Administrative Notice
November 25, 2025

To: Pharmaceutical Affairs Section,
Prefectural Health Department (Bureau)

Medical Device Evaluation Division,
Pharmaceutical Safety Bureau,
Ministry of Health, Labour and Welfare

Evaluation for shedding associated with gene therapy products using viruses/vectors

For evaluation strategies for shedding of viruses/vectors used in gene therapy products from the treated living bodies, our basic concept has been presented in the "ICH Considerations 'General Principles to Address Virus and Vector Shedding'" (Administrative Notice dated June 23, 2015, by the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau and Counsellor to the Minister's Secretariat in charge of review management of medical devices and regenerative medical products, Ministry of Health, Labour and Welfare, hereinafter referred to as "ICH Considerations").

To present more specific considerations and principles for evaluation for shedding associated with gene therapy products using viruses/vectors based on the latest scientific knowledge, the document provided in the attachment has been compiled by the Project of Research on Regulatory Science of Pharmaceuticals and Medical Devices of the Japan Agency for Medical Research and Development (AMED) conducted between fiscal years 2022 and 2024 "Development of Quality, Efficacy, and Safety Evaluation Systems for AAV Vector-derived Gene Therapy Products using Patient Specimens in *In Vivo* Gene Therapy" (Research and Development Representative: Masafumi Onodera, Director of Gene & Cell Therapy Promotion Center, National Center for Child Health and Development). Please inform the relevant business operators in your jurisdiction thoroughly to ensure that in addition to the ICH Considerations, this administrative notice is consulted to evaluate shedding associated with the concerned products. The presented evaluation strategies only represent the strategies potentially applicable at present and are not necessarily required to be implemented. Please additionally inform them that for selection of the evaluation strategy, etc., they should consult with the Pharmaceuticals and Medical Devices Agency where necessary.

Attachment

Evaluation for shedding associated with gene therapy products using viruses/vectors

(Onodera Group, AMED)

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1.0 Objective of this document

The objective of this document is to present considerations given to preparation of protocols for shedding studies in nonclinical and clinical studies using virus-derived gene therapy products as well as principles applied to risk assessment for shedding based on results from the shedding studies. This document also provides examples of analytical methods for shedding studies and presents considerations given according to characteristics of the product. The principles for evaluation for shedding associated with gene therapy products presented herein are expected to allow estimation and assessment of a risk of transmission to third parties and potential impacts on public health. Furthermore, information on shedding associated with the products collected according to this document is expected to be utilized in pharmacovigilance activities in post-marketing clinical use and development of the post-marketing information collection plan. The presented evaluation strategies are not necessarily all assumed to be implemented only by developers and marketing authorization holders and thus may be used as references in research to evaluate shedding profiles conducted by investigators solely or jointly with marketing authorization holders.

1.1 Background

In recent years, gene therapy products have been actively developed, and several products have been approved for marketing in Japan. In addition, gene therapy products under development are diverse in type, and not only recombinant viruses for gene delivery but also wild-type and recombinant oncolytic viruses have undergone many clinical studies (trials). These wild-type viruses or recombinant viruses (hereinafter referred to as “viruses/vectors”) may be shed from the treated patients, posing a risk of transmission to third parties. In addition, if the transmission from humans to humans occurs and adversely affects health of third parties, etc. around the patient, it should be regarded as a serious public health problem. In this document, of risks related to shedding of viruses/vectors, which are used as gene therapy products, a “transmission risk” and a “third party risk” are separately defined. The transmission risk represents a risk of transmission of viruses/vectors to third parties around the patient mediated by discharges or excrements. In basic principles, unintended transmission of the viruses/vectors to third parties should be avoided. On the other hand, the third party risk refers to undesirable effects including diseases that occur when viruses/vectors are transmitted from the patient to third parties. The third party risk is defined in view of the risk of causing public health hazards as a consequence of transmission of viruses/vectors to third parties, who can further spread them across society.[1] In particular, the third party risk greatly varies in its impact magnitude, depending on the health status of the third parties potentially exposed to transmitted viruses/vectors, such as pregnant women and immunocompromised patients. Mitigation of the third party risk is considered critical. The reduced transmission risk can mitigate the undesirable effects on health of third parties and the public health risk.

Because the third party risk largely depends on characteristics of the viruses/vectors, which are supposed to be distributed to medical institutions across Japan as products, viruses/vectors with a low third party risk should be used if possible. If modification of the virus/vector characteristics relevant to the transmission capability is not possible, strict control of the transmission risk should be applied in general to mitigate the third party risk that emerged as a consequence of the transmission.

The shedding study approaches presented in this document can be applied to assessment of both transmission risk and third party risk associated with the shed viruses/vectors.

[1] Potential consequences of the third party risk may include extensive vertical and horizontal transmission occurring after transmission from the treated patient to household contacts as well as disadvantages such as a loss of opportunity of treatment using the concerned viruses/vectors because of presence of antibodies against them produced after the exposure.

1.2 Scope

This document is applied to the gene therapy products that use viruses/vectors as the main component, but not ones that use nucleic acids, including plasmid DNA and mRNA, as the main component or preventive vaccines against infections. Cell processed products used for *ex vivo* gene therapy are not included in the scope of this document either. Principles described in this document, however, may be applicable to some of these products.

In addition, this document is not intended to present requirements for protocols of shedding studies to be conducted as a part of the environmental risk assessment defined in the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Act No. 97 of 2003, hereinafter referred to as the "Cartagena Act"). However, results from the study conducted based on this document may be utilized as data in a "Biological Diversity Risk Assessment Report" when it is prepared in accordance with the Cartagena Act.

1.3 General Principles

In development of gene therapy products, generally, behavior of viruses/vectors such as shedding is evaluated by measuring amounts of viruses/vectors excreted or discharged in body fluids (urine, feces, saliva, tears, semen, exudate, etc.) of experimental animals or participants treated with the product in nonclinical or clinical studies. Thus, it should be noted that the shedding studies have a different purpose from that of biodistribution studies that are intended to investigate distribution from the administration site to organs (see ICH S12). Although blood (serum, plasma, etc.) specimens are supposed to be handled in biodistribution studies but not shedding studies in a strict sense, amounts of viruses/vectors in blood are often measured as a part of a shedding study, because for healthcare professionals, family members, home caregivers, and third parties who come into contact with the patients, a risk of exposure to viruses/vectors via the patient's blood cannot be ruled out. In addition, the shedding study does not necessarily have to be conducted independently and is generally conducted as a part of other nonclinical and clinical studies, such as biodistribution studies.

