Guideline on Nonclinical Assessment of Gene Therapy Products

[for industry]

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

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

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Cell and Gene Therapy Products Division
National Institute of Food and Drug Safety Evaluation (NIFDS)

Tel : +82-43-719-3538
Fax : +82-43-719-3530

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1. Background

Gene therapy products (GTPs) require submission of preclinical data for investigational New Drug Applications (INDs) as specified in Article 5 and Annex 1 of *Regulation on IND Approval of Medicinal Products*, and for marketing authorization as stated in Article 6 and Annex 3 of *Regulation on Review and Authorization of Biological Products*.

However, since GTPs, unlike other biological products, present unique safety issues associated with their inherent properties, such as potential for integration of viral genomes into the host DNA, replication potential of viral vectors, etc., this guideline is published for the purpose of providing detailed evaluation principles applicable for the preparation of nonclinical data specifically for GTPs.

2. Introduction

Article 2-15 of *Regulation on Review and Authorization of Biological Products* defines a GTP as 'a genetic material administered into the human body to affect gene expression' or 'cells that are genetically modified or transduced with a genetic material'; in other words, GTPs are divided into 1) *in vivo* GTPs, a direct method of transferring a genetic material into the human body, and 2) *ex vivo* GTPs, an approach of administering cells that are genetically modified *in vitro* into the human body. In general, vectors used for delivery of genes can be classified into 3 types as follows:

- Viral vector (i.e., retrovirus, adenovirus, adeno-associated virus (AAV), vaccinia virus, herpes virus, etc.)
- DNA vector (i.e., plasmid DNA, chromosome-based vector such as transposon, etc.)
- Bacterial vector (i.e., modified *Lactococcus sp.*), *Listeria sp.*, *Streptococcus sp.*, etc.

The type of vector that is most commonly used for developing *in vivo* or *ex vivo* GTPs is viral vector, and it is important to obtain a sufficient understanding of the characteristics of each viral vector since properties unique to each vector warrant different preclinical considerations. Additionally, for *ex vivo* GTPs, characteristics expected not only from GTPs but also from cell therapy products (CTPs) should be considered.

As an overview of this guideline, the document is intended to provide general considerations and science-driven evaluation principles for nonclinical studies, such as toxicity studies, pharmacology studies, etc., and vector-specific considerations in the Appendix.

3. Scope

This guideline is applied to products that meet the definition of GTPs in Article 2 of *Regulation on Review and Authorization of Biological Products*.

4. Considerations for Preclinical Evaluation of Gene Therapy Products

GTPs display unique characteristics that stem from their structural components such as a vector, etc. As characterization of GTPs should be considered as part of not only nonclinical studies but also quality evaluation or clinical studies, developers should have a thorough understanding of the characteristics of their products in development (especially safety-related issues), and be able to submit relevant data.

Please, refer to Appendix at the end of the document for detailed special considerations based on the structure of GTPs.

Biological effects of GTPs are mostly conferred by delivery system, transgene, and transgene product. Therefore, in principle, nonclinical studies should include assessment of not only the delivery system/vector used for the GTP but also the gene introduced for a therapeutic purpose.

Test results obtained using an analogous product may be used as a reference for designing appropriate nonclinical/clinical studies, but generally such data are deemed insufficient to be accepted as definitive study data to permit the initiation of a clinical trial.

Appropriateness of nonclinical animal models should be explained considering exploration of pharmacological effects and therapeutic functions of the genes expressed. The pharmacological effects anticipated in patients should be able to be assessed in the selected animal model to the extent possible.

The following should be considered in nonclinical studies:

- Efficacy in the preclinical model (proof of concept)
- Biodistribution
- Starting dose and dose escalation scheme for clinical studies
- Identification of potential target organs in toxicity studies
- Identification of potential target organs that display biological activity
- Identification of target markers and specific target patients for clinical studies

5. Toxicology Data

According to Annex 3 of *Regulation on Review and Authorization of Biological Products*, toxicity data that must be submitted are single-dose and/or repeated-dose toxicity data unless otherwise justified scientifically. For other toxicity studies, characteristics of the GTP will determine the necessity and scope of data submission, and therefore developers should obtain thorough scientific knowledge of the properties of their products under study thereby securing validity of the conduct of selected studies.

