体外基因修饰系统药学研究与评价技术指导原则（试行）

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I. Introduction

In recent years, technological approaches such as cell therapy and gene editing have developed rapidly, and

The exploration of clinical medical treatment continues to deepen, providing new solutions for serious and refractory diseases.

Treatment concepts and methods. Increasing clinical needs promote new genetic manipulation technologies

Application and update of technology.

Outside the human body, modification systems constructed using genetic engineering technology can effectively

Transfer genetic material, etc., into specific target cells to modify the genes of the target cells.

Transferring substances, changing gene expression patterns, or regulating cell biological characteristics, etc. at present,

Lentiviral vectors, gamma-retroviral vectors, etc. are commonly used to combine chimeric antigen receptors

(Chimeric Antigen Receptor, CAR) gene is introduced into T cells to achieve

Nowadays, CAR-T cells can target tumors; free carriers (Episomal

Vector), Sendai virus vectors, etc. can be used to introduce transcription factors into cells.

Obtain induced pluripotent stem cells through reprogramming to provide opportunities for the production of derived cell products.

Provide starting raw materials. In the future, it is expected that more diverse carrier designs will be available for different

Products of the same type.

Various types of genetic modification systems, vector design, preparation process and quality

Differences in control and other aspects directly affect the safety and effectiveness of the final product,

And their sources may be different, and their quality management systems may be different. To ensure genetic modification

The quality of the decoration system meets the requirements of clinical application, and sufficient quality research needs to be conducted on it.

Research. Therefore, it is necessary to refine the techniques for pharmaceutical research on different types of gene modification systems.

technical requirements.

This guideline is based on current scientific understanding and is specific to in vitro genetic modification.

The system proposes suggested technical requirements for the listing stage, aiming to provide R&D units with

It provides guidance and serves as an important reference for the evaluation of regulatory agencies. Book

The guiding principles are not mandatory. If there are alternative or applicable other research methods,

If there are inapplicable contents in these guidelines, the applicant/holder can provide relevant information.

The supporting reasons and basis for relevant alternative research should be stated. with the development of technology,

With in-depth knowledge and accumulation of experience, the content of this guiding principle will be gradually

Revised and improved.

2. Scope of application

In this guideline, genetic modification system refers to the conversion of foreign genes outside the human body

etc. are introduced into cells to add, replace, compensate, block, and correct specific genes.

To obtain cell therapy products or seed cells for the production of cell therapy products

And the modification system used. Possible mechanisms of action include intracellular expression of functional

target gene, or use nucleotide editing methods such as gene knockout, repair, and insertion

Change specific gene sequences, etc.

Currently, gene modification systems include lentivirus, gamma-retrovirus, adenovirus

virus, adeno-associated virus, Sendai virus and other viral vectors, as well as DNA, RNA,

Non-viral gene modification systems such as proteins and protein-RNA complexes. This refers to

The guideline is to conduct research on two types of genetic modification systems: viral vectors and non-viral vectors.

Line discussion. With the continuous changes in technology in this field, new genetic modification systems are also

Possible applications and, if applicable, pharmaceutical research may also refer to this guidance.

3. General principles

Design rationality, process stability, and quality controllability of genetic modification systems

It can directly affect the safety and effectiveness of final cell products and is an important issue in pharmaceutical research.

point. The pharmaceutical research that needs to be carried out may include upstream design, preparation technology,

Quality research and control, stability research and many other aspects. genetic modification system

In principle, the entire preparation process should comply with Good Manufacturing Practices (GMP)

requirements. The specific requirements may vary depending on the specific use cases.

analyze.

Although the quality risks of genetic modification systems are similar to those of in vivo gene therapy products,

Similar, but different from in vivo gene therapy products. After in vitro genetic modification

The cells may also undergo in vitro culture, medium replacement and cleaning steps before being used in the human body.

The final cell product must be tested before release. Therefore, genetic modification systems compared to

For in vivo gene therapy products, the modifying properties may be controlled prior to reinfusion,

Some related impurity residues can be released after quality control and need to be

Carry out the design, quality research and control of in vitro genetic modification systems according to their characteristics.

(1) General requirements for different R&D stages

Like the development of other drugs, pharmaceutical research on genetic modification systems also has

It has characteristics such as stage and gradualness, and depends on the non-clinical and different clinical aspects of the final cell product.

changes as the pilot phase of research progresses. Developers should develop research plans in advance and

strategy to encourage relevant research in accordance with the concept of "quality by design".

In-depth research and development, gradually optimizing the preparation process, and strengthening quality control.

During the clinical trial application stage, it is necessary to identify and control the relevant aspects of the genetic modification system.

risk, clarify its molecular design, and complete the construction of a library of production seeds (if applicable)

and verification, preliminary assessment of the rationality and safety of the selection of raw materials for production, and through

Establish a relatively stable preparation process through process research and carry out corresponding quality research.

Establish appropriate quality standards to ensure that genetic modification systems and the cells they modify

Safety in clinical applications.

When the genetic modification system is applied to cell products in the marketing stage, as

With a deepened understanding of process and quality attributes, the process has been continuously optimized, and after sufficient

Process development and validation studies determine the commercialization process for genetic modification systems.

If the process of the genetic modification system changes during clinical trials,

Comparability studies on gene modification systems and corresponding cell products need to be completed; it is recommended that

Before confirmatory clinical trials, major changes are completed and the process is determined. based on sufficient

In-depth quality research and multi-batch data accumulation to formulate reasonable quality control strategies

Strategy, clarify critical quality attributes (CQA),

Determine appropriate analytical methods and conduct comprehensive methodological verification; at the same time, relevant

Note modification system-related or process-related impurity studies and develop corresponding risks

Control Strategy. Standardize and complete stability studies and packaging material compatibility studies,

Establish reasonable storage conditions and times.

(2) General requirements for gene modification systems from different sources

Gene modification systems may include self-production, commissioned production, purchase, etc.

sources, genetic modification systems from different sources follow the same technical requirements and quality

Control principles, pharmaceutical research can be carried out with reference to these guiding principles.

Marketing authorization holders of cell end products should address the quality of genetic modification systems

take the main responsibility for quality, through strengthening internal quality control or improving the production of genetic modification systems

Control corresponding quality risks through the manufacturer's review and formulation of quality agreements. If base

Due to changes in the modification system, risks should be assessed in a timely manner and corresponding pharmaceutical safety procedures should be carried out.

Comparative studies, and in some cases non-clinical or/and clinical bridging may be required

Research.

4. Risk Assessment and Control

(1) Overall risk identification and control strategy

The risks involved in genetic modification systems mainly include viral vector reverse mutation,

Integration of vectors into the genome causes cancer or disease, off-target risks, contamination by exogenous factors,

Impurity residues, etc.

Pharmaceutical research can start from the design, preparation process and quality control of modified systems, etc.

Carry out risk factor analysis from multiple aspects to identify and determine issues related to product quality and safety.

Risk factors related to safety and determine the number of risk assessments required during R&D

According to the scope and focus, risk prevention, control and treatment measures will be formulated. At the same time, it is necessary to combine

Cell type, dose, route of administration, user population, mechanism of action, in vivo distribution

Risks should be considered comprehensively from aspects such as distribution and time of action in the body.

In terms of genetic modification system design, typical risk factors may include: Risk

The use of elements, sequence recombination that may result from homologous sequences, and viral vector

After complementation with wild-type or helper virus, reverse mutation occurs, and the vector is latent in the cells.

Voltage/reactivation and/or mobilization, the degree and integration site of the vector in the cell chromosome

Point preference, etc. In terms of preparation processes, relevant risk factors may include: high

The use of risky raw materials, contamination by external factors during the preparation process, and intermediate products

storage and quality control, introduction and generation of harmful impurities, and modification systems

gene sequence stability, etc. In terms of quality control, relevant risk factors may include

Including: applicability of detection methods, rationality of standard limits, etc.

In recent years, enzyme-based gene editing systems have been gradually used in cell therapy products.

