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Supplementary Data
An example of detailed scientific considerations for the regulatory evaluation of cell therapy products

1. Introduction

The primary goal of cell therapy products (CTPs) is to provide new opportunities to patients suffering from severe diseases for which there may be no effective treatment, or conventional medicine may require costly long-term treatment with significant adverse effects. Because a wide variety of cell-based products are in development or are already approved, encompassing a variety of situations and circumstances, this document is intended to describe principles, concepts, and some basic technical elements rather than product-specific points in order to provide greater flexibility with respect to specific issues related to particular products. This document is intended to facilitate the rapid development and availability of CTPs.

The present document should be interpreted and employed in a flexible and scientifically sound manner, taking into consideration that the application of the principles is not all-inclusive or definitive. Scientific progress in this field is continuing to advance at a rapid pace as is experience and knowledge, all of which need to be taken into consideration at any specific point in time when testing and evaluating an individual product.

The product itself should not be considered in isolation. The whole process, including evaluation of donor cells, raw materials, bioprocessing, testing, storage, shipment and delivery, as well as factors involving the patient such as dose, frequency and route of administration will all need to be considered to assess the safety of the product.

The main purpose of evaluating quality and safety of the desired cell products before conducting investigational clinical trials is to determine: 1) whether certain quality attributes (QA) of the product are understood sufficiently to establish a relationship between the clinical findings and the QA; 2) whether consistency of the QA can be ensured within a defined range; and 3) whether there are any quality and/or quality-related safety problems that would obviously hinder initiating human clinical trials of the products in question.

Simultaneously, it is important to identify and control as much as possible any presumed known risk factors associated with product quality and safety using up-to-date science and technology and to describe the scientific appropriateness of the results of such action. The possibility of unidentified risk factors should be weighed against the risks associated with not performing the trials in patients who suffer from diseases that are serious and life-threatening, that involve

marked functional impairment or a marked decreased in quality of life (QOL) resulting from the loss of a certain degree of physical function or form, or for which existing therapies have limitations and do not provide cures.

As in all clinical studies, the patient has the right to make a decision after he/she has reviewed all of the information provided by the clinical investigator. In the case of clinical trials for CTPs, applicants may submit a provisional non-clinical data package, taking into account product aspects and patient aspects including a balance between risk of the investigational product *vs* risk to the patient without the experimental treatment in question. Determining whether it is reasonable to initiate a clinical trial with a CTP is often based in part on the premise that the data package submitted at the time of a marketing application/registration to ensure quality and safety will be enriched and developed as the clinical trial progresses.

There is a desire in many countries to accelerate the product regulatory review process in order to ensure efficient access to new therapies, and unique systems have been developed in several countries/regions. In spite of clear potential benefits, the pressure to accelerate review must be balanced against the need for a suitable and robust scientific review of each product to ensure that it does not represent hazards to patients.

2. Scope

The general principles outlined in this document should apply to all viable and replication competent cells that are in clinical development as well as commercial products, recognizing that both the process and the QC strategy may be further developed/validated and completed in the later stages of product development.

Cell components and replication-incompetent cells are outside the scope of this document. Although in principle the same considerations would apply to all cells, there may be a different emphasis on specific tests and/or additional tests based on: 1) the species of origin of the cells; 2) whether the cells are allogeneic or autologous; and 3) the degree to which the cells have been manipulated. All of these considerations fall outside the scope of this document, and should be addressed within the guidance for specific products.

Some of these principles also may be useful in the identification/qualification and quality control of specific biological products used during the manufacturing process, but it is beyond the scope of this document to recommend quality control release tests. Like-wise, risk-based assessments related to product approvals are beyond the scope of this document.

Cells used for therapy should be developed and used in accordance with applicable requirements and recommendations of the National Regulatory Authority (NRA) / National Control Laboratory (NCL) for individual products.

Cells such as stem cell lines (SCLs) used for the production of biological products such as growth factors and vaccines fall outside the scope of this document, and should comply with WHO recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks [1].

Some NRAs, in addition to defining a CT based on its biological activity, provide a regulatory and/or legal classification for CTs. It is possible that depending on the particular definition used by the NRA, additional testing may be required. These regulatory, ethical, and legal issues are specific for each NRA and are beyond the scope of this document.