In a shedding study, presence of a specimen positive for viruses/vectors does not directly represent a risk of transmission to third parties. Assessment of the risk of transmission to third parties requires comprehensive considerations on infectivity and amount of shed viruses/vectors as well as a contact possibility of third parties. Furthermore, assessment of the third party risk requires a comprehensive view on hazards of diseases, etc., potentially occurring as a consequence of transmission to third parties coming into contact and a possibility of onward transmission from the exposed third parties to other third parties. In particular, the transmission risk may increase in pregnant women, infants, or immunocompromised individuals such as cancer patients if they are third parties potentially exposed to transmitted viruses/vectors. Thus, the third party risk should be carefully investigated. Findings in the assessment of the transmission risk or third party risk will be used to develop methods of handling gene therapy products and to inform healthcare professionals and patients. In addition, they may be included in the post-marketing risk management plan, depending on the situation.

If the risk assessment suggests a third party risk based on the potential transmission to third parties which can cause diseases in them, actions for strict control of the transmission risk should be taken to minimize the third party risk wherever possible. In this case, presence or absence of transmission may have to be evaluated in individuals who need to come into close contact with the patient (healthcare professionals, household contacts, etc.) in an ongoing or start-ready clinical study. If applicable, consultation with regulatory authorities should be made, including whether the study to evaluate presence or absence of transmission should be conducted.

2.0 Basic matters

2.1 Quality attributes of viruses/vectors to be considered for data on shedding

2.1.1 Quality attributes of viruses/vectors

Information on properties of wild-type viruses, etc. used as the source of viruses/vectors (hereinafter referred to as "parental viruses")[2] is important in designing a shedding study. In addition, key properties to be particularly considered are replication competence, persistence, tropism toward specific cells/tissues, and pathogenicity acquired by viruses/vectors through genetic modification of the parental viruses. For oncolytic viruses derived from wild-type viruses, on the other hand, information about a selection history of the viruses to be used as the main component in their preparation (screening, etc.) is of special importance.

Characterization of viruses/vectors may be corroborated by information on similar recombinant viruses.

1) Replication competence and associated risk assessment

Replication competence of viruses/vectors is a property important in considering shedding and transmission. Replication competent viruses/vectors can propagate in the patient body, thus may extend duration of shedding, potentially reaching the final amount of shed viruses/vectors greater than the dose, and may result in an increased transmission risk. Furthermore, propagation of viruses/vectors in the patient body may raise a concern of potential mutations in the viruses/vectors (including reverse mutations), which could result in shedding of viruses derived from more infectious viruses/vectors. Replication incompetent viruses/vectors, on the other hand, would not propagate in the patient body, and the amount of shed viruses is not expected to exceed the dose. Even replication incompetent viruses/vectors, however, may pose a new transmission risk, for example, in the case where replication competent viruses (RCVs) are generated during the manufacturing process of replication incompetent viruses/vectors and remain in the product. Quality control should be applied to viruses/vectors to minimize generation of RCVs wherever possible.

2) Persistence in the body and associated risk assessment

Persistence of viruses/vectors in the body is an important property to be considered because of its impact on duration of shedding and amount of shed viruses. Viruses/vectors highly persistent in the body may be accompanied by further extended duration of shedding. Persistence is largely determined by replication competence of viruses/vectors described in 1) but substantially affected by properties of parental viruses, particularly immunogenicity. Some replication incompetent viruses/vectors can be more persistent than replication competent viruses/vectors, staying in the body longer. For example, replication competent viruses/vectors derived from adenovirus (AdV), which is highly immunogenic, are generally less persistent than replication incompetent viruses/vectors derived from adeno-associated virus (AAV). However, this does not imply that replication competent viruses/vectors derived from AdV have a lower transmission risk.

For example, viruses/vectors derived from viruses that can stay latent after infection and may be reactivated, such as herpes simplex virus (HSV) known to cause latent infection, may start shedding upon reactivation.

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[2] Parental viruses used as the source of viruses/vectors are not necessarily wild-type viruses from which the viruses/vectors are derived. For example, if viruses/vectors are produced by further modifying recombinant viruses, the original recombinant viruses are also deemed as parental viruses of the concerned viruses/vectors. In addition, when low-pathogenic wild-type viruses obtained from nature are developed as oncolytic viruses, common wild-type viruses of the same species can be regarded as parental viruses in designing a shedding study.

For this reason, to evaluate extended shedding or post-reactivation shedding, an appropriate long-term study plan may be needed.

3) Tropism, route of administration, route of infection, and associated risk assessment

Cell and tissue tropism and route of administration of viruses/vectors may affect shedding profiles and route of infection.

Information on cell and tissue tropism and route of infection of parental viruses is useful. If cell and tissue tropism of viruses/vectors remains unchanged from that of their parental viruses, information on route of infection of the parental viruses would be useful in designing shedding studies. For example, if the parental viruses cause infection via contact with blood, body fluids, etc., a transmission risk via contact with blood, body fluids, etc. should be considered. If the parental viruses cause infection without contact (airborne or droplet infection), the viruses/vectors may cause transmission without contact as well. If such viruses/vectors are shed into the patient's body fluids and essentially share cell and tissue tropism and route of infection with the parental viruses, saliva, nasal discharge, etc., which facilitate infection without contact, are anticipated to have a higher transmission risk than the other routes of shedding.

Viruses/vectors with cell and tissue tropism modified by genetic technique may have biodistribution and shedding profiles different from those of the parental viruses. In addition, it should be noted that administration to the site not expected from the route of infection of the parental viruses may also affect biodistribution and shedding profiles.

4) Pathogenicity and associated risk assessment

In general, wild-type viruses used as the source of viruses/vectors are infectious for humans but often non-pathogenic or low-pathogenic in humans. Viruses/vectors derived from non-pathogenic viruses are less likely to raise concerns than those derived from pathogenic viruses when shed. Still, the third party risk should be assessed comprehensively in view of the other properties of viruses/vectors such as replication competence and target gene in addition to the above.

When vectors are prepared from pathogenic viruses, genetic technique is applied to eliminate or attenuate the pathogenicity. Viruses/vectors genetically modified to eliminate or attenuate the pathogenicity should be tested in the manufacturing process to rule out any reverse mutation at the concerned modified sites. In addition, viruses/vectors with the pathogenicity eliminated by deletion of the replication competence warrant close attention to development of RCVs. Furthermore, for replication competent viruses/vectors with the pathogenicity eliminated or attenuated, assessment of the transmission risk and third party risk should include tests to rule out reversion of the pathogenicity in the patient or third parties exposed to the transmitted viruses/vectors.

