The interaction and compatibility between genes, cells and/or tissues and the structural components shall be described.

3.4.2. *Combined advanced therapy medicinal products as defined in Article 2(1)(d) of Regulation (EC) No 1394/2007*

For the cellular or tissue part of the combined advanced therapy medicinal product, the specific requirements for somatic cell therapy medicinal products and tissue engineered products set out in section 3.3 shall apply and, in the case of genetically modified cells, the specific requirements for gene therapy medicinal products set out in section 3.2 shall apply.

The medical device or the active implantable medical device may be an integral part of the active substance. Where the medical device or active implantable medical device is combined with the cells at the time of the manufacture or application or administration of the finished products, they shall be considered as an integral part of the finished product.

Information related to the medical device or the active implantable medical device (which is an integral part of the active substance or of the finished product) which is relevant for the evaluation of the combined advanced therapy medicinal product shall be provided. This information shall include:

(a) information on the choice and intended function of the medical device or implantable medical device and demonstration of compatibility of the device with other components of the product;

(b) evidence of conformity of the medical device part with the essential requirements laid down in Annex I to Council Directive 93/42/EEC (**), or of conformity of the active implantable device part with the essential requirements laid down in Annex 1 to Council Directive 90/385/EEC (***);

(c) where applicable, evidence of compliance of the medical device or implantable medical device with the BSE/TSE requirements laid down in Commission Directive 2003/32/EC (****);

(d) where available, the results of any assessment of the medical device part or the active implantable medical device part by a notified body in accordance with Directive 93/42/EEC or Directive 90/385/EEC.

The notified body which has carried out the assessment referred to in point (d) of this section shall make available on request of the competent authority assessing the application, any information related to the results of the assessment in accordance with Directive 93/42/EEC or Directive 90/385/EEC. This may include information and documents contained in the conformity assessment application concerned, where necessary for the evaluation of the combined advanced therapy medicinal product as a whole.

4. SPECIFIC REQUIREMENTS REGARDING MODULE 4

4.1. **Specific requirements for all advanced therapy medicinal products**

The requirements of Part I, Module 4 of this Annex on the pharmacological and toxicological testing of medicinal products may not always be appropriate due to unique and diverse structural and biological properties of advanced therapy medicinal products. The technical requirements in sections 4.1, 4.2 and 4.3 below explain how the requirements in Part I of this Annex apply to advanced therapy medicinal products. Where appropriate and taking into account the specificities of advanced therapy medicinal products, additional requirements have been set.

The rationale for the non-clinical development and the criteria used to choose the relevant species and models (*in vitro* and *in vivo*) shall be discussed and justified in the non-clinical overview. The chosen animal model(s) may include immuno-compromised, knockout, humanised or transgenic animals. The use of homologous models (e.g. mouse cells analysed in mice) or disease mimicking models shall be considered, especially for immunogenicity and immunotoxicity studies.

In addition to the requirements of Part I, the safety, suitability and biocompatibility of all structural components (such as matrices, scaffolds and devices) and any additional substances (such as cellular products, biomolecules, biomaterials, and chemical substances), which are present in the finished product, shall be provided. Their physical, mechanical, chemical and biological properties shall be taken into account.

4.2. **Specific requirements for gene therapy medicinal products**

In order to determine the extent and type of non-clinical studies necessary to determine the appropriate level of non-clinical safety data, the design and type of the gene therapy medicinal product shall be taken into account.

4.2.1. *Pharmacology*

(a) *In vitro* and *in vivo* studies of actions relating to the proposed therapeutic use (i.e. pharmacodynamic "proof of concept" studies) shall be provided using models and relevant animal species designed to show that the nucleic acid sequence reaches its intended target (target organ or cells) and provides its intended function (level of expression and functional activity). The duration of the nucleic acid sequence function and the proposed dosing regimen in the clinical studies shall be provided.

(b) Target selectivity: When the gene therapy medicinal product is intended to have a selective or target-restricted functionality, studies to confirm the specificity and duration of functionality and activity in target cells and tissues shall be provided.

4.2.2. *Pharmacokinetics*

(a) Biodistribution studies shall include investigations on persistence, clearance and mobilisation. Biodistribution studies shall additionally address the risk of germline transmission.

(b) Investigations of shedding and risk of transmission to third parties shall be provided with the environmental risk assessment, unless otherwise duly justified in the application on the basis of the type of product concerned.

4.2.3. *Toxicology*

(a) Toxicity of the finished gene therapy medicinal product shall be assessed. In addition, depending on the type of product, individual testing of active substance and excipients shall be taken into consideration, the *in vivo* effect of expressed nucleic acid sequence-related products which are not intended for the physiological function shall be evaluated.

(b) Single-dose toxicity studies may be combined with safety pharmacology and pharmacokinetic studies, e.g. to investigate persistence.

(c) Repeated dose toxicity studies shall be provided when multiple dosing of human subjects is intended. The mode and scheme of administration shall closely reflect the planned clinical dosing. For those cases where single dosing may result in prolonged functionality of the nucleic acid sequence in humans, repeated toxicity studies shall be considered. The duration of the studies may be longer than in standard toxicity studies depending on the persistence of the gene therapy medicinal product and the anticipated potential risks. A justification for the duration shall be provided.

(d) Genotoxicity shall be studied. However, standard genotoxicity studies shall only be conducted when they are necessary for testing a specific impurity or a component of the delivery system.

(e) Carcinogenicity shall be studied. Standard lifetime rodent carcinogenicity studies shall not be required. However, depending on the type of product, the tumourigenic potential shall be evaluated in relevant *in vivo/in vitro* models.

(f) Reproductive and developmental toxicity: Studies on the effects on fertility and general reproductive function shall be provided. Embryo-foetal and perinatal toxicity studies and germline transmission studies shall be provided, unless otherwise duly justified in the application on the basis of the type of product concerned.

