Points to Consider for Human Gene Therapy and Product Quality Control

State Food and Drug Administration of China

This document by Shenzhen SiBiono GeneTech Co., Ltd. and the National Institute for the Control of Pharmaceutical and Biological Products, China, is now SFDA's official guidance regarding gene therapy development. It was translated from the Chinese by Shenzhen SiBiono GeneTech Co., Ltd. (Also see the interview with SiBiono founder, Dr. Zhaohui Peng, page 42).

INTRODUCTION

Gene therapy is a medical intervention based on the modification of the genetic material of living cells. Currently, gene therapy is restricted in application to somatic cells.

Based on transferring methods, gene therapy can be classified into two categories: *ex vivo* and *in vivo*. *Ex vivo* gene therapy refers to cells being modified *ex vivo* for subsequent administration to humans, while *in vivo* refers to cells being altered *in vivo* by giving gene therapy directly to the subject. The products of *ex vivo* gene therapy are cells that are modified and are intended to be administered to the patient. *Ex vivo* gene therapy is expected to be performed in well-established medical care establishments with specially trained medical professionals and GMP facilities. The products of *in vivo* gene therapy are recombinant DNA or RNA in the form of naked DNA, DNA complex, or viral vectors that are manipulated by genetic technologies. Both *ex vivo* and *in vivo* gene therapy products are subject to the regulations in this guidance. Because of the complexities of the different modalities of gene therapy, it is not possible to generalize a common guidance that is suitable for all kinds of products. However, the following basic principles should be followed when sponsoring the development of a gene therapy product:

1 The safety and efficacy of the product should be guaranteed. A comprehensive assessment of the benefit and risk of the product should be conducted.

2 New and innovative ideas should be promoted when sponsoring gene therapy product development. Considering the uniqueness of gene therapy relative to traditional chemically synthesized and genetically engineered protein medicines, there will be certain flexibilities for the regulation of novel gene therapy products. Gene therapy as a form of medical intervention is still in its early phase of development. SFDA expects the sponsors to not just follow this guidance but also to conduct rigorous scientific study to ensure the development of a safe and efficacious gene therapy product.

When applying to conduct a clinical trial of an investigational new gene therapy drug (IND) from the State Food and Drug Administration (SFDA), the sponsor should prepare the application following the guidance in this document. In addition, the following information should be provided:

1 Review of the research and development of the relevant gene therapy field

The review should cover the following topics:

- therapeutic gene
- delivery vehicle
- gene delivery system and method
- *in vitro* study efficacy data
- preclinical animal study, including toxicity, safety, and efficacy data
- clinical trial investigation plan, including safety and efficacy study
- overview of production process
- overview of quality control
- discussion of the novelty of the product
- discussion of the product commercialization strategy.

The review should be comprehensive and up to date.

2 Review of the intellectual property status of the product

The review should cover the following topics:

2.1 Overview of the intellectual property status of the proposed product

2.2 Reference and patent search report

The reference and patent search should cover the therapeutic gene, delivery vehicle, delivery product, and other components in the final product, producer cell and production process, and related topics.

APPLICATION CONTENT AND PRODUCT QUALITY CONTROL

The following should be included in the application proposal:

1. Construction of the DNA expression cassette and the gene delivery system

1.1 Therapeutic target gene

A detailed description of the clonal origin of the therapeutic target gene, including a patent search on the gene, should be provided. Method of gene cloning and sequence identity should be provided.

1.2 Vector

Information supplied should include restriction mapping and gene-bank data for the vector. Known regulatory elements such as promoters, enhancers, and PolyA should be identified. If there is any change in the vector backbone gene structure (such as deletion, mutation, or insertion), the DNA sequence data should be provided. For a new viral vector, it is necessary to provide information on the material, method of construction, and testing of the new vector.

For non-viral gene delivery systems, plasmid is needed to express the target gene in human cells. In addition to naked DNA, another component is generally used to complex with the DNA. This guidance does not cover oligoribonucleotide (such as antisense RNA, ribozyme, and siRNA) products.

1.3 DNA expression cassette

A detailed description of the cloning procedure, the methods and materials used, and DNA sequence data should be given. Known regulatory elements such as promoters, enhancers, and PolyA should be identified. Restriction mapping of the gene expression cassette and the kinetics of gene expression should be provided.