In summary, CTs included in the scope of this document are: a) cells isolated and cultured for a limited period of time *in vitro* (and possibly modified artificially) before being administered, b) cells derived from SCLs that can proliferate indefinitely *in vitro* and can differentiate into various cell types to give rise to a lineage-specific CTP, and c) cells derived from SCLs that are obtained through relevant induction or dedifferentiation from SCs and that can proliferate indefinitely *in vitro* before being differentiated into various cell types to give rise to a lineage-specific CTP.

3. Manufacturing and quality

As with other biological products that are complex mixtures that cannot be completely defined, close monitoring and in process quality control of the manufacturing process for CTPs, as well as the end-product quality control, are necessary to ensure proper quality profile and the resulting safety and efficacy profile of the intended product. Poor control of production processes can lead to non-reproducible critical steps, as well as the risk of introduction/reactivation of adventitious agents or other contaminants, resulting in inadvertent (and sometimes undetected) changes in the product quality profile that may have an adverse effect on its safety and/or efficacy. For these reasons, the methods and reagents involved in the production process should have been defined prior to routine manufacturing. Also, cell banks and key intermediates in the production process should be subject to quality control. Lot-to-lot reproducibility of both the final product and of critical materials should be examined. All these technical aspects should be covered during the quality/non clinical development stages.

Only when the process is deemed sufficiently developed and evaluated (with the understanding that for CTPs, the quality development may be stepwise and parallel to clinical development) that a detailed description of where and how the CTP is manufactured, including all of the components and materials used during the manufacturing process, such as cells, cell bank systems, and any reagents or excipients can be proposed for production purpose (for clinical trial materials or routine commercialization).

Specific guidance documents on raw materials have been published [2-5].

In addition, all procedures used during the manufacturing process should be described. Examples of these procedures may include recovery and processing of tissues or cells, purification, and other preparation of cells, donor screening and testing, including final formulation of the product. This information should include the identity, purity, and potency of the product. The development of a potency assay is perhaps the most difficult to address for a CTP, and is discussed in more detail below. The consistency of the manufacturing process should be demonstrated and in-process controls should be described.

Variation in cells or tissues from different donors can introduce significant variation in starting material. This variation should be carefully considered and the process of donor selection and acceptability of starting materials should be suitably documented as discussed elsewhere (1, 6).

It is also important to keep in mind that when a qualified and validated process is translated to new manufacturing sites careful reevaluation is needed. Demonstration of comparability of product in such cases will be especially dependent on the selection of suitable cell characterisation to confirm the final product has not been changed significantly.

Specifications as well as storage and transport conditions are important elements that should be set.

In 2010, WHO revised its recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks [1]. Many of those recommendations should apply to CTPs. For example: principles of good cell culture practices, selection of source materials, cryopreservation, cell banking, and GMPs. In addition, that document includes recommendations on the characterization of cell banks, including: identity, stability, sterility, viability, growth characteristics, homogeneity, tumourigenicity, cytogenetics, and microbial agents. However, it is important that such guidance is interpreted with the special nature of cell-based medicines in mind.

Other sources of detailed guidance on manufacturing and quality are available [7-22].

3.1 Potency

Only product lots that meet defined specifications or acceptance criteria should be administered during all phases of clinical investigation and following market approval. Among the quality attributes that are verified during the process and/or at release of a batch, the potency assay is a key parameter, for any biological product, to verify that the final product has kept its biological properties and activities for its intended use in humans.

National and international organizations responsible for providing regulatory guidance for biological products have developed definitions of potency that are meant to apply to a broad range of products. The International Conference on Harmonization (ICH) has defined potency as "the measure of biological activity, using a suitably quantitative biological assay, based on the attribute of the product that is linked to the relevant biological properties" [23]. In November 2003 the WHO ECBS modified its definition of potency by adopting the ICH definition [24]. WHO also has commented that "Potency tests measure biological activity of a vaccine but do not necessarily reflect the mechanism of protection in humans" [25]. The FDA (USA) has defined potency in the U.S. Code of Federal Regulations as: "the specific ability or capacity of a product to effect a given result" [26]. Of all the definitions, this is the most general, but it is consistent with that of the ICH and WHO.