(g) *Additional toxicity studies*

— Integration studies: integration studies shall be provided for any gene therapy medicinal product, unless the lack of these studies is scientifically justified, e.g. because nucleic acid sequences will not enter into the cell nucleus. For gene therapy medicinal products not expected to be capable of integration, integration studies shall be performed, if biodistribution data indicate a risk for germline transmission.

— Immunogenicity and immunotoxicity: potential immunogenic and immunotoxic effects shall be studied.

4.3. **Specific requirements for somatic cell therapy medicinal products and tissue engineered products**

4.3.1. *Pharmacology*

(a) The primary pharmacological studies shall be adequate to demonstrate the proof of concept. The interaction of the cell-based products with the surrounding tissue shall be studied.

(b) The amount of product needed to achieve the desired effect/the effective dose, and, depending on the type of product, the frequency of dosing shall be determined.

(c) Secondary pharmacological studies shall be taken into account to evaluate potential physiological effects that are not related to the desired therapeutic effect of the somatic cell therapy medicinal product, of the tissue engineered product or of additional substances, as biologically active molecules besides the protein(s) of interest might be secreted or the protein(s) of interest could have unwanted target sites.

4.3.2. *Pharmacokinetics*

(a) Conventional pharmacokinetic studies to investigate absorption, distribution, metabolism and excretion shall not be required. However, parameters such as viability, longevity, distribution, growth, differentiation and migration shall be investigated, unless otherwise duly justified in the application on the basis of the type of product concerned.

(b) For somatic cell therapy medicinal products and tissue engineered products, producing systemically active biomolecules, the distribution, duration and amount of expression of these molecules shall be studied.

4.3.3. *Toxicology*

(a) The toxicity of the finished product shall be assessed. Individual testing of active substance(s), excipients, additional substances and any process-related impurities shall be taken into consideration.

(b) The duration of observations may be longer than in standard toxicity studies and the anticipated lifespan of the medicinal product, together with its pharmacodynamic and pharmacokinetic profile, shall be taken into consideration. A justification of the duration shall be provided.

(c) Conventional carcinogenicity and genotoxicity studies shall not be required, except with regard to the tumourigenic potential of the product.

(d) Potential immunogenic and immunotoxic effects shall be studied.

(e) In the case of cell-based products containing animal cells, the associated specific safety concerns such as transmission to humans of xenogeneic pathogens shall be addressed.

5. SPECIFIC REQUIREMENTS REGARDING MODULE 5

5.1. **Specific requirements for all advanced therapy medicinal products**

5.1.1. The specific requirements in this section of Part IV are additional requirements to those set in Module 5 in Part I of this Annex.

5.1.2. Where the clinical application of advanced therapy medicinal products requires specific concomitant therapy and involve surgical procedures, the therapeutic procedure as a whole shall be investigated and described. Information on the standardisation and optimisation of those procedures during clinical development shall be provided.

Where medical devices used during the surgical procedures for application, implantation or administration of the advanced therapy medicinal product may have an impact on the efficacy or safety of the advanced therapy product, information on these devices shall be provided.

Specific expertise required to carry out the application, implantation, administration or follow-up activities shall be defined. Where necessary, the training plan of health care professionals on the use, application, implantation or administration procedures of these products shall be provided.

5.1.3. Given that, due to the nature of advanced therapy medicinal products, their manufacturing process may change during clinical development, additional studies to demonstrate comparability may be required.

5.1.4. During clinical development, risks arising from potential infectious agents or the use of material derived from animal sources and measures taken to reduce such risk shall be addressed.

5.1.5. Dose selection and schedule of use shall be defined by dose-finding studies.

5.1.6. The efficacy of the proposed indications shall be supported by relevant results from clinical studies using clinically meaningful endpoints for the intended use. In certain clinical conditions, evidence of long-term efficacy may be required. The strategy to evaluate long-term efficacy shall be provided.

5.1.7. A strategy for the long-term follow-up of safety and efficacy shall be included in the risk management plan.

5.1.8. For combined advanced therapy medicinal products, the safety and efficacy studies shall be designed for and performed on the combined product as a whole.

5.2. **Specific requirements for gene therapy medicinal products**

5.2.1. *Human pharmacokinetic studies*

Human pharmacokinetic studies shall include the following aspects:

(a) shedding studies to address the excretion of the gene therapy medicinal products;

(b) biodistribution studies;

(c) pharmacokinetic studies of the medicinal product and the gene expression moieties (e.g. expressed proteins or genomic signatures).

5.2.2. *Human pharmacodynamic studies*

Human pharmacodynamic studies shall address the expression and function of the nucleic acid sequence following administration of the gene therapy medicinal product.

5.2.3. *Safety studies*

Safety studies shall address the following aspects:

(a) emergence of replication competent vector;

(b) emergence of new strains;

(c) reassortment of existing genomic sequences;

(d) neoplastic proliferation due to insertional mutagenicity.

5.3. **Specific requirements for somatic cell therapy medicinal products**

5.3.1. *Somatic cell therapy medicinal products where the mode of action is based on the production of defined active biomolecule(s)*

For somatic cell therapy medicinal products where the mode of action is based on the production of defined active biomolecule(s), the pharmacokinetic profile (in particular distribution, duration and amount of expression) of those molecules shall be addressed, if feasible.

5.3.2. *Biodistribution, persistence and long-term engraftment of the somatic cell therapy medicinal product components*

The biodistribution, persistence and long-term engraftment of the somatic cell therapy medicinal product components shall be addressed during the clinical development.

5.3.3. *Safety studies*

Safety studies shall address the following aspects:

(a) distribution and engrafting following administration;

(b) ectopic engraftment;

(c) oncogenic transformation and cell/tissue lineage fidelity.

5.4. **Specific requirements for tissue engineered products**

5.4.1. *Pharmacokinetic studies*

Where conventional pharmacokinetic studies are not relevant for tissue engineered products, the biodistribution, persistence and degradation of the tissue engineered product components shall be addressed during the clinical development.