5.1 Single-dose/Repeated-dose Toxicity Studies

Animal models chosen for toxicity studies should display biological responses that mimic those expected in humans induced by the investigational GTP, so appropriate animal models that can provide relevant information for designing a clinical trial should be used. Toxicity assessment for GTPs can possibly be conducted in one animal species, but more than one animal species may be required for safety evaluation of other affecting factors (i.e., characteristics of the GTP, intended clinical route of administration).

For the selection of appropriate animal species, the following should be considered:

1. Assessment of permissiveness/susceptibility to infection by, and replication of, viral vectors in various animal species
2. Pharmacological response of the animal species to the transgene product expressed by the GTP
3. Sensitivity of the animal species to the biological actions of the *ex vivo* genetically modified cells

If a viral vector is used, sensitivity of the animal species to its parental virus should be taken into account. Since standard animal species in common use may not be appropriate, known specialized animal species can be considered for use. Although it is ideal to adopt an animal model that is sensitive not only to viral infection but also to pathological outcomes that may occur in humans with respect to the virus, 'humanized' rodents can be used that express human target receptors via genetic modification or cell/tissue transplantation. For the safety assessment of genetically modified human cells, immunodeficient animals have been used.

If the expressed transgene is found biologically inactive in the selected animal species, a vector expressing a homologous transgene that will show biological activity in the test species can be used. In this case, comparability of the intended investigational GTP to the product containing the homologous transgene should be documented (i.e., sequence, target specificity, expression level, etc.).

It is recommended that appropriate parameters and biomarkers be used for the assessment of potential toxicity of the transgene product in animal models. For toxicity

assessment, comprehensive consideration is required on the entire components of the GTP (vector/delivery system, transgene, etc.). Transgene product is often overlooked, so toxicity assessment should be conducted to investigate overexpression of the transgene product, and/or possible results from immunogenicity or unwanted pharmacological effects.

Purity of raw materials should also be considered. If quality test results indicate that generation of aberrant gene products is expected, toxicity assessment may be required to address this concern.

In vivo effects exerted by proteins associated with the expression vector but unrelated to the intended therapeutic activities (i.e., antibiotic resistance gene in the plasmid, viral protein expressed in the vector, etc.) should also be evaluated.

5.2 Genotoxicity Studies

If there is a possibility of genetic modification or chromosomal insertion through direct interaction with DNA or chromosomal elements, genotoxicity studies should be considered. While *Regulation on Review and Authorization of Biological Products* does not require conventional toxicity studies, they may be warranted for the assessment of toxicity of possible unusual impurities or a new delivery vehicle that is contained in the drug product.

Insertional mutagenesis and subsequent oncogenicity should be assessed in relevant *in vitro/in vivo* models, and integration of the vector DNA into the host genome, detection/expression of the viral gene outside the target tissue or organ, persistence of the expression should be studied. Even for GTPs that are not intended for integration into the host genome, data from *in vivo* or *in vitro* studies may still be required for detection of viral vector integration to rule out any possible safety concerns. When the expression of a therapeutic gene is lasting over a prolonged period of time, persistence of the gene and the potential for the gene/vector to integrate into the host genome should be carefully investigated. For example, for plasmid DNA, if assessment of *in vivo* persistence in biodistribution studies shows that the plasmid DNA measured at the site where its maximum amount was detected (i.e., administration site) exceeds 30,000 copies/1μg host DNA at 60 days post-administration and onwards, genotoxicity studies should be considered. If integration is confirmed, copy number determination, integration site identification, and monitoring of subsequent biological toxicity and changes in cell behavior are necessary. Depending on the characteristics of the vector used, extended *in vitro* and *in vivo* studies to further investigate insertional oncogenesis may be required prior to first administration in humans.

Genotoxicity studies can be undertaken following the 3-step approach as described below:

1. Investigate occurrence of genomic modification and detect subsequent abnormal cell behavior
2. Evaluate toxicity arising from insertional mutagenesis and investigate the mechanism that gives rise to the toxicity.
3. Identify genomic integration sites and evaluate possible cross-talk between the transgene and neighboring sequences.

