standards, the formulation of standard limits (acceptance standards) for each inspection item

The determination should have a reasonable basis, generally considering the target product quality profile (QTPP),

Clinical trial exposure, product quality attribute characteristics, and batch release test results

and stability studies, etc. During the establishment of standard limits, based on representative processes

When analyzing batch data to determine the ability of the process to control product quality, it is also necessary to comprehensively

Consider product stability trends, sample batches in non-clinical and clinical studies

Quality exposure among study subjects, quality of products produced at commercial scale should

Consistent with the samples used in critical clinical studies, the standard limit requirements should generally not be lower

The worst-case exposure scenario for subjects in clinical studies.

2.3 Analysis methods

In order to achieve effective control of product quality, advanced and mature testing methods should be used.

And through analytical methods optimized for applicability, we encourage the use of analysis methods with multiple complementary principles.

Analytical methods are used for quality control. Methodological verification should generally be completed before marketing application.

However, based on the needs of product quality control and comparative research at different R&D stages, it is recommended

Try to complete the development and verification of methodology before conducting confirmatory clinical trials, and

Quality control methods related to safety and content testing need to be developed during clinical trials.

Conduct necessary methodological confirmation studies before the exhibition. If any methodological changes occur during R&D

For changes, a bridging study should be conducted on the methods before and after the change. For short validity period or

For products with a small sample size, new rapid and micro-volume detection methods may be considered.

It is an alternative method to the pharmacopoeial method, but it should be demonstrated that this method has better performance than the pharmacopoeial method.

Equivalence or superiority.

2.4 Control/Standard

In the absence of national and international standards, analysis based on method

For analysis needs, you can prepare the corresponding activity standards by yourself according to the preparation requirements of the standard products.

products or physical and chemical reference materials, and conduct corresponding quality research and release testing on them.

At different R&D stages, representative processes of the corresponding stages can be selected based on quality control needs.

Prepare standards/controls from the samples produced, but the bridge of standards at different stages should be done well.

Take research. The establishment and preparation of reference substances/standard substances should comply with the "Chinese Pharmacopoeia"

"General requirements for the preparation and calibration of national reference materials for physical products", and

Perform calibration and conduct necessary storage stability studies. In order to make good reference materials/standards

For the traceability of products, it is generally recommended to carry out hierarchical management of reference products/standard products.

9. Stability research

Stability research can refer to the "Technical Guiding Principles for Stability Research on Biological Products"

(Trial)" and the relevant requirements of ICH Q5C. The research plan should be based on the product

The characteristics of the product itself, clinical medication regimen and other conditions are set. Research projects generally include

Including long-term stability, accelerated stability, research on influencing factors, transportation stability,

Stability in use, etc., research conditions should be based on specific storage, transportation and use conditions.

conditions, and determine the research purpose under corresponding conditions. The research projects should be comprehensive

Comprehensive and reasonable, especially the stability, change trend, safety and effectiveness of the product

For items with important indicating significance, the detection method should be verified and capable of sensitive detection.

Measure the stability change characteristics of the product. Representative production processes should be used during research

product or intermediate sample, placed in the actual product/sample storage container, or with the actual

Other specifications of the same material as actual storage containers and representative of the worst-case exposure conditions

Proceed in container. The expiration date of the product/sample should be set based on the results of the stability study,

The study period should normally cover the actual storage or use period of the product/sample.

Viral vector products are generally stored at low temperatures due to their poor stability.

During the research process, close attention must be paid to the storage, transportation and use of viral vectors.

changes in titers (especially infectious titers), aggregates and biological activity, limiting

The number of freezing and thawing times required to produce viral vectors is to avoid exposure to conditions that can easily inactivate and degrade viral vectors.

Influence conditions for solution or aggregation.

The stability of DNA nucleic acids is relatively good, but severe environmental conditions and nuclear

Exposure to acidase may still damage the higher-order structure and integrity of DNA.