5) Development of recombinants with wild-type viruses and associated risk assessment

In the patient coinfected with viruses/vectors and wild-type viruses of the same species, recombinants with wild-type viruses may develop theoretically. If viruses/vectors have biodistribution that does not overlap that of the wild-type viruses and are rapidly eliminated from the body, such recombinants are considered unlikely to develop. However, if viruses/vectors have biodistribution similar to that of the parental viruses and persist long, development of recombinants should be considered after administration. For such developed recombinants, the transmission risk and third party risk should be assessed in view of lifecycle of the parental viruses, administration route of the viruses/vectors, results of biodistribution studies, and persistence in the living body.

2.1.2 Insertion of the target gene or deletion of gene

If viruses/vectors have been genetically modified to include the target gene, expression of the

target gene may affect shedding of viruses/vectors. If viruses/vectors are constructed by deletion of the gene of which the expression product (protein) on the parental viruses causes immune reactions, such deletion may affect shedding. For example, if viruses/vectors have been modified to avoid the immune system, duration of shedding may be extended. Contrarily, if viruses/vectors have the inserted target gene of which the expression product stimulates the immune system, elimination of the viruses/vectors may be accelerated.

Expression of the target gene may cause hazards to third parties around the patient such as diseases, overexpression of the gene, and immunological overreactions. Developers should carefully evaluate functions of the target gene and investigate a third party risk of the transmitted viruses/vectors.

2.2 Analytical procedures

Selection of appropriate analytical procedures is a key to successful shedding studies. Quantitative analytical procedures are the most useful for quantitative assessment of a transmission risk. To detect shed viruses/vectors, molecular biological techniques such as nucleic acid amplification tests (NATs) including quantitative polymerase chain reaction (qPCR) and biological techniques such as infectivity assay are generally used. NAT-based assays are highly sensitive but do not characterize the detected viruses/vectors in terms of infectivity. Infectivity assays, on the other hand, are capable of detecting infectious viruses/vectors but are relatively less sensitive in detection. Although selection of appropriate assays is important, a combination of molecular biological and biological techniques can overcome limitations of each assay in general. In view of the recent advances in science and technology, the above guidance does not intend to exclude selection of appropriate assays using new technologies for detection of viruses/vectors.

Any selected assays should be validated for justification, because specimens in shedding studies contain not only intact viruses/vectors but also fragments and defective particles of the viruses/vectors, and the adopted assays should be characterized in terms of what state of viruses/vectors are detected and demonstrated to have adequate specificity, sensitivity, precision, and reproducibility. If sensitivity of the quantitative analytical procedures is weighed, the detection limit and lower limit of quantitation must be established. For establishment of these limits, qPCR assays require appropriate NAT reference standards, and infectivity assays require appropriate virus/vector reference standards and cell lines highly susceptible to viruses/vectors.

The assay procedure should be established in view of characteristics of specimens. Whether biological substances contained in specimens could interfere with the assay should be evaluated, and depending on the situation, measures to avoid the interference should be considered. For example, the specimens may be diluted before analysis to prevent biological substances from extensively interfering with the assay. Furthermore, means to remove inhibitors may be required if such specimen dilution does not resolve interference caused by biological substances.

In general, NAT results in biodistribution studies are expressed as amounts per microgram of genomic DNA for each tissue, but results on viruses/vectors shed into body fluids are recommended to be expressed as amounts per unit volume, and those in feces are often expressed as amounts per unit weight.

Principles outlined in the ICH Q2 and Q14 guidelines may be informative for appropriate qualification of analytical procedures.

2.2.1 Methodology

1) NAT-based analytical procedures

NAT achieves detection of viral/vector genomes by amplifying specific genomic regions. This procedure is much easier than that of infectivity assays and can be performed quickly and allow simultaneous measurement of many specimens. The concerned NAT is thus adopted as the first step of specimen analysis. To understand shedding of viruses/vectors thoroughly, NAT targeting multiple genome sequences in the viruses/vectors is recommended. For example, if NAT is performed by amplifying the genome sequence only at a single target site, extra detection of non-infectious genomic fragments including the target site would occur, leading to potential overestimation of the amount of shed viruses/vectors. For this reason, to evaluate shedding of viruses/vectors with the intact genome, use of multiplex NAT and droplet digital PCR (ddPCR) capable of amplifying sequences at multiple target sites simultaneously should be considered where possible. Efforts should be exerted to obtain more accurate information.

Although improvement of the NAT technology is constantly occurring, the established NAT-based detection procedure should be provided with high specificity, adequate sensitivity, reproducibility, and quantitative capability for the target viruses/vectors. The main recommendations for validation of these characteristics at present are provided below.

- Detection of nucleic acids by their amplification should be validated using control specimens spiked with the NAT reference standard containing a known amount of virus/vector sequences.
- Sensitivity of NAT for each tissue specimen should be determined using the specimens spiked with the NAT reference standard containing a known amount of virus/vector sequences and documented.
- Selection of amplification regions, primers, and probes for NAT should be justified with the rationales.
- NAT should be performed using multiple samples for each specimen to be analyzed.
- The sample size for NAT should be justified with the rationale.

2) Infectivity assays

In an infectivity assay, specimens of discharge from the patient or animals are added to culture of the cell line highly susceptible to infection *in vitro*, and the concerned culture is analyzed for viral/vector genome to check its presence after infection procedure. For replication incompetent viruses/vectors, cell lines appropriate for properties of the viruses/vectors, such as ones having the gene capable of restoring the replication competence and ones having a reporter gene, which can be expressed in response to infection as a marker gene, should be used. Infectivity is often tested in plaque assays or, if the viruses/vectors exhibit cytopathic effect (CPE), in assays on the basis of 50% Tissue Culture Infectious Dose ($TCID_{50}$ assays). In plaque assays, specimens are added to culture of indicator cells, and then plaques caused by degeneration of infected cells are counted. This assay method is widely used to measure infectivity of viruses/vectors in a specimen. $TCID_{50}$ assays are commonly performed on viruses/vectors that do not form plaques, using CPE of the viruses/vectors as an indicator.

Infectivity assays are required to have adequate sensitivity and reproducibility and should be performed with a sufficient number of specimens. In addition, to ensure sensitivity, appropriate positive controls for the viruses/vectors of interest should be included. Selection of the indicator cells should be justified with the rationale. The indicator cells used for detection of replication incompetent viruses/vectors express the complementing genes that can restore the replication competence.

