

Design and Analysis of Shedding Studies for Virus or Bacteria-Based Gene Therapy and Oncolytic Products

Guidance for Industry

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I. INTRODUCTION

The Center for Biologics Evaluation and Research (CBER)/Office of Cellular, Tissue, and Gene Therapies (OCTGT) is issuing this guidance to provide you, sponsors of virus or bacteria-based gene therapy products (VBGT products)¹ and oncolytic viruses or bacteria (oncolytic products)² with recommendations on how to conduct shedding studies during preclinical and clinical development. For purposes of this guidance, the term “shedding” means release of VBGT or oncolytic products from the patient through one or all of the following ways: excreta (feces); secreta (urine, saliva, nasopharyngeal fluids etc.); or through the skin (pustules, sores, wounds). Shedding is distinct from biodistribution because the latter describes how a product is spread within the patient’s body from the site of administration while the former describes how it is excreted or released from the patient’s body. Shedding raises the possibility of transmission of VBGT or oncolytic products³ from treated to untreated individuals (e.g., close contacts and health care professionals). This guidance represents FDA’s current thinking on how and when shedding data should be collected for VBGT and oncolytic products during preclinical and

¹ Gene therapy products are all products that mediate their effects by transcription and/or translation of transferred genetic material and/or by integrating into the host genome and that are administered as nucleic acids, viruses, or genetically engineered microorganisms. The products may be used to modify cells in vivo or transferred to cells ex vivo before administration to the recipient. See section III. of FDA’s guidance entitled “Guidance for Industry: Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events” dated November 2006. <http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/cellularandgenetherapy/ucm072957.htm>.

² Oncolytic products refer to replication competent viruses or dividing bacteria that are used as therapeutic agents to mediate lysis of tumor cells. Some oncolytic products carry foreign genes (immune modifying genes, genes that enhance oncolysis etc.), and mediate part of their anti-tumor effect by transcription and/or translation of these foreign genes in the host. Hence, oncolytic products that carry foreign genes can also be classified as gene therapy products.

³ Transmission could occur if the VBGT or oncolytic product is shed in the form of intact viruses or bacteria but not when shed as viral or bacterial degradation products such as nucleic acid fragments.

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clinical development and how shedding data can be used to assess the potential for transmission to untreated individuals. This guidance finalizes the draft guidance of the same title dated July 2014.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. SCOPE

The products covered by this guidance are VBGT and oncolytic products that OCTGT reviews. The focus of this guidance is shedding studies, including both how and when shedding data should be collected and how shedding data can be used to assess the potential for transmission to untreated individuals.

This guidance does not cover plasmids, peptides, and genetically modified mammalian cells that OCTGT also reviews because, unlike VBGT and oncolytic products, there is no potential for plasmids, peptides, and genetically modified mammalian cells to be infectious or transmissible. This guidance also does not address collection or submission of adverse event information, including those adverse events that could be attributed to shedding. Please see the regulations at Title 21 of the Code of Federal Regulations (CFR) Part 312, specifically 21 CFR 312.32 and 21 CFR Part 600, specifically 21 CFR 600.80, for information on the collection and submission to FDA of adverse event information.

Finally, while assessment of shedding can be utilized to understand the potential risk to the environment, the scope of this guidance does not include shedding as it may relate to potential environmental concerns with respect to a specific VBGT or oncolytic product. For more information on this topic, you may wish to consult FDA's guidance document entitled "Determining the Need for and Content of Environmental Assessments for Gene Therapies, Vectored Vaccines, and Related Recombinant Viral or Microbial Products; Guidance for Industry" dated March 2015.⁴

III. BACKGROUND

VBGT and oncolytic products are derived from infectious viruses or bacteria. In general, these products are not as infectious or as virulent as the parent strain of virus or bacterium because of, in part, the derivation methods and/or modifications made during product development that lead to attenuation. Hence, it is likely that these products are shed to a lesser extent than during

⁴ Available at

<http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm401869.htm>.