5.4.2. *Pharmacodynamic studies*

Pharmacodynamic studies shall be designed and tailored to the specificities of tissue engineered products. The evidence for the "proof of concept" and the kinetics of the product to obtain the intended regeneration, repairing or replacement shall be provided. Suitable pharmacodynamic markers, related to the intended function(s) and structure shall be taken into account.

5.4.3. *Safety studies*

Section 5.3.3 shall apply.

(*) OJ L 102, 7.4.2004, p. 48.
(**) OJ L 169, 12.7.1993, p. 1.
(***) OJ L 189, 20.7.1990, p. 17.
(****) OJ L 105, 26.4.2003, p. 18.'

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COMMITTEE FOR MEDICINAL PRODUCT FOR HUMAN USE (CHMP)

GUIDELINE ON HUMAN CELL-BASED MEDICINAL PRODUCTS

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EXECUTIVE SUMMARY

This guideline replaces the Points to Consider on the Manufacture and Quality Control of Human Somatic Cell Therapy Medicinal Products (CPMP/BWP/41450/98). It takes into account the current legislation and the heterogeneity of human cell-based products, including combination products. A risk analysis approach can be used by the applicants to justify the development and evaluation plans and can be a basis for the preparation of a risk management plan.

In the quality and manufacturing section, guidance is provided on the criteria and testing of all starting materials, on the design and validation of the manufacturing process, on characterisation of the human cell-based medicinal products, on quality control aspects, on the development programme, traceability and vigilance* and on comparability issues. Guidance specific to the matrix/device/scaffold component in combination products is provided.

The guideline acknowledges that conventional non-clinical pharmacology and toxicology studies may not be appropriate for cell-based medicinal products. Therefore the guideline addresses which non-clinical studies are necessary to demonstrate proof-of-principle and to define the pharmacological and toxicological effects predictive of the human response.

Special problems might be associated with the clinical development of human cell-based medicinal products. Guidance is therefore provided on the conduct of pharmacodynamic/pharmacokinetic studies, dose finding and clinical efficacy and safety studies. The guideline describes the special consideration that should be given to pharmacovigilance aspects and the risk management plan for these products.

1. INTRODUCTION (background)

Rapid development in the fields of biology, biotechnology and medicine has led to the development of new treatments and highly innovative medicinal products, including medicinal products containing viable cells. These new cell-based medicinal products have a high potential in the treatment of various diseases where there is a previously unmet medical need.

Human cell-based medicinal products are heterogeneous with regard to the origin and type of the cells and to the complexity of the product. Cells may be self-renewing stem cells, more committed progenitor cells or terminally differentiated cells exerting a specific defined physiological function. Cells may be of autologous or allogeneic origin. In addition, the cells may also be genetically modified. The cells may be used alone, associated with biomolecules or other chemical substances or combined with structural materials that alone might be classified as medical devices (combined advanced therapy medicinal products).

2. SCOPE

This multidisciplinary guideline will address development, manufacturing and quality control as well as non-clinical and clinical development of cell-based medicinal products (CBMP) including somatic cell therapy medicinal products as defined in Directive 2001/83/EC, Part IV, Annex I[1] and tissue engineered products as defined in Regulation 1394/2007/EC[2]. This guideline is intended for products entering the Marketing Authorisation (MA) procedure. However, the principles laid down in the guideline should be considered by applicants entering into clinical trials.

Cell-based medicinal products discussed in this document have the following characteristics:

- They contain viable human cells[3] of allogeneic or autologous origin undergoing a manufacturing process;

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* Vigilance as described in Article 11 of Directive 2004/23/EC.

- They may be combined with non-cellular components;
- The cells may be genetically modified. The present document applies only to the cellular component of the cell based medicinal products containing genetically modified cells.

Although this document does not cover non-viable cells and cellular fragments originating from human cells, the underlying scientific principles may be applicable.

This guideline does not cover xenogeneic cell-based medicinal products.

3. LEGAL BASIS

This guideline should be read in conjunction with the introduction and general principles (4) and part 4 of the Annex I to Directive 2001/83/EC[1] as amended and the Regulation on Advanced Therapy Medicinal Products 1394/2007/EC[2].

Also, donation, procurement and testing of cells from human origin must comply with overarching Directive 2004/23/EC[4] and technical directives drawn from it, Directives 2006/17/EC[5] and 2006/86/EC[6].

4. MAIN GUIDELINE TEXT

4.1 *Risk analysis*

The risk posed by the administration of a cell-based medicinal product is highly dependent on the origin of the cells, the manufacturing process, the non-cellular components and on the specific therapeutic use. The variety of cell-based medicinal products can lead to very different levels of risks for the patients, the medical personnel or the general population. Therefore the development plans and evaluation requirements need to be adjusted on a case by case basis according to a multifactorial risk based approach (see Annex I to Directive 2001/83/EC[2]).

At the beginning of the product development, an initial risk analysis may be performed based on existing knowledge of the type of product and its intended use. This should be updated by the applicant throughout the product life cycle as data are collected to further characterise the risk.

The comprehensive risk analysis should be used to justify the product development. It should also serve as a basis for the preparation of a risk management plan in accordance with the guideline on risk management systems for medicinal products for human use (EMEA/CHMP/96268/2005)[7]. In particular, the results of the comprehensive risk analysis should be used:

- to identify risk factors associated with the quality and safety of the product;
- to determine the extent and focus of the data required during non-clinical and clinical development;
- when establishing the need for risk minimisation activities;
- when determining the post market risk management activities specified in the pharmacovigilance plan.

The following general risk criteria (non-exhaustive) can be used in the estimation of the overall risk of the product:

- origin (autologous-allogeneic);
- ability to proliferate and/or differentiate;
- ability to initiate an immune response (as target or effector);
- level of cell manipulation (in vitro/ex vivo expansion/activation/ differentiation /genetic manipulation/ cryo-conservation);
- mode of administration (e.g. ex vivo perfusion, local or systemic surgery);

- duration of exposure or culture (short to permanent) or life span of cell;
- combination product (cells and bioactive molecules or structural materials);
- availability of clinical data on or experience with similar products.

