ICH Considerations

General Principles to Address Virus and Vector Shedding

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1.0 Introduction

For the purpose of this ICH Considerations document, shedding is defined as the dissemination of the virus / vector through secretions and/or excreta of the patient. Virus / vector shedding should not be confused with biodistribution, e.g., spread within the patient's body from the site of administration.[1] Virus / vector[2] includes gene therapy vectors[3] and oncolytic viruses.

Assessment of shedding can be utilized to understand the potential risk associated with transmission to third parties and the potential risk to the environment. The scope of this document excludes shedding as it relates to environmental concerns because it is regulated differently in various regions.

The focus of this document is to provide recommendations for designing non-clinical and clinical shedding studies when appropriate. In particular, emphasis will be on the analytical assays used for detection, and considerations for the sampling profiles and schedules in both non-clinical and clinical studies. The interpretation of non-clinical data and its use in designing clinical studies is also within the scope of this paper, as well as the interpretation of clinical data in assessing the need for virus / vector transmission studies.

2.0 Biological Properties of the Virus / Vector

Information on the known properties of the wild-type strain from which the virus / vector under consideration was derived is essential in guiding the design of shedding studies.

Replication competence is an important property to consider. Replication-competent virus /vector might persist in the patient for extended periods and can increase in amount. Therefore, the potential for shedding can be higher with replicating virus / vector and could result in a greater likelihood of transmission. For replicating virus / vector, analysis of molecular variants will also be important and could impact virus / vector shedding[4].

In practice, most viral / vector products currently under investigation are replication incompetent or conditionally replicative. It is likely that virus / vector shedding in these cases would be of a much shorter duration, and, depending on the route of administration, would display a different shedding profile as compared to shedding following infection with the wild-type counterpart. Additionally, information regarding the known route of transmission of the wild-type strain will help in the interpretation of data from shedding studies and the estimation of the likelihood of transmission. For replication-incompetent products, characterization of any potential replication-competent

[1] Results from biodistribution studies may be used to address blood shed in situations such as surgery or an open wound.
[2] While not described within this document, the principles also apply to replicating bacteria used in oncology applications.
[3] Excluding genetically modified cells, except when there is a risk of mobilisation of the virus / vector.
[4] ICH Considerations on Oncolytic Viruses

recombinants that might be generated during manufacturing is important from both a product quality standpoint and for consideration of shedding.

Other property of the replication-competent virus / vector that should be considered when designing shedding studies would be whether infection is expected to be short- or long-term. Considerations include whether the virus / vector itself has been genetically modified to alter the cellular / tissue tropism compared to the wild-type strain, and whether the immune status of the patient impacts virus / vector shedding. For example, in some cases a greater immune response to the virus / vector might result in faster clearance of the virus / vector and therefore reduce the duration and extent of viral / vector shedding.

3.0 Analytical Assay Considerations

Having suitably qualified analytic assays in place for shedding studies is very important. Assays should be specific, sensitive and reproducible. Quantitative assays are preferred as these will aid in quantifying the probability of transmission. Assessment of interference from the biological sample matrix is important and it might be appropriate to dilute the sample prior to analysis to avoid extensive interference.

Polymerase chain reaction (PCR) and infectivity are the two assays typically used for the detection of shed virus / vector. Use of a quantitative PCR (qPCR)-based assay to detect viral / vector genetic material is recommended. The advantage of qPCR assays is that they are sensitive, reproducible, and rapid. The main disadvantage of qPCR-based assays is that they will not differentiate between intact virus / vector and non-infectious or degraded virus / vector. Infectivity assays involve *in vitro* culture of shed material with a permissive cell line followed by sensitive endpoint detection.

Infectivity assays have the advantage that they will detect only virus / vector that is intact and has the potential of being transmitted. For replication-incompetent virus / vector, these assays will involve the transduction and detection of transgene in an *in vitro* culture system. The main disadvantage of infectivity assays is that they are inherently less sensitive than PCR-based assays. It might be appropriate for the first line of sample analysis to be based on the detection of nucleic acid fragments by quantitative methods specific for the virus / vector product. Other assays such as immunoassays or Southern blots have also been used; as with PCR assays, the main disadvantage is that they do not differentiate between intact virus / vector and non-infectious or degraded virus / vector. Whatever analytical method(s) is selected, it should be justified.

However, to accurately assess the potential for transmission of shed material, the use of an infectivity assay is considered important as this will allow for an accurate assessment of the nature of the shed material (e.g., intact virus / vector vs. fragments of virus / vector). Infectivity assays are commonly used as the primary assay for detection of shed replication-competent virus / vector. When the virus / vector is replication-incompetent, infectivity assays can also be informative in determining whether the shed material is a replication-incompetent virus / vector or a replication-competent recombinant. This can be done by the use of cell lines with and without complementing genes. For example, with adenoviral vector, one can use non-complementing A549 cells and complementing HEK293 cells, which contain regions of E1a, to characterize shed material. A positive culture from HEK293 cells and negative culture from A549 cells will demonstrate that the shed material is likely defective virus / vector and not a replication-competent recombinant. Molecular characterization could be appropriate to confirm the identity of what was detected in the positive culture.

