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*Evaluation of Medicines for Human Use*

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# COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

# POSITION STATEMENT ON THE USE OF TUMOURIGENIC CELLS OF HUMAN ORIGIN FOR THE PRODUCTION OF BIOLOGICAL AND BIOTECHNOLOGICAL MEDICINAL PRODUCTS

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## 1. INTRODUCTION

### Background

A wide variety of phenotypically distinct cell types may be used for the production of biological and biotechnological medicinal products (biologicals) for human use. This includes primary cultures of simian and avian origin, diploid cell lines of human and simian origin and continuous cell cultures of murine, primate, insect and other origin. The use of primary and diploid cells is currently limited to the manufacture of viral vaccines whilst continuous cell lines (CCLs) are used in the production of a variety of products including recombinant biopharmaceuticals, monoclonal antibodies, vaccines and products for gene therapy.

Continuous cell lines are those which have the potential to be sub-cultured *ad infinitum*. Some continuous cell lines (e.g. CHO and Vero cells) developed spontaneously by sequential passage of primary cultures of normal tissue, some lines were derived from normal tissue but were treated specifically to transform or immortalise them, for example, transformation by an oncogenic virus whereas others are of direct neoplastic origin. Cell lines may also be transformed by specific genetic modification.

There is a wide range of authorised biological medicinal products derived from CCLs and regulatory guidelines have been developed which provide recommendations to assure the safety and quality of such products. Most CCLs in use are of non-human origin and tumourigenic cells of human origin have not been widely used for the production of biologicals for human use. However, they are increasingly being considered and this document therefore addresses the issues presented by their use.

### Current regulatory guidelines

Current regulatory guidelines provide broad guidance on appropriate standards for the use of human and animal cell lines and microbial cells for the preparation of biologicals. However, the Biotechnology Working Party (BWP) of the CPMP considers that the use of tumourigenic cells of human origin raises issues which may not have been adequately covered in these guidelines.

This position statement has been developed by the BWP to address the quality and biological aspects specific to the use of such cell lines, taking into account:

- current scientific thinking with regard the use of neoplastic or transformed cell-lines for the production of biologicals and
- existing regulatory guidelines (see references)

### Scope

The scope of this position statement is:

i) purified products derived from human continuous cell lines obtained from solid tumours or haematological malignancies,

ii) purified products derived from human cells that have been transformed *in vitro* by biological, chemical or genetic engineering techniques.

Biological products derived from tumourigenic cells of human origin which receive no or minimal purification or consisting of replicative systems, e.g. live vaccines, are not within the scope of this document.

This statement sets out the current EU regulatory thinking with regard to risk assessment of tumourigenic cells of human origin and emphasises certain aspects of the characterisation of these cell-lines already detailed in the relevant guidelines. It addresses:

- characterisation of the cell substrate and related issues,

- genomic and phenotypic stability,
- residual cell substrate DNA,
- biologically active growth factors with transforming potential.

This document is not legally binding on applicants and may evolve with new scientific developments.

## 2. POSITION STATEMENT

### 2.1 General considerations

For products derived from tumourigenic cells of human origin, a risk assessment should be properly carried out and risk factors should be defined, as far as possible, in a quantitative manner. However, with respect to novel cell substrates, risks may be difficult to define qualitatively or quantitatively because the end-points are either theoretical or cannot be measured.

Although this document focuses on risk assessment, it should be noted that the potential risks associated with the use of transformed cells in manufacturing medicinal products should be evaluated against the intended clinical use of such products. Inevitably, in any decision-making process with regard to the suitability of using a given cell type, this will call for a properly conducted risk/benefit assessment including the rationale for choosing the use of a human CCL instead of a non-human cell substrate.

Residual cellular DNA is perceived to be the principal risk associated specifically with CCL derived products. It is not DNA *per se* which is a concern but the actual sequence encoded by the residual DNA. Tumourigenic cells of human origin are likely to contain sequences not present in the non-tumourigenic state, e.g. a mutated p53 gene or a translocation event which generates a novel gene such as *bcr-abl* or *myc*. Furthermore, a tumourigenic cell line may contain a biologically active viral oncogene or a virus genome and this may present a greater concern than a mutated cellular oncogene. A viral oncogene, if expressed, has the potential of causing a greater biological effect due to the multi-functional properties of the encoded protein than a cellular oncogene whose product generally has a single biological property. With regard to tumourigenic cells of human origin, the sequences of concern are those encoding a growth-promoting factor or those controlling expression (e.g. promoters) and which have the potential to be expressed once integrated into a recipient's cell.