Genetic modification of products, common systems include transcription activator-like effector nucleic acids

ÿÿTranscription activator-like effector nucleasesÿTALENÿÿ

Clustered regularly repeating short palindromic sequence-related proteins (Clustered regularly

interspaced short palindromic repeats-CRISPR associated

protein, Cas), etc. The risks of clinical use of this type of system mainly include genetic

Autotoxicity and immunogenicity of tool enzymes, gene on-target and off-target during gene editing

The resulting toxicity and impurity residues, etc.

Therefore, it should be based on many factors and combined with the characteristics of the final cell product.

Risk assessment and control of the properties of different types of genetic modification systems. For example,

Based on the risk assessment, rationally design the structure and sequence of the modification system to avoid

Use high-risk components such as carcinogens and conduct relevant testing to reduce the risk as much as possible

Possibility of homologous recombination and viral vector reverse mutation; for high-risk manufacturing agents

Conduct quality control on materials and set process control inspections at appropriate preparation links.

Test items and acceptance criteria; according to the mechanism of action of the modification system, target the gene sequence

Conduct research on stability and other safety-related risk factors. end products in cells

Track, analyze and update the modification system during its life cycle, and collect data for further

Determine its risk characteristics and formulate control strategies in one step.

(2) Risks in different production and use situations

The use of genetic modification systems in the production of cell therapy products

It may be different. Current research may include direct preparation of cells after in vitro genetic modification.

Products (Scenario 1) and cell products prepared after in vitro genetic modification to establish lines/banks

(Scenario 2) Two usage scenarios. Gene modification systems corresponding to two use cases

The risks are different, and the requirements for pharmaceutical research may be different. Specific questions need to be asked.

Detailed analysis of the problem.

For case 1, the genetic modification system is recommended to be prepared under GMP conditions.

The genetic modification system used in this case is in direct contact with the cells that are reinfused into the human body.

Attention should be paid to the quality control of raw materials used in the production of this system, quality differences between batches,

Research on impurity levels and other factors that may affect the safety and effectiveness of cell therapy products

Allow. The rest of this guideline focuses on this situation.

For scenario 2, the genetic modification system is used for the establishment of cell lines/banks, which

Sequence design, production materials, preparation process, quality, stability, insourcing

Material requirements can be determined based on specific circumstances and combined with the quality research results of cell lines/libraries.

Conduct a comprehensive assessment. Due to the complexity of the use of genetic modification systems (e.g. once

use or multiple uses), can be combined with production use and cell line/library research

and detection conditions, and formulate risk control strategies for modifying the system. Good genetic modification

Quality research and control of the decoration system is conducive to screening and establishing cells of good quality.

department/library, which is beneficial to subsequent production research. During the cell line/library establishment process, establish

It is recommended to conduct monoclonal screening, characterize cell lines/libraries, and conduct passage stability studies.

Research, pay attention to whether the function of the cell line/library conforms to the theoretical design and expectations, and whether the safety

Whether it is controllable, if necessary, conduct genetic modification-related quality studies on the final cell product.

To confirm the suitability of the genetic modification system.

(3) Change risk

With the continuous updating of genetic modification technology and the gradual accumulation of research experience, researchers

The development process is often accompanied by the upgrading of genetic modification systems and optimization of processes, so research and development

The genetic modification system may change at each stage of development.

Changes in genetic modification systems may significantly affect the safety and

Effectiveness is one of the important risks in pharmaceutical research of cell therapy products. Developers

The impact and risks of introducing changes need to be assessed. Based on risk assessment, genes

Quality, process control, stability of the modified system and its cell end product (if applicable)

Conduct in-depth research in qualitative and other aspects, and rationally design and change the comparability research plan.

5. Design, preparation and quality control of genetic modification systems

(1) Viral vector gene modification system

1. Design and construction of viral vectors

1.1 Target genes and regulatory elements

The target gene is the key sequence in the gene modification system to achieve the expected function.

Regulatory elements are important sequences that affect the transcription, translation and stability of the target gene sequence.

List. Developers should consider the risks based on the safety and effectiveness of the final cell product.

Evaluation, design and construction of various components of the genetic modification system.

Target gene: In this article, it refers to the inheritance and expression of gene modification system.

Substances, developers should be cautious in considering their role or mechanism of action in disease treatment

Select and design the target gene sequence, pay attention to the source of the target gene and sequence screening

and optimization process to clarify the complete sequence information of nucleotides and amino acids (if applicable).

information, and it is recommended to conduct sequence comparison with related sequences included in the database. purpose base

In the optimization of factors, the scientific evaluation and verification research considerations of molecular modification should be elaborated.

Such as humanized reconstruction, knockout elements, suicide markers, and the regulation of high-level structures
Formation or sequence modification and deletion to adapt to the vector size, etc. At the same time, build
Discuss the specific binding ability of the target gene or its expression product to the target molecule,
Assess potential non-specific effects, etc. can be combined with the sequence characteristics of the target gene,
The interaction between the target protein and the cell genome, etc., fully consider the relationship between the target gene and
Effects on genome stability of target cells. If the target gene is of non-human origin or modified
structural sequence, etc., and can also be combined with the sequence differences and expression products of the target gene between species.
immune properties and evaluate the immunogenicity of the target gene.

Regulatory elements: Functional elements play an important role in the transcription and expression of target genes.
To control the effect, developers should pay attention to its design principles and conduct confirmation studies. Can
Based on the expression level of the target gene, the expected effect and duration, and the purpose details
Reasonable selection and design of regulatory elements based on the type of cells, etc. The relevant regulatory elements may
Including signal peptide, transcription promoter, enhancer, terminator, isolator, 5'
Untranslated region, 3' untranslated region, polyadenylation signal region, intron, others
Elements related to enhancing transcription and translation efficiency, replication origin sites, additionally introduced
Gene sequences, etc. The sequence source and selection basis of each regulatory element should be clear. example
For example, in terms of promoter design, it is recommended to combine the target cell type and target gene expression.
Analyze the use of promoters in terms of time and expression requirements, human experience with promoters, etc.
The safety of use should be rationally selected based on risk assessment. Control element design
It is necessary to fully consider the safety and effectiveness of components, and pay attention to the introduction of relevant sequences.
Necessity and rationality, try to avoid using high-risk components, if necessary,
Security modifications of relevant sequences should be performed. For sequence-reconstructed or optimized elements,

The basis and safety considerations for sequence changes should be clearly stated. In addition, it is also recommended to pay attention to

Functional element design interferes with endogenous genes in the cell genome.

1.2 Viral vectors

Viral vectors can usually stably and efficiently transduce target genes to target cells.

play a role in the cells. The selection and design of viral vectors can take into account the target gene

Expression time, expression amount, pathogenicity, integration ability, infection history of viral vector

process, transduction efficiency, cytotoxicity, and cell type and mechanism of action of cell products

preparation, clinical indications, administration methods, etc. Structural optimization of viral vectors

Strategies include improving virus stability, enhancing cell transduction rate, broadening the range of transducible cells

Cell type, etc.

Currently commonly used viral vectors are usually tested for virulence, pathogenicity or replication ability.

Deletion of force-related genes to ensure safety of use. In the design, as much as possible

Reduces any pathogenicity associated with wild-type virus and minimizes viral repopulation

The risk of group and reversion mutations is minimized. Attention needs to be paid to modified viral vectors

The source, culture history and biological characteristics of the parent virus, etc., and the materials used for reconstruction,

Methods, procedures and identification are fully studied. When viral vectors undergo modification, they do not

New security risks should be introduced.

The following is an example of common viral vectors in current research.

1.2.1 ÿ-Retroviral Vector (ÿ-Retroviral Vector)

Gamma-retroviruses can reversely transcribe their RNA genome into DNA copies,

Viral DNA is randomly integrated into the genome of mitotically active cells. at present,

Common gamma-retroviral vectors used for in vitro gene modification include murine leukemia virus

(Murine Leukaemia Viruses, MuLV), feline leukemia virus (Feline

Leukaemia Virus (FeLV) and Gibbon Ape

Leukaemia Virus, GALV) and other vectors. Its genetic design and modification mainly

Including improving vector safety and gene transduction effectiveness.