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natural infection by the parent strain. Nonetheless, the possibility that the shed VBGT or oncolytic product may be infectious raises safety concerns related to the risk of transmission to untreated individuals. To understand this risk, shedding studies that are conducted in the target patient population(s) may be appropriate before licensure.

Typically, clinical shedding studies are not stand-alone studies but are integrated into the design of a safety or efficacy trial. Because there are many product-specific factors and patient-specific factors that can influence the design of a shedding study, sponsors should consult with OCTGT in the early stages of product development for specific recommendations as to their product.

IV. WHY COLLECT SHEDDING DATA DURING PRODUCT DEVELOPMENT?

Shedding studies should be conducted for each VBGT or oncolytic product to provide information about the likelihood of transmission to untreated individuals because historical data alone may not be predictive of the shedding profile. Shedding data can be used to evaluate measures to prevent transmission. Shedding data collected during product development should provide a clear and comprehensive understanding of the shedding profile of VBGT or oncolytic products in the target patient population(s). Note that it may be appropriate to describe these data in the package insert for an approved Biologics License Application (BLA).

To inform the design of human shedding studies, shedding data may be collected in animals following administration of the VBGT or oncolytic product. These data can help estimate the likelihood and potential shedding profile in humans, particularly when there is concern about transmission to untreated individuals. However, such data cannot substitute for human shedding studies for several reasons. For example, a VBGT or oncolytic product may be derived from a human-specific strain; therefore, animals may not adequately predict the shedding profile in humans. Similarly, various animal species/models may not adequately address patient-specific factors, such as differences in the immune status at the time of product administration, which may contribute to the potential for shedding in humans (for more details refer to section VII.B. of this guidance).

Product-specific variables may also affect shedding. For example, the biological characteristics and route of administration (entry) of VBGT or oncolytic products can be different from that of the parent strain of viruses and bacteria. Specifically, these products may be:

- Derived from laboratory-adapted wild-type, attenuated or engineered strains that may not have been characterized in humans in prior studies.
- Replication competent or incompetent viruses; viruses that can infect a host cell and amplify to produce progeny are replication competent and those that can infect a host cell but cannot establish an infection, amplify, and produce progeny are replication incompetent.

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- Dividing and/or auxotrophic bacteria; auxotrophic bacteria are unable to synthesize an organic molecule required for their growth and division but when this molecule is available with the other nutrients they require, growth and division of the bacteria may occur.
- Introduced into the human body through unnatural routes and hence, the infectivity, replication, persistence and shedding from the human body may be different than that of the parental strains.
- Engineered to carry transgenes, e.g., tropism-altering genes, immune modifying gene(s) or genes that enhance oncolysis.

V. COLLECTION OF SHEDDING DATA IN PRECLINICAL STUDIES

The decision to assess shedding in preclinical studies⁵ is based on the biological characteristics, derivation, and genetic make-up of the VBGT or oncolytic product. For example, preclinical shedding data may be requested for an oncolytic or a replication competent VBGT product, if:

- Humans have not been previously exposed to the product, as in the case of a non-human bacterial or viral strain.
- The product has been administered to humans, but has been modified to achieve a different in vivo tropism than the parent strain.
- The product has been previously administered to humans; however, a change in the route of administration is proposed.
- Humans have not been previously exposed to the product, and the route of administration differs from the natural route of exposure/infection.

The use of the animal species/model(s) is an important factor that can affect the biological relevance of the shedding profile generated in the animal. Considerations include the permissiveness or susceptibility of the animal to infection from the VBGT or oncolytic product under investigation, and any preexisting immunity that may affect infectivity or product clearance.

Collection of shedding data is an endpoint that can be included in preclinical studies designed to collect other data, such as safety and biodistribution. The decision to include an assessment of the shedding profile of a VBGT or oncolytic product in an animal study will depend on various

⁵ For general information about preclinical assessment, including study design, of these products see FDA's guidance entitled "Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products" dated November 2013, <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm376136.htm>.