5.3 Oncogenicity Studies (Tumorigenicity Studies)

Vectors used in GTPs, or resultant transgene products have the potential to induce tumor formation. For example, in case where the vector/gene enters cells that are susceptible to viral infection/replication, this insertion may activate a proto-oncogene in the host cells, or diminish the activation of a tumor suppressor gene, which causes changes in cell morphology, and, in turn, results in tumor formation. Integration of vector/gene may possibly be triggered by ① random chromosomal integration, ② recombination events that may occur when the endogenous sequence in the host chromosome and the sequence delivered by the vector are similar, or ③ direct insertion mechanism of retrovirus.

To determine whether tumorigenicity studies are necessary, the following should be considered: mechanism of action of the GTP (i.e., mechanism related to growth factor), potential for vector integration into the host genome, histopathology results from repeated-dose toxicity studies that indicate tumorigenic potential, immunosuppressive activity that can be a contributor to tumorigenicity, and abnormal hormonal regulation, etc. In addition, if the vector displays extensive sequence homology with human genome, or if any sequence with oncogenic potential is present in the vector, tumorigenicity studies should be considered. If the GTP shows a very high level of insertional activity, or if it is used to treat a non-life threatening condition for a prolonged period, tumorigenicity studies may also be considered.

If tumorigenic potential is detected, it may be more appropriate to conduct studies using a suitable *in vivo*/*in vitro* model based on the characteristics of the GTP, rather than conventional oncogenicity studies using rodents. *In vitro* studies may include analyses on proliferation competence, dependence on external stimuli, response to apoptotic stimuli, and genetic alteration, etc. For detailed information on *in vivo* tumorigenicity studies, refer to *Considerations in Tumorigenicity Assessment of Stem Cell Therapy Products*.

For GTPs developed as oncology therapeutics, oncogenicity data may not be required as outlined in *ICH Guideline S9*, but submission of these data should be taken into consideration if long-term survival of treated patients is expected or if the indication of the GTP is expanded beyond cancer.

5.4 Developmental and Reproductive Toxicity Studies

Necessity of developmental and reproductive toxicity studies may be determined by data on the expression of the GTP, or its persistence, in gonads and reproductive organs. If the GTP persists in the reproductive organs beyond a certain period of time, where it begins to affect gene expression, alteration may occur in gametes. Therefore, the possibility of gamete distribution of the GTP or its integration and/or subsequent expression therein should be investigated. Presence and persistence of the GTP can be assessed in biodistribution studies.

If the GTP contains a vector with integration capacity, or if there is evidence showing that persistent distribution of the vector/gene increases possibility of integration and that it can be distributed in the reproductive organs, then additional tests, such as investigation on sperm and ova or testing on possible impacts on fertility potential and general reproductive potential, may be required. If the target population includes women of reproductive potential, who are to be exposed to the GTP, fetus-embryo and prenatal developmental toxicity tests may be required. However, requirement for developmental and reproductive toxicity data can possibly be waived for first in human studies, if justification is provided, that is, consent to use contraceptives given by a patient group with a life-threatening condition.

5.5 Immunotoxicity (Immunogenicity) Studies

For GTPs intended to express growth factors, cytokines, or proteins known to have an impact on the immune system, immunotoxicity (immunogenicity) assessment should be considered, including humoral or cell-mediated immunity.

Especially when quality data show occurrence of anomalies detected in gene expression, immunogenicity of the transgene product should be studied. Also, testing is required on vector/gene immunogenicty following repeated dosing of the viral vector.

For certain GTPs, inherent differences between animal studies and human clinical applications may render immunotoxicity (immunogenicity) findings from animal studies inappropriate to be extrapolated to humans. Under these circumstances, a more deliberate study should be designed (i.e., a test using an animal homolog of the human gene).

6. Pharmacology Data

6.1 Efficacy Studies

Efficacy studies are critical to generating nonclinical evidence to support purported clinical effects or relevant biological effects, and the selected mechanism of action. The

outcomes of efficacy studies may be used as the grounds for 1) identifying the dose range of pharmacological activity by establishing the optimal dose and minimum effective dose, 2) determining the potential optimal route of administration for the GTP, and 3) identifying the dosing regimen for early stage clinical studies.

For this, *in vivo* and *in vitro* studies can be conducted, and if the goal is to demonstrate the mechanism of action via *in vivo* testing, it is necessary to use suitable animal species for the analysis. Relevant animal species will be those that will display the expected level of gene expression and functional activity by the transgene sequence acting on the target gene. Accurate expression of the transgene should also be explained. If the quality data of the GTP indicate the possibility of aberrant gene expression, biological consequences arising from the aberrant gene product should be evaluated.

