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GUIDELINE ON VIRUS SAFETY EVALUATION OF BIOTECHNOLOGICAL INVESTIGATIONAL MEDICINAL PRODUCTS

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EXECUTIVE SUMMARY

The purpose of this document is to provide scientific guidance relating to the viral safety of biotechnological medicinal products used in clinical trials. Guidance is provided with respect to:

(i) the criteria for and the extent of viral safety evaluation studies, that are required prior to and during clinical development.

(ii) the extent to which manufacturers are able to refer to in-house experience concerning virus safety evaluation.

(iii) the risk assessment which should form part of the safety evaluation.

1. INTRODUCTION

Assuring the viral safety of biotechnological medicinal products is a complex process and a reliable assessment of the viral safety of an investigational medicinal product (IMP) is critical.

This guideline provides advice on the viral safety data and documentation that should be submitted in a request for authorisation of a clinical trial of a human biotechnological medicinal product. Reference is made to ICH Q5A (see references), which defines data requirements for marketing authorisation applications (MAA). Although Q5A does not provide specific guidance for biotechnological products in clinical development, the basic principles remain pertinent and applicable.

The guideline provides for a harmonised approach throughout the European Union, for both sponsors and regulators, with regard to assessment of viral safety of biotechnological IMPs during clinical development. This will be especially beneficial for multi-centre studies, potentially involving several different member states.

2. SCOPE

This guideline applies to human biotechnological IMPs prepared from cells cultivated *in vitro* from characterised cell banks of human or animal origin as described in Q5A. Many IMPs are derived from well-characterised rodent cell lines such as CHO, NS0 or SP2/0, although a variety of other cell lines are in use and under development and should be considered on a case-by-case basis.

The guideline covers monoclonal antibodies and recombinant DNA derived IMPs including recombinant subunit vaccines. It does not apply to IMPs that contain recombinant viruses or bacteria (both replicative and non-replicative), or live attenuated or inactivated vaccines. IMPs derived from hybridoma cells grown *in vivo* are also excluded from the scope of this guideline.

This guideline outlines the viral safety requirements applicable to all stages of clinical development of an IMP. The guideline does not apply to IMPs to be used solely for non-clinical testing. ICH Q5A provides guidance on data required to support MAAs.

3. LEGAL BASIS

Clinical trials within the EU are regulated by Directive 2001/20/EC (see references) and investigational medicinal products used in trials should be manufactured according to the principles of Good Manufacturing Practice Approval of trials is the responsibility of individual Member States, which are required to evaluate the products used in clinical studies.

4. MAIN GUIDELINE TEXT

4.1 General Principles

The aim of virus safety studies for biotechnological IMPs is to demonstrate an acceptable level of safety for clinical trial subjects.

The viral safety of a licensed biotechnological medicinal product is assured by three complementary approaches involving (i) selecting and testing cell lines and other raw materials of human or animal origin for viral contaminants, (ii) assessment of the capacity of downstream processing to clear

infectious viruses and (iii) testing the product at appropriate steps for contaminating viruses (see ICH Q5A).

For a biotechnological IMP, due to the developmental nature of the manufacturing process and of the product, a reduced programme of studies on assuring viral safety is envisaged compared with the data requirements for marketing authorisation; firstly, for testing for viruses in end of production cells/unprocessed bulk (see Section 4.2.3) and secondly, for studies on the validation of virus reduction (see Section 4.2.4). Such a reduced programme would only be applicable for cell lines classified in ICH Q5A as 'Case A' or 'Case B'. Demonstrated in-house experience (see Section 4.2.4) could also contribute to a reduced package of studies on virus reduction.

In addition to the provision of data, a risk assessment should be made taking into account some or all of the following factors:

- the nature and history of the cell line,
- the extent of characterisation of the cell line,
- use of raw materials of human and/or animal origin during manufacture and their control,
- potential for product exposure to adventitious contamination,
- experience of the manufacturer with the cell line involved,
- experience of the manufacturer with specific virus reduction procedures to be used,
- published data.

