

## **Quality and Safety Evaluation of Gene Therapy Products in Japan**

### **Review Mechanism for Gene Therapy in Japan**

The review mechanism for gene therapy in Japan was partially amended to simplify the necessary procedures and to shorten the review time, aiming at promoting gene therapy clinical trials.

Procedures necessary to start gene therapy clinical research are as follows.

First, the director of the clinical research should submit the protocol to the institution head and the protocol should be evaluated by the Institutional Review Board (IRB). After the IRB approves the protocol, the institution head must submit the protocol to the Minister of Health, Labor and Welfare (MHLW). The Health Science Division of the Minister's Secretariat at MHLW consults with the Health Sciences Council concerning the protocol. All clinical research protocols in Japan are evaluated by the Health Science Council, and the Council's approval is necessary for conducting any clinical research in order to perform the clinical research appropriately with respect to clinical and ethical propriety.

In cases where the gene therapy product to be used is produced and provided by the manufacturer and the clinical research is planned as a part of the future development of a gene therapy product intended for marketing authorization of a pharmaceutical, the product will be reviewed more carefully with respect to its quality and preclinical safety attributes. In order to assure the quality and safety of the gene therapy product, manufacturers or importers of gene therapy products are encouraged to submit a confirmatory assessment to the Minister of MHLW stating that the gene therapy product conforms to the guideline. The Pharmaceutical and Food Safety Bureau of MHLW consults with the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) concerning the application.

### **Summary of the “Guidelines for Gene Therapy Clinical Research” (Health Science Div.)**

This guideline outlines matters to be followed in clinical research on human gene therapy and discusses the procedures for conducting research so as to ensure the scientific validity and ethics of gene therapy clinical research and to promote the smooth development and proper conduct of gene therapy.

Important points described in the guideline are as follows.

- 1) In principle, diseases targeted by gene therapy clinical research should be limited to fatal hereditary diseases or life-threatening diseases such as cancer and AIDS except for thromboangiitis obliterans etc.
- 2) The prerequisites for clinical research are i) the effectiveness of treatment should be sufficiently predicted to be better than currently available alternative methods.
- 3) Gene therapy clinical research for the purpose of genetically altering human germ cells and gene therapy in which there is a possibility of genetic alteration of human germ cells is prohibited.
- 4) Gene therapy clinical research should be limited to those types of research whose effectiveness and safety can be predicted based on sufficient scientific knowledge.
- 5) In order to protect the human rights of research subjects, informed consent should be obtained when conducting gene therapy clinical research.
- 6) The director of the research should prepare a project protocol for the procedures for conducting clinical research.

### **“Guidelines for Assuring the Quality and Safety of Gene Therapy Products”**

**(Notification No. 1062 of the Pharmaceutical Affairs Bureau, MHW, dated Nov. 15, 1995)**

The quality, efficacy and safety of gene therapy products, especially the safety of those used clinically, and the achievement of therapeutic objectives should be reasonably predicted and confirmed based on scientific evidence. This guideline identifies the major issues concerning the assurance of quality and safety of the gene therapy products and outlines the data and information to be addressed by manufacturers when filing an application with respect to the quality and safety of gene therapy products intended for clinical use.

**1. Origin or Progress of Discovery and Situation in Foreign Countries**

When an application for clinical research on a gene therapy product is submitted, information concerning the stage of its development, specific features, and effectiveness of the product should be described as follows:

- 1) Descriptions of the target diseases and current therapy, and the rationale why gene therapy is applicable, based on theoretical considerations and/or scientific evidence, should be described.
- 2) When a clinical study for a person using a similar product has already been conducted, the outline, results and the relations between the two products should be described.
- 3) The gene transfer method should be described. The rationale for selecting the gene transfer method should be described, including the type of gene transfer (viral vectors or non-viral vectors, or direct transfer of naked nucleic acid) and the administration method (*ex vivo* or *in vivo* method).
- 4) The structure, process, and characteristics of products administered for gene therapy should be described.

In addition, the name, patent, filing information (state of the application and clinical use in foreign countries) and a summary of the specific features and effectiveness of the gene therapy products from the results of non-clinical studies should be documented.