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product-specific factors, as described above and in sections I. and IV. of this guidance. We recommend that sponsors initiate communication with the Pharmacology/Toxicology staff in OCTGT early in their product development program to discuss the need for generating a shedding profile for their VBGT or oncolytic product in animals, and the planned methodology to collect this shedding data.

VI. DESIGN OF SHEDDING STUDIES: GUIDING PRINCIPLES

The key aspects in the design of shedding studies are: the choice of clinical samples that are collected from subjects in a trial (e.g., feces, urine, nasal swabs); the frequency of sample collection and duration of the monitoring period; and the assay methodology selected to test for the presence of the shed VBGT or oncolytic product in the clinical sample (Ref. 1).

To guide the design of shedding studies, the following should be considered:

A. Biological Characteristics

- Replication competence: The ability of the VBGT or oncolytic product to multiply and amplify in the human host greatly affects how it is disseminated in the body and may increase the extent and duration of shedding.
- Immunogenicity: When the VBGT or oncolytic product is derived from viruses or bacteria that elicit a strong immune response, the product may be more rapidly cleared from circulation than a poorly immunogenic product, and may be shed for a shorter duration. Similarly, when a product is administered multiple times, the product may be shed for a shorter duration in the later dose cycles than after the immune-priming first or early doses.
- Persistence and latency: The duration of a shedding study may be longer if the VBGT or oncolytic product exhibits persistence or latency-reactivation in the host, as in the case of an oncolytic herpes virus product that is capable of latency (period of time during which a virus is present in the host without producing overt clinical symptoms). Shedding of such products may be intermittent and unpredictable.
- Tropism: Tropism of the product may affect what samples should be collected to assess shedding. For example, VBGT or oncolytic products that are engineered to carry tropism modifying gene(s) or mutation(s) may exhibit an altered shedding profile than the parent virus because of retargeting of the product to different tissues or organs.

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- Stability of product attenuation: It is common for VBGT or oncolytic products to be attenuated either for replication in normal (non-tumor) cells, or for loss of virulence or latency in the human host. However, for some products that have a higher potential for recombination or reversion in the patient, the shedding pattern and/or what is shed may change.

B. Route of Administration

In addition to the tropism of the VBGT or oncolytic product, the route of product administration should be considered in the selection of sample types to collect in a shedding study. For example, to assess shedding in patients administered an oncolytic virus by the intradermal route, we recommend the collection of skin swabs at the site of injection, in addition to the other samples routinely assessed for shedding (e.g., urine, feces, and saliva). Similarly, we recommend the collection of nasopharyngeal washes when an oncolytic virus is administered by inhalation or via the intranasal route.

VII. COLLECTION OF SHEDDING DATA IN CLINICAL STUDIES

Shedding data collected in clinical studies provides a shedding profile of a product in the target patient population and is used to estimate the potential of transmission to untreated individuals. Depending on the shedding profile, it may be appropriate to include the information on shedding in the Investigator Brochure and in the Informed Consent for Investigational New Drug (IND) studies. Depending on the shedding profile, it also may be appropriate to include shedding data in the package insert for licensed products. This information will inform patients and physicians if shedding could occur with the use of a VBGT or oncolytic product, the potential for transmission of the product to untreated individuals, and of the measures to take to prevent such transmission.

A. When to Collect Shedding Data in Clinical Studies?

- For VBGT and oncolytic products classified as replication competent, we recommend that sponsors begin collecting shedding data in Phase 1 trials. Considering that replication competent products are associated with a higher potential for release as infectious viruses or bacteria, sponsors may need to continue collecting shedding data during Phase 2 and Phase 3, after a dose and regimen have been determined, to better characterize shedding.
- For VBGT products that are classified either as replication incompetent or replication deficient, we recommend that sponsors collect shedding data later in product development (e.g., during Phase 2 studies), after a dose and regimen have been determined. Compared to shedding of replication competent products, shedding of replication incompetent or replication deficient products is expected to be low, for a limited duration, and associated with a lower potential for release as infectious viruses or bacteria.