1.4 Construction of the gene delivery system (including viral and non-viral gene delivery systems)

1.4.1 Viral gene delivery system including adenoviral vector, retroviral vector, and adeno-associated viral (AAV) vector

A thorough description of the clonal origin of the viral vector should be provided. The methods and materials used for the construction of the viral vector should be included. Testing methods and results should also be provided. General testing should include structural analysis (for example, restriction mapping and PCR), complete sequencing of the viral genome (≤ 40kb), gene expression and bioactivity analysis, SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), DNA sequencing of the gene expression cassette, Western blot analysis of the expressed protein, transduction efficiency analysis, negative-staining transmission electron-microscopy of the purified viral vector, replication-

competent virus detection, and analysis for residual process contaminants.

1.4.2 Non-viral gene delivery system

Non-viral gene delivery systems encompass naked DNA, mammalian cell carrier systems, and other carrier systems such as liposome, polypeptide, and gold particles. The nature and characteristics of the non-viral delivery system should be described adequately. To prevent allergic reaction to penicillin in some patient populations, it is recommended that a kanamycin- or neomycin-resistant gene be used as the drug selection gene.

For the physical delivery system, a detailed description of the delivery method, procedure, efficiency of gene delivery and expression, gene stability after delivery, and bioactivity should be included. Evidence of absence of gene arrangement and mutation should be provided. Detailed testing results should be given, including plasmid restriction mapping, PCR analysis, DNA sequencing of the gene expression cassette, SDS-PAGE, and Western blot analysis of the expressed protein.

2. Generation and characterization of cell banks and engineered bacteria banks

A three-tiered bank system should be established. These typically include a primary seed bank, a master cell bank, and a working cell bank. For engineered bacteria, these typically include a primary bacteria bank, a master bacteria bank, and a working bacteria bank.

2.1 Cell bank

2.1.1 Primary seed bank: The origin, cell passage history, and cell characterization, as well as cell culture and banking procedures should be described.

2.1.2 Master cell bank: The master cell bank should be derived from one or more ampoules of the primary seed bank by serial subculture to a specific passage number. The passage number should be identified.

2.1.3 Working cell bank: The working cell bank is derived from one or more ampoules of the master cell bank by serial subculture to a specific passage number. The passage number should be identified.

2.1.4 Characterization of cell banks: The master cell bank or working cell bank should be characterized according to guidelines in "Guidance for Application of Clinical Trials for Human Gene Therapy."[1]

Additional testing should include:

a) testing of susceptibility of virus infection and production
b) testing the status and stability of the transduced gene and level of gene expression
c) testing for generation of replication-competent viruses.

If a helper virus is needed in the testing, the origin, methods of preparation and separation, and virus passage history should be provided.

2.2 Bacteria bank

A master bacteria bank and a working bacteria bank should be established and tested according to the specifications of the "Chinese Pharmacopoeia."[2]

2.2.1 Testing of the master bacteria bank

a) Uniformity and the identity of bacteria host strain: Testing should include the origin, genotype and phenotype of the bacteria strain. The genotype should be tested by RAPD (random amplified polymorphic DNA). The phenotype should be tested for the specific marker or antibiotic resistant gene marker of the engineered plasmids.
b) Purity: The bacteria should be shown to be free of other adventitious agents, such as fungi and bacteria.
c) Stability: The ratio of transformed bacteria, propagation condition, copy numbers, level of gene expression, and passage number should be qualified.
d) Sequencing of gene expression cassette: The inserted gene of interest and associated regulatory elements should be sequenced.

2.2.2 Working bacteria bank

Testing of the working bacteria bank should be performed in accordance with the requirements for the master bacteria bank mentioned above with the exception of the sequencing of the gene expression cassette.

3. Manufacture of gene therapy products

3.1 General requirements

3.1.1 Description of manufacturing facility and environment

GMP regulations should be followed in the manufacturing of gene therapy products. This applies to both *ex vivo* cell products and recombinant viral products. Recombinant viral products intended for preclinical and clinical studies should be manufactured using a production process that can be validated.

3.1.2 A detailed description of the manufacturing process, raw materials, and the components used should be provided.

3.1.3 Manufacturing process controls should be provided. Critical process parameters should be identified, monitored, and recorded.