ÿ-retroviral vector can be transferred by plasmid (containing the gene of interest),

Helper plasmids are transiently transfected into cells for viral vector packaging, or integrated transfer

Preparation of stable toxin-producing cell lines based on plasmid sequences and auxiliary packaging elements. to enhance the base

Due to the safety of the modification vector, it is recommended to optimize the modification system, for example using

Fully restructured, high-security gamma-reversal with unnecessary components removed

Record viral vectors to minimize the risk of viral recombination and reverse mutation.

Specific optimization operations may also include the use of heterologous promoters and heterologous polyA signals.

No. to express auxiliary packaging elements, split the auxiliary packaging elements into different plasmids for expression,

Modify the long terminal repeat (LTR) sequence so that the final

Self-inactivating (SIN) and other methods.

In terms of the effectiveness of gene transduction, developers are encouraged to use viral vector packaging components

Optimize to improve viral vector packaging efficiency, structural stability and transduction activity

etc., such as replacing the envelope protein of gamma-retroviral vector with other envelope proteins

To improve virus stability, etc. In view of the transformation situation, sufficient research and

verify.

1.2.2 Lentiviral Vector

Lentiviral vectors work by mediating the integration of target genes into the cell genome.

function, unlike gamma-retroviral vectors which can only infect mitotically active cells but not

At the same time, lentiviral vectors can infect mitotically active and inactive cells. at present,

Common lentiviral vectors used for in vitro gene modification are human lentivirus, non-human primate and

Non-primate lentivirus and other vectors. Using non-human primates and non-primate lentiviruses

It is recommended to pay attention to the generation of recombinant virus chimeras and/or cross-species transmission when using vectors.

about risks. The design and modification of lentiviral vectors mainly include improving vector safety

and gene transduction effectiveness.

The main risk points for lentiviral vector preparation and clinical use include: the production of complex

Replication-competent lentivirus (RCL), occurs

In vivo recombination and insertion of proviral DNA in or near relevant genes from

And may cause or promote tumorigenesis and other cell lesions. Therefore, chronic diseases

In terms of viral vector design, it is recommended to use all possible methods to reduce the risk of lentivirus pathogenicity.

measures, including separate packaging genes expressed on different plasmids (e.g. *gag/pol*

and *rev* separation), reducing the sequence homology between the helper plasmid and the transfer plasmid,

Delete unnecessary regulatory elements, modify LTR to make the terminal self-inactivating, etc. right

For transferring plasmids, sequence optimization is encouraged to improve safety, such as reducing startup

The probability of proto-oncogene activation caused by insertion into target cells.

In order to improve the efficiency of gene transduction, replacement of envelope proteins,

Transformation strategies such as improving fixed-point integration capabilities. For example, human immunodeficiency virus

(HIV)-derived lentiviral vectors can be used to encode heterologous viral envelope proteins.

Prepared by replacing the HIV envelope protein gene sequence with a white (such as VSV-G) gene

To enhance the affinity and stability of the carrier. By sequencing integrase and LTR

The column was modified to improve the site-specific integration performance of lentiviral vectors. Modify the carrier

At the same time, it is recommended to pay attention to changes in viral vector stability and integration site preferences.

Risks that may be introduced by sex.

1.2.3 Sendai Viral Vector

Sendai virus vector expresses the target gene in the cytoplasm and usually does not integrate into

Single-stranded antisense RNA viral vectors in cellular genomes. There are currently studies that consider it

It is used in the process of cell reprogramming to establish induced pluripotent stem cell lines (iPSC).

When designing and constructing, while stably expressing the gene of interest, in order to prevent the production of specific

For virus particles with replication capabilities, deletion or modification of virus assembly-related proteins may be considered

Whites, such as nucleocapsid structural proteins and matrix proteins involved in virus assembly.

To ensure effective control of the residual amount of Sendai virus vector in iPSCs, one can consider

Consider modifying replication-related elements, such as through mutations of RNA polymerase,

Construct a Sendai virus vector with temperature-dependent replication ability; at the same time, it is necessary to construct

Independently related methods are used to detect and verify whether the Sendai virus vector remains in iPSC clones.

Stay to control risk.

Other viral vectors may also include adenovirus, adeno-associated virus and other vectors,

Its molecular design and construction can combine specific viral structures and target gene sequence characteristics.

Comprehensive consideration must be given to the safety of the packaging sequence and other symptoms. The basic principles can be referred to the above

Allow.

2. Materials for production

2.1 Plasmid

For viral vectors prepared by transient co-transfection of multiple plasmids, it is necessary to

Carry out corresponding plasmid design, construction, and production based on risks, binding vector characteristics, etc.

Research on production technology, quality control and stability.

Design and construction: Based on research, select a reasonable plasmid backbone, plasmid complex

Edit the starting point, promoter, selectable marker and other components, and delete unnecessary

elements (especially sequences with a higher risk of carcinogenesis), and complete confirmation of the plasmid

Nucleotide sequence. Beta-lactam antibiotics should generally be avoided during plasmid construction

resistance gene, and it is recommended to minimize the insertion of resistance gene sequences when designing plasmids.

into the viral vector genome, and encourage the development and research of non-resistance gene screening

plasmid. When using additional elements that enhance transcription or translation, their use should be fully evaluated.

Functionality and safety, if necessary, carry out corresponding safety modifications, such as soil

Woodchuck hepatitis virus post-transcriptional regulatory element (Woodchuck hepatitis virus

posttranscriptional regulatory element, WPRE), etc. Suggestions are possible

Different packaging elements can be split into multiple plasmids for expression to reduce homologous duplication.

probability of group occurrence.

Production process: According to the process research situation, through the analysis of key process parameters

Explore and optimize to determine a stable large-scale plasmid production process. The production process should

Have clear production scale, process flow, detailed description of process steps and process

process control strategies, etc. The use of human and animal sources should be avoided as much as possible during plasmid production.

Source materials, avoid using beta-lactam antibiotics, use other types if necessary

For antibiotics, the antibiotic residues should be controlled and safety assessed. base

During the R&D stage and risk assessment, conduct plasmid process verification or validation studies, such as

Process control confirmation, intermediate product storage stability research, multi-batch quality

Analysis and impurity removal research, etc.

Quality control: Reasonable quality standards need to be established and release inspection carried out. quality

Quality control items for particle release generally include: pH value, appearance, identification (restrictive

Endonuclease analysis and sequencing), plasmid concentration/content, purity and impurities

(A260/A280, supercoil ratio, host DNA residue, host RNA

Residues, host protein residues, antibiotic residues (if applicable), sterility and bacteria

Endotoxin etc.

Stability research: Choose reasonable and sensitive inspection items (such as superspiral ratio

(e.g., etc.) conduct stability monitoring and formulate reasonable storage conditions based on the research results.

and validity period.

2.2 Bacterial seed batch

This guideline refers to bacterial seed batches that transform host cells with viral packaging plasmids.

The main bacteria are seed banks established after single clone screening and culture and passage. For selection

host bacteria, the source, genotype, phenotype, and past history of the host bacteria need to be fully considered.

Factors such as usage experience and production needs. If new strains are used, attention should be paid to their feasibility.

can introduce additional risks.

Establishment of bacterial seed batches: Establish bacterial seed batches at all levels based on research and clarify

Correct preparation scale, amplification conditions, storage conditions, etc. Focus on target clones in research

The situation of screening, focusing on the source and production process of the materials used to generate the seed batch

Etc. to assess and analyze security risks.

Quality control of bacterial seed batches: establishing appropriate release testing items, standards

Accuracy and detection methods. Testing items generally include bacterial morphological identification, staining microscopy

detection, biochemical characteristic analysis, resistance examination, plasmid restriction map analysis, target

Sequence accuracy analysis of genes and other elements, etc. Detection methods need to be studied
Confirmation and/or verification. Quality control should ensure the absence of other bacteria, genuine
contamination by bacteria and phages, as well as ensuring the sequence of the target gene and other elements
accuracy.