Infectivity assays have several limitations. For example, because humans may be infected with a wide variety of viruses, whether the detected genome is derived from the target viruses/vectors or from wild-type viruses already existing in the patient owing to previous infection should be

clarified. If the culture exhibits positive signals, assays such as NAT using sequences specific to the target viruses/vectors should be performed to clarify whether the signals are raised by the target ones. Variability of infectivity assays caused by heterogeneity of the indicator cells is also an issue that warrants investigation.

Infectivity assays are commonly used to detect RCVs. That is, infectivity assays can determine whether the signals detected in NAT for replication incompetent viruses/vectors are derived from the viruses/vectors or suggest development of RCVs. For example, AdV vectors, which are replication incompetent viruses/vectors, are added to cultures of both complementary HEK293 cells bearing the E1a region and non-complementary A549 cells lacking the E1a region, and a finding of virus propagation only in HEK293 cells suggests presence of the viruses/vectors and a finding of virus propagation in both HEK293 cells and A549 cells suggests development of RCVs.

2.2.2 Interpretation of data obtained

In some cases, genome was detected by NAT, but the amount of viruses/vectors estimated from the detected genome is less than the detection limit of infectivity assays, making it difficult to determine infectivity of the shed viruses/vectors because of the limitation of analytical sensitivity. In such cases, the shed viruses/vectors should be assumed to have infectivity although the amount is minimal, and a transmission risk should be assessed.

If NAT performed alone without infectivity assays provides a positive result, shedding of potentially infectious viruses/vectors should be assumed. In addition, presence of the transmission risk should be assumed until NAT provides negative results at multiple consecutive timepoints.

Developers should evaluate NAT and infectivity assay results in light of known characteristics of parental viruses (e.g., route of infection, biodistribution, route of shedding, incubation period, reactivation) and take actions to reduce the transmission risk appropriately. In addition, if a potential third party risk causing hazards such as diseases is presumed, the manufacturing process including the structure of viruses/vectors should be thoroughly reviewed, and efforts should be made to minimize the transmission risk.

2.3 Nonclinical studies

This section provides guidance for planning and implementing shedding studies of viruses/vectors in nonclinical studies. This guidance covers selection of species/models, design of nonclinical shedding studies, data analysis, and interpretation.

A purpose of nonclinical shedding studies is to elucidate shedding profiles of viruses/vectors, thereby estimate what shedding may occur in humans, and reflect the estimate in design of clinical shedding studies. Conduct of nonclinical shedding studies for each product is desirable. However, if the other viruses/vectors with characteristics similar to those of the viruses/vectors under development already have adequately accumulated knowledge (such analogues viruses/vectors are, for example, ones that are derived from the same virus and not expected to have a different shedding profile because of different target genes), it should be noted that additional conduct of similar shedding studies would provide data with limitations of their novelty. Furthermore, it should also be noted that information obtained from nonclinical shedding studies will not lead to exemption from clinical shedding studies.

Shedding of viruses/vectors found in a nonclinical study is generally evaluated in other nonclinical studies. Results from previous studies conducted with other analogous viruses/vectors may also help estimation of shedding profile of the target viruses/vectors and be utilized as a part of the information justifying a plan of shedding studies in clinical studies.

However, if the plan of shedding studies in clinical studies is developed only based on the past information of the other analogous viruses/vectors without conduct of nonclinical shedding studies, the accountable evidence should be presented, showing that the viruses/vectors to be studied and the other analogous viruses/vectors have similar shedding profiles. The desirable

evidence is results from biodistribution and shedding studies with multiple analogous viruses/vectors.

2.3.1 Test products used in nonclinical shedding studies

The test product used in nonclinical shedding studies should be consistent with the investigational product in terms of quality attributes such as genomic identity, virus titer/activity, and product formulation. In addition, the test product should be desirably manufactured by the same process as that for the investigational product wherever possible, but in general, the test product is often manufactured at a smaller scale than that for the investigational product and unlikely to be comprehensively characterized as done for the investigational product. If results from nonclinical shedding studies with such test product are used as information justifying a plan of shedding studies in clinical studies, accountable evidence for quality consistency should be presented by comparing quality attributes of the test product with those of the investigational product.

2.3.2 Animal species

In nonclinical shedding studies, animal species in which reactions to viruses/vectors are similar to those in humans should be desirably selected as done in other nonclinical studies such as Proof-of-Concept (PoC) and safety studies.

Many of the viruses/vectors used in clinical development of gene therapy products are unlikely to be infectious to non-human animal species and may have parental viruses with weak replication competence. Because of such characteristics, infectivity of viruses/vectors in the selected animal species must be evaluated before start of nonclinical shedding studies. Cells and tissues susceptible to viruses/vectors may differ depending on animal species. In particular, differences in expression level and tissue distribution of the virus receptor between humans and study animals may affect infectivity in each tissue and consequently shedding profile of viruses/vectors. For appropriate selection of animal species/models, information about differences in shedding of specific viruses/vectors between healthy animals and disease models is desirable if any. For this reason, a plan of nonclinical shedding studies should be developed based on prior knowledge from nonclinical PoC, safety, and biodistribution studies available to date, including data justifying the selected animal species/models. If the target disease is expected to affect the shedding profile, animal models of this disease may have to be used in some of shedding studies to optimize evaluation on the shedding profile. For example, oncolytic viruses may have to be studied in immunocompromised tumor-bearing animal models, which potentially represent the condition most suitable for replication of viruses/vectors and thus allow the optimized evaluation. On the other hand, such immunocompromised condition may affect biodistribution and elimination rate of viruses/vectors and consequently evaluation on shedding.

Shedding studies in animal models of the disease are supposed to have difficulty complying with the Good Laboratory Practice (GLP) when conducted. Such studies, which may inevitably finish in a non-GLP status, should be conducted in compliance with the GLP wherever possible to ensure the data integrity to the extent possible.