If the amount of shed material detected by qPCR is below the limit of detection of the infectivity assay, one might not choose to further characterize the shed material by infectivity assays due to the constraints of assay sensitivity.

4.0 Non-Clinical Considerations

Non-clinical shedding studies help guide the design of clinical shedding studies. The aim of a non-clinical shedding study is to determine the secretion / excretion profile of the virus / vector. Information collected from these non-clinical studies can then be used to estimate the likelihood and extent of shedding in humans. The non-clinical shedding study can be integrated into the design of other non-clinical studies (e.g., might not be a stand-alone study). It might be helpful to consider the results of studies conducted with virus / vector products that display similar characteristics (e.g., the same virus strain or a strain of the same virus / vector expressing a marker gene) prior to initiating non-clinical shedding studies.

When designing and interpreting non-clinical shedding studies the following points should be considered:

4.1 Animal Species

One of the difficulties of investigating virus / vector products in non-clinical studies is the relevance of the animal species as a large number of virus / vector products under clinical evaluation are derived from parental strains which do not readily infect and rarely replicate in non-human species. The permissiveness of the animal will clearly be important for interpreting data from non-clinical animal studies. The expression and tissue distribution of viral receptors in a specific animal might affect the profile of shedding of the virus / vector. Thus the profile of shedding might not directly correlate with that in humans, as cellular and tissue sequestration could be different. The use of an animal model to mimic a disease condition can be helpful to best assess shedding. For example, the use of a tumour bearing model might be appropriate to support the replication of oncolytic viral products. The impact of immunity to the virus / vector should be taken into consideration as this can affect the rate of viral / vector clearance and therefore shedding. See also sections 4.3 and 4.4 on sampling.

4.2 Dose and Route of Administration

Wherever possible the dose and route of administration used in non-clinical shedding studies should reflect those intended for use in the clinical setting. The non-clinical protocol should be designed for maximum exposure taking into account the clinical route of administration and dose(s) selected. For example, the use of dose levels bracketing the anticipated clinical dose range could be considered for the evaluation of virus / vector shedding in the non-clinical study.

4.3 Sampling Frequency and Study Duration

Known biological properties of the wild-type strain can be used to guide the frequency of sampling after virus / vector administration. In general, one might need to take samples more frequently in the first days following administration in order to detect a transient shedding profile. Practical considerations should determine the number and frequency of samples, depending on the type of excreta or secreta.

Several factors should be considered when determining the duration of the study. These include the natural course of infection of the parental virus, pre-existing immunity and the replicative capacity of the virus / vector under investigation.

The course of a natural infection with the wild-type strain will give some indication of the expected duration of shedding. It is of particular importance if the virus / vector under investigation is replication-competent, as the duration should be sufficient to detect a possible secondary peak of virus / vector suggestive of viral / vector replication. If the virus / vector persists for a long duration in certain tissues, i.e., kidney, lungs or the blood stream, it is recommended that the duration of shedding analysis should follow a similar time course. However if multiple consecutive negative samples are observed it might be appropriate to shorten the study duration. It is important to note that for certain viral / vector products where latency or reactivation is a concern, negative tests performed in a pre-specified time period might not accurately capture virus / vector shed at later time points. It is recognized that reactivation cannot always be modeled in non-clinical studies. Furthermore, immune

responses might be expected to reduce the duration of shedding due to removal of the virus / vector from the circulation.

4.4 Sample Collection

The characteristics of the virus / vector, the route of administration, and animal species should be taken into consideration in determining the samples to be collected. Examples of collected samples most commonly include urine and faeces, but could include other sample types such as buccal swabs, nasal swabs, saliva, and bronchial lavage.

It is worth considering the samples that should be taken and the volumes that should be collected in order to perform quantitative, suitably qualified analytical assays. For certain secreta or excreta, such as urine, it can be difficult to collect sufficient sample material. Pooling of samples from several animals at the same time point receiving the same dose might be an option so that sufficient sample size or volume can be obtained.

4.5 Interpretation of Non-Clinical Data and Transmission Studies

It is important to keep in mind that data from non-clinical shedding studies are useful in guiding the design of clinical shedding studies, particularly as to sample types, sampling frequency, and duration.

If the shedding detected in a non-clinical study indicates the possibility of transmission, performing cage mate transmission studies might be useful in predicting the possibility of human-to-human transmission in clinical studies. See also section 5.3.