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- After shedding data are collected in early phase studies, it is not uncommon that dose, route, regimen (frequency of product administration, concomitant therapy, preconditioning regimens, etc.), or indication are modified. These changes can alter how product is shed. In such cases, shedding data collected in early clinical trials may not be adequate or relevant to predict the shedding profile of the product in its current state. Additional data should be collected in subsequent clinical trials where the route, dose regimen, and indication are the same as in the pivotal trial.

B. Study Design

The plan to collect shedding data in clinical studies can be based on prior clinical experience with the same or similar product, but when there is no such experience, as in the case of first-in-human VBGT or oncolytic products, the shedding profile generated in animals can be informative. We recommend that sponsors prospectively design and incorporate the sampling plan in the clinical study to collect shedding data.

There are four critical choices in the design of a sampling plan:

- Frequency of sample collection;
- Duration of sample collection;
- Type(s) of samples collected; and
- Storage conditions for types of samples collected.

However, there are many aspects that can influence these choices, as described earlier in section VI. of this guidance and further elaborated below.

- **Frequency of sample collection:** Shedding is most likely to occur in the period immediately following product administration, irrespective of replication competence of the VBGT or oncolytic product. A second peak of shedding may be noted in the days/weeks after administration of a replication competent product as a result of its multiplication/amplification in vivo. Accordingly, sampling should start immediately after product administration, with frequent sampling during the initial weeks following treatment to capture the shedding pattern accurately (e.g., sampling on day 1, 3, 7, 10 and then weekly). Analysis of samples should continue until three consecutive data points are obtained at or below the limit of detection (LOD) of the shedding assay. If the level of shedding does not reach the LOD of the assay but there is a continual decreasing trend, collection should continue until the results demonstrate that a plateau has been reached in at least three consecutive data points.

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- Duration of sample collection (monitoring period for shedding):
 - In general, when the VBGT or oncolytic product is a replication competent virus or bacteria, the monitoring period for shedding is longer (for sample collection) as compared to when the product is replication incompetent or replication deficient. This is because you will want to capture the second peak of shedding associated with multiplication or amplification in vivo.
 - The immune status of the patient population should be considered. Patients who are immune compromised may have an extended or even different shedding profile than their immune competent counterparts. For replication competent products, the immune competence of the patient population is a relevant factor because many of these products are used in cancer patients who have had immunosuppressive chemotherapy. When treated with replication competent VBGT or oncolytic products, immunosuppressed patients may become persistently infected and may shed the product for extended periods of time (Ref. 2). Therefore, the monitoring period for shedding may be longer for immunosuppressed patients treated with a replication competent VBGT or oncolytic product.
 - When a VBGT or oncolytic product is administered in multiple cycles, or when there is pre-existing immunity, the duration of shedding may be shortened because of product-specific immune responses. Data from single dose administration of product may be used to guide the timing of sample collection following multiple administrations.
 - If an oncolytic product is based on a virus that has the potential for latency reactivation, we recommend the collection of additional samples for shedding analysis when clinical signs warrant, i.e., when patients show signs of infection due to reactivation.
- Type(s) of samples collected: The types of clinical samples (e.g., urine, fecal swabs, saliva, etc.) collected to assess shedding depend on a variety of factors including the route of administration of the product, the tropism of the virus or bacteria, the natural route of transmission and shedding of the parent virus or bacterium from which the product is derived (as described in section VI. of this guidance), and biodistribution or shedding data from preclinical studies. For example, if an oncolytic herpes virus product is administered intradermally for treatment of skin cancers, there is the potential of transmission of the oncolytic herpes virus product through infected scabs/skin secretions because that is a natural route of transmission for herpes viruses. In this case, skin swabs or dressing from injection sites should be analyzed for shedding. Likewise, if an oncolytic adenovirus product is administered intranasally, there is the possibility of transmission of the adenovirus product through respiratory secretions, therefore, nasopharyngeal swabs or washes should be collected in the shedding study. For tropism-modified products, knowledge about the route of transmission

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and shedding of the parent virus or bacterium may not be relevant or sufficient to guide in sample collection.