6.2 Biodistribution Studies

Characterization of vector biodistribution is one of the most important components in nonclinical studies of GTPs. Biodistribution data are used to determine the potential presence of the vector/gene and the resulting gene product in the target organs, non-target organs, and the reproductive system. Data on the presence, persistence, and clearance profile of the vector will provide information essential in determining dosing schedule, observation timeline, and animal sacrifice time points. Especially when biodistribution studies are conducted in parallel with evaluation of other nonclinical safety endpoints, such as histopathology, the data obtained may be utilized to determine whether vector presence or gene expression in animals are correlated with tissue-specific detrimental effects.

Biodistribution should be evaluated in all organs regardless of their target organ status (i.e., blood, heart, brain, liver, kidney, bone marrow, ovary/testis, mesenteric lymphatic vessels, spleen, adrenal gland, and tissues in injection site, etc.), including persistence and mobilization of the GTP, and, if applicable, results of viral shedding. The duration of the studies should be long enough to evaluate transgene expression and persistence of its activity, with a proper design that will enable determination of time points for the detection of the maximum level of the vector/gene, and its loss or persistence, in order to characterize pharmacodynamics of the vector/gene biodistribution and persistence. If toxicity is detected in non-target organs or if evaluation/proof of concept is needed for the vector/gene design, the transgene product should also be analyzed. Dosage should be within an appropriate range, using the planned clinical dose or maximum tolerated dose. If biodistribution studies are conducted using animal models different from those used in toxicity studies, appropriate safety endpoints should be evaluated in distribution studies, including clinical observation, body weight, and histopathology, to measure potential association between vector/gene presence and adverse reaction. Please refer to *Guideline on Considerations in Validation of Analytical Methods for*

Biodistribution Studies of Gene Therapy Products Using qPCR for harvest and analysis of tissues for biodistribution studies.

As techniques for PCR assay are constantly evolving, we recommend that developers reach out to relevant MFDS staff for consultation on alternative test methods. The test method intended to use should be validated, and the detection limit should be set at $\geq$ 100 copies/1μg host DNA for therapeutic DNA vaccines, and, if applicable, $\geq$50 copies/1μg host DNA for other products using a gene/vector.

- **Viral Shedding**

Viral shedding may be evaluated as part of toxicity or biodistribution studies in nonclinical studies. Viral shedding that should be measured in biodistribution studies is defined as dissemination of vector/virus through secretions or excreta. Especially if oncolytic virus or replication competent virus is used, assessment of viral shedding should be given significant weight, and shedding studies should be of a duration long enough to detect the second viremia following the primary replication of the viral vector. The types of samples to be collected should be chosen based on vector characteristics, intended route of administration, and animal models to be used; generally, urine and stool are included, and salivary, oral, nasal, and tracheal specimens, etc. may also be considered.

For oncolytic virus, conduct of shedding studies can be determined based on its biological properties, origin, and genetic factors. Shedding studies may be necessary if any of the following is applied:

- Vectors without prior human exposure to them (i.e., a virus that does not infect humans)
- Vectors previously administered to humans but whose parent virus is modified to develop a different *in vivo* tropism
- Vectors previously administered to humans, for which significant changes in the ROA are expected
- Vectors without prior human exposure to them, but with an ROA different from their natural route of exposure/infection

[Appendix] Vector-Specific Considerations for Gene Therapy Products

1. Plasmid

If plasmid DNA is used for a GTP, detailed considerations can be found in *Guideline for Assuring Quality and Preclinical Evaluation of Therapeutic DNA Vaccines* (2015).

2. Viral Vector

For viral vectors designed to be fully replication competent or conditionally replication competent, it should be investigated whether they perform their intended role(s) in target and non-target organs and cells. If a replicating oncolytic vector is designed for a GTP, considerations should be given to the potential for viral infection and replication in normal cells, and for transmission and replication when it is administered to immunosuppressed patients or used in concomitant treatment with radiation therapy, chemotherapy, and precursor materials.

For vectors designed to be replication incompetent, it may be necessary to investigate the potential for inadvertent restoration of replication competence through complementation by wild-type viruses. The possibility of viral vectors displaying virulence by permanently regaining replication competence through recombination process should be studied.