3.1.4 Batch production records and testing records of one lot of product should be provided.

3.1.5 One lot of product should be tested by institutes that are designated by SFDA for lot-release testing. The testing report should be provided.

3.2 Recombinant viral vector as gene therapy product

A master virus bank (MVB) and a working virus bank (WVB) should be established and tested. The WVB should be derived from the MVB. A detailed description of the origin, construction, cloning, passage, and storage of the virus bank should be provided. Quality control testing procedures outlined in this document should be followed. Production should be initiated from producer cells derived from the working cell bank. Non-qualified cells should not be used for production purposes. For a viral vector product, the producer cell infection should be carried using a virus from the WVB. Infection should not be performed using a non-qualified virus. Production can be performed using large-scale attachment-dependent or suspension cell culture. Virus banks can be prepared directly from a cell culture without further purification. Buffer formulation for virus banks should be qualified to protect virus infectivity during storage.

3.3 Non-viral plasmid DNA complex as gene therapy product

A detailed description of the following aspects should be provided:

3.3.1 Manufacturing process and quality control of the recombinant plasmid DNA

3.3.2 The origin, characteristics, and method of preparation of the liposome

3.3.3 The origin, characteristics, and method of preparation of the polypeptide

3.3.4 The origin, characteristics, and method of preparation of other components in the product complex

3.3.5 Method of preparation and quality control of the final product complex

For gene therapy products in the form of gene gun and other physical gene delivery approaches, detailed description of the following items should be provided:

a) origin and preparation of therapeutic gene
b) gene expression
c) propagation of the naked DNA
d) DNA purification
e) equipment characteristics
f) method of gene delivery.

3.4 Genetically-modified somatic cells as products

This category encompasses *ex vivo* gene therapy products. Manufacture of this category of products should follow guidelines in "Guidelines for Study of

Somatic Cell Therapy and Quality Control."[3] Detailed description of the process for cell propagation, media used for cell culture, method of cell collection, method of *ex vivo* cell transduction and selection, and formulation used for washing and storage of the transduced cells should be provided.

4. Quality control

Extensive quality control testing should be performed during the manufacture of gene therapy products. Product release testing should be performed on the final drug product. However, depending on the nature of the manufacturing process, some testing should be performed on process samples if the excipients included in the final product formulation interfere with the testing.

4.1 Recombinant viral vector as gene therapy product

In reference to the domestic and international viral vector development status, testing of a recombinant adenovirus (rAd) product is used as an example of the quality control testing for viral vector products. The testing can be used as a reference for other viral vector products.

4.1.1 Quality control for rAd gene therapy product

4.1.1.1 Crude harvest

a) sterility tests to be performed in accordance with "SFDA Guidance for Biological Products, subpart — Sterility Testing," 2000 edition[4]
b) measurement of virus particles
c) titration of infectious virus titer

4.1.1.2 Bulk product (drug substance)

a) sterility test
b) measurement of virus particles
c) endotoxin testing

4.1.1.3 Final product (drug product)

a) particulates
b) physical appearance
c) recoverable volume
d) pH
e) identity testing by restriction mapping analysis of the gene expression cassette
f) purity: A260/280nm or HPLC
g) virus particle concentration: spectrophotometric OD260 method or infectious titer (TCID50)
h) potency: testing level of gene expression and bioactivity testing
i) replication-competent adenovirus (RCA), A549 cell-culture detection method (no more than 1 RCA in 3 x 10^{10} viral particles)
j) adeno-associated virus (AAV), testing of the presence of AAV using PCR
k) testing of residuals: residuals, such as host cell DNA, host cell protein, bovine serum albumin for process using fetal bovine serum, and other residuals specific to the production process should be tested and quantified
l) sterility test
m) general safety testing
n) endotoxin test
o) stability study: stability of three batches of final product during real-time storage, freeze, and thaw; and accelerated stability study
p) mycoplasma testing

4.2 Quality control for *ex vivo* gene therapy using retrovirus gene therapy product

Manufacture of gene therapy products for *ex vivo* transduction of somatic cells should follow guidelines in "Guidelines for Study of Somatic Cell Therapy and Quality Control."[3] Testing for replication-competent retroviruses (RCRs) should be emphasized. Testing should be performed for the master cell bank, the working cell bank, end-of-production cells, and final virus products. Five percent of the total supernatant should be tested for RCR. One percent of total pooled end-of-production cells or 10^8 cells, whichever is less, should be co-cultured with a permissive cell line (such as *Mus dunni* cells) for a minimum of five passages in order to amplify any potential RCR present. Then, the amplified material is detected in a combination of two appropriate indicator cell assays, such as PG4 S+L- cells, maker rescue, or the RT/PCR method. All assays should include positive (such as supernatant from COS4070A cells) and negative controls to assess specificity, sensitivity, and reproducibility of the methods used.