- Blood is not typically analyzed for shedding but should be collected as part of pharmacokinetic analysis to understand the extent of product dissemination from the site of administration and the rapidity of product clearance. This information can be particularly useful to assess the extent of product shedding when limited vascular spread is expected for products administered locally (e.g., intratumoral, intramuscular, intracranial, subretinal routes).
- Storage conditions for type(s) of samples collected: The appropriate storage conditions for different type(s) of samples need to be established in order to minimize degradation of product-specific nucleic acids or loss of product-specific infectivity or bacterial viability. Multiple aliquots of samples may be needed for different tests, such as for a product-specific infectivity assay after a sample is scored positive for product-specific nucleic acids.

VIII. ANALYTICAL ASSAYS TO MEASURE SHEDDING

An analytical assay that measures shedding is designed to detect product in the clinical sample, either by detection of nucleic acids or for the presence of infectious viral particles or dividing bacteria. Based on the design and output (nature of the assay readout), shedding assays can vary greatly in their performance and suitability. Hence, the choice of a shedding assay can greatly affect the quality of the data collected, and is important in the generation of meaningful shedding data, i.e., data that provides a complete shedding profile for a product and can be used to estimate the potential for transmission to untreated individuals.

We recommend that sponsors consider the following in the selection of the analytical assay to measure shedding:

- At least one of the assays used to measure shedding should be quantitative. We recommend that sponsors report the extent of shedding of VBGT or oncolytic products in terms of the number of genome copies or infectious units to provide a quantitative assessment of shedding. Often an assay with a quantitative readout, like quantitative polymerase chain reaction (qPCR), is used because of the ease of performing/standardizing the assay, high throughput format, rapid turnaround time, and assay sensitivity.
- Because detection of nucleic acids by qPCR may not indicate the presence of infectious virus, for replication competent products, detection of nucleic acids should be followed up with infectivity or growth-based assays. Replication competent products are capable of growth or multiplication in humans and if shed, can be infectious. Since only infectious viruses or bacteria are potentially transmissible, we recommend that sponsors follow a step-wise approach for the analysis of shedding of replication competent products. Specifically, a steady rise in the PCR signal for

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product-specific nucleic acids in clinical samples collected over successive time-points is suggestive of bacterial growth or viral multiplication in vivo. In such cases, clinical samples should be further analyzed for infectivity in cell culture (for viruses) or growth (for bacteria).

- If shedding of conditionally replicating VBGT or oncolytic products is noted by qPCR assay at a level above the LOD, we recommend that sponsors further characterize the shed material to confirm infectivity or growth because such products could be shed in their infectious form even if replication is confined, mostly, to tumors or to a particular tissue-type. The assessment of infectivity for conditionally replication competent products, such as conditionally replicating adenovirus or auxotrophic bacteria, should take into account product-specific in vitro cell culture or growth conditions; for example growth in differential media for auxotrophic bacteria followed by a selective method for product identification.
- There are many different approaches to assess infectivity or growth with assays that have a quantitative read-out (Ref. 3). For example:
 - For detection of infectious viruses: Assays that measure infectivity in terms of Tissue Culture Infectious Dose 50 (TCID₅₀), plaque-forming units (PFU), focus-forming units (FFU).
 - For detection of dividing bacteria: Assays that measure bacterial growth in colony forming units (CFU).

Sponsors may justify limiting the shedding analysis of a replication competent or conditionally replicating product to qPCR assay, if:

- A correlation between qPCR and the infectivity assay or growth-based assay is established, and the signal noted in the qPCR assay is at or below the LOD of the infectivity or growth-based assay; or
 - The cell culture step in an infectivity assay is demonstrated to be unsuitable for the analysis of clinical samples with complex composition such as excreta due to adverse effects on cell viability.
- Shedding analysis of replication incompetent or deficient products by qPCR may be adequate. Most VBGT products are replication incompetent or replication deficient; for example, adeno-associated virus (AAV) vectors, E1-deleted adenovirus (Ad) vectors, and some herpes virus vectors (HSV). Replication incompetent or replication deficient products are incapable of multiplying in humans, and therefore, are shed to a lower extent and in a form that is incapable of establishing an infection. Hence, qPCR may be adequate as the primary assay to assess shedding of replication incompetent or replication deficient products.