**2. Manufacturing Process for Gene Therapy Products**

Clarification of the details of the manufacturing processes for the gene therapy products and their validation are critical components for the assurance of quality and safety of the products. Relevant information concerning the manufacturing process, which differs depending on the type of gene transfer and administration method, should be described. The type of gene transfer can be categorized into three classes; (1) viral vectors, (2) non-viral vectors, (3) direct transfers, and the administration method can be categorized into (1) *ex vivo* and (2) *in vivo*. Examples of the information, test items and data necessary for evaluation of the product in each category are listed below.

**2.1 Viral Vectors**

For viral vectors, at least the following data and information should be provided.

- (1) The reasons why a specific type of gene transfer was selected and its specific features.
- (2) Biological features of the wild type virus and its harmful effects on humans.
- (3) Construction, structural analysis and characterization of DNA or RNA introduced into humans, and the structure and biological activity of expression products from

transgenes.

- (4) Manufacturing procedure, structure, and characterization of other DNA used for the production process.
- (5) Culture methods and biological features of cells used for packaging and potential harmful effects of the cells on humans.
- (6) Culture methods and biological features of packaging cells and potential harmful effects of the packaging cells on humans.
- (7) Potential harmful effects of viral vector-producing cells on humans.
- (8) Features of particle structure of the viral vector.
- (9) Biological features of the viral vector.
- (10) Manufacturing method for the viral vector.
- (11) Cell banking system.

As for the reasons for selection of the viral vectors as a specific type of gene transfer and its specific features, the rationale for the selection of a specific virus and a helper or a packaging cell should be described; the structural features of the viral vector and helper should be explained.

As for the biological features of the viral vector, descriptions are required, for example, into what types of cells is gene transfer possible using a viral vector; species specificity and tissue specificity of gene transfer; and the possibility of gene transfer to resting cells. In addition, information regarding the transfection efficiency and expression efficiency of the gene, the existence state (potential for integration) of the transgene and its stability should be provided. When the transgene is integrated into a chromosome, whether the integration locus is specific or unspecific should be clarified.

The following items should be included concerning the manufacturing methods for virus vectors.

- 1) The complete manufacturing process of viral vectors should be provided on the basis of data from the construction of transfected DNA or RNA to the production of viral vectors.
- 2) The purification method should be described.
- 3) In the case of scaling up for manufacturing, a detailed description should be provided with suitable validation data.
- 4) When a packaging cell is used, the manufacturing procedure, selection and identification method, and cloning history until the seed cell clone is established, should be described. For MCB and WCB of the packaging cells, the methods of preparation and storage, control and renewal of MCB and WCB, as well as their characterization, should be provided. Stability of DNA or RNA transferred into the packaging cells is also described.
- 5) The testing method(s) and the results to confirm that cell characteristics such as phenotype have not been changed during the cultivation period should be documented. The constancy of the cell characteristics between the lots should also be demonstrated.
- 6) The period, method(s) and results of the safety test necessary for quality control of the viral vector, including a replication competent virus, should be provided.

In the case of viral vectors, it is expected a cell banking system will be used for, 1) cells

used to produce viral vectors (DNA or RNA transferred into humans), a gene of interest, plasmid and other DNA used for constructing viral vectors, and the cells used for producing viruses, 2) cells used for the packaging of a viral vector, 3) packaging cells and the viral vector producing cells. When cell banking systems are used, the preparation, storage, controlling, and renewal methods, etc. should be described in detail in the paragraph on the manufacture of each substance and each cell. With regard to cells used for packaging and packaging cells, the procedures for freezing and thawing, an identity test after thawing and after cultivation, and the freezing period should be also clarified. The concept and the requirements of the cell banking system are the same as the cell banking system used for the manufacture of biotechnology-derived products.

## **2.2 Non-Viral Vectors**

In the case of gene transfer by non-viral vectors, at least the following data and information should be provided

- 1) Theoretical and experimental rationale for selection of non-viral vectors as the gene transfer method.
- 2) Transferred DNA or RNA.
- 3) Manufacturing procedure, structure, and characteristics of other DNAs.
- 4) The manufacturing procedures and purification methods of non-viral vectors.
- 5) Analysis of structure and composition of non-viral vectors.
- 6) Biological features of non-viral vectors.
- 7) Cell banking system.

The rationale and experimental data for the selection of a specific non-viral vector as a specific type of gene transfer should be described, including an explanation of the structural features of the non-viral vector.

Information concerning the manufacturing procedures and purification methods of non-viral vectors such as methods for controlling non-viral vectors, all constitutive components (proteins, carbohydrates, lipids, etc.) of the vector, their derivation (origin, source), preparation method, purification method, quality and any other relevant information should also be described in detail. When the materials of the vector are derived from biological sources, potential contamination of infectious microorganisms should be tested in advance.