2.3.3 Dose and route of administration

The doses and routes of administration used in nonclinical shedding studies should reflect clinical use. A plan of nonclinical shedding studies should include 1) the intended route of administration in humans, 2) the maximum dose in clinical studies or corresponding dose, and 3) number of doses administered and be designed in view of dosing schedule planned in clinical settings. Furthermore, to evaluate shedding of viruses/vectors for dose-dependency, multiple doses covering the dose range anticipated in clinical settings may be established. To evaluate the shedding profile when viruses/vectors are distributed throughout the body after administration,

intravenous administration may be included in the study design in addition to the intended route of administration in humans. The intravenous administration is often deemed as the “worst-case scenario” route for exposure, but it should be noted that this idea is not always applicable, depending on characteristics of viruses/vectors and the route of administration. For example, if the intended route is intracranial administration, which leads to local infection and propagation of viruses/vectors, intravenous administration may not allow appropriate evaluation on shedding profile in nonclinical shedding studies. If the intravenous administration is used in nonclinical studies as an alternative to the intended route in clinical studies, such use including the dose should be justified.

2.3.4 Sampling frequency and study period

Biological properties of parental viruses can be used as a guide to develop sampling schedules after administration of viruses/vectors. In general, samples are collected frequently during a period of the first several days post dose to capture a transient shedding profile just after administration. Because collection of excrements and discharges may be restricted, the number of samples and sampling frequency should be established based on feasibility. Practically, the sampling schedule in a multiple-dose study including the frequency should be developed based on results on shedding from a single-dose study, which thus should be conducted first.

Biodistribution study results are useful in evaluating persistence of viruses/vectors in specific tissues. If viruses/vectors persist in specific organs or tissues such as kidneys, lungs, intestinal tract, and blood for an extended period, the shedding studies should be conducted for a period covering the durations in these organs. If shedding of viruses/vectors lasts longer than the biodistribution study, another study covering a period long enough to capture shedding should be considered. If the viruses/vectors are replication competent, the period should be long enough to capture the secondary peak suggestive of virus/vector replication in the body. If negative results are observed at multiple consecutive timepoints, the shedding study may be terminated leaving planned subsequent sampling operations undone.

If the viruses/vectors are derived from viruses, such as HSV, that can be latent in cells and reactivate, the latent period and results of reactivation in animals may not reflect those in humans. In addition, immune reactions in experimental animals are expected to accelerate elimination of viruses/vectors from the body, shortening the duration of shedding. Because the immune reactions in animals are not necessarily analogous to those in humans, interpretation of data on shedding warrants adequate consideration on the immunological responses in animals in nonclinical studies and understanding that data on shedding of intended viruses/vectors in nonclinical studies cannot be fully extrapolated to clinical studies.

2.3.5 Specimens to be collected

To select specimens to be collected for shedding studies, characteristics of viruses/vectors, route of administration, and animal species should be considered, and their selection should be justified. Specimens are most commonly collected from urine and feces but can be collected from buccal swabs, nasal swabs, saliva, tears, and exudates.

Specimens should be collected for each dose group and each dosing interval. In addition, the number of specimens to be collected should be large enough to ensure consistency and reliability of assays. To perform appropriate assays in a quantitative manner, types and amounts of specimens to be collected should be examined. For example, in small animals such as mice, adequate amounts of specific discharges or excrements are sometimes difficult to collect. In such cases, specimens collected from multiple animals in the same dose group at the same timepoint may be pooled.

The specimens should be handled and stored to ensure appropriate preservation of viruses/vectors potentially contained.

2.3.6 Interpretation of nonclinical data

As described in Sections 2.3.4. “Sampling frequency and study period” and 2.3.5. “Specimens to be collected,” the specimen type and frequency and period of sampling in the nonclinical shedding studies are particularly important in interpreting study results and can serve as the firm basis for development of protocols of shedding studies in clinical studies. For example, sampling in clinical studies may be of little significance for viruses/vectors that are theoretically unlikely to shed and actually did not shed in nonclinical studies. In addition, if the specimen type (body fluids) and timing of the secondary peak of replication competent viruses/vectors are identified, the concerned information would contribute to not only development of protocols of shedding studies in clinical studies but also guidance to healthcare professionals, etc. during the clinical studies.

Extrapolation of results from nonclinical shedding studies to humans, if applicable, requires consideration that the selected animal species/models may exhibit responses different from those in humans, and the obtained data should be carefully interpreted.

If viral/vector shedding has been captured in nonclinical shedding studies, and the shed viruses/vectors are confirmed to be infectious in appropriate infectivity assays, development must be accompanied by consideration that human specimens of the same type collected at the same timing may contain shed viruses/vectors and pose a risk of infection to third parties. To evaluate the transmission risk, co-housing transmission studies may be conducted using animals in nonclinical studies. In such studies, it should be noted that the route of infection of shed viruses/vectors differs between humans and animals. For this reason, to evaluate a transmission risk, clinical studies require careful observation for transmission potential at the timing corresponding to when shedding was captured in nonclinical shedding studies. Measures to prevent third parties from readily coming into contact with body fluids potentially containing shed viruses/vectors are useful. In this regard, 2.4.3 “Interpretation of clinical shedding study results” should be referred to. On the other hand, it should be also kept in mind that shedding of viruses/vectors does not necessarily result in infection to third parties.

2.4 Clinical studies

Points to consider for planning of clinical shedding studies are many, including a) at what stage of development the shedding studies will be conducted, b) how the study should be designed, and c) how persistence in the patient and transmission to third parties should be evaluated based on biological properties of the viruses/vectors. However, shedding studies are not necessarily required to be conducted independently and thus may be included as a part of the original clinical study protocol.

Many of the viruses/vectors under development are derived from replication incompetent virus or conditionally replication competent viruses, which acquire replication competence under a specific condition. For such viruses, the duration of shedding can be shorter than that of the parental viruses after natural infection. Depending on the route of administration, the shedding profile may be different from that of the wild-type viruses after infection. Still, information on known infection cases of parental viruses will help interpretation of data obtained from the shedding studies and estimation of transmission potential.

The period observed for shedding, specimen type, and frequency of sampling discussed in Section 2.3 “Nonclinical studies” can be highly informative for planning shedding studies in clinical studies. In addition to the above, key elements to be considered for design of shedding studies in clinical settings include known biological properties of parental viruses, replication competence of viruses/vectors, dose, route of administration, and health status of the patient population.

Shedding profiles are affected by the dose of viruses/vectors and route of administration. For example, intravenous administration leads to more extensive distribution than local administration

of similar viruses/vectors and thereby more types of body fluids potentially containing shed viruses/vectors. In addition, high doses of viruses/vectors are more likely to affect not only duration of shedding but also distribution in tissues and organs than low doses. Furthermore, to evaluate duration of shedding of viruses/vectors, the disease and immune status of patients enrolled in the clinical study should be considered.