4.2 Quality and manufacturing aspects

This part of the guideline describes activities by manufacturers following procurement of the cells and tissues. The manufacture of cell-based medicinal products should be in compliance with the principles of good manufacturing practices, as set out in Directive 2003/94/EC[8] and its Annex 2[9].

The active substance of a cell-based medicinal product (CBMP) is composed of the engineered (manipulated) cells and/or tissues. Additional substances (e.g. scaffolds, matrices, devices, biomaterials, biomolecules and/or other components) when combined as an integral part with the manipulated cells are considered part of the active substance and are therefore considered as starting materials, even if not of biological origin.

CBMP often contain, or consist of cell samples of limited size and many are intended to be used in a patient-specific manner. This can raise specific issues pertaining to quality control testing designs for each product under examination. Since this document covers a variety of CBMPs, processes involved can vary from very simple to highly complex. For certain CBMPs, the starting material, the active substance and the finished product can be closely related or nearly identical. For such products, some requirements listed below could be inadequate and in those cases only relevant sections and items should be addressed.

4.2.1 Starting and raw materials

The manufacturing process of CBMP usually does not include terminal sterilisation, purification steps, viral removal and/or inactivation steps. Therefore, stringent sourcing requirements and acceptance criteria for all materials derived from human or animal origin should be adequately defined according to their intended use.

4.2.1.1. Cells

Donated cellular material from single or pooled donors, once processed (see 4.2.2.1) may be:

- A single primary cell isolate used directly for the CBMP;
- Primary cells cultured for a few passages before being used for the CBMP;
- Cells based on a well-defined cell bank system consisting of a master cell bank and a working cell bank.

An adequately controlled cell storage system should be established to allow proper maintenance and retrieval of cells without any alteration of their intended final characteristics. Storage conditions should be optimised to ensure cell viability, density, purity, sterility and function. Identity should be verified by relevant genotypic and/or phenotypic markers and the proportion of cells bearing these identity markers evaluated as an indicator of the intended cell population.

A. Cells of primary origin

The specific requirements for donation, procurement and testing laid down in Directive 2006/17/EC[5] shall be met.

Procedures and standards employed for the selection of appropriate donors and the exclusion of high-risk or otherwise unsuitable candidate donors should be clearly delineated and justified. If it is necessary to pool cells from different donors, the risk analysis should address the possibility that pooling of allogeneic cell populations may increase the risk of undesired immunological responses in the recipient and compromise its therapeutic activity. In addition, pooling of cells may increase the

risk of disease transmission. Depending on the nature of the source of the cells and tissues, other risk factors, e.g. previous radiation exposure, should be also considered and addressed.

On receipt of the cells for use in a medicinal product, a specific microbiological screening programme should be in place, adapted to the type of cells, with validated assays capable of detecting human infectious agents with appropriate sensitivity and taking into consideration the medium components that might interfere with the assays (e.g. antibiotics). When cells originate from non-healthy tissues, the product specific acceptance criteria should be defined according to the intended use.

Quality parameters aimed at the definition of acceptance criteria for a given organ or tissues should be specified, taking into consideration general aspects such as shipment and storage conditions.

In the case of autologous donation, the testing regimen of the starting material should be justified, taking into account the autologous use.

Where allogeneic primary cells are collected and expanded for use in multiple patients, the cell lot should be appropriately characterised. The same characterisation programme shall be applied to each new cell lot.

B. Banking system for established cell lines

Where cell lines are used, an appropriately characterised Master Cell Bank (MCB) and Working Cell Bank (WCB) should be established, whenever possible. Cell banking and characterisation and testing of the established cell banks should comply with the ICH guideline Q5D[10].

4.2.1.2. Other materials, reagents and excipients

Various materials are needed for collection, selection, culture or even genetic or phenotypic modification of cells, such as other cells, enzymes, antibodies, cytokines, sera and antibiotics. Exposure to such materials can also impact on the quality, safety and efficacy of the final therapeutic product. As a consequence, each substance used in the procedure should be clearly specified and evaluated as to its suitability for the intended use. The microbial purity and low endotoxin level of these materials should be ensured.

Materials, including cells that function as support for growth and adhesion e.g. feeder cells should be evaluated and/or validated as to their suitability for the intended use.

The quality of biologically active additives in culture media such as growth factors, cytokines and antibodies, should be documented with respect to identity, purity, sterility and biological activity and absence of adventitious agents. It is recommended to keep the use of such materials to a minimal and to avoid the use of reagents with sensitisation potential e.g. β-lactam antibiotics.

For viral safety aspects, the guidelines on viral safety[11, 12] and Eudralex vol. 2B[13] should be taken into consideration. The principles laid down in the general text of the European Pharmacopoeia on viral safety[14] should be followed for every substance of animal and human origin that is used during the production.

Measures should be taken to reduce the risk of transmissible spongiform encephalopathy according to the relevant European legislation and guidelines[15].

Where appropriate, the Note for Guidance on the "Production and quality control of medicinal products derived by recombinant DNA technology"[16] and the Note for Guidance on the "Production and quality control of Monoclonal Antibodies"[17] should be taken into account.

When the raw materials, reagents and/or excipients have a marketing authorisation or mentioned in a Pharmacopoeia, appropriate references may be given.

The following information must be added for materials of human or animal origin:

A. Human derived materials

Reagents of human origin (e.g. albumin, immunoglobulins) should be evaluated for their suitability in a manner identical to that employed for plasma-derived products as recommended in the CPMP Note for guidance on plasma-derived medicinal products[18]. The use of synthetic alternatives should be investigated. If serum is required in the culture media, the use of serum isolated from the same individual who donated the cells is preferred, where possible, to alternate allogeneic serum.