As for the analysis of structure and composition of non-viral vectors, (1) the structure and composition of a vector, and (2) the structure or composition of each constitutive component (proteins, carbohydrates, lipids, etc.) of a vector before and after manufacturing of the vector should be elucidated. In addition, (3) when a new lot of each constitutive component is used, the consistency between lots should be verified. For example, where a recombinant protein or a monoclonal antibody is a part of the constitutive component of the vector, documents describing the following items are required: establishment of a seed cell clone for the production of the target protein; preparation, storage, control and renewal method of a cell bank; cell culture method for the protein production; purification method, structure and composition analysis, characterization, specifications, test method, and stability during storage of the target protein; etc.

### **2.3 Direct Transfer of DNA or RNA**

In the case of direct transfer of DNA or RNA, the most critical concern is how to manipulate the naked nucleic acid so as to introduce it into target cells or humans. Thus, full details of the gene transfer procedure, reagent to be used, apparatuses and any other elements in practical use should be clarified. Another concern is the biological features of the naked nucleic acid introduced by the direct transfer method. The types of cells in which gene transfers are conducted by direct transfer (that is, species specificity and tissue specificity of gene transfer; and possibility of gene transfer to resting cells) should be demonstrated. Information regarding the efficiency of transfer and expression efficiency of the gene, the existence state of the transgene and its stability should be provided. If the transgene is integrated into a chromosome, whether the integration locus is specific or non-specific should be clarified.

In addition to the two concerns mentioned above, information concerning the rationale for the gene transfer methods, transferred DNA or RNA (manufacturing method, structural analysis and characterization of transferred DNA or RNA, DNA sequences, structure and biological activity of products expressed from transgene, expression mechanism of transgene and its regulation), and the cell bank system, should be provided as is the case with the other gene transfer methods.

### **2.4 Classification by Administration Methods**

The administration methods of the gene therapy product should be appropriately designed and justified taking into account various factors such as the nature of the target diseases, the treatment strategy, the biological features of the target cells, and technical applicability.

In the case of *ex vivo* methods, the following items should be described.

- 1) Source of target cells, biological features, and rationale for selecting the cells as the target.
- 2) Donor selection criteria.
- 3) Cell culture procedures (procedures for the collection of cells, cell culture methods, gene transfer method, culture period, prevention of microbiological contamination, etc.).
- 4) Acceptance criteria of transduced cells including tests for the amount of residual reagent, sterility test, mycoplasma test, test for replication competent virus, etc..
- 5) Method of administration of transduced cells to the patients (method for transplantation of the cells, frequency and interval of administration).

In the case of *in vivo* methods, the following items should be described.

- 1) Biological features of the target cell and rationale for selecting the cell as a target.
- 2) Administration method (gene transfer method, dosage, frequency of administration, interval etc.).
- 3) Possibility of transfer of genes into non-target cells (especially germline cells).

### **3. Specifications and Formulation**

In order to assure the quality of gene therapy products, validation of the production process and the quality control of the raw materials as well as the setting of specifications of the final products should be performed appropriately. In particular, purity tests for raw materials, contaminants, process-related impurities, product-related impurities, should be set, and freedom from bacteria, adventitious viruses, mycoplasmas, fungi and endotoxin should be

described. If there are special formulations of the drugs for gene therapy, the appropriateness of such formulations should be explained rationally. Procedures for lot-to-lot production control of bulk and final product should be provided. Examples of specifications and test methods are as follows: 1) properties, 2) the suitable identification tests (physicochemical tests, biological tests, immunochemical tests), 3) a limiting test for impurities and toxic contaminant denial test, 4) the functions of expression products of transgenes or related products, where possible, the potency or quantitative biological activity, and 5) cell viability in the case living cells should be included. It is important to set the most suitable test items for each product.

#### **4. Stability of the Gene Therapy Products**

For the stability of the gene therapy products, taking into consideration the distribution and usage period, appropriate stability tests should be performed, and the storage conditions and expiration period should be set on the bulk and final products. The storage in other conditions prescribed or beyond the expiration period should be examined to define the limits of their stability. The rationale for the appropriateness of the lot number used in each test should be provided. When carrying out the stability tests, the relevant guidelines should be consulted where necessary and appropriate.