Ideally, the potency assay will represent the product's mechanism of action (i.e., relevant therapeutic activity or intended biological effect). However, many CTPs have a complex and/or not fully characterized mechanism of action (MOA), making it difficult to determine which product attributes are most relevant to measuring potency. Nonetheless, attempts should be made to develop potency measurements that reflect the product's relevant biological properties. Detailed considerations of potency tests for CTPs have been published [7, 8, 14, 27]. It should be noted that potency may not be a valid surrogate marker of efficacy, and it should be used in that regard only after data have been developed to justify it.

Specific consideration should be given on how to document the potency question during the development of a CTP from the very first batch produced for non-clinical purposes up to the batches intended for clinical trials and marketing.

3.2 Tumorigenicity and genetic stability

The tumorigenic potential of cells used for the manufacture of biological products has been a concern from the earliest days when such cells were excluded as acceptable substrates for viral vaccines. That position was modified in the 1980s when CHO cells were accepted for the manufacture of highly purified recombinant DNA products.

A critical feature regarding pluripotent stem cells (PSCs), even though they usually display a diploid karyotype, is that they form tumors in immunocompromised animals, and that there is, in fact, a potential risk for humans who might receive PSC-based CTPs. Specifically, the development of cystic structures, primitive tissue grafts and teratomas in animal models have been reported [28]. This was seen as a hurdle to their use as candidate starting material to produce an investigational CTP [29]. Specific concerns relate to the generation of iatrogenic teratomas or tumors from residual immature or non-

terminally differentiated cells in the final cell product. Tumorigenicity testing is therefore a key preclinical safety test in PSC-derived cell therapy [14, 30, 31].

Concerns regarding the potential tumorigenicity of mesenchymal stromal cells have been expressed. However, CTP-derived tumor formation has yet to be reported in both animal models and humans [32].

PSC tumorigenicity has been divided into two separate categories: malignant transformation involving the development of teratocarcinoma cells from undifferentiated PSCs and benign teratoma formation from residual undifferentiated PSCs, either of which can produce tumours consisting of either one or all three germ layers, respectively [28]. Some factors that might influence the tumorigenic phenotype have been described [28], but this is an active area of investigation, as are the specifics of an acceptable test for tumorigenicity. Guidance documents have been published by FDA, EMA, WHO, and South Korea [1, 7, 8, 33].

Cells passaged and or differentiated in vitro may also undergo genetic changes and in some circumstances altered clones may have a growth advantage and can become predominant over the original cells. This has been observed in different types of stem cell lines [34], and extended passage cultures of mesenchymal stromal cells. The exact impact of these genetic changes on CTP safety and the most appropriate assays to assess genetic stability are difficult areas from which to draw conclusions. At this stage, it continues to be the subject of ongoing discussions [35].

3.3 Microbial agents

Human and animal cell substrates are subject to contamination with and have the capacity to propagate some extraneous, inadvertent, or so-called adventitious organisms such as mycoplasma and viral agents. In addition, animal cells contain endogenous agents such as retroviruses that also may be of concern. Testing for both endogenous (e.g., retroviruses, hepatitis viruses) and adventitious agents (e.g., bacteria, fungi, mycoplasmas) is an important part of the characterization of the CTP. In general, cells contaminated with microbial agents are not suitable for the production of biological products. However, there are exceptions to this general rule. For example, the CHO and other rodent cell lines that are used for the production of highly purified recombinant proteins express endogenous retroviral particles. Risk versus benefit must be considered when determining the suitability of cells for the production of a CTP.

The potential for infection from transplanted cells is one of the greatest risks to recipients of cell-based medicines. Established risk assessment and donor testing procedures for donated tissues provides a level of safety appropriate for serious conditions treated by transfusion or transplantation, and will assure the absence of the most serious blood borne viral pathogens in of cell-based medicines. However, the very broad range of diseases that may be covered by regenerative cell-based medicines involves new risk/benefit relationships which

will require that manufacturers revisit how they assure microbiological safety of these new products. The presence of many infectious agents can be revealed by a cytopathic effect on the cells or cell line used and thus such tissues can be eliminated from the supply chain. However, it is not difficult to compile a list of potential contaminants which may not be covered by normal donor screening, including opportunist pathogens and possibly others which are emerging or yet to be recognised. Some of these are known to be able to establish persistent infection of cells and could thus persist in a cell-based therapeutic product (Table 1). A broader range of opportunist pathogens also may need to be considered in recipients of cell-based medicines who may be immunocompromised by reason of their disease or treatment.