B. Animal derived material

Where cells or tissues of animal origin are used e.g. as supportive cells, the guidance given in "Points to consider on Xenogeneic Cell Therapy Medicinal Products"[19] should be followed.

Animal derived reagents may harbour infectious agents and may increase undesirable immunological responses in the recipient. When applicable, the use of animal reagents should be avoided and replaced by non animal derived reagents of defined composition.

When bovine serum is used, the recommendations of the Note for Guidance on the "Use of Bovine Serum in the Manufacture of Human Biological Medicinal Product"[20] should be followed. The use of irradiated sera and/or alternative synthetic media is encouraged and should be considered.

For viral safety testing of materials of other animal species, the table of extraneous agents to be tested for in relation to the general and species-specific guidelines on production and control of mammalian veterinary vaccines[21] and Note for Guidance on Production and Quality Control of Animal Immunoglobulins and Immunosera for Human use[22] should be consulted.

C. Special considerations

Special recommendations for the starting materials of cell-based Gene Therapy Medicinal Products

When the cells in the active substance are genetically modified, the "Note for Guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products"[23] should be followed, which gives details on the quality control, characterisation and preclinical testing of gene transfer vectors. Cell populations which are transformed should be assayed for appropriate and reproducible expression of the newly acquired characteristics. Special attention should be paid to the level and length of expression and quality of the gene product(s) produced by the cells. As far as applicable and practicable, the new characteristics of the cells should be quantified and controlled.

Special recommendations for matrix/device/scaffold components of combined products

Cell-based medicinal products may incorporate structural components which independently are medical devices or active implantable medical devices. Those devices should meet the essential requirements laid down in Directive 93/42/EEC[24] concerning medical devices and Directive 90/385/EEC[25] on the approximation of the laws of the Member States relating to active implantable medical devices, respectively, and this information shall be provided in the marketing authorization application. In the case where a Notified Body has evaluated the device part, the result of this assessment shall be included in the dossier. Cell-based medicinal products may also incorporate structural components which are not identical to, or used in the same way as in a medical device. All structural components should be appropriately characterised and evaluated for their suitability for the intended use (See sections on Characterisation and Development Pharmaceutics).

Any matrices, fibers, beads, or other materials that are used in addition to or in combination with the cells should be described and their function underpinned by means of chemical, biological, physical (e.g. structure and degradation) and mechanical properties. Inclusion of additional bioactive molecules should also be described and their impact should be evaluated.

4.2.2 Manufacturing process

The manufacturing process of cell-based medicinal products should be carefully designed and validated to ensure product consistency. The requirements should be defined and justified.

A detailed description of the manufacture of the active substance and of the finished product should be provided. The type of manipulation(s) required for cell processing and the physiological function of the cells shall be described. A flow diagram of the entire process starting from biological fluid/tissue/organ or from cell banks should be prepared indicating critical steps and intermediate products (e.g. intermediate cell batches), as well as operating parameters, in-process controls and acceptance criteria. Manufacture of combined medicinal products consisting of cells and matrices/devices/scaffolds, require additional consideration regarding the cell-matrix/scaffold interactions and quality issues raised there from. Attention should be paid to biodegradable materials, which may possess the potential for environmental changes (e.g. raising pH) for the cells during the manufacture or after administration.

Information on procedures used to transport material during the manufacturing process of the product, including transportation and storage conditions and holding times, should be provided.

The manufacturing area should be physically separated from the procurement area If different tissues and cellular products are processed and stored in the same manufacturing area there is an increased risk of cross contamination during each step of the procedure, e.g. via processing equipment or in storage containers such a liquid nitrogen tanks, and therefore, adequate control measures to prevent cross-contamination should be put into place.

Equipment and premises used for manufacturing of CBMP should be suitable and qualified for aseptic production. It is recommended that dedicated, product-specific or single-use equipment are used in the production, whenever possible.

1. Cell preparation procedures

All cell preparation procedures should be justified in terms of their intended purpose.

Inappropriate handling and improper processing of cells/tissues must be avoided as they can impair or destroy the integrity and/or function of the cells and thus result in therapeutic failure. Microbiological control is a pivotal aspect of process control and quality evaluation of all cell preparations. Monitoring of in vitro cell culturing at selected stages of the production should be performed where feasible. The culture should be examined for any microbial contamination in accordance with the culturing procedure and growth characteristics of the cells.

After appropriate controls have been performed / implemented, the biological fluid / tissue /organ can undergo one or more of the following steps:

<u>Organ/tissue dissociation</u>

The procedure to obtain the cells from the organ/tissue has to be described (with respect to the type of enzyme, media, etc.) and validated. Consideration should be given to the degree of disruption applied to the tissue in order to preserve the intended functional integrity of the cellular preparation and to minimize cell-derived impurities in the product (cell debris, cross contamination with other cell types).

<u>Isolation of the cell population of interest</u>

Any procedure used to isolate and / or purify the cell population of interest should be described. Its effectiveness should be addressed in relation to the intended use and the method(s) should be validated.

Cell culture

During in vitro cell culture, consideration should be given to ensure acceptable growth and manipulation of the isolated cells. The processing steps should be properly designed to preserve the integrity and control the function of the cells. The procedures for any manipulation should be documented in detail and closely monitored according to specific process controls. The duration of cell culture and maximum number of cell passages should be clearly specified and validated. The relevant genotypic and phenotypic characteristics of the primary cell cultures, of the established cell lines [10] and the derived cell clones should be defined and their stability with respect to culture longevity determined. Consistency/repeatability of the cell culture process should be demonstrated and the culture conditions including the media and the duration should be optimised with respect to the intended clinical function of the cells.

Special consideration should be given to the growth potential of cells in response to growth factors since cell subpopulations may gain a growth advantage under defined in vitro culturing conditions.

Cell modification

Various treatments (physical, chemical or genetic) can be applied to cells. The method used to modify the cells should be fully described. In the case of genetic modification of cells, requirements set up in the Note for guidance on Quality, preclinical and clinical aspects of gene transfer medicinal products[23] should be followed.