Stability study of bacterial seed batches: Passage stability generally includes plasmid size,
Plasmid sequence accuracy, plasmid restriction map, plasmid retention rate, plasmid copy
The number of shellfish, etc. is determined based on the research results and the limited number of generations of seed batches. stable storage
Qualitative recommendations focus on the viability of seed batches under storage conditions and duration.

2.3 Production/Packaging of Cell Banks

The cell matrix involved in the preparation of viral vectors includes cells for packaging viral vectors.
Cell matrices and cell matrices that can stably produce viral vectors. Select cell matrix
When doing so, it is necessary to consider the feasibility of viral vector packaging and preparation, combined with the cell source
(including species origin), growth characteristics and vector preparation capabilities, as well as possible effects on
Select the appropriate cell matrix according to the cell characteristics that affect the safety of the final product. wind
When assessing risk, it is necessary to fully consider whether there are endogenous virus particles in the cell matrix,
Carcinogenic sequences, etc. For newly established cell matrices or cells with corresponding risks
stroma (e.g. cells from which tumor cells are derived), cells should be assessed where applicable
tumorigenicity and tumorigenicity. In some cases, modification of the cell matrix is required
(e.g. inserting specific viral protein expression sequences to allow viral replication or packaging),
Research should focus on the genetic, epigenetic and growth characteristics of the modified cells as well as
Preparation of viral vectors, etc. After cell matrix selection and/or modification, cells need to be established
cell bank to ensure the stability and consistency of production. Please refer to "Chinese Pharmacopoeia",

ICH Q5A, ICH Q5D and other relevant requirements require testing of cell banks. Detection items

The purpose needs to be determined based on risk assessment, including at least identification, sterility, mycoplasma, snail

Primary organisms (insect cells) as well as endogenous and exogenous viral agents, species-specific viruses

wait. Carry out cell bank passage stability research, including cell growth stability, genetic

Transmission stability, viral vector packaging ability stability, etc., it is recommended that the virus be included in the study

To produce terminal cells or control cells for parallel production of viral vectors, develop a formulation suitable for the disease.

Cells prepared with toxic vectors are limited to passage times.

Cell bank for packaging viral vectors: Cell bank cells are amplified and transfected with plasmids

Then synthesize the viral vector gene sequence, express the vector protein, and finally form the virus

carrier particles. For example, HEK293T is currently commonly used for lentiviral vector packaging.

cells, the DNA fragment between the LTR of the plasmid transferred into the cell is transcribed into

RNA, which is packaged into viral vector particles by proteins expressed from the helper plasmid.

It is important to note that when viral vectors are combined with non-vector DNA sequences (e.g. plasmid

DNA, helper virus sequences, cellular DNA, etc.) when co-packaged, non-vector

The DNA sequence may homologously recombine with the viral vector, or its residues may produce pathogenic

cancer risk, it is recommended to carry out risk analysis and research to evaluate the appropriate selection of cell matrix.

reason. For example, the use of viruses containing risk elements such as adenovirus E1 and SV40LT antigens

When packaging lentiviral vectors in cell matrix, attention should be paid to the adenovirus E1 and

Residues of the SV40LT antigen sequence.

Cell bank that can stably produce viral vectors: the cell matrix is genetically modified,

After screening and library construction, proteins or components for virus packaging can be stably expressed or produced.

Virus packaging and preparation. For example, gamma-retroviral vector preparation is commonly used

Mouse PG13, HEK293-Phoenix cells, etc. Cells used for production need to have stable expression

Reach gag-pol, envelope proteins, target genes, etc. It is recommended to pay attention during the construction process

Its virus packaging efficiency and the quality of the virus vector produced, and reduce the risk of internal and external factors

safety risks such as subcontamination and the generation of replicating viruses. Stable toxin-producing cell lines require

Monoclonal cell lines obtained through genetic modification and monoclonal screening

A cell bank needs to be established and comprehensively tested. At the same time, cell passage was carried out to stabilize

Qualitative study, focusing on the inheritance of inserted gene fragments in stable toxin-producing cells of different generations

transmission stability as well as the yield and quality of viral vectors.

2.4 Virus seed batch

To prepare virus vectors or helper viruses from virus seeds, it is necessary to establish virus

Poison seed batch. The source and historical cultivation status of virus seeds must be clear and clear.

And the risks are controllable. For unclear culture history and the risk of other virus contamination,

Or virus seeds whose monoclonality cannot be confirmed are not recommended for use. If necessary,

Using it, multiple rounds of plaque purification and limiting dilution purification can be performed during the construction process.

Or through DNA/RNA cloning rescue, etc. to ensure the purity and single clone of the strain

sex.

Information on the establishment process, generation, storage and maintenance of seed batches should be clear and

Clear; if applicable, the human/animal source materials used in seed batch preparation should be stated

materials and conduct safety assessments.

Viral seed batches should be fully tested, and recommended testing items include: sterility,

Identification and detection of mycoplasma, exogenous viral factors, viral vectors and target genes

sequence, viral titer or concentration, biological activity, impurities, antiviral drug

Sensitivity (if applicable), reverse mutation (if applicable), etc. It is recommended that virus species

Perform whole-gene sequencing on sub-batches, and compare the sequencing results with expected sequences.

analysis and, if any, all differences need to be evaluated. For viral vectors with longer sequences

body, it is recommended to perform sequence analysis to the greatest extent. It is recommended that the analyzed sequence include the base

Because inserts, flanking regions, and vectors are modified or may be susceptible to recombination

area.

Virus seed batches need to carry out comprehensive passage stability studies, and the research process should

Can represent or simulate the actual preparation process, and focus on the genetics of virus seed batches in research

Stability and production stability, etc. Establish limits for virus seed batches based on research

Passed down from generation to generation.

If helper viruses are used in the viral vector preparation process, sufficient research should be carried out

This study explains the necessity and selection basis for the use of helper viruses, combined with scientific understanding and

Production experience demonstrates the safety of helper viruses. Design and construction of auxiliary viruses

Recommendations for library, production preparation and testing refer to the general requirements for viral seed lots above.

2.5 Other production materials

Other production materials include raw materials (such as reagents, culture media, etc.), consumables

Materials, culture containers, etc. Combined with process research, select appropriate raw materials for production,

Develop reasonable raw material quality control standards and conduct strict supplier audits,

Clarify the sources, components, functions, stages of use, and quality standards of raw materials for production

Wait. Try to avoid using ingredients of human or animal origin during preparation if

If you really need to use it, you can refer to the relevant provisions of the Chinese Pharmacopoeia and/or ICH Q5A, etc.

Guidelines for conducting safety-related risk assessment and research on exogenous factors and other factors. Need to be heavy

Raw materials of concern include: materials of human or animal origin used for cell culture (e.g.

bovine serum, digestive enzymes), plasmid transfection reagents (such as polyethylenimine, cationic

lipids, etc.), nucleases, etc. For people using viral vectors during preparation or storage

For products with higher risks such as albumin, it is recommended to use products approved by regulatory authorities as much as possible.

Products that are accurate and comply with relevant national technical requirements and management specifications. For consumables

and culture containers, it is recommended to conduct analysis and research to evaluate their suitability and minimize

Low safety risks during viral vector preparation.

3. Preparation process

The preparation process of viral vectors refers to the recovery and amplification of production cells, virus

The entire process from viral vector harvesting to viral vector filling and storage (if any). right

A viral vector system that directly prepares cell products after genetic modification in vitro, because

The quality of viral vectors can directly affect the quality of the final product, so research on its preparation process

should be more fully improved. With a full understanding of the overall process and the viral vector material

Formulate the preparation process based on accumulated experience and establish standardized process operation steps.

steps, process control parameters and discard standards, and clarify key process parameters. preparation

The process should be suitable to ensure that the cell product meets the quality objectives for the corresponding development stage

Product profile requirements. In addition, measures should be taken to prevent viral vectors from being

Confusion, contamination and cross-contamination may occur during the entire process of storage and transportation.