4.2 Assuring the viral safety of biotechnological IMPs

4.2.1 Cell line qualification: testing for viruses

Testing of the master cell bank (MCB) for viral contaminants should be performed as described in Q5A prior to the initiation of a Phase I trial.

A working cell bank (WCB) might only be set up during clinical development and thus, for some biotechnological IMPs being used early in clinical development, it may not yet have been established. When established, the first WCB should in principle be tested as outlined in ICH Q5A. However, where unprocessed bulks are tested as described in Section 4.2.3/Table 1, testing of cells at the limit of *in vitro* cell age is not required.

Since endogenous retroviral viruses or particles are present in most cell lines currently in use and there is a probability that they will be present in a novel cell line, particular attention should be paid to investigating the cell line for their presence.

4.2.2 Raw materials of biological origin

The viral safety evaluation of biotechnological IMPs should take into account biological raw materials (especially animal or human derived) used in production. A risk-based assessment focusing on the type and origin of the raw material, its process conditions and testing, as well as its use in the manufacture of the medicinal product and tests applied to the unprocessed bulk material, is an acceptable approach to the evaluation of its viral safety (see also Section 4.2.3).

Appropriate documentation should be provided regarding the viral safety of raw material of biological origin. Reference is made to guidance documents on bovine sera as well as on minimising the risk for transmission of animal spongiform encephalopathy (see references).

4.2.3 Testing for viruses in unprocessed bulk

Independent of the stage of development, each batch of unprocessed bulk material that will be used to manufacture clinical trial material should be tested as per Q5A. The sample to be tested should include cells, when appropriate, and tests should include *in vitro* and PCR-based screening tests for adventitious agents and an estimation of retroviral particles, where applicable. No further testing is required for bulks deriving from CHO cell lines. For manufacture based upon NS0 or Sp2/0 cell lines, tests for infectious retroviruses should be applied on a one-off basis but should be repeated if there is a significant change in production cell culture, e.g. manufacturing scale. For manufacture based upon

any other cell line, tests for infectious retroviruses and *in vivo* tests (as per section 3.2.3 of ICH Q5A) should be applied on a one-off basis, but should be repeated if there is a significant change in production cell culture, e.g. manufacturing scale. These testing recommendations are shown in Table 1. Consideration should be given to the inclusion of a test for MMV if the cell line is permissive for this virus.

The source and viral safety of the raw materials used during cell culture should be taken into account when devising the unprocessed bulk testing (see also Section 4.2.2). Additional specific tests may be required if human or animal derived raw materials are used, e.g. bovine serum.

Table 1. Testing requirements for unprocessed bulks

	***In vitro* testing**	**Tests for infectious retroviruses***	***In vivo* testing***
CHO	Yes, all bulks[§]	No	No
NS0 and Sp2/0	Yes, all bulks[§]	Yes, once for given scale	No
All other cell lines	Yes, all bulks[§]	Yes, once for given scale	Yes, once for given scale

* Where possible, test material should contain cells or cellular fragments in order to detect cell-associated viruses. For perfusion cell cultures, manufacturers should determine and justify the most appropriate stage at which to derive samples containing cells for testing. It is also acceptable to derive test material from cells that have been cultured beyond the scale used to generate the batch of product; in these circumstances, the approach taken should be justified. Testing for infectious retroviruses may be omitted when more sensitive tests have shown negative results.

[§] Quantification of retroviruses or retroviral-like particles need only be performed for the first three bulks for a specific stage of development (or less, if less than three bulks are prepared).

4.2.4 Validation of virus reduction

The objective of the validation is two-fold; firstly, to characterise and evaluate process steps that can be considered to be effective in inactivating/removing viruses and secondly, to estimate quantitatively the overall level of reduction of any virus/viral particle, e.g. endogenous retroviral particles. A case-by-case approach will be required taking into account the characterisation of the cell line, the use of raw materials of biological origin, as well as the nature of the process steps that may be effective in inactivating/removing viruses.