Cells cultured in or on a matrix/device/scaffold

If the cells are grown directly inside or on a matrix/device/scaffold, the quality of the combined advanced therapy medicinal product relies predominantly on the properly controlled manufacturing process. For such products, the cell culture process has to be thoroughly validated and the effect of the device on the cell growth, function and integrity has to be taken into account. The effect that the cells may exert on the device (e.g. on rate of degradation) should also be considered (see also 4.2.6. Development Pharmaceutics).

2. In-process controls

The manufacturing process needs to be controlled by several in-process controls at the level of critical steps or intermediate products. Intermediate cell products are products that can be isolated during the process; specifications of these products should be established in order to assure the reproducibility of the process and the consistency of the final product. Tests and acceptance criteria should be described. If storage occurs, it is necessary to validate the storage conditions (e.g. time, temperature).

3. Batch definition

The purpose of the batch definition is to ensure consistency and traceability. A clear definition of a production batch from cell sourcing to labelling of final container should be provided (i.e. size, number of cell passages/cell duplications, pooling strategies, batch numbering system). In the autologous setting, the manufactured product should be viewed as a batch.

4. Container and closure system

A description of the container closure system should be provided. Compatibility with the product should be demonstrated. It should be indicated if the container closure *per se* has a CE marking under the Medical Devices Directive 93/42/EEC[24]. Information on the sterilisation procedures of the container and the closure should be provided.

The choice of packaging materials should be addressed as part of the development pharmaceutics. Additional data may be required if packaging components are used in the transport and/or application procedure.

4.2.3 Characterisation

The characterisation of a CBMP should encompass all the components present in the finished product. Characterisation may prove particularly challenging for products containing cells together with matrices, scaffolds and innovative devices. Characterisation data are likely to be necessary for single components as well as for the combined final product. Characterisation data could encompass data obtained throughout the development and/or manufacturing process. It should be noted that in a combined product the characteristics of both the cellular and the non-cellular components may be altered by the process of integration.

An extensive characterisation of the cellular component should be established in terms of identity, purity, potency, viability and suitability for the intended use, unless justified.

The expected biological function of a CBMP encompasses complex interactions that may range from a biochemical, metabolic or immunological action to the structural replacement of damaged tissue or organ. Therefore, the requirements for a complete characterisation of the active substance in terms of biological function could be very taxing. Moreover the specific mechanism of action is often difficult to pinpoint to specific molecular entity but it is more dependent on the functionality of the cellular components acting in a "tissue-like" fashion as a whole. Therefore, when considering the extent of characterisation, the following issues should be taken into account: i) autologous cells vs. allogeneic cells, ii) extensively or minimally manipulated in vitro, iii) immunologically active or neutral, iv) proliferative capacity of the cells, v) cell-like or tissue-like organisation and dynamic interactions amongst cells and with the structural component, vi) intended use.

Non-cellular components should be characterised in the context of their required function in the finished product. This includes structural components designed to support the cellular components such as scaffolds or membranes which should be identified and characterised in chemical and physical terms such as porosity, density, microscopic structure and particular size according to the type of substances and intended use according to EN/ISO 10993-18[26] and EN/ISO 10993-19[27].

The characterisation should be designed to allow setting up the routine controls that will be applied for release of the active substance and finished product as well as those to be performed at several steps of the process to guarantee batch consistency.

If biologically active molecules (e.g. growth factors, cytokines etc.) are present as components of the cell-based products, these have to be described adequately and their interaction with the other components of the product and the surrounding tissues after administration should be characterised. This should involve an appropriate range of *in vitro* and where necessary *in vivo* methods.

1. Identity

Cellular Component

The identity of the cellular components, depending on the cell population and origin, should be characterised in terms of phenotypic and/or genotypic profiles.

When addressing the phenotype of the cells, relevant markers could be used, where justified. These markers may be based on gene expression, antigen presentation, biochemical activity, response to exogenous stimuli, capability to produce biologically active or otherwise measurable molecules, etc. For adherent cells, morphological analysis may be a useful tool in conjunction with other tests. Where applicable, a description of the procedures which could lead to a modification of the characteristic of the product, including adhesion, absorption, degradation, presentation of components of the culture media, should be provided.

For cellular components of allogeneic origin, identity should include histocompatibility markers, where applicable, and identification of genetic polymorphisms with specific reference to the intended use.

Non-cellular Components of the active substance

All non-cellular components should be appropriately characterised as such and identity parameters established.

Should the finished product contain a distinct active substance in addition to the cellular component, then that active substance should be characterised with respect to identity in accordance to relevant CHMP guidelines, depending on the nature of the active substance, whether it be of chemical or biological origin.

Structural components designed to support the cellular components such as scaffolds or membranes should be identified and characterised with respect to their composition and structural characteristics.

Combination Products

In a combination product the active substance may be formed by the integration of cellular and non-cellular components to form a single entity. In such a case the identity of both the cellular and the non-cellular components may be altered by the process of combination. Consequently a distinctive way to define identity should be established for the components in the combination, unless justified.

2. Cell purity

The cellular population of interest could contain other cells that are of different lineages and/or differentiation stage or that may be unrelated to the intended population.

Where a specific cell type is required for the indication, the unwanted cells should be defined and their amount in the final product should be controlled by appropriate specifications, i.e. acceptance criteria for the amounts of contaminating cells should be set.

In cases, where the desired biological activity and efficacy of the product requires a complex mixture of cells, the cell mixture needs to be characterised and its composition controlled by appropriate in-process controls and release testing.

Irrespective of the cell type, the cell population can be contaminated with non-viable cells. Since cell viability is an important parameter for product integrity and directly correlated to the biologic activity, the ratio between non-viable and viable cells should be determined and specifications should be set.