3.1 Preparation scale and batch definition

Cell types, growth characteristics, viruses used for production of different types of viral vectors

There are large differences in vector yield and stability, the maturity of the preparation process and the disease

The amount of virus vector used is also different. It is recommended to combine the characteristics of the cells to be genetically modified.

properties, viral vector technology and clinical use needs, etc., to reasonably determine the viral vector

scale of preparation. The scale of viral vector preparation needs to be in line with the research stage of final cell products.

(clinical trials, commercial preparation). The process may include production

Cell culture, plasmid transfection or virus infection, harvesting, purification and other steps, preparation

The scale of the upstream and downstream processes in the process must be matched and reasonable as much as possible. for smaller

scale, it is recommended to focus on quality consistency between batches.

According to the process characteristics of viral vectors, batch definitions and numbering rules are formulated. like

If necessary, the different process steps in the preparation process can be clarified, paying special attention to any differences.

Batch definition and numbering rules for batch and combined batch operation steps. Ensure viral load

Traceability of individual batches.

3.2 Process research and development

There are a variety of processes used to prepare viral vectors, including via plasmid DNA

Transient transfection packaging cell matrix preparation, prepared from stable production cell lines,

or prepared by infecting cell matrices with viral seeds.

Encourage the integration of new concepts such as quality by design and full process control, as well as the

Conduct process research on general requirements related to risk control. With the accumulation of production experience

Deepen the understanding of quality attributes, continuously optimize the process, and finally complete the laboratory

Process conversion to commercial scale processes. The preparation process can generally be divided into upstream,

The two downstream stages are the upstream viral vector harvesting stage and the downstream viral vector purification stage.

transformation stage. Production cell types, cell culture conditions, and transformation during the preparation process

Contamination or infection conditions, harvest time, purification steps and storage conditions will all affect

Packaging efficiency and quality of viral vectors. In the research, it is necessary to understand the process steps and key processes.

Research, confirm or verify process parameters and their control ranges, and establish corresponding

Process control standards. For example, replication competent virus (RCV) is one of the security risk concerns in process

control and should be based on

Carry out risk assessment based on viral vector types, process characteristics, etc., during the preparation process

Choose a reasonable detection point (such as viral vector harvest fluid, production end-stage cells or

Purified viral vector stock solution, etc.), and use validated methods for testing.

In addition, based on risk and the virus's tolerance to physical and chemical conditions, if necessary,

It also needs to be based on the type of cells used for production, the use of helper viruses, the characteristics of the purification process, etc.

Establish appropriate virus removal/inactivation procedures and fully validate them.

The following uses gamma-retroviral vectors and lentiviral vectors as examples to analyze the stability of

Two preparation processes, namely fixed toxin production and plasmid transfection, are introduced. other viral vectors and

Other preparation methods, if applicable, may also be considered.

3.2.1 Research on stable toxin-producing preparation process

(1) Preparation

Taking gamma-retroviral vectors as an example, they are prepared from stable toxin-producing cells.

Secreted into the culture medium, the viral vector can be purified through steps such as clarification and filtration.

It is recommended to formulate a suitable method based on the structural characteristics, packaging mechanism, stability, etc. of the viral vector.

process steps and parameters, pay attention to the cell culture volume, contact during the preparation process

Seed density, culture conditions, harvest time, etc. For example, it has been reported that due to ÿ-

Some envelope proteins of retroviral vectors are under normal cell culture conditions (37°C)

The stability under the condition is weak. It is recommended to carry out research on this situation and formulate corresponding disease measures.

Virus preparation and harvesting strategies to ensure viral vector activity and recovery.

(2) Purification

According to the structural characteristics of the gamma-retroviral vector, the type of host cells, the components of residual impurities, etc., a reasonable purification process is designed to improve the efficiency of the viral vector. purity and reduce the safety risk of impurity residues.

For example, the envelope proteins of some viral vectors may be sensitive to chromatography processes. sense (such as shear force), therefore, the development of advanced purification processes is encouraged to meet On the basis of the structural stability and functional activity of viral vectors, try to improve the viral The purity of the vector enables large-scale purification of viral vectors.

3.2.2 Research on plasmid transfection preparation process

(1) Packaging and preparation

Taking lentiviral vectors as an example, virus packaging cells used for plasmid transfection usually Adopt adherent or suspension culture methods. The cell culture method is recommended based on scale and production. Options include preparation process design and quality research to meet the needs of commercial preparation. sick Research on the virus packaging process includes plasmid concentration, plasmid ratio, transfection reagents and concentration, induction reagent concentration (if any), transfection time, cell density, cell Medium components, cell culture environment (temperature, pH, dissolved oxygen), harvest time, Optimization of parameters such as harvest times. Based on research, confirm process steps, key processes Process parameters and their control ranges, and establish corresponding process control standards. For example in During the cell culture process, cell viability and bioburden are regularly detected, and carrier dripping is carried out. Detection of viruses, mycoplasma and exogenous viruses, and replication lentivirus (RCL) Testing etc. If the vector harvest solution needs to be stored, corresponding studies need to be carried out to confirm the storage storage conditions, storage methods, etc. For the detection of exogenous factors, it is recommended to maximize the detection

The sensitivity of the detection method at the appropriate detection point (such as the stage of exogenous factor enrichment)

Perform testing.

(2) Purification

Currently, the purification process of lentiviral vectors usually uses endonuclease removal

Large fragments of nucleic acid impurities attached to the surface of viral vectors are clarified, filtered and ionized

Purification steps such as chromatography or size exclusion chromatography are used to remove impurities, and finally the

agent, sterilization, filtration, and filling to prepare viral vectors for use. According to research, pure

The purification process can be appropriately adjusted and optimized, and each purification process step can be studied and determined.

steps to remove impurities and purify the virus. If applicable, process under study

It is recommended to adjust the nuclease concentration, purification method, media selection, dynamic capacity, flow rate,

The recovery rate, viral vector storage conditions, filling process parameters, etc. were studied, and

For nuclease residues, BSA residues, risk element residues (such as adenovirus E1 and

SV40LT antigen sequence residue), plasmid DNA residue, host protein residue,

The removal rate of impurities such as host DNA residues and transfection reagent residues was studied.

During the purification process, process control testing or quality research needs to be strengthened, such as biochemical

Charge, endotoxin, physical titer, transduction titer, etc.

3.3 Process verification

After the preparation process is locked, process verification needs to be carried out to conduct process verification of each step.

Confirmation, including, if applicable, individual steps of cell expansion and vector preparation

Verification, intermediate product storage conditions and time verification, impurity removal verification, culture

Base simulation filling verification, chromatography column and ultrafiltration membrane recycling times and sterilizing filters

verification and transportation verification, etc. Prove the preparation process and its performance through process validation

Sustainable and stable production within the set process parameters, virus vectors

The yield and recovery rate should be relatively stable, and residual nucleases, host cell proteins

White, host cell DNA, plasmid DNA, cell debris, transfection reagents and other impurities

Quality should be effectively removed to levels below the quality standard range or validated studies.

Encourage the establishment of a preparation process that matches the upstream and downstream scales. If the preparation process

If batching and/or batching occurs during the process, it needs to be combined with the actual preparation situation.

Sufficient research verification, and formulating principles for batching and/or batching based on research results

and specific operating specifications. Samples used for batching should be inspected and confirmed before batching

for qualified samples.

4. Quality research and quality standards

4.1 Quality research

Encourage the use of advanced analysis methods to carry out quality work from multiple angles and levels

Research. Analytical methods need to be researched and confirmed to ensure accurate and reliable results.

It is generally recommended to use multiple representative batches to conduct quality studies. Common research

Research items include appearance, viral vector morphology, identification, integration characteristics (if applicable),

Viral vector titer, biological activity, purity, impurities (such as replicating viruses,

Risk component residues, exogenous factors), etc. Based on the research results, determine the key qualities

Quantitative attributes.

4.1.1 Identification and sequence confirmation

It can be detected from the overall level, protein level and nucleic acid level of the viral vector.