In the development, appropriate timing when clinical shedding studies of viruses/vectors should be conducted differs depending on characteristics of the investigational product and patient population. Usually, shedding studies are conducted before start of a confirmatory clinical study, which is intended to verify the product efficacy. Although regulatory guidelines for marketing approval applications do not particularly specify the timing when shedding studies should be conducted, the early stage of development (phase I or I/II) would be the most reasonable timing for conduct of shedding studies in view of the following points: a) phase I or I/II studies have the small sample size; b) these studies are thus readily manageable; and c) if found necessary in these studies, appropriate actions can be taken in the subsequent development activities. Particularly, viruses/vectors have a high transmission risk and potentially pose a third party risk, shedding studies should be conducted during the first-in-human clinical study. On the other hand, if the third party risk is unlikely, shedding studies may be started in the late stage of development. If shedding studies have already been conducted in foreign clinical studies, and shedding of the concerned viruses/vectors is unlikely to differ among ethnic groups, conduct of shedding studies in Japan is of little need. Complete omission of shedding studies in clinical studies throughout the development in and outside Japan is discouraged. However, developers may be exempted from conduct of shedding studies in clinical studies if the following actions are possible: a) to evaluate a transmission risk associated with the investigational product adequately based on data of known similar viruses/vectors; b) to justify handling of the product during review for marketing approval; and c) to provide information about the appropriate handling to healthcare professionals (physicians, etc.) and patients in post-marketing settings.

At the time of planning a shedding study in a clinical study, developers should consider enrolling an appropriate number of patients to obtain data representative of typical shedding profile expected in post-marketing clinical use. In shedding studies, collection and assays of patient specimens must be performed under appropriately controlled conditions. For example, patient samples should be collected in a manner that prevents contamination and be subjected to assays within a predetermined period of time to minimize specimen degradation. If assays are performed after end of the above period, specimens should be appropriately processed such as freezing immediately after collection and stored under conditions that will not compromise infectivity of the viruses/vectors or reduce the genome copy number in the specimens. Of note, performing assays collectively at the same laboratory is desirable, but if a shedding study is conducted across a wide geographical region, transportation means to minimize specimen degradation should be considered.

2.4.1 Sampling frequency and period

Data obtained from nonclinical shedding studies and shedding studies in already conducted clinical studies may serve as the basis for determination of the timing, period, and frequency of sampling in clinical studies to be conducted. As described in Section 2.3.4 “Sampling frequency and study period,” specimens are generally collected frequently for the first few days after administration followed by sampling at a decreasing frequency with time. If the detection target is replication competent viruses/vectors, the period of sampling should be long enough to detect the secondary peak of viruses/vectors potentially caused by post-dose replication in the body.

The frequency and period of sampling may be affected by the patient population, clinical application, concomitant therapy, and immune status of patients, and thus the sampling plan should be designed with these factors taken into account. In addition, immune reactions to viruses/vectors can alter the shedding profile. Strong immune reactions can accelerate elimination

of the viruses/vectors, thereby shortening the duration of shedding of them and resulting in a reduced amount of shed viruses/vectors. It should be noted that the immune response to viruses/vectors may differ from that to the wild-type viruses, depending on the target gene inserted in the viruses/vectors.

If viruses/vectors such as oncolytic viruses are replication competent, frequency and period of sampling should be further carefully established, because patients immunocompromised by cancer, etc. may not only exhibit immune functions directing the viruses/vectors toward elimination unlike immunocompetent patients but also allow the viruses/vectors to replicate more actively than usual in the body, increasing a risk of transmission to third parties. In addition, oncolytic viruses may be used concomitantly with drugs involving immune functions. It should be noted that such concomitant drugs may alter the shedding profile.

For clinical shedding studies, criteria for termination should be considered. In shedding studies, usually, sampling and detection of viruses/vectors need to be continued until negative results are obtained at multiple consecutive timepoints. Whether the shedding study may be terminated with negative results at multiple consecutive timepoints should be determined in view of replication competence of the viruses/vectors, sampling interval, and the detection method. For example, even if negative results for replication competent viruses/vectors are obtained twice in infectivity assays performed at a short interval, potential overlook of the secondary peak may not be ruled out. In such cases, assays may have to be performed after a certain period of time to reconfirm the negative results. For viruses/vectors that may go into a long-term latent period, need of additional shedding studies should be considered when clinical symptoms suggestive of reactivation are observed. In this case, prompt consultation with regulatory authorities is encouraged.

On the other hand, for viruses/vectors such as ones derived from AAV that may persist for a long period of time but are not expected to replicate in the body, sampling and assays may be terminated when assay results show a consistent decreasing trend or a stable plateau phase, even though the amount of shed viruses/vectors has not reached the lower limit of detection of the assay.

A multiple-dose regimen of viruses/vectors requires a longer sampling period than a single-dose regimen, and a sampling plan should be developed to cover a certain period of time after each dose in general. Of note, shedding data collected with a single-dose regimen of the same or similar viruses/vectors, if available, may be informative for development of a sampling plan with a multiple-dose regimen. If elimination of viruses/vectors after the second or subsequent doses is confirmed to be faster than that after the first dose, sampling after the second or subsequent doses may not be needed.

2.4.2 Specimen collection

Results from nonclinical shedding studies can be used as a guide to determine what specimens should be collected in shedding studies in clinical studies. Specimens collected in shedding studies in clinical studies are generally urine, saliva, and feces, but the other specimens may be appropriate depending on characteristics of the viruses/vectors and route of administration used in the clinical studies. For example, for viruses/vectors to be administered intravitreally or subretinally to treat an eye disease, tear fluid may be collected. For intratumoral administration to treat head and neck cancer, nasopharyngeal lavage fluid and swabs may be collected. For viruses/vectors to be administered intradermally or subcutaneously, cotton swabs may be applied to the injection site to check for infiltration in the administration site. If the parental viruses are known to cause infection via nasal aerosols, nasal swabs can be included as specimens to be collected. For viruses/vectors to be administered to treat cancers in digestive organs such as esophagus and stomach, feces may have to be evaluated. In addition, after intravenous administration of viruses/vectors, shedding into digestive organs often occur, and thus potential shedding into feces should be evaluated. For any of the above regimens, however, feces may not necessarily have to be collected in some cases where shedding into feces is not expected based on results from nonclinical shedding studies and route of administration.