Non-clinical shedding studies should not be viewed as a substitute for clinical studies. Even if shedding is not observed in non-clinical studies, it might not preclude assessing virus / vector shedding during clinical development.

5.0 Clinical Considerations

The considerations raised above for non-clinical studies are relevant to the design of virus / vector shedding studies in a clinical setting (i.e., route of administration, duration of shedding observed, sample types to be taken and frequency). The known biological properties of the parental virus / vector, the replication competence of the product, dose, route of administration, and patient population will be key factors to consider in the design of clinical shedding studies.

The exact timing of the conduct of virus / vector shedding studies will depend on the nature of the viral / vector product and the patient population and should be discussed with regulatory authorities. If sufficient data on shedding are obtained during initial clinical studies it might be possible to justify the omission of shedding analysis in confirmatory clinical trials. Factors that will influence the need to perform additional shedding studies in confirmatory clinical trials include the quality of the data collected and the consistency of the shedding pattern seen in patients. . Assessment of shedding might be appropriate to continue even after market authorization

5.1 Sampling Frequency and Duration

Data obtained from non-clinical and any relevant clinical studies can help guide the duration and frequency of sample collection. As discussed in the non-clinical study section, sampling will be more frequent in the first days following administration and can become less frequent with time after administration. It will also be dependent on the replication capacity of the virus / vector under investigation. If the virus / vector is replication-competent the duration of sample collection should take into consideration detection of a possible secondary round of virus / vector shedding after administration. The immune status of the patient population might impact the clearance of the virus / vector and thus could be considered in the shedding study. Consideration should also be given to the possible impact that treatment with immunosuppressive agents might have on virus / vector shedding.

Another point to consider is when to stop collecting and analyzing samples for shed virus / vector. Sample collection and analyses should continue until multiple consecutive negative samples are detected. In the case of a virus / vector derived from a wild-type that displays latency, it can be challenging to sample for shed virus / vector for the time periods necessary to see reactivation. This situation should be discussed with the regional regulatory authority.

5.2 Sample Collection

In addition to using data from non-clinical studies to guide the decision on what samples to collect, one should also consider the characteristics of the virus / vector and the route of administration to be used in the clinical study. For the example of intratumoral injection in head and neck cancer, one might consider the collection of nasopharyngeal lavage or swab in addition to common samples such as urine, faeces, and saliva. Additionally, if the virus / vector is administered intra-dermally or subcutaneously, one might also sample the injection site by swabbing.

5.3 Interpretation of Clinical Shedding Data

There are a number of factors to take into account when assessing the clinical shedding data and the potential risk associated with transmission from shed virus / vector. An important factor to consider is to identify and characterize what is being shed. Specifically, if the assay used does not distinguish intact from degraded or non-infectious virus / vector, then the data might not be informative as to the potential risk associated with transmission. Therefore, assays such as qPCR may be coupled with the use of infectivity assays. If the amount of shed material detected by qPCR is below the limit of detection of the infectivity assay, one might not choose to further characterize the shed material by infectivity assays due to the constraints of assay sensitivity. If one relies solely on assays that do not distinguish intact and non-infectious or degraded virus / vector, then one should assume that the shed material is infectious.

Determining how virus / vector is shed is an important factor when assessing the potential risk associated with transmission. Therefore one should take into account the natural route of infection of the wild-type strain. For example, some viruses are spread through aerosols and in this case if the virus / vector product is shed through saliva or found in nasopharyngeal swabs, this could pose a higher likelihood for transmission as compared to a different route of shedding such as through urine.

One should also consider how much is being shed and the duration of shedding. Replication competent virus / vector might persist in the patient for extended periods and can increase in amount and consequently might result in a greater likelihood of transmission.

A virus / vector derived from a non-pathogenic strain might be of less concern than one derived from a pathogenic strain when shed, but this will depend on the other biological properties of the virus / vector, i.e., replication-competence and the extent of attenuation. If the virus / vector is genetically modified to contain a transgene, one should take into account the safety profile of the expressed transgene product. Furthermore, the potential effect of the transgene on the phenotypic characteristics of the virus / vector should be considered. Ultimately safety information should come from clinical studies.

6.0 Third Party Transmission

In some cases, when shedding is observed, the potential for transmission to third parties might need to be investigated. These investigations would involve evaluation of persons that come into close contact with virus / vector recipients (e.g., family members, healthcare workers) for evidence of transmission. The immunological status of the third party should be considered. A high proportion of the population might already have pre-existing immunity to the virus / vector; in this case, clearance should be effective in those individuals. However, the immune status of the third party contacts could

be compromised, e.g., in the elderly or very young, and so clearance mechanisms might be inefficient. Thus the consequences of infection might be more significant in these individuals.

It might be appropriate to instruct the patient and family members in ways to minimize the exposure of others. This could also include the advice to use specific sanitation measures.

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