Measurement. At the overall level, electron microscopy observation, immune serology, etc. can be used

Method analysis and identification. In terms of protein level, protein electrophoresis and immunoblotting can be used

Trace and other analysis methods are used to analyze the structural proteins, expression products, protein expression profiles,

Analyze immune markers, phenotypic characteristics, etc. In terms of nucleic acid levels, it is recommended to

Whole-genome sequencing of the viral vector was performed to confirm the sequence. Special attention should be paid to the target gene

and complete analysis of regulatory element sequences, and comparative analysis of the measured sequence and the expected sequence.

consistency. For integrated viral vectors, the viral vector can also be used to transduce the target

After the cells are harvested, the genome of the cells integrated with the vector sequence is sequenced to verify the vector.

The accuracy of the body skeleton and target gene sequence. Alternatively, restriction enzymes can be used

Cut pattern analysis, polymerase chain reaction (Polymerase chain reaction,

PCR) and other methods to identify viral vectors and target genes. The identification test should

Set up appropriate positive and negative controls.

4.1.2 Integrated features

For integrated viral vectors, it is recommended to use appropriate methods to study vectors

Typical characteristics of integration into the target cell genome, including dominant insertion sites, insertion

Input copy number, abnormal growth of dominant clones, etc. Pay attention to whether there are viral vectors

First integrated into the genome of the target cell near the oncogene and other potential

Cancer risk.

4.1.3 Viral vector titer

Titer is an important test item for the biological activity and content of viral vectors.

For process monitoring and release testing, sensitivity, accuracy, and precision need to be selected.

Conduct research on density and other testing methods that meet the requirements. Encourage a variety of approaches to

Conduct titer detection and explore the correlation of detection values with different methods. Titer test

The tests include physical titer (total number of viral vector particles) and transduction titer (viral load

number of infectious particles in the body). Standards or controls need to be used to calibrate drops during research.

Degree test results. The ratio of infectious particles to total particles in the viral vector can be

Examples for comparing quality between different batches and between different stages of vector preparation

quantitative characteristics.

Physical Titer: Quantification of vector specific proteins/nucleic acids can be used on vector particles

Estimate of quantity (physical titer). For example, HIV-1-derived lentiviral vectors,

Commonly used enzyme-linked immunosorbent assay (ELISA) to detect p24 in carrier samples

The protein is thus tested for physical titer, and the test results can be used to determine the p24 protein content.

/ml or number of particles/ml indicates that the results of free p24 protein should be paid attention to during detection.

Impact. Additionally, quantification of vector genome copy number can also be used for physical titers

estimate, it is recommended to pay attention to the interference of exogenous DNA when testing. If new technology is used

Detection, such as virus counter method, nanoparticle tracking analysis method, field flow separation-

Multi-angle laser light scattering method, etc. It is recommended to pay attention to the method of virus vector type to be tested

applicability.

Transduction Titer: Viral load can be detected using cell-based in vitro assays

body's ability to infect. Usually, after the virus vector to be tested infects the cells for a certain period of time,

Cell-based genomic quantitative PCR, flow cytometry, tissue/cell lesions or morphology

Plaque formation and other methods were used to detect, and the transduction titer of the viral vector was calculated, and the test results

Usually expressed in transduction units (TU/ml) and half tissue culture infectious dose (TCID50)

Or plaque forming units (PFU) and other expressions.

4.1.4 Biological activity

Generally refers to the ability to transfer the target gene to the target cell and the target gene

Due to the biological effects of the expression product, the gene transfer ability is also related to the viral vector.

related to the transduction titer. Biological activity research runs through the overall research and development process

, it is recommended to establish biological activity detection methods in the early stage of research and development; in the marketing stage,

It is recommended to determine and implement predictions as much as possible based on the mechanism of action and quality attributes of the carrier.

related to phase functions (replacement, compensation, blocking, modification of specific gene effects, etc.)

Biological activity detection methods, and when necessary, establish appropriate standards. due to illness

The biological activity of the toxic carrier may be reflected in the final product of the cell. Therefore,

The biological activity of the viral vector can also be comprehensively evaluated based on the biological activity of the final product.

Academic activity.

4.1.5 Purity and Impurities

(1) Vector-related impurities: Typical impurities related to viral vectors may

Including mispackaged particles, defective interfering particles, non-infectious particles, and empty capsids

For particles, aggregates or replicating viruses, it is recommended to use applicable methods, e.g.

High performance liquid chromatography, electrophoresis, capillary electrophoresis, ultraviolet absorption spectrometry, etc.

research. By the ratio of total particles to infectious particles in the viral vector, it is also

It can reflect the purity and impurities of the viral vector. In addition, at the nucleic acid level,

Consider identifying vectors with deleted, rearranged, hybridized or mutated sequences, etc.

For impurities, the test value should be reported in the form of content ratio, and the quality should be included if necessary.

Quantity standards.

(2) Detection of replicating viruses: using replication-deficient or conditional replication

When using viral vectors, possible production during the preparation process needs to be detected at appropriate stages of the process.

viruses with replication capabilities, and the dosage and disease will be determined based on the final cell product.

To determine the standard limits for residues, such as the risk of toxic vectors. Replicating viruses and products

Safety-related, it is necessary to refer to relevant research experience or literature to study and establish sensitive

detection method. Common detection methods include indicator cell culture methods, direct

qPCR method, etc. Samples to be tested include viruses harvested during viral vector preparation

Vector harvesting solution, production of final cells and cell final products, etc. When developing methods,

It is necessary to pay attention to the operating procedures, sample sizes, negative controls, positive controls corresponding to each detection method.

Setting and testing of sex controls, detection markers, detection limits, judgment standards, etc.

evidence research. It is recommended to design specific detection markers based on the virus packaging system used.

If applicable, the study is encouraged to adopt two or more methods based on different principles or targeting

Detection methods of different markers can improve the detection rate of replicating viruses. when

When using the indicator cell culture method, it is necessary to select reasonable amplification cells and indicator cells.

The inhibitory effect of the sample to be tested on the growth of indicator cells should also be considered when researching and designing

Set a reasonable titer range for the sample to be tested, and it is recommended to set up an interference group control.

Replicating retrovirus (RCR) detection: based on current research technology

level, it is recommended to use sensitive indicator cell culture methods to detect gamma-retroviral vectors.

body for RCR testing. RCR amplified cells are co-cultured with the sample to be tested to

To maximize the expansion of RCR, after a certain number of passages and time of subculture

Take an appropriate amount of supernatant and inoculate it into RCR indicator cell culture to observe and count cell diseases.

Change colonies or detect RCR markers.

Replicative lentivirus (RCL) detection: According to the current level of research technology,

It is recommended to use indicator cell culture method for RCL detection of lentiviral vectors. right

For HIV-derived lentiviral vectors, the positive control used in RCL detection can be considered

Consider using HIV viruses that meet the testing requirements, such as those lacking accessory genes, and viruses that are replication-competent at the time, study and evaluate the structure and Preparation methods, and proper operation and use in an environment that meets the requirements. RCL In terms of detection indicators, it is usually considered that p24 protein, reverse transcriptase activity, psi-gag The detection of VSV-G sequences and the like can reflect the presence of RCL, combined with viral vectors Select appropriate detection indicators based on the specific situation and research situation.

(3) Process-related impurities: may include residual host cell proteins, non-Target nucleic acid sequence, auxiliary viral contaminants (such as infectious viruses, viral DNA, viral proteins, etc.) and residues of reagents used in the process, such as cytokines, Growth factors, antibodies, transfection reagents, magnetic beads, nucleases, serum and solvents, etc.

Take non-target DNA residues as an example, which may include co-purification with viral vectors Residual host DNA, plasmid DNA, etc. are common process-related impurities. During the packaging process, non-target DNA may also be co-packaged inside the capsid of the viral vector. These impurities may adversely affect product quality and safety, and optimization is recommended process to reduce its pollution. If necessary, remove residual non-target DNA sequences Carry out confirmation and content monitoring. When producing/packaging cells for tumor-derived cells cells, tumorigenic cell lines, or cells carrying oncogenic gene sequences that require high attention When removing cells, on the basis of optimizing the process and reducing their residues, it is also recommended to control unintended DNA fragment size (recommended to be below 200bp), and be sensitive to known high risk Conduct special monitoring of risk genes. For example, using HEK293T cells to treat chronic diseases When preparing toxic vectors, it is necessary to use methods with sufficient sensitivity and specificity to detect Detection of adenovirus E1 and SV40LT antigen sequence residues.