### 2.2 Specific considerations

**Characterisation of cell substrates**

In line with the general principles as laid down in various guidelines, it is important to characterise the cells with respect to their biological and morphological characteristics.

In addition, the following is emphasised: a human tumour may have been the result of a viral infection and so the presence of a latent viral genome, or part thereof, should be investigated taking into account the association of certain viruses with specific tissues and tumours. Whilst the presence of viral sequences does not necessarily preclude the use of a cell-line, consideration should be given to investigating the possibility of viral genome reactivation, especially under the conditions of cell culture during manufacture.

In any risk assessment, a distinction should be made between transformation mediated by known or by unknown factors. The latter scenario may present a higher level of uncertainty in so far as risk assessment is concerned.

Consideration should also be given to the possible consequences of the expression of biologically active proteins by the cell substrate. CCLs may express potent proteins involved in the regulation of cell division and the potential effects of them on the cells of the recipient

of the biological product should be considered. However, it is recognised that in a highly purified product, the biological effects of such proteins are transient and likely to be insignificant due to their extremely low levels.

Viral safety should be addressed taking into consideration the pertinent principles as set out in current guidelines. Emphasis is given to the expression of endogenous retroviral elements although their presence is generally independent of the tumourigenic state of a cell-line.

**Genomic and phenotypic stability**

The stability of the cells should be evaluated with respect to their suitability for manufacturing products for therapeutic and prophylactic use. However, particular attention should be paid to the possibility that changes to genotypic or phenotypic characteristics may induce or result from the expression of quiescent sequence(s) within the cells, for example the expression of a viral sequence or a protein. The biological consequences of such an event should be considered and properly investigated. Reference is made to the ICH Guideline on "genetic stability" (8).

**Residual cell substrate DNA**

In the case of tumourigenic cells of human origin, residual cellular DNA is a key safety issue to be addressed in any regulatory submission. There is at present no incontrovertible evidence to show that DNA extracted from human tumours is capable of inducing tumours *in vivo*. However, there is published literature showing that DNA from highly tumourigenic cells of human origin is capable of transforming certain mouse cell-lines *in vitro*. Also, it has been reported that nucleic acids extracted from oncogenic viruses such as polyoma can induce tumours in animals *in vivo*. However, in these cases, this is likely to be due to the biological activity of the DNA in establishing a viral infection.

The WHO has concluded that levels of up to 10 ng of residual host cell DNA per purified dose can now be considered acceptable. However, it has stated that instances do occur where CCL DNA is considered to pose a greater risk, e.g. where it could include infectious retroviral provirion sequences. The CPMP/BWP considers that tumourigenic cells of human origin is another such instance. With these considerations in mind, the level of residual cell substrate DNA permitted in the final product should be as low as possible and should be based on a properly conducted risk assessment taking into account the following factors:

- the nature and origin of the cell substrate
- the nature of any viral sequences present in the cell substrate
- the molecular size and nature of the fragments of any residual DNA found in the purified bulk material
- the capability and robustness of the purification process to remove residual cell substrate DNA
- the sensitivity, selectivity and precision of the assay method to estimate the quantity of cell substrate DNA
- the dose and dosing regimen of a given medicinal product

## 3. CONCLUSION

This document outlines the relevant factors that need careful consideration when using tumourigenic cell lines of human origin for the production of medicinal products. It is important to define the risk factors attendant to the use of such cell substrates with a view to addressing such concerns in a properly conducted risk assessment for any regulatory submission.

References


1. CPMP/ICH Note for Guidance on Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products (CPMP/ICH/294/95))
2. World Health Organisation (WHO) Technical Report [1998] "Requirements for the use of animal cells as in vitro substrates for the production of biologicals"
3. CPMP/BWP Position Paper on DNA and Host Cell Proteins (HCP) Impurities, Routine Testing versus Validation Studies
4. CPMP Note for Guidance on Production and Quality Control of Monoclonal Antibodies
5. CPMP Note for Guidance on Production and Quality Control of Medicinal Products Derived by Recombinant DNA Technology
6. CPMP 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)
7. CPMP/ICH Note for Guidance on Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (CPMP/ICH/295/95)
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