3. Impurities

Product or process-related

During the production of a CBMP, variable amounts of impurities, product- and process-related, may be introduced into the final product. Any reagents known to be harmful in humans should be analysed in the final product (or in individual components if otherwise not possible) and acceptance criteria should be set. The specification limits should be justified by levels detected in batches used for toxicological and/or clinical studies.

Any material capable to introduce degradation products into the product during the production, e.g. biodegradable materials, should be thoroughly characterised in this respect and the impact of the degradation products to the cell component(s) should be addressed.

If genetically modified cells are used in the product, any additional proteins expressed from the vector, e.g. antibiotic resistance factors, selection markers, should be analysed and their presence in the product should be justified.

Adventitious agents

A critical aspect is to establish that CBMP are free from adventitious microbial agents (viruses, mycoplasma, bacteria, fungi). The contamination could originate from the starting or raw materials (see above), or adventitiously introduced during the manufacturing process. A risk assessment should be performed to evaluate the possibility of reactivation of cryptic (integrated, quiescent) forms of adventitious agents. A thorough testing for the absence of bacteria, fungi and mycoplasma shall be performed at the level of finished product. These tests should be performed with the current methodologies described in the European pharmacopoeia for cell based products [28]. In cases where the short shelf life of the CBMP is prohibitive for the testing of absence of bacteria under the Eur Ph. requirements, alternative validated testing methods may be acceptable, if justified.

4. Potency

It is strongly recommended that the development of a suitable potency assay be started as soon as possible. Preferably, a suitable potency assay should already be in place when material for the first clinical trial is produced and it should be validated prior to pivotal clinical trials unless otherwise justified. Lot release and shelf life specifications for potency should be determined and amended during product development, if appropriate.

According to the ICH guideline 6QB[29], potency is the quantitative measure of biological activity based on the attribute of the product, which is linked to the relevant biological properties. The assay demonstrating the biological activity should be based on the intended biological effect which should ideally be related to the clinical response.

Basically, two types of potency assays can be envisioned: 1) *in vitro* assays using cell systems and 2) *in vivo* assays using animal models. Major cellular functions as viability, self renewal, death and differentiation are pivotal to the quality, function and sustainability of the CBMP and may need to be monitored during production and at release using surrogate markers and appropriate technology (e.g. gene expression profiles by microarrays, flow cytometric immunofluorescent analysis, cell cloning, PCR and many others). In vivo assays for potency may also be useful especially when experimental animal models are available.

Markers for purity and markers for potency should not be mixed in the same assay. .

Reference is made to the Guideline on "Potency testing of cell-based immunotherapy medicinal products for the treatment of cancer"[30]. Although this guideline focuses on cell-based immunotherapy medicinal products, the principles, including on reference preparations, apply for all CBMP. As described in the referred guideline, a combination of multiple methods may be needed to adequately define the potency of these products during the development. Certain assays may be needed to control process changes, whereas others are more suitable for release testing.

Tissue repair and regeneration

An *in vivo* test can either be performed in an animal model mimicking the intended clinical tissue repair/ regeneration or can otherwise be based on the mode of action (e.g. an ectopic model). An *in vitro* assay can be based on the expression of markers that have been demonstrated to be directly or indirectly (surrogate markers) correlated to the intended biological activity, such as cell surface markers, activation markers, expression pattern of specific genes. Also a physiological response under defined conditions such as differentiation in specific cell types and/or secretion of tissue specific proteins (e.g. extracellular matrix components) can be used as a basic principle for a potency test. The manufacturers should, however, ensure that the method of characterisation is relevant for the intended biological effect *in vivo*.

The potency assay should be performed by using a specified number of cells and, when possible, quantified against a qualified reference preparation. The potency should be defined as the required time to obtain a predefined effect (e.g. restoration of function or repair of anatomical structure) or the potency is calculated from the measured effect in a defined time period.

Metabolic or pharmacological activity

Cells contained in a CBMP can be chemically treated or genetically modified *in vitro* to express certain desirable proteins like growth factors, cell surface antigens or other molecules in order to sustain the biological response as long as needed in the new microenvironment. Therefore, the potency assays to be developed should be able to assess the activity-related aspects of the active substance that may be composed not entirely of intact viable cells but also of other components.

If the intended biological function of the CBMP is mainly based on the capacity of cells to secrete specific molecule(s) e.g. to repair a metabolic disorder, to promote growth, to release a metabolite, then its potency assay will be based on the detection of the active molecule(s) produced and the biological activity expected. This can be carried out by conventional reliable qualitative and quantitative analytical methods (protein analysis, nucleic acid identification, HPLC chromatography etc.). The same molecule can be also assessed for function in animal model systems assuming that the active substance is released from the cell-based medicinal product into biological fluids (plasma, CSF, urine or interstitial fluid).

Immunotherapy

Potency assays of cell-based medicinal products intended for immunotherapeutic use will be based on complex immune mechanisms which may be complicated by multi-antigen formulations and inherent variability of the starting material. Special guidance for cell-based immunotherapy medicinal products is provided in Guideline on "Potency testing of cell-based immunotherapy medicinal products for the treatment of cancer"[30].

5. Tumourigenicity

The tumourigenicity of CBMP differs from the classical pharmaceutics as the transformation can also happen in the cellular component of the product (e.g. chromosomal instability) and not only in the treated individual. If the risk of cellular transformation and subsequent potential for tumourigenicity can be foreseen, the cellular components should be evaluated for their tumourigenic potential by analysing e.g. their proliferative capacity, dependence on the exogenous stimuli, response to apoptosis stimuli and genomic modification. Testing of chromosomal integrity and tumourigenicity of cells derived from a cell culture / cell banking system will be required. Reference is made to the ICH Q5D[10] and to the Ph. Eur. Monograph on cell substrates for the production of vaccines for human use[31].

4.2.4 Quality control

For proper quality control, the active substance and/or the final product should be subjected to release testing, whenever possible. If justified, it would be acceptable to have reduced testing at one level provided an exhaustive control is performed at the other. All release testing should be performed using methods validated at the latest at the time of submission of an application.