#### **5. Preclinical Safety Studies of the Gene Therapy Products**

As for the preclinical safety studies of the gene therapy products, the appropriate tests on the products using suitable animals and/or *in vitro* systems should be performed. The safety tests should reflect the routes of administration of the products in humans. In particular, the following items should be considered.

- 1) Potential for appearance of a replication competent virus.
- 2) Cytotoxicity (the possibility that the products cause damage to cells and/or tissues).
- 3) Genetic integration to chromosomes (the number of copies per cell, whether the integration locus is specified or not, in case transgenes are integrated into a chromosome. Information about whether activation/inactivation of intracellular genes and their variation by genetic insertion have ever been recognized in experiments shall be described).
- 4) Abnormal expression of the transgenes (the safety range of the products expressed from the transgenes).
- 5) Carcinogenicity (the possibility of a change in the proliferation potency of genetically modified cells, tumor formation and malignant alteration).
- 6) Immunogenicity (the possibility that the components of the products, products expressed from transgenes and/or transduced cells may cause undesirable immunological reactions).
- 7) General preclinical toxicity tests, when appropriate and possible.

#### **6. Tests for Effectiveness of the Gene Therapy Products**

As for the tests of effectiveness, the following examinations are required.

- 1) To examine the efficiency of gene transfer, structure and stability of the transgenes, the expression efficiency from transgenes and their stability, biological activities of the expression products, intended effects on cells, tissues and individuals, etc. should be examined by appropriately designed tests using cultured cells and experimental animals

- 2) When appropriate model animals for the intended disease are available, the therapeutic effects using them should be examined.

### **7. Pharmacokinetics and Pharmacodynamics of Gene Therapy Products**

For the pharmacokinetics and pharmacodynamics of gene therapy products, 1) it is necessary to estimate the life span of genetically modified cells in humans and explain that the intended effects can be accomplished sufficiently, through tests on the *in vivo* kinetics including absorption, distribution, etc. of gene therapy products or transduced cells in test animals, 2) especially, in the case where gene therapy products or genetically modified cells are targeted to reach a specific site (tissues, etc.), their localization should be sufficiently explained.

In performing pharmacokinetic tests, refer to the attached papers of the "Guideline for Pharmacokinetic Tests" of the "Guideline for Pharmacokinetic Tests Required for Application for Approval of Manufacturing (Import) of New Drugs" (New Drug Division Notification No.6 of January 29, 1991).

### **8. Summary of Non-clinical Test Results and Outline of Clinical Studies on Gene Therapy**

Data from the manufacturing process to pharmacokinetics and pharmacodynamics described so far, should be summarized; the safety of gene therapy products is adequately guaranteed based on current knowledge, and conducting the clinical studies is justified from the viewpoints of quality, safety, and expected efficacy.

For the outline of clinical studies, the following information should be described.

- 1) Current knowledge about the diseases selected as indications.
- 2) Plan for the clinical study on gene therapy.
- 3) Justification of conducting gene therapy clinical research. (the mechanism of action of the gene therapy product should be clarified. The justification and validity of conducting gene therapy in comparison with existing therapeutic methods should be described.)
- 4) Suitability of the facility and organization with respect to performing gene therapy clinical research.
- 5) Patient selection and exclusion criteria.
- 6) Method of obtaining patient consent.
- 7) The number of patients required for clinical evaluation and the terms of clinical research, and their rationales.
- 8) Methods used in the gene therapy clinical research.
- 9) Schedule for follow-up of patients.
- 10) Potential for gene transfer to persons who are not patients.

### **9. Manufacturing Facilities and Equipment**

The manufacturing area for gene therapy products should be separated from other areas and provided with a well-arranged culture apparatus. Facilities for the analysis and testing of the physicochemical, biological and immunological properties of the recombinant and gene therapy products handled should be provided. There should be facilities for the storage of genetically modified cells, the preparation of culture media, the washing and sterilization of equipment, utensils, containers, and other items used in the manufacturing process, and dressing facilities for manufacturing personnel.

## **10. Miscellaneous Provisions**

In order to assure the quality and safety of a gene therapy product, the manufacturer and/or importer of the gene therapy product may ask the Minister of Health and Welfare whether the product concerned conforms to the Guidelines. The manufacturer and/or importer of the gene therapy product should collect information about gene therapy, and in cases where there is some information which might influence the evaluation of the gene therapy product in question, the manufacturer and/or importer should immediately submit a report on that information to the Minister of Health and Welfare.