Regardless of the extent of direct virus testing of the production cell line, due to limitations in viral detection assays, there remains a potential for unknown contamination of the cells with a virus originally present in the cells or arising from materials of biological origin that are used during cultivation of the production cells. Consequently, even when no raw materials of biological origin have been used and the cell line is fully tested, the downstream process for all IMPs should be evaluated for virus inactivation/removal.

Validation of virus reduction should be performed prior to the onset of the clinical trial. Potential contaminants may be enveloped or non-enveloped viruses and virus reduction studies should include both an enveloped virus and a small non-enveloped virus, preferably a parvovirus. Especially, it must be demonstrated that any virus or viral particle known to be present in the bulk harvest has been effectively inactivated or removed during downstream processing. Case B cells (as defined in Q5A) contain endogenous retroviruses or retrovirus-like particles and a retrovirus should be used in validating the inactivation/removal of viruses to demonstrate full clearance of particles present in the bulk harvest.

Virus reduction studies should be performed according to the principles of Q5A although a demonstration of robustness (i.e. influence of process parameters on virus reduction) may not always be warranted as outlined below. The relevant steps in product purification that contribute to virus reduction should be described. The capacity of these steps to inactivate/remove potential virus

contaminants should take into consideration the viral safety of the production cell line, e.g. the type and level of endogenous retroviral contamination, or the use of human or animal derived materials during manufacture and possible levels of contamination. The CHMP Note for guidance on virus validation studies (see references) also provides useful detailed information on such studies.

It is desirable to investigate the contribution of more than one production step for virus reduction and at least two orthogonal steps should be assessed. Orthogonal steps are defined as process steps where different mechanisms are responsible for virus inactivation/removal. The criteria for an effective step have been outlined in Note for Guidance on Virus Validation Studies (CPMP/BWP/268/95). It is not necessary to investigate process steps where no significant virus reduction can be expected.

The reproducibility of an effective virus reduction step should be demonstrated by at least two independent experiments.

In performing the validation study, the limits of (i.e. worst-case) process parameters should be used, whenever such conditions are known. However, during development, such worse case limits may not have been defined for a new manufacturing process. In these cases, use of representative (i.e. set-point) conditions is justified as long as the manufacturer can demonstrate that the actual manufacturing process ran at these set-points.

Circumstances to support a reduction of the above studies:

- Investigation of a single specific inactivation/removal step might be sufficient whenever effective virus reduction of a broad range of viruses, including small non-enveloped viruses such as parvoviruses, can be demonstrated for such a step. However, for case B cells, it will usually be necessary to evaluate more than one step in order to demonstrate adequate clearance of retroviral particles.

- Prior experience of the manufacturer with a specific downstream processing step. In the event that a manufacturer is developing similar types of products by established and well-characterised procedures, virus reduction data derived for these other products might be applicable to the new product for an equivalent processing step.

  In general, in order to make use of data from such a step, the step should have been carefully evaluated, including a thorough study of the process parameters that affect virus reduction. If data for more than one product is available for the specific step, the effectiveness of virus reduction should be comparable in each case. Processing prior to the specific step for the new and the established product(s) should follow a similar strategy.

  A rationale should be provided why prior in-house data can be applied to the new product, e.g. referring to viral reduction data of a particular process step would be possible when the product intermediate at the stage before such a step has comparable biochemical properties and is purified by identical methods. The manufacturer should provide a critical analysis of the manufacturing step for which in-house data will be applied and on the composition of the respective product intermediate. This should include, for example, the type of filter, load per filter area, flow rates, pressure and composition of product intermediates for virus retention filters, or the column dimensions including bed height, load, composition of buffer and product intermediates, and linear flow rates for chromatographic methods. In addition, each new product might have components not present in previous products and so the potential influence of product-specific components should be considered. The analysis should provide complete confidence in the conclusion that in both cases the established manufacturing step is similar in its capacity to inactivate/remove potential virus contaminants. If the comparison of the step is not entirely convincing, or if the database is not convincing enough to rule out a product-specific effect on virus reduction capacity, at least a single run with an appropriate virus is needed to confirm that the step is indeed performing as expected. If the process performance is clearly different, e.g. different chromatographic profiles are obtained using the same equipment, then the step should be validated as above and according to the principles of Q5A.