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- Use of a qualitative assay to assess shedding may be justified. When a qualitative shedding assay is demonstrated to be more sensitive than a quantitative assay in the detection of a specific product, a sponsor may justify the use of the former. However, if there is detectable shedding with a qualitative assay, a follow-up quantitative analysis should be performed to understand the extent of shedding in positively scored samples.
- Shedding assay(s) should be demonstrated to be specific, sensitive, reproducible and accurate. We recommend testing of clinical samples in a shedding assay in replicates to determine reproducibility. The specificity of the assay should be well understood to avoid false-positive or false-negative results, particularly since retesting is not always feasible with clinical samples that are limited in quantity, such as nasal or skin swabs. The sensitivity of the assay should be determined in terms LOD and the limit of quantitation (LOQ), if using a quantitative assay. While the Agency does not expect shedding assays to be validated, the assays should be qualified to meet minimal performance capabilities and be suitable for the intended purpose.

The effect of sample type and composition on assay performance should be well understood. Clinical samples such as feces and urine are rich in complex organic matter that can adversely affect the performance of an assay and lead to an underestimation of shedding. Also, samples such as feces, saliva, and nasal swab are rich not only in host proteins and nucleic acids but also in the body's natural flora and in circulating strains of viruses and bacteria from the environment. Thus, the assay conditions should be optimized to selectively analyze for the product under investigation. For that, the specificity of the reagents used in the assay should be assessed and the quality of the reagents should be controlled. Certified and contaminant-free reagents should be used in the analysis of clinical samples in a shedding assay.

Interference from clinical sample matrix can lead to a false-negative result or an underestimation of the amount of shedding. For example, clinical samples like urine, saliva, and feces are rich in proteases, nucleases, ions and salts that can affect the amplification process in a PCR; specifically, nucleases in saliva/feces can degrade template DNA, bile salts in feces or urea in urine can affect the activity of thermostable DNA polymerases in the PCR mixture. When PCR inhibition is suspected due to interference from components in a clinical sample, the clinical sample can be diluted to a limited extent to reduce the interfering component. Since dilution of the clinical sample also leads to template dilution, the sensitivity of PCR assay should be assessed in each assay run. For that, each diluted sample should be tested in parallel with one that is spiked with a reference standard or positive internal control prior to dilution. If interference cannot be decreased by limited sample dilution, alternative or additional extraction procedures should be considered to remove the interfering component(s) in the clinical sample.

An underestimate of the level of shedding may also result due to degradation of viral or bacterial nucleic acids in enzyme-rich clinical samples such as feces and saliva during storage, handling/shipping and nucleic acid extraction. To account for such effects, we recommend that mock/donor sample types be spiked, soon after collection, with the reference standard or internal

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positive control and the percent recovery of the reference standard or internal positive control should be determined on a one-time basis. Sample collection, storage, shipping, extraction and analysis should be performed with the same methodology as that planned for the clinical (test) samples.

IX. ANALYSIS OF SHEDDING DATA

In order to assess the potential of transmission to untreated individuals due to shedding, the analysis of shedding data for VBGT or oncolytic products should address the following:

A. The Nature of the Shed Material

When clinical samples are scored positive for product in a shedding assay, the subsequent analysis of these samples should provide answers to the following questions:

1. Do the clinical samples contain product-specific nucleic acids (full-length genomes) suggestive of the presence of infectious viruses or bacteria or do the clinical samples contain mostly degraded product-specific nucleic acid (genome) fragments found in the absence of infectious viruses or bacteria?