Microbes known to grow well in cell cultures include Mycoplasma and Acholeplasma and whilst established pharmacopeia reference methods for the detection of these organisms can be applied, manufacturers should be aware of the potential risk from related genera such as Spiroplasma, and other organisms which have been known to cause infection in mammals. Broth culture sterility tests used to screen for bacterial and fungal contamination can be useful, but species which may not be detected in such conditions (e.g. Leptospira, Mycobacteria) should also be considered in risk assessments, and testing regimes should be developed and applied as appropriate. Such assessments should include the potential for the presence of parasites (e.g., Onchocerca, Brugia, Leishmania, Trypanosomes, Ancathomeoba, Pneumocystis) where some species can be sustained under certain cell culture conditions.

A range of organisms are known to cause infection in immunocompromised patients or to be able to establish sustained infection in human cell cultures and in some cases may cause cell transformation. Examples of organisms not addressed in regular donor screening that have been reported in the literature to cause infection in immunocompromised patients include: N. meningitides, Mycobacteria, Nocardia, Histoplasma, and Herpes viruses such as CMV, EBV, and HSV.

This is not intended to present a complete list of potential hazardous organisms which may need to be considered in screening donor cells. Product-specific risk assessment will remain an essential component to ensure product safety. Scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine, provided by WHO [36], may serve as a starting point for developing national guidance and strategy for regulatory risk evaluation to ensure safety of cell therapies.

Tests should be undertaken to detect, and where possible identify, any endogenous or exogenous agents that may be present in the cells. Guidance documents are available that describe in detail tests for microbial agents in cells used to produce biologicals [1, 37, 38]. Adoption of national donor selection procedures used for tissues for transplantation may be required, and other

guidance specific to donor cells and CT preparations may be useful to consider [6].

It should be noted that the cells, as a starting material, are not the only source of risk of microbial contamination. Other materials used in the process (raw materials) also may be sources of contamination, and WHO guidance on the evaluation of cell substrates will be useful in that regard [1]. Feeder cell cultures are generally considered to be raw materials although under certain regulatory jurisdictions they may be called ancillary materials [39]. Banks of such cells should be created and safety tested according to robust risk assessment [1].

3.4 Ongoing characterization

In the absence of significant knowledge or understanding of the active cell, its quality attributes and its mode of action, a range of data on the CTP during clinical development (including production batch data) should be collected to enable associations to be made with clinical outcomes over time and to support enhanced safety and efficacy. For example, cell markers and biophysical data may be collected for information only until patterns emerge that enable a more efficient determination of batch-to-batch consistency. Establishing product comparability after a process change presents an especially difficult challenge if relevant data have not been gathered in the initial development steps (both at the quality/non-clinical and clinical level). The selection of appropriate parameters to measure the impact of the change is a critical step [23, 40].

Product consistency is managed through approaches adopted for other biologicals and is standardised through the establishment of specifications and assays for product identity, purity and potency.

4. Nonclinical

The three primary goals of nonclinical studies are to assess safety, demonstrate potential biological activity, and gain an understanding of possible MOA. The general scientific principles within the fields of pharmacology and toxicology apply to CTPs; however, the traditional, standardized approaches for nonclinical toxicity testing that were developed for drug development and device testing, are often not appropriate for evaluating the safety of CTPs. Nevertheless, the objectives of the nonclinical studies are: to provide information to select safe doses for clinical trials, to demonstrate proof-of-principle, to define the pharmacological and toxicological effects predictive of the human response; to assess biodistribution of therapeutic cells, to provide information to support the route of administration and the application schedule, to provide information to support the duration of exposure and the duration of the follow-up time to detect adverse reactions, and to identify target organs for toxicity and parameters to monitor in patients receiving these therapies.

The nonclinical studies should be performed in relevant animal models if they are available or can be developed. If relevant animal models are not available or cannot be developed, *in vitro* studies may replace animal studies. Justification for the selection of a specific animal model should be provided. The

experimental design of nonclinical studies should reflect the intended clinical use in humans.