Published data can be useful in indicating the potential of a step to inactivate/remove viruses and can provide an insight to the mechanisms involved. This facilitates an exploration of the key process

parameters that affect virus reduction and in setting worst-case limits for specific steps to be validated. Nevertheless, the application of published reduction factors to a specific product would require extensive demonstration of comparability of the processes involved, of the product intermediates, and an assurance that product specific process factors do not affect virus reduction. Virus reduction may depend on various process parameters and from the specific composition of a product intermediate. Furthermore, the assigned reduction capacity may be specific for selected viruses (e.g. chromatographic methods). Therefore, published data have to be carefully assessed.

Due to the use of dedicated columns and the small number of batches manufactured during early stage development, specific column re-use and sanitisation studies are generally not required for IMPs. However, whenever columns are extensively re-used for production of IMPs, this should be considered in the investigation of the virus reduction capacity.

Revalidation of virus reduction

The data generated for IMPs used in a previous trial (e.g. a first phase I trial) may be used for subsequent trials. However, significant changes in manufacture might have been implemented during development of an IMP and it has to be considered that such changes may influence directly or indirectly (by changes other than in the evaluated process steps) the capacity for virus reduction. Therefore re-evaluation before the start of the next clinical trial will be necessary whenever the available data do not reflect production of the IMP to be used in the forthcoming clinical trial. Depending on the introduced changes, the selection of viruses should be reconsidered and additional viruses used if needed, to provide confidence in the virus reduction capacity of the process. Even if a complete validation according to Guideline Q5A is not required in extended clinical trials at late stages (e.g. phase III), manufacturers should justify the approach taken considering the selection of model viruses and evaluated process steps. Full viral validation studies according to Q5A should be undertaken as soon as the final production and purification process has been established.

4.2.5 Description and Qualification of Analytical Procedures

Different types of analytical procedures can be used to test for viruses in the starting materials and intermediate products, or in assessing the virus reduction capacity of the manufacturing process. Virus detection assays include broad screen *in vitro* tests evaluating both cytopathic effect (cpe) and hemadsorption in multiple indicator cell lines, *in vivo* tests and specific viral testing using for example PCR. To test for retroviruses, transmission electron microscopy (TEM), co-cultivation assays using different cell lines and assays for RTase such as Product Enhanced Reverse Transcriptase (PERT) may be used.

Irrespective of the clinical trial phase, the suitability of the analytical methods used for viral testing, either as a qualitative or a quantitative method, should be substantiated. Basically, ICH Q5A Chapter 3.2 “Recommended Viral Detection and Identification Assays” and Chapter 4 “Testing for Viruses in Unprocessed Bulk” are applicable. A sufficiently detailed description of the analytical procedures should be provided, including reagents, assay controls, test procedure, and validity criteria, to allow for a clear understanding of the assay used and how it is controlled. Where compendial procedures are used, clear references should be given.

For analytical procedures supporting the qualification of the cell bank system and other starting materials as well as testing of unprocessed bulk for viruses, a tabulated summary of the analytical qualification/validation results of these procedures should be provided, as appropriate (e.g. results of values found for specificity using appropriate positive and negative controls, sensitivity, quantification and detection limit). It is not necessary to provide a full qualification report for each method; however such reports should be held available and submitted upon request.

For analytical procedures supporting the viral reduction studies, full details should be provided which show the suitability of these procedures to quantify the (model) virus particles. These should include studies to assess, for example, quantification limit, specificity, intrinsic assay variability, buffer/matrix interference with viral infectivity, and product and buffer cytotoxicity that might affect the ability of the selected model viruses to infect the indicator cells. Statistical considerations for assessing virus

assays can be found in ICH Q5A Appendix 3. Where applicable, a report from a contract laboratory which conducted the viral testing may be acceptable.