An example of a clinical sample containing product-specific nucleic acid (full-length/complete genomes) suggestive of the presence of infectious viruses is one in which product-specific nucleic acids are amplified (by PCR) after treatment with nucleases. Under such conditions, the only genomes that are amplifiable are the full-length/complete genomes protected within intact nuclease-resistant viral particles that may be infectious. When nuclease treatment is not feasible, amplification of full length/complete viral genome by long PCR may suggest the presence of infectious viral particles. If only small product-specific nucleic acid fragments are amplifiable, then the clinical sample is not likely to contain infectious viruses.

2. Can viral or bacterial growth attributable to the shed product be detected in the clinical sample?

Ideally, the shedding assay should be able to discern infectious from non-infectious viruses, or dividing from non-dividing bacteria. We refer you to section VIII. of this guidance for details on shedding assays and for our recommendations. If qPCR is the only assay you have relied on in your shedding analysis of replication competent products, or the shedding assay screens for small genome fragments of the product, then we will assume that the shed material in the positively scored samples is infectious.

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B. The Extent of Shedding

In the analysis of shedding data, the extent of shedding noted for each sample type as a factor of time, dose (amount of product administered) and regimen (number of doses) should be reported for all the patients monitored in the study. Raw data in the shedding report should be accompanied by a corresponding analysis that is comprehensive and describes the following:

1. The number of patients that are shedding as a percentage of the total patients in the study for each sample type, dose and regimen studied.
2. The duration of shedding, including the first and last day of shedding, and the peak period(s) of shedding in each sample type. The period when shedding stops in most patients in the study also should be clearly identified.
3. The clinical sample(s) where shedding was consistently noted (type and time point) and samples that were consistently negative for all the patients in the study.
4. The quantity of product shed in a clinical sample. The amount shed should be reported taking into account the final volume/mass of the clinical sample, (e.g., 10 PFU of virus per mL of urine, or 10 CFU of bacteria per mL of urine, or 10 genome copies per microgram of stool). When assessing the quantity of shedding, you should factor in the stability of the product in the clinical sample, and whether there could be an underestimate of the level of shedding because of loss during sample storage, handling and shipping (for details, please refer to section VIII. of this guidance).

Note that your analysis of shedding data should be accompanied by a summary of the shedding profile of the product in patients treated for a specific indication. While it is common practice in clinical development of VBGT and oncolytic products to study a product for different indications in multiple trials, the shedding pattern may be distinct in each study population. We recommend against pooling shedding data from multiple trials in which the same product is studied for different indications because results from a shedding study in a given indication may not be generalizable to other indications.

Finally, we recommend that the shedding data be submitted in a format as described in the next section.

X. WHAT TO INCLUDE IN A CLINICAL SHEDDING STUDY REPORT

In order to address the potential for transmission to untreated individuals due to shedding of VBGT or oncolytic products from the patient, a full shedding report should be provided in the BLA.⁶ The following should be provided in the report:

⁶ Interim data and study reports can be submitted in your IND annual report, for FDA review, if guidance is desired.

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- A. A comprehensive shedding profile of the product in the target patient population(s) which includes the following:
1. Background information on the product: Derivation history; biological characteristics of parent viral or bacterial strain; route of transmission of parent strain; replication competence; attenuation; and tropism of the product.
 2. A summary of the biodistribution profile in animal models and the findings from preclinical shedding studies, if conducted.
 3. The rationale for both the clinical shedding study design (i.e., choice of clinical samples, frequency and procedures of collection and storage), and the analytical method selected to assess shedding.
 4. Your data collection/sampling plan and your procedures for storage, shipping, and handling of the product.
 5. An assay description that includes the following:
 - (a) Test sample preparation or nucleic acid extraction procedures including the dilution factor and amount of nucleic acid extracted per sample.
 - (b) If you use qPCR assays, provide for each assay the sample volume, amount of nucleic acid per reaction, cycle numbers, primers, and size of the amplified DNA.
 - (c) If you use infectivity/growth-based assays, provide the permissive cell line/growth media, the conditions for adsorption and infection or growth, and the nature of the read-out (TCID₅₀, FFU, PFU or CFU; or Cycle threshold (Ct) for assays with qPCR read-out).
 - (d) Assay qualification, controls and sensitivity: Description of the qualification studies, standards, spikes, controls, number of replicates, assay variability and sensitivity (LOD and LOQ, if applicable).
 6. Analysis of shedding data:
 - (a) Tabulation and/or graphical representation of the shedding data.
 - (b) Analysis of the data and summary of the findings from the study.
 7. Your estimate of the potential for transmission to untreated individuals of the product.