4.1.6 Others

Microbial contaminants: detects possible introduced contaminants, including exogenous viruses,

Bacteria, fungi, mycoplasma, bacterial endotoxins, etc.

Physical and chemical testing: Routine physical and chemical testing items may include appearance (color,

Transparency), visible foreign matter, insoluble particles, pH, content, osmotic pressure, etc.

4.2 Quality standards

Quality standards are an important part of quality control, including testing items, testing

Use methodology and acceptance criteria for each test indicator. The testing phase generally includes

Perform testing and/or process control, etc.

According to existing research knowledge, quality standard testing items for viral vectors usually

Including appearance, identification, purity, content/titer, biological activity, impurities,

Sterility, bacterial endotoxins, mycoplasma and exogenous viral factors, etc. Testing method

The method needs to be researched and verified to ensure that the test results are reliable and accurate. If applicable,

Try to establish reference substances/standards and carry out corresponding quality research, including

Calibration of quantity/activity, determination of storage conditions, etc. Generally, bids are acceptable

The standard formulation basis includes product quality design, quality research, process development, inspection

Validation studies, methodological studies and validation, multi-batch testing and stability results, and

Reasonable statistical methods, etc.

(2) Non-viral vector gene modification systems

Non-viral vector gene modification systems can be transformed through physical, chemical or biological transformation.

Delivered into cells in a guided manner. After entering the target cell, through transcription, cutting,

It functions through translation and other methods, and its active components are DNA, RNA or protein.

Combinations of nucleic acid and protein components are also possible.

1. Molecular design

Design of non-viral vector gene modification systems affects target gene modification

or the specificity, accuracy and effectiveness of target gene expression, which also affects gene modification.

The safety and effectiveness of the final cell-decorated product requires design and restructuring during construction

Conduct a trade-off analysis of the pros and cons.

For DNA gene modification systems, attention needs to be paid to gene transfer during the development process.

Guidance efficiency, off-target efficiency, insertional mutation status, target gene in target cells

Integration site and copy number, etc. The design strategies adopted include genetic codon optimization

ionization, modification of chromosomal homologous sequences, modification of GC-rich region sequences, signaling

Application of peptides and reasonable promoters, etc. Currently, the commonly used cyclic

DNA gene modification systems, including plasmids, minicircle DNA,

Nanoplasmid and other different types. Different types of circular DNA

The selection can focus on the ease of preparation of high-purity vectors, vector recombination,

Epigenetic modification of bacterial-derived DNA sequences in target cells affects the target gene

Due to the influence of expression, etc. In addition, circular DNA gene modification systems can induce

Inject special DNA fragments to form episomal vectors, such as introducing EBV sources

The expression of cis-acting DNA fragment OriP and trans-acting EBNA1 gene

For such vectors, attention should be paid to the impact of species and cell types on the function of free vectors.

The effect of vector recombination and cis/trans-acting DNA fragments on the expression of target genes

Impact etc.

For RNA gene modification systems, taking mRNA as an example, according to current

Research progress, taking into account the stability of RNA design and its performance in target cells

The biological activity within may have a significant impact, and you can pay attention to the bases when constructing

Modification types and proportions, 5'-cap or cap analog structures, untranslated region sequences,

Poly (A) tailing structure and self-amplification elements (if any), etc., and proceed at the same time

Improvements in codon optimization, regulation of interactions between bases, and advanced structures, etc.

to achieve the expected functionality.

For gene editing systems, it is recommended that targeting sequences, target gene sequences (such as

Yes) and optimize the sequence and ratio of gene editing enzymes. through specific

Gene editing effects in cells confirm the characteristics of gene editing enzymes and targeting sequences

heterosexual, screen the best target binding sequence (such as sgRNA sequence), and take

Measures to reduce the probability of gene off-target and insertional mutations and stabilize the target cell genome

Qualitative adverse effects. For transposon systems, it is recommended to consider the characteristics of the integration site

Sex and distribution trends, and the movement of transposons through the genome

(genomicmobilization) and other characteristics, conduct transposon sequences, transposase

and the optimization of corresponding regulatory elements, rationally setting the ratio of transposable sequences/transposase,

Sequence distribution, etc.

2. Materials for production

For basic principles, please refer to the relevant section on viral vector gene modification systems.

Require.

For production purposes prepared by recombinant technology or biological/chemical synthesis technology

For raw materials, the process and quality control situation need to be clarified, especially the production needs to be analyzed

Safety-related impurities that may be introduced during the process. For use in the preparation process

For enzyme reagents, it is recommended to focus on the functional activity of the enzyme, such as the

Fidelity and activity of DNA polymerase, RNA polymerase, enzymatic digestion of digestive enzymes

function, non-specific digestion conditions of enzymes, etc. At the same time, attention needs to be paid to the purity of the enzyme,

Impurities introduced during the production process, etc. For nucleotides, 5'-cap or cap analogs

The overall quality of raw materials should meet the requirements for preparation. It is recommended to pay attention to identification,

Concentration, purity and impurities, etc. It is recommended to avoid the use of cesium chloride and bromide during the preparation process.

Toxic substances such as ethidium and chloroform, avoid using animal-derived tryptone and nuclease

and other raw materials that may introduce risks such as external factors.

For materials used as delivery systems, key raw materials involved in their preparation

(Lipids, cationic polymers, etc.) require adequate screening and quality control.

3. Preparation process

The basic requirements are the same as those of viral vector gene modification systems. For in vitro genetic modification

When cell products are prepared directly after decoration, multiple, large-scale preparations are required.

The specific situation is introduced below.

3.1 DNA-like gene modification system

Taking plasmid DNA as an example, its preparation steps generally include microbial culture and

Fermentation, cell collection, cell lysis, plasmid purification, concentration, filling, etc. work

The process of process research and determination requires exploring and optimizing key process parameters and establishing

Stable preparation process. Key process parameters may include fermentation medium composition,

Fermentation culture temperature, feeding medium composition, feeding time and feeding amount, dissolved oxygen

Amount, composition of alkali lysis buffer and neutralization buffer, alkali lysis time, chromatography

Column loading, chromatography flow rate, etc. In the study, it is recommended to pay attention to the impact of the entire preparation process on the plasmid structure.

Possible effects on structure and function, such as the alkaline cleavage step that may produce irreversible plasmids
Transsexual situations, etc. During the development of the purification process, the actual conditions of the plasmid can be
Select the appropriate column chromatography packing material in terms of size and properties to maximize host removal
Impurities such as RNA, host DNA, DNA fragments, and bacterial endotoxins. According to research
Research, set reasonable process control indicators and acceptance standards, such as plasmid intermediate production
concentration, superhelical ratio, residual impurities, etc.

3.2 RNA-based gene modification system

Based on the current research status, the preparation of RNA generally includes in vitro chemical synthesis and
Two process routes for DNA transcription. For in vitro chemical synthesis process, please refer to Chemical Drugs
Technical guidance related to the object. The DNA transcription process route is based on the preparation process of mRNA.
Take this technology as an example. This process generally uses DNA transcription templates to transfer mRNA in vitro.
transcription, mRNA capping, dephosphorylation, DNase treatment, mRNA purification, etc.
step. It is recommended to research and optimize process parameters to develop a robust process,
Ensure the correctness of the mRNA sequence, structural integrity, biological activity and differences
Consistency of quality from batch to batch. Conduct research on potential impurities introduced during the process,
Clarify the source of impurities, removal steps and removal capabilities. In process research, it is necessary to
Confirm key process parameters and control ranges, such as NTP concentration, transcription time
time, reaction temperature, capping reaction material feeding ratio, chromatography medium, dynamic loading capacity
etc., pay attention to product recovery rate, impurity removal rate, capping rate, poly(A) length
degree and distribution (if applicable), integrity of the mRNA fragments, and accuracy of the sequence
Sexual aspects, etc. Reasonable process control indicators need to be set during the preparation process, such as
mRNA concentration, double-stranded mRNA content, incomplete mRNA content, residue

DNA, sterility, bacterial endotoxins, etc.