4.3 Virus safety risk assessment

In addition to the derivation and provision of data on the viral safety of the product, a virus safety risk assessment should be provided with an application for clinical trial authorisation. The factors noted under Sections 4.1 and 4.2.1 to 4.2.4 should be taken into account as the primary factors. In accordance with Q5A, testing of the cell line and of all raw materials of human or animal origin for viral contaminants, validation of virus reduction and testing of the product at appropriate steps of the manufacturing process for absence of contaminating infectious viruses should be considered.

The risk assessment should include the calculation of estimated particles per dose (see ICH Q5A, Appendix 5) and encompass all steps of the production process.

In particular cases it may be reasonable to consider clinical parameters such as the indication, the dose, the frequency of administration, the number of people exposed, the study duration and the immunological status of the patients in the overall risk assessment for a clinical trial. In this context, it should be considered that several of these parameters might change between Phases I, II and III. The clinical parameters should not be considered as primary decision parameters, but they can contribute to the final decision on whether to authorise a clinical trial from the viral safety point of view.

Each situation will be considered on a case-by-case basis.

4.4 Re-evaluation of viral safety during development

Process changes are often introduced during development of an IMP and some of them could impact on a previously determined viral safety assessment. Whenever changes are introduced in the production process of an IMP for which a viral safety risk assessment has been performed, the manufacturer should document all changes introduced and for each of them should consider if a re-assessment of the risk is required. In some cases it will be clear that the change has no impact on the viral safety risk assessment. However where there is a clear impact or the outcome is uncertain, the risk assessment should be re-evaluated and where necessary appropriate practical studies performed. All aspects of viral safety assurance should be taken into account in these considerations.

For changes that might compromise the validity of virus reduction studies, see paragraph ‘Revalidation’ in Section 4.2.4.2

4.5 Format of clinical trial authorisation documentation

The overall programme of assuring viral safety should be carefully and clearly presented, with explicit justification for any deviation from the minimum recommendations made in this guideline.

The format, as required by the “Detailed guidance for the request for authorisation of a clinical trial on a medicinal product for human use to the competent authorities, notification of substantial amendments and declaration of the end of the trial” includes a specific attachment, i.e., Attachment 2: 2.1.A Appendices, 2.1.A.2, Adventitious Agents Safety Evaluation, dedicated to the data on TSE agents, virus safety of biotechnological IMPs and other adventitious agents. All the data should be brought together in this Attachment in order to be self-standing and understood in its entirety with minimum references to the other sections of the main dossier. Full reports including raw data of cell line testing and viral reduction studies should be available upon request. During evaluation of the submitted data, it may be necessary to request such reports to ensure as clear an understanding as possible of the viral safety of an IMP. Raw data might be provided by contract laboratories or internal labs as part of the reports. When the applicant makes use of prior in-house data (i.e. data from other products), an adequate package of data should be provided to allow an assessment of the in-house data and to provide confidence that these data are valid or supportive for the specific product under development.

For general consideration on virus safety documentation, information to be submitted can take into consideration the items stated in volume 2B of the Notice to Applicants, Part II V: v*irological documentation*.

REFERENCES (scientific and/or legal)

Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use

ICH Q5A: ICH harmonised tripartite guideline on Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin

CHMP Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses (CPMP/BWP/268/95)

Detailed guidance for the request for authorisation of a clinical trial on a medicinal product for human use to the competent authorities, notification of substantial amendments and declaration of the end of the trial (ENTR/F2/BL D(2003)).

Volume 2B of the Notice to Applicants, Part II – IX. concerning chemical, pharmaceutical and biological documentation for biological medicinal products, Part II V: virological documentation (http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/vol-2/b/pdfs-en/part2_3en.pdf)

CHMP Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01 in its current version)

CHMP Note for guidance on the use of bovine serum in the manufacture of human biological medicinal products (CPMP/BWP/1793/02).

Ph. Eur. monograph on “Bovine serum” (01/2008:2262)