4.2.1 Retroviral vector producer cells as product

Testing should include:

a) morphology, karyology, surface marker, and homogeneity of the cells
b) cell viability and concentration
c) stability, copy number, and level of expression of the inserted gene (particularly, describe the risk of insertional mutagenesis in target cells and method of evaluation).

4.2.2 Quality control of thawed cells

4.2.2.1 These tests should be performed for thawed cells:

a) percentage of cell recovery and viability after thaw
b) transduction efficiency.

4.2.2.2 These tests should be performed for supernatant from thawed cells:

a) sterility test
b) endotoxin test
c) general safety testing
d) transduction efficiency
e) mycoplasma testing.

4.2.3 Retroviral vector supernatant as product

The following testing should be performed:

a) virus titer
b) restriction mapping
c) transduction efficiency
d) RCR testing (it is critically important to test the level of RCRs in the product considering the chromosomal integration nature of the retrovirus and associated potential genetic toxicity)
e) testing for sterility and endotoxin
f) detection of residual BSA
g) mycoplasma testing.

For cell products, the expiry is very short. In those cases, the cell product can be released pending results from testing that requires a longer time. If testing results are positive, the cell product should be immediately recalled and investigation should be initiated for the positive results.

4.3 Non-viral DNA vectors as gene therapy product

The following testing should be performed:

4.3.1 Purity: testing for percentage of supercoiled DNA, residual bacterial RNA and DNA, and residual bacterial host protein

4.3.2 DNA restriction mapping

4.3.3 The origin and characteristics of liposome, polypeptide, and gold particles used (due to the inherent variation in the production of liposome and polypeptide, measures should be implemented to ensure the consistency of the production process)

4.3.4 Sterility test

4.3.5 Endotoxin test

4.3.6 Allergy testing

4.3.7 General safety testing

4.3.8 Bioactivity testing

4.3.9 Testing for process residuals

5. Evaluation of efficacy for gene therapy product

5.1 *In vitro* testing

Data should be provided to show therapeutic effect when the exogenous gene is delivered into the target cells. For *ex vivo* cell therapy, data should be provided to show the gene expression level in the transduced cells.

5.2 *In vivo* testing

Animal study data should be provided to show the gene expression level in the target tissue cells, biodistribution of gene expression, and therapeutic effect. The selection of the animal model should be justified. The route of administration should simulate that of clinical situation. If limited by the animal model, and the clinical route of administration cannot be used, justification should be provided for selection of other routes of administra-

tion. If efficacy cannot be produced in animal model studies, surrogate indicators for efficacy should be provided.

6. Safety evaluation

In addition to the above quality control requirements, the following product safety information should also be provided:

6.1 Overall safety evaluation: For *ex vivo* somatic cell therapy products, the following information should also be provided:

6.1.1 For growth-factor-dependent cells, cells cannot be used if loss of growth-factor-dependence is observed during cell expansion.

6.1.2 For allogeneic somatic cell therapy products, immunological safety data should be provided.

6.1.3 For xenogeneic somatic cell therapy products, the survival of the xenogeneic cells and *in vivo* safety data should be provided.

6.1.4 Tumorigenicity testing should be performed for all somatic-cell therapy products. For cancer vaccine products, procedures taken to attenuate the growth potential of cells derived from the tumor tissue should be provided. The tumorigenicity testing should include testing in nude mice and colony formation in soft agar.

6.1.5 Testing for cell homogeneity: For cancer vaccine products, measures should be provided detailing the separation of non-tumor cells from tumor cells. If non-tumor cells are selected from tumor cells (for example, lymphocytes), measures used to eliminate tumor cells should be provided.

6.1.6 Testing for replication competent viruses: see related parts of this guidance.

6.1.7 Additives: In addition to the additives used for cell culture and product storage, additives are used in some *ex vivo* or *in vivo* gene therapy products. The safety profile of those additives should be provided from animal studies.