RNA nucleic acids are less stable and resistant to RNase (Ribonuclease, RNase)

It is relatively sensitive and its production and storage should be in a strict RNase-free environment.

Nucleic acid delivery complexes formed by combining with chemical delivery materials/media need attention

Changes in the physical and chemical properties and biological activities of nucleic acids and complexes, such as encapsulation efficiency,

Content, purity, particle size and distribution, surface potential, particle integrity, and particle

Polymerization and aggregation of particles, leakage of encapsulated drugs, etc. In addition, attention should be paid to nucleic acid delivery

Stability of chemical delivery material/medium components such as liposomes in the delivery complex.

Bacterial vector products should be stored in appropriate freezing solutions and freezing temperatures.

Generally relatively stable, it is necessary to pay attention to the viability of the bacterial vector and the efficiency of the transgene under the research conditions.

Changes in stability and biological properties.

In addition to the above characteristics, other defects may occur during storage, transportation and use.

Physical and chemical properties that undergo changes (such as pH value, osmotic pressure, concentration, insoluble particles

etc.), key ingredients and microbial contamination should also be carried out during the research process.

Appropriate inspection.

10. Packaging and sealed container systems

Packaging and container sealing systems generally include raw liquid (if any), semi-finished products (such as

There are), preparations, and packaging containers equipped with diluents. Containers and closure systems

The selection should be based on a reasonable basis to fully ensure the storage stability of the sample or product.

Qualitative. To avoid storage containers or sealing systems that may have unintended consequences on product quality

Impact, compatibility studies and sealing studies should be conducted on containers and sealing systems.

The setting of research conditions should consider the relative characteristics of the container and sealing system under special conditions.

Capacitance and sealing properties, such as sealing performance under freezing conditions, phase resistance under accelerated conditions

Capacity etc. For biologically active active ingredients such as viral vectors, attention needs to be paid to

Note the influence of leachables on its activity during storage and use. For those with special

For secondary packaging materials with special functions (such as light-shielding materials), their corresponding functions should be

Researched and verified. Products involving special drug delivery devices, such as electroporation devices, nasal

spray devices, needleless syringes, etc., the research and development requirements of related medical devices need to be considered.

and drug delivery device compatibility with the product.

11. Explanation of terms

Starting raw materials: used to generate product active ingredients, or for product active ingredients

Provide raw materials for components, such as virus seed batches and transfer for viral vector production

Chromatin plasmids, production/packaging cell banks, plasmids and hosts for non-viral vector production

Bacteria, bacterial seed batches, etc.

Hybrid viruses: refers to those produced during the production process and mixed with other foreign viruses.

Genetic virus.

Viral vector infectious particle ratio: refers to the viral vector infectious activity titer

The ratio to the number of carrier particles.

Control cells: refers to the cells that are reserved for production/packaging in a certain proportion during virus production.

cells, without plasmid transfection or inoculation with the target virus, with plasmid transfection or inoculation

Other cells targeting viruses use the same media composition and are cultured in the same

Temperature and culture site, parallel culture to the specified time. adopt the prescribed method

method, by judging the detection of exogenous factors in control cells, the production

Contamination of cell batches by exogenous agents.

Nucleic acid complex: Nucleic acid is mixed with chemical delivery auxiliary materials/media to form

formed polymer.

12. References

[1] National Pharmacopoeia Commission. "Pharmacopoeia of the People's Republic of China" (2020 Edition). 2020.

[2] European Medicines Agency . Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products [EB/OL] 2018.

[3] European Medicines Agency. Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells [EB/OL]. 2020.

[4] U.S. FDA. Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) Guidance for Industry [EB/OL]. 2020.

[5] U.S. FDA. Recommendations for Microbial Vectors used for Gene Therapy [EB/OL]. 2016.

[6] CDE. Technical Guiding Principles for Human Gene Therapy Research and Preparation Quality Control [EB/OL] 2008.