3.3 Others

If the genetic modification system contains recombinant protein components, depending on the specific circumstances,

You can refer to the relevant technical requirements for the production of recombinant protein biological products.

4. Quality research and quality standards

4.1 Quality research

Quality studies typically include identification, structural characterization, physical and chemical properties, biological

Activity, purity and impurity analysis, etc. Multiple representative batches are recommended in studies

Research. Such as containing chemically synthesized components and/or recombinant protein components,

Quality research can be carried out with reference to relevant guiding principles.

Identification and sequence confirmation: In identification studies, restriction endonucleases can be used for

Perform enzyme digestion and perform electrophoresis analysis on the enzyme digested product to observe whether there are any characteristic

Banding pattern; PCR method can also be used to amplify and analyze whether the fragment size is consistent with

Methods such as theoretical size consistency. For RNA, it can be reverse transcribed into DNA,

Use the above methods for identification, or consider other applicable methods. sequence

In the confirmation study, it is recommended to carry out full sequence determination, focusing on the target gene and regulatory

The sequence correctness of the control element. For RNA, if any, it is recommended to also pay attention to the poly

(A) Correctness of sequence.

Structure: Appropriate methods are recommended for structural integrity and uniformity of size

research. For example, for DNA, you can pay attention to whether there are single strands, double strands,

Various structural forms such as linear/open ring, cyclic and superhelical, as well as possible high

hierarchical structure, etc.; for mRNA, you can focus on the integrity of its different region structures

(such as 5'-cap or cap analog structure, poly(A) length), base modification structure

structure, degree of dephosphorylation, and possible higher-order structures (such as stem-loop structures), etc.

If functional activity is related to higher-order structure, it is recommended to analyze and study higher-order structural features.

For complex nucleic acid gene modification systems, it is recommended to carry out structural analysis, such as paying attention to

The particle size, particle size distribution, particle aggregation, etc. of the composite structure.

Physical and chemical properties: It is recommended to carry out molecular weight, nucleic acid concentration/content, modification sites

Research on points and proportions (if any), physical properties (such as pH, osmotic pressure), etc.

Research.

Biological Activity: Depending on the mechanism of action, biological activity studies typically include

Gene modification efficiency, target gene expression level, expression product function or

Measurement of simulated physiological functions in vitro, etc. It is recommended that quantitative detection methods be preferred, if possible

By analyzing the expression level and function of the target gene or gene editing product, the relevant

Note whether the expression product is consistent with expectations or whether protein expression/gene is inhibited,

Whether the spatial structure conforms to the design (such as polymer), etc. When using in vitro transfection assay

When using methods to detect cells, attention should be paid to whether the selected detection cells are representative and

rationality.

Purity: Agarose gel electrophoresis, high performance liquid chromatography, capillary

Conduct purity studies using tube electrophoresis and other methods. For complex nucleic acid gene modification systems

System, it is recommended to pay attention to the encapsulation rate, free nucleic acid content, etc.

Impurities: On the one hand, impurities may come from raw materials, preparation processes, preparation

and dissolved substances in direct contact with containers and materials during storage. Such as DNA template,

Enzyme reagents, magnetic beads and other raw materials and added ingredients; ethanol, isopropyl alcohol, etc.

Organic solvent; host protein residue, host DNA residue, host RNA residue

wait. On the other hand, impurities associated with genetic modification systems may include deletions,

Relevant impurities such as rearrangement, hybridization or mutation sequences are recommended for qualitative and quantitative analysis.

Related research. For DNA-based genetic modification systems, relevant research can include

Open/linear DNA content, molecules modified by hypermethylation, etc. for

mRNA gene modification system, related research can include degradation/fragmentation

RNA fragments, incompletely capped mRNA, excessively modified RNA,

RNA mismatch sequences, RNA oxidation products, etc. Impurity removal combined with preparation process

removal capacity, use applicable methods to analyze and evaluate the residual levels of impurities

Related security risks.

Microbiological safety: can be combined with the process to detect possible contamination introduced

substances, including bacteria, fungi, mycoplasma (if applicable), bacterial endotoxins, etc.

Other characterization studies: For modification systems using gene editing tool enzymes,

In quality studies, it is recommended to continue to pay attention to the residues of modified systems in corresponding cell products.

situation, using bioinformatics tools to analyze changes in target cell genome structure, single

Point and small-scale genetic mutations and the insertion location of foreign DNA in the genome,

Copy number, etc., monitor the sustained expression time of gene editing enzymes in cells, and investigate

Off-target effects and corresponding safety impacts, etc.

4.2 Quality standards

Based on risk analysis, combining process research and validation, clinical trials and commercial

Develop reasonable quality standards based on batch quality analysis, stability research data, etc.

Clarify the analysis methods and standard limit ranges corresponding to each test item and establish standards

/Reference products, etc. For general process-related impurities, if it is fully verified that the process

The technology can remove it effectively and stably, and can be controlled in combination with the technology. The relevant residues

The remaining testing may be considered not included in the verification items.

Quality control items may include physical and chemical properties, purity and impurities, biological

activity, microbial safety, etc. Among them, DNA quality standards can include

Enter appearance, pH value, content, identification, sequence analysis, purity, superhelical ratio

For example, biological activity, sterility testing, bacterial endotoxin, impurity residue testing, etc.

project. Quality standards for mRNA can include appearance, pH value, content,

Identification, sequence analysis, mRNA integrity, capping rate, poly(A) length

and distribution, biological activity, sterility testing, bacterial endotoxin, double-stranded RNA

Test items such as residue and solvent residue. Common complex nucleic acid gene modification systems

Systematic quality control items include appearance, pH, particle size and dispersion coefficient,

Osmotic pressure, identification, sequence determination, content/concentration detection, biological activity,

Purity, sequence integrity, encapsulation efficiency, content of each component of the composite material (such as lipids

quality identification and content), free nucleic acids, visible foreign matter, impurities, microbial safety

Sex etc.

In terms of methodological research and verification, the basic principles are the same as those for genetic modification of viral vectors.

decoration system requirements.

6. Stability research and direct contact container/material research

(1) Stability research

If the genetic modification system involves storage, corresponding stability studies need to be carried out.

Research. Studies are conducted using representative batches of samples from the intended storage period, which generally include

Storage, transportation (if applicable) and use stability studies, etc. Before the research is carried out,

It is necessary to coordinate the formulation of stability research plans and pay attention to the samples used in each stability study,

Direct contact containers/materials, testing time points, testing conditions and analysis items

wait.

In research, it is necessary to study sensitive features that can reflect quality changes, such as

Content, integrity, purity, microbial safety and biological activity, etc. Research

The proposed conditions need to be covered, such as temperature, light, repeated freezing and thawing (freezing

during storage), shaking, etc. Based on actual usage conditions, carry out stable operation during use

Qualitative studies, such as reconstitution or thawing, compatibility with reconstitution diluent, etc.

The research needs to use direct contact containers/materials made of the same material as those actually used.

For viral vector gene modification systems, it is recommended to focus on stability studies

Examine the titer, purity, impurities, microbial safety indicators, and production of viral vectors

Key quality attributes such as physical activity. For non-viral vector gene modification systems,

It is recommended to focus on key quality attributes such as physical and chemical properties, structural integrity, and impurities.

For example, the ratio of DNA superhelical structures may affect DNA transfection efficiency,

The mRNA capping rate may affect the structural stability and translation efficiency of mRNA.

It is recommended to focus on investigation in stability studies.

(2) Research on direct contact containers/materials

If storage is involved, corresponding packaging materials must be provided for packaging containers that are in direct contact.

Compatibility studies. Based on the compatibility study results and combined with the stability study, select

Reasonable packaging containers.

In addition, containers and disposable materials that come into contact with the sample during the preparation process should be

Materials (such as storage bags, filter membranes, chromatography media, pipelines, etc.) need to carry out risk management evaluation and/or corresponding compatibility studies.

7. References

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