Many oncolytic viruses are mainly administered intratumorally. For oncolytic viruses, an

appropriate sampling strategy needs to be developed depending on the tumor type to be treated. In addition, if administration of viruses/vectors is followed by tumor resection, tissue biopsy may be useful in evaluating a potential presence of replicated viruses/vectors spreading to surrounding tissues. The biopsy may provide useful information about a possibility of further transmission and third party risks of viruses/vectors.

For appropriate assays for detection of viruses/vectors, the amount of specimens to be collected should be sufficient. Wherever possible, the amount to be stored should be large enough to repeat assays for detection as necessary. Specimens should be stored in such a manner as to prevent cross-contamination and maintain stability during the assay period, thereby ensuring the quality.

Capturing behavior of viruses/vectors in blood after administration is useful. In common practice, amounts of viruses/vectors in blood are measured over time simultaneously with the shedding study or separately. Presence or absence of viruses/vectors in blood can be useful information in estimating to what extent the viruses/vectors would spread from the administration site across the body and will be highly informative for preparing a manual for proper handling of patient specimens to be used by physicians, nurses, etc.

2.4.3 Interpretation of clinical shedding study results

To estimate a transmission risk of shed viruses/vectors, their characteristics should be identified in terms of infectivity or gene sequence. However, even if viruses/vectors are detected, infectivity assays may not be feasible depending on the amount and storage conditions of specimens, and thus evaluation of infectivity of the viruses/vectors remaining in the body fluids may be difficult. If virus/vector genome is detected by NAT, which cannot distinguish between infectious viruses/vectors and inactivated viruses/vectors or their non-infectious degradation products, a transmission risk should be assessed on the assumption that infectious viruses/vectors are present in the body fluids even though the detected amount is extremely minimal.

A key to the transmission risk assessment is to characterize the shedding profile including the route of shedding. Natural route of infection of parental viruses is very important information for evaluation. For example, if viruses/vectors derived from parental viruses that cause infection via aerosols are detected in saliva or nasopharyngeal swabs, such viruses/vectors can have an increased transmission risk compared with viruses/vectors that are shed into urine, etc.

Amounts of shed viruses/vectors and duration of shedding should be considered. Replication competent viruses/vectors may persist in the body for a long period of time and expand in quantity, consequently posing an increased transmission risk.

If viruses/vectors derived from non-pathogenic viruses are shed, the third party risk may be lower than that of viruses/vectors derived from pathogenic viruses, but the ultimate third party risk will depend on other biological properties of the viruses/vectors such as replication competence and the extent of pathogenicity attenuation. If viruses/vectors contain the target gene, effects of the expression product of the target gene on safety of third parties must be considered. Furthermore, potential effects of the target gene on phenotype and characteristics of the viruses/vectors must be considered in assessment of the third party risk. Because a third party risk greatly depends on properties of viruses/vectors, use of viruses/vectors with a low third party risk is encouraged in view of post-marketing distribution across medical institutions in Japan. If switchover to viruses/vectors with a low third party risk is difficult, the third party risk must be minimized by controlling the transmission risk strictly.

Even if no direct third party risk occurs, unwanted transmission to third parties should be avoided in principle. In particular, the third parties exposed to the transmitted viruses or their children may lose an opportunity of the same or similar gene therapy owing to increased antibody titers against the transmitted viruses. The transmission risk to third parties should be minimized wherever possible.

2.5 Assessment of transmission risk and third party risk

Even if shedding of viruses/vectors from the treated patient is confirmed, this finding does not necessarily mean transmission of the viruses/vectors to third parties, but the transmission and third party risks should be continuously assessed. If developers consider it possible to assess the transmission risk and third party risk based on available results from shedding studies in clinical studies and prior knowledge, they will not necessarily have to conduct additional clinical shedding studies. However, justification for such decision will be a desirable subject of consultation with regulatory authorities. For example, if shedding of replication incompetent viruses/vectors is captured in specimens from a body part not involved in the route of infection, thus ruling out co-infection with wild-type viruses, and furthermore the detected amount is very minimal, they may be able to assess the transmission and third party risks without conducting additional clinical shedding studies. On the other hand, if the transmission risk and third party risk cannot be adequately explained based on data from clinical shedding studies and prior knowledge, additional shedding studies may have to be included in the next-phase clinical study plan to evaluate presence or absence of transmission.

2.5.1 Transmission risk

In assessment of a transmission risk, amounts of shed infectious viruses/vectors and the duration should be evaluated. The concerned information should be evaluated to address the following separate questions about transmission caused by shedding: 1) whether the amount of shed viruses/vectors is large enough to enable transmission to third parties; and 2) when transmission is most likely to occur or whether transmission potentially occurs soon after administration (infection to healthcare professionals), or whether transmission potentially occurs even after a certain post-dose period of time (infection to family members, other inpatients, and public).

Biological properties of viruses/vectors are important basic information for evaluation. The most significant biological property is replication competence of shed viruses/vectors. However, even if shed viruses/vectors are found replication competent, they do not always replicate in the body of third parties. On the other hand, even if shed viruses/vectors are found replication incompetent, they may be transiently transmitted to third parties, leading to expression of the target gene in them and thereby inducing production of antibodies against the viruses/vectors. For assessment of a transmission risk, information about the pre-existing immunity against parental viruses in the general population may be useful. For example, if vaccination against the parental viruses is extensively implemented, a large part of the population has immunity against the viruses/vectors, and thus a transmission risk of the viruses/vectors can be low. Caution should be always exercised concerning the patient's contact with the population unvaccinated against the parental viruses and immunocompromised patients. Of note, if characteristics of the target gene raise no public health concern, a third party risk caused by product transmission to healthy third parties is considered unlikely, except for cases where the amount of shed viruses/vectors is excessive.

A viewpoint of whether effective preventive measures against transmission of viruses/vectors can be taken is another key to transmission risk assessment. For diapers, tissue paper, vomitus wipes, towels, etc. that may be contaminated with viruses/vectors, appropriate handling and disposal methods should be established to ensure that such wastes will not cause transmission to third parties. If minimal shedding of viruses/vectors is found in saliva, a transmission risk can be reduced by the patient's wearing a mask for an appropriate period of time. A measure to establish methods for inactivating viruses/vectors physically and chemically will be useful when preventive measures against transmission to third parties are taken. For example, if viruses/vectors are shed into urine, precluding inactivation of them and thus posing a transmission risk, addition of appropriate virus inactivating agents into the used toilet bowl may be able to prevent transmission to third parties. In addition, procedures for handling contaminated bedding should be predetermined based on the methods for inactivating viruses/vectors desirably, including actions to quarantine the bedding to prevent contamination with the patient's body fluids from spreading and to wash the bedding separately.