In this guidance, see section VIII. for a discussion of analytical assay types and conditions, section VII.B. for a discussion of clinical sample collection, section IX. for

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analysis of shedding data, and section XI. for details on assessing the potential for transmission to untreated individuals due to shedding.

- B. As needed, data and analysis of clinical monitoring for transmission to untreated individuals in the target patient population (refer to section XI.B. of this guidance for additional information).
- C. Other relevant information on the ability of the VBGT or oncolytic product, or its parental/related strain of viruses or bacteria to potentially infect humans and cause disease. When there is the potential to cause disease in humans, the following should be discussed:
 - 1. The spectrum of disease symptoms caused by the parental strain, including atypical presentation of the disease, or occurrence of asymptomatic shedding;
 - 2. The attenuation of the product compared to the parent strain of the virus or bacterium circulating in the community;
 - 3. The natural or acquired immunity of the general population that could potentially protect against infection from the shed product;
 - 4. The therapeutic options to treat the infection/disease in case of transmission to untreated individuals of the shed product; and
 - 5. Preventive/containment measures that can limit spread of the shed product beyond the treated individual to minimize exposure of third parties, particularly, immune-compromised adults, neonates and seniors. Note that the data you collect on onset and duration of shedding can inform appropriate preventive/containment measures. For example, if peak shedding occurs soon after treatment when the patient is monitored in a health-care setting, then the possibility of transmission is mainly confined to health care professionals (HCP) and individuals that come into close contact with the patient. If shedding is prolonged or if there is a second peak of shedding in the days following discharge of a patient from a health care setting, there is the possibility of transmission to contacts beyond the health care and home setting.

XI. ASSESSING THE POTENTIAL FOR TRANSMISSION TO UNTREATED INDIVIDUALS DUE TO SHEDDING

Our current understanding is that in most cases, the potential for transmission to untreated individuals is extremely low when VBGT or oncolytic products are shed because of the derivation methods and/or modifications that are designed to attenuate the product when compared to the parent strain of virus or bacterium. Nevertheless, you should discuss the potential for transmission based on the analysis of the shedding data collected in the clinical studies and taking into consideration the factors described below.

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A. What Information in the Shedding Data Can be Used to Assess Potential for Transmission to Untreated Individuals?

- Whether the VBGT or oncolytic product was shed.
- Whether the shed product was determined to be infectious.
- Whether the amount of infectivity in the clinical samples was comparable to that needed to initiate infection in a third party. For example, adenovirus infective dose is reportedly >150 PFU when given intra-nasally (Ref. 4) or lower when aerosolized (Ref. 5). The minimum infectious human dose may vary when viruses or bacteria are administered or acquired through different routes, or among different strains, but for many disease-causing viruses and bacteria, the minimum infectious dose in humans may be undefined.
- Whether the clinical sample containing the shed product represents the natural route of transmission. For example, a respiratory virus that is shed in feces may not be as infectious and transmissible when compared to that shed in nasopharyngeal secretions.

B. Monitoring Untreated Individuals for Transmission

Because transmission to untreated individuals is an extremely low probability event, monitoring such individuals for transmission is usually not required during the clinical development of a product. However, if there is a potential for transmission, additional data will be needed to assess that possibility; in which case, we recommend that sponsors consult with OCTGT in connection with developing a monitoring plan.

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XII. REFERENCES

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