1. Release criteria

The release specifications of the active substance and finished product should be selected on the basis of parameters defined during the characterisation studies. Selection of tests is product-specific and has to be defined by the manufacturer.

Specifications for release testing should include identity, purity, potency, impurities, sterility, potency, cell viability and total cell number, unless otherwise justified. If the structure is an essential characteristic of the product, the structural characteristics of the active substance or finished product shall be defined and justified. In case the primary function of the CBMP is the excretion of specific proteins, specifications regarding these excreted proteins should be set.

If certain release tests cannot be performed on the active substance or finished product, but only on key intermediates and/or as in-process tests, this needs to be justified. In these cases an adequate

quality control has to rise from the manufacturing process, supported by the results of the clinical studies. These exceptions may include the following:

- Some release tests might not be feasible on the combined components of the active substance/ finished product for technical reasons.

- A complete release testing cannot be finalised before the product is administered to the recipient due to time restrictions (e.g. in case of autologous products, which are administered immediately after completion of the production and initial testing). However, a critical set of essential tests that can be performed in the limited time prior to clinical use must be defined and justified. Whenever feasible, retention samples should be stored for future analysis.

- The amount of available product is limited to the clinically necessary dose (e.g. due to very limited cell numbers at collection or low proliferation rates). The release of the product should be justified by the validation of the cell manipulation process and the in-process controls.

2. Stability testing

A shelf life for the cells under specified storage conditions shall be determined for the following materials: i) all intermediates subject to storage if applicable, ii) components of the combined CBMP, iii) the active substance, iv) the finished product. Furthermore, a valid in-use shelf life (after opening from the transport container) should be assigned to the CBMP. Also, all storage conditions including temperature range should be defined. Transportation and storage conditions should be supported by experimental data with regard to the maintenance of cell integrity and product stability during the defined period of validity. If relevant, appropriate methods for freezing and thawing should be documented.

Due to the complex nature of the active substance of a CBMP, requirements for stability should be defined on a case-by-case basis. Whenever possible, stability should be assessed for both the cellular as well as the non-cellular component prior to combination and together as a finished product in the final packaging.

3. Special quality requirements for cell-based medicinal products containing genetically modified cells

If cells have been genetically modified, quality control must be performed in compliance with guidance available on gene transfer medicinal products[23]. This information is in addition to control of the cells according to the guidance presented elsewhere.

4. Special quality requirements for combination products

Specifications for structural components of the product shall be defined. Impurities and degradation product that originate from the structural component (matrix, scaffold, device) shall be described and specifications for the relevant impurities should be set. Testing of the structural/mechanical properties and biological activity with reference to the anticipated conditions for use and potential for degradation may be difficult to conduct as part of release testing. Thus, it is anticipated that these parameters could be explored through proper testing of raw materials and characterisation studies of the final product. In extremely limiting conditions (e.g. for autologous products with small cell numbers), the analysis of structural/functional characteristics of a combination product may necessitate the development of a model product composed of same non-cellular components combined with cell component(s) of equal characteristics but with proven availability.

4.2.5 Validation of the manufacturing process

The entire manufacturing process, including cell harvesting, cell manipulation processes, maximum number of cell passages, combination with other components of the product, filling, packaging, transport, storage etc., should be validated. Validation of the production process of a combined

product should encompass all steps from separate components up to the final combination to ensure consistent production.

It should be demonstrated that each step of the manufacturing process of the active substance, supportive components and final product is well controlled. The selection and acceptance criteria of the operational parameters and the in-process controls should be justified. Putative variability, related to starting materials and biological processes, should be taken into account in the validation. Furthermore, the critical points of the manufacturing process should be defined and validated, especially the aseptic processing.

Any preservation steps, holding periods and/or transportations of the active substance, final product, supportive structures or intermediate products during the manufacturing process should be validated.

In case of limited sample sizes (e.g. autologous preparations for one single administration), it is recommended that a more extensive validation is performed with cell preparations of comparable characteristics but available in sufficient amounts for validation purposes. It is recommended that validation of such a manufacturing process is performed depending on the product characteristics, for adventitious agents, identity, potency, viability, purity/impurities and other product specific parameters.

4.2.6 Development Pharmaceutics

The general principles set out in Note for Guidance on Development Pharmaceutics for Biotechnological/Biological Products[32] can be applied to human CBMP. The potential complexity of composition and the dynamic nature of a product containing living cells will result in very specialised pharmaceutical and biopharmaceutical requirements for each development programme from the individual cell components into the final product.

1. Cellular Components

The development programme should address the choices of materials and processes to be used in production. This should be addressed from the point of view of the biological/therapeutic function, the maintenance and the protection of the cell population.

Integrity of the cellular component is most critical for the CBMP and must be assessed by the ability of cells to survive, and maintain the genotype or phenotype needed for the intended functions. However, detection of possible changes in cellular nature that may influence the intended function, can be feasible by analysis of cellular surface antigens, proteomics and functional genomics analysis (e.g. microassay for gene expression profile, flow cytometry etc.). Cell viability can be easily assessed in culture by employing widely applied assays. For combined products, where structural components are an integral part of the active substance, such assays may be more difficult to apply. Alternative approaches could be sought such as combination of other suitable assays (e.g. detection of pH and O_2 / CO_2), where needed.

The ability of cells to continue to produce or express products should be evaluated as part of the stability programme. Such stability studies should be carried out as long as the defined period of validity requires.

2. Non-Cellular Components

A CBMP may contain non-cellular components, such as biomaterials, bioactive molecules, proteins or chemical entities. These may supply structural support, suitable environment for growth, biological signalling or other functions. They may also be used during the *ex vivo* manipulation process.

Matrices, scaffolds, devices, biomaterials or biomolecules which are not an integral part of the active substance, are considered as excipients of the finished product. For excipient(s) used for the first time in combination with cells and/or tissues, the requirements for novel excipients, as laid down in part I,