Information about treatment of patients infected with parental viruses should be extensively collected to establish methods for addressing transmission cases. If appropriate therapies are available, they may be applied to address a third party risk after infection with viruses/vectors.

2.5.2 Third party risk

If shedding is observed in clinical studies, and the amount of shed viruses/vectors and duration of shedding indicate a transmission risk, and characteristics of the viruses/vectors suggest a third party risk, the transmission and third party risks should be carefully assessed, and where necessary studies to assess the third party risk should be conducted. These studies may be conducted as a part of clinical studies, but they are not considered to capture a possibility of transmission of the investigational product perfectly in the clinical studies in view of frequency of occurrence of the risk. In this case, continuous monitoring may be required after an approval for marketing as a part of the post-marketing surveillance plan.

If third party risk assessment suggests critical concerns, but the development is considered to be worth ongoing, consultation with regulatory authorities concerning the subsequent actions is strongly encouraged. At least, during the development, not only should the patient's activities be restricted wherever possible, but those at the greatest risk of infection, including healthcare professionals, home caregivers, family members, and other close contacts, should be kept under close supervision. For the supervision, signs suggestive of infection with viruses/vectors and those potentially resulted from expression of the target gene should be separately defined, and the information collection system should be established.

Information collection from third parties is basically expected to be performed in a non-invasive manner (questionnaires on health status, etc.), but if any sign suggestive of transmission is observed, full examination should be performed. This type of information collection is most useful in situations where overt clinical consequences of infection are anticipated, such as transmission of viruses/vectors derived from vaccinia virus, which results in skin lesions on third parties.

Full examinations, if any, are mostly expected to involve blood drawing and include detection of viral/vector genome, which can provide evidence of relevant viremia, assays for antibody titers, and antibody responses to exogenous proteins, expression products of the viruses/vectors and target gene. Sampling of body fluids should be also considered to assess shedding status of viruses/vectors. The type of body fluids selected for sampling should be based on observation data on shedding in the original clinical study, and viruses/vectors should be detected by appropriate assay methods.

2.5.3 Actions based on assessment of transmission risk and third party risk

Importance is attached to the following activities: 1) to quantify the risk of transmission to third parties based on assessment results of available clinical data; and 2) to interpret the assessment results from various viewpoints, including one of whether a transmission risk would cause a third party risk. If a transmission risk is observed, appropriate measures to prevent transmission to third parties should be taken. The measures should be increasingly strict and extensive with an increasing third party risk. The appropriate measures to prevent transmission to third parties include education using guides for proper use for healthcare professionals and brochures for patients or patients' families.

Because patients are kept under certain control with the limited number of patient contacts in clinical studies, extensive transmission to third parties is considered unlikely to be identified during clinical studies even if the viruses/vectors have a transmission risk owing to the properties. Consideration should be given to inclusion of evaluation about the transmission in product pharmacovigilance activities and post-marketing surveillance. On the other hand, if transmission to third parties is identified during a clinical study but found to have a minimal effect on health, consideration should be desirably given to inclusion of evaluation about the transmission in post-marketing surveillance as done above.

Appendix 1Evaluation of shedding of bacterial vectors

Many of the vectors under development are of viral origin, but bacterial vectors are also being developed. This appendix provides specific considerations for bacterial vectors.

1 Considerations for characteristics of bacterial vectors

Many issues that need to be considered to understand behavior of viruses/vectors also apply to bacterial vectors. Because bacterial vectors are generally replication competent or capable of replicating under specific conditions, evaluation of replication competence is a key element. Characteristics specific to bacterial vectors such as *in vivo* persistence and antimicrobial resistance should also be considered.

1) Replication competence

Because bacterial vectors are usually replication competent, the principle of replication competent viruses/vectors described in Section 2.1.1 “Quality attributes of viruses/vectors” is applicable. To specific bacterial vectors that are replication incompetent in the body and after shedding, the principle of replication incompetent viruses/vectors is applicable.

2) Dormancy and persistence

Because certain types of bacteria (such as *Salmonella*, *Listeria*, and *M. tuberculosis*) replicate in cells (intracellular infections), shedding of bacterial vectors involved in intracellular infections may persist longer than that of bacterial vectors that replicate and proliferate extracellularly. To estimate the route and duration of shedding, consideration should be given to types of cells allowing replication of the target bacterial vectors and characteristics of the tissues and organs where bacteria may stay or live in dormancy.

3) Antimicrobial susceptibility/resistance

Susceptibility to antimicrobial agents is a key factor when removal of bacterial vectors from the patient's body after administration and treatment methods in third parties exposed to transmitted bacterial vectors are considered. It should be also taken into account when transmission and third party risks of bacterial vectors are assessed. In addition, because drug resistance genes may limit use of antimicrobial agents that are supposed to prevent transmission of bacterial vectors and be transferred to other bacteria in nature, bacterial vectors with antimicrobial resistance factors introduced by genetic modification should not be used wherever possible. Characterization of bacterial vectors should include information on antimicrobial susceptibility/resistance of the product.

2 Shedding studies of bacterial vectors

Human discharges and excrements are highly likely to contain bacteria similar to bacterial vectors. To distinguish between bacterial vectors and similar bacteria, NAT targeting the sequence unique to the bacterial vector may be used. To detect bacterial vectors by distinguishing them from similar gram-positive or gram-negative bacteria, bacterial culture using a growth attribute specific to the bacterial vectors should be performed. The obtained culture may have to be subjected to infectivity assays using indicator cells, NAT, and gene sequencing to identify bacterial vectors.

In some cases, when administered bacterial vectors are confirmed to have exerted adequate therapeutic effects, the bacterial vectors are removed by antimicrobial agents. Such antimicrobial agents should be evaluated for their capability and efficiency of removing bacterial vectors in nonclinical studies, and their short-term effects (up to several months) may be evaluated in animals/models. It should be noted that repeated use of antimicrobial agents may induce acquisition of drug-resistance genes in the administered bacterial vectors, leading to altered

shedding profile. In addition, the acquisition of drug resistance in bacterial vectors may affect transmission and third party risks.

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“Development of Quality, Efficacy, and Safety Evaluation Systems for AAV Vector-derived Gene Therapy Products using Patient Specimens in *In Vivo* Gene Therapy,” Project of Research on Regulatory Science of Pharmaceuticals and Medical Devices of the Japan Agency for Medical Research and Development

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