6.2 Genetics study

For *in vivo* gene therapy products, the biodistribution of the viral and non-viral DNA products and expression of the recombinant DNA in target and non-target tissue should be provided. For *ex vivo* gene therapy products, the biodistribution and gene expression of the transduced cells should be provided. Risks for stem cell transduction should be carefully evaluated.

6.3 Toxicology study

Toxicity evaluation is an important part of the safety testing.

6.3.1 For *in vivo* gene therapy products, a detailed description of the toxicology study should be given. In addition to the study of potential toxicity of the therapeutic gene product, a safety evaluation of the delivery system should be performed.

The toxicology study should include an acute toxicity study (maximum tolerated dose) and a chronic toxicity study. A relatively wide range of doses should be tested, such that a reasonable dose-response curve for toxicity can be obtained. The dose range should include at least a dose equivalent to that to be used in a clinical trial and higher doses.

The routes of product administration should simulate that to be used in clinical trial study. Justification should be provided if any other route of administration is used. Product doses based on either body weight or total body surface area are preferred to facilitate comparisons across species. In addition to observations for standard toxicities, genetic toxicity specific to gene therapy products should also be included.

6.3.2 For autologous cancer vaccine, lymphocytes, or macrophage *ex vivo* gene therapy products, a toxicity study in an animal model is not required. However, if specific additives (for example gelatin, suture, or special catheters) are included in the *ex vivo* gene therapy product, or the gene therapy product is delivered to critical organs (for example heart, brain, or liver), a toxicity study in an animal model is required. If cell growth factors are supplemented in the *ex vivo* cell expansion process, a safety study is required on the growth factors. For subcutaneous and intramuscular administration, information on any injection site reaction should be provided.

6.4 Immunology study

The immunological response, including anaphylaxis, repellency, and autoimmune reaction, should be evaluated for both viral and non-viral gene therapy products after administration. A strategy to deal with potential immunological reactions should be established. For gene therapy products that are used to elicit immunological responses, such as cancer vaccines, cytokines, or genetically modified immune cells, changes to the immunological system and their potential side effects should be described. A strategy for monitoring and resolving the potential side effects should be established. Refer to "Guidelines for Study of Somatic Cell Therapy and Quality Control" for details.[3]

6.5 Tumorigenicity study: see related section in this guidance.

7. Clinical trial of gene therapy products

Clinical trials of gene therapy products are different from those with other biotechnology products. First, gene therapy products tend to be very complex. For example, *ex vivo* gene therapy products require a medical specialist to administer the product to the targeted tissues. In some cases, surgery is required in order to administer the product. Secondly, gene therapy product development is still in its early phase, and there is a possibility of high risk. Intensive patient monitoring should be implemented during clinical trials. The following information should be included when clinical trial plans are submitted.

7.1 Sponsor GMP compliance certificate
7.2 Clinical study site and biography of the principal investigator
7.3 The route of administration, dose, time, and period of treatment (if surgery is required for product administration, detailed description of the surgery procedure is required)
7.4 General clinic-status index and laboratory test
7.5 The signed consent forms of patients and family members
7.6 The molecular biology analysis of target and non-target tissues
7.7 Recording and reporting of side effects
7.8 Follow-up strategy and plan
7.9 A medical intervention plan should be provided to deal with unexpected immune reactions

8. Ethics study

Special attention should be paid to medical ethics during clinical trials of gene therapy products. Details can be found in SFDA's GCP (Good Clinical Practice) regulations.[5] The study plan and potential risks associated with the clinical study should be clearly communicated to the patient and family members. Patients have the right to choose medical treatment options and terminate participation in the gene therapy clinical trial. The patient's medical history should be kept private. The patient cannot be enrolled in the study until the patient or family member signs the study consent form.

9. References

1. SFDA. Guidance for application of clinical trials for human gene therapy. Beijing: 1999 May 1.
2. SFDA. Chinese pharmacopoeia. Beijing: 2000.
3. SFDA. Guidelines for study of somatic cell therapy and quality control. Beijing: 2003 March 20.
4. SFDA. SFDA guidance for biological products, subpart — sterility testing. Beijing: 2000.
5. SFDA. Guidelines for good clinical practice. Beijing: 1999 Sep 1.