2.1 Adenovirus

For adenovirus vectors, attention should be given to the potential to elicit immune response and inflammatory response. For replicating adenovirus, in particular, as standard test animals commonly used for nonclinical studies may not be relevant, specialized species need to be considered depending on their responsiveness/susceptibility, such as cotton rats or Syrian hamsters. If *in vivo* replication does not occur in the selected animal species, repeated dosing can induce circumstances where replication occurs. If non-replicating adenovirus vector is designed, potential for adverse reaction resulting from contamination with replicating adenovirus should be taken into account.

2.2 Adeno-associated Virus (AAV)

AAV remains episomal in infected cells, but its random integration into the host chromosome may lead to insertional mutation, which, in turn, induces unexpected biological consequences. In addition, the possibility of AAV capsid proteins triggering immune response should be considered in designing nonclinical studies. With regard to the potential of AAV vector integration, germline transmission, genotoxicity, and tumorigenicity should be explained. For products based on AAV vector, long-term follow-up of study subjects is recommended for monitoring of delayed long-term adverse events in clinical

trials.

2.3 Retrovirus and Lentivirus

As retroviral and lentiviral vectors are designed to be non-replicating, careful attention should be given to the possibility of inadvertent production of replication-competent vectors. If replication-competent vectors are manufactured as needed, potential for insertional mutagenesis and oncogene activation, germline integration, and aberrant expression of host genes should be studied. With regard to the potential for viral integration, germline transmission, genotoxicity, and tumorigenicity should be explained. In general, long-term follow-up of study subjects is recommended for monitoring of delayed long-term adverse events in clinical trials.

2.4 Pox Virus

As pox virus is able to replicate in many different types of human tissues and cells, studies should be designed in consideration of patients with compromised immunity, such as cancer patients.

2.5 Herpesvirus

Herpesvirus exhibits tissue tropism, and can be reactivated after the latent phase. For GTPs with herpes virus vector, long-term follow-up of study subjects is generally recommended for monitoring of delayed long-term adverse events in clinical trials.

3. Genetically Modified Cells

Biodistribution, cell migration, and persistence (or life-span) and expression of the transgene should be investigated as part of efficacy and safety assessment. Depending on circumstances, effects on cellular phenotype, including differentiation or proliferation, or immune response potentially induced by modified cells should also be examined.

3.1 Release of *In Vivo* Transfer Vector

The possibility of vector/plasmid release by genetically modified cells should be studied regardless of whether or not the cells are designed as intended. Potential for interactions with infectious agents or disease-related drugs may be assessed, if applicable. The extent of these studies will vary depending on the vector/plasmid used to transduce cells, its replication capacity, and integration status in the cells. Dissemination of vectors to various tissues and organs (gonads, in particular), and their release into the environment should be investigated, and infectivity, persistence and activity of the disseminated

agents should also be identified.

3.2 Induced Cellular Transformation

It is necessary to assess the effects of the cellular phenotype transformed in efficacy studies on cellular morphology, phenotype, function and behavior (i.e., proliferation, differentiation, immortalization or induction). Serious consideration should be given to any unintended or unexpected changes that may occur following vector transfer in comparison with unmodified cells. In addition, the degree of transgene expression and the quality of the transgene product should be evaluated.

When cells with replication potential (i.e., progenitor cells) are transduced with integrating vectors (i.e., retroviral or lentiviral vector), the copy number of the integrated genetic material should be investigated, which should be discussed in association with clinical use. The integration sites should be classified based on adjacent genes and their functions, if applicable. The biggest concern will be activation of oncogenes or inactivation of tumor-suppressing genes. The impact of copy number in single cells should also be evaluated in view of quality requirements, that is, consistency. In addition, the possibility of reactivation of latent viruses (i.e., herpes zoster, Epstein-Barr virus, and cytomegalovirus), which can result in the production of infectious viruses, may also be investigated depending on the types of vectors and cells used.

4. Genetically Modified Microbial Vector

GTPs may target immune-compromised or suppressed patients. So, for a GTP containing microbial vector, the level of attenuation and the stability of the attenuated phenotype should be evaluated. Besides, the potential for vector DNA replication in non-target organs and excessive induction of proinflammatory cytokines should be considered while antibiotic sensitivity testing is necessary to examine bacterial susceptibility of the product to various antibiotics

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