The principles of Reduction, Refinement, and Replacement of Animal Use (the "3Rs") should be considered during the development of a nonclinical program for a CT product, as is the case for other medicinal products. Opportunities for reducing, refining and replacing animal use during the process of designing a preclinical development program should be encouraged. For example, whenever practical, a single study might be used to gather both pharmacological and toxicological data.

Major specific questions or issues and special considerations for nonclinical studies have been described elsewhere [7, 8, 9-14, 20, 31, 41, 42].

Compliance with good laboratory practice (GLP) requirements may not be possible or feasible for some toxicology assessments. However, toxicology nonclinical studies should be in substantial compliance with GLP principles, particularly regarding the recording and traceability system put in place to guarantee the potential for a possible retrospective examination and the identification of deviations. Guidance on development of *in vitro* toxicological assays (Good In Vitro Methods Practice) is currently in preparation by the Organisation for Economic Co-operation and Development (OECD).

5. Clinical evaluation

Early-phase clinical trials of CTPs are usually different from other types of biological products in terms of their design and the nature of the risks. For example, there may be prolonged biological activity after a single administration and a high potential for immunogenicity. In addition, the preclinical data may not be as useful as for other biologicals.

In comparison to traditional biologicals, there currently is a limited amount of clinical experience with CTPs, and there usually is considerable uncertainty about the nature and frequency of safety issues. The evaluation of safety might require observation of subjects for a substantial period of time to more fully understand the safety profile.

Characteristics of CTPs that should be considered include but may not be limited to:

- Persistence of cells
- Immune response (allogeneic cells)
- Effect of the microenvironment on the expression of cell factors/molecules
- Differentiation into undesired cell types
- Transformation into tumor cells (stem cells)
- Multiple cell types with different potential for toxicity or therapeutic effect
- Cell migration to unintended sites

- Non-active ingredient cells/contaminants (these may actually have unknown positive or negative impacts)

5.1 Clinical Trial Considerations

For all early-phase clinical trials, especially first-in-human trials, the primary objective should be an evaluation of safety. In the case of CTPs, the human subjects in such trials will generally, if not always, be patients with no alternative therapeutic options.

In addition to general principles of Good Clinical Practice for medicinal products, ethical principles and the importance of inclusion in clinical trial registry, there are some special considerations that are briefly summarized below.

5.2 Dose

Selecting the study dose(s) of a CTP can be challenging. Dosing to target a therapeutic effect might be based on one cell type, but adverse reactions might depend more on a different cell type that is present in the same product. Another complication is that the active cell subset may not be known, so the dose may need to be based on a specific subset that is thought to be the best representation of the desired activity even though it has not been confirmed. Because implanted cells may persist and replicate *in vivo*, the concept of dose is very different from that which has been applied to traditional medicines.

An additional objective for some CTPs may be to identify the maximum tolerated dose (MTD), the highest dose that can be given with acceptable toxicity using a dose-escalation protocol. In other cases, the objective of dose exploration may be to determine the range of biologically active or optimal effective doses. There may be significant practical limits on the dose of the CTP that can be produced or delivered. In such cases, the trial objectives may focus on characterizing the safety profile of the feasible dose or doses, rather than finding the MTD.

5.3 Activity

A common secondary objective of early-phase trials is to obtain preliminary assessments of product activity, using either short-term responses or longer-term outcomes that could suggest a potential for efficacy.

5.4 Study Population

As in all clinical studies, the choice of the subjects to include in the trial depends on the expected risks and potential benefits, recognizing that there will be considerable uncertainty about those expectations in an early-phase trial. The objective is to select a trial population with an acceptable balance between the anticipated risks and potential benefits for the study subjects, while also achieving the study's scientific objectives. Subjects with severe or advanced disease might have confounding adverse events, due to underlying disease, that could make the safety or effectiveness data difficult to interpret. Thus, while severely affected subjects are often included in early-phase CT trials, they

should not be an automatic choice. On the other hand, it would be an unusual circumstance that would justify the exposure of healthy volunteers to allogeneic cells or to harvest and manipulate their own cells.

5.5 Monitoring and Follow-up

In addition to general tests and monitoring to look for unanticipated safety issues, evaluations might include acute or delayed infusion reactions, immune response to the product, autoimmunity, graft failure, graft-versus-host-disease (GVHD), new malignancies (of donor or recipient origin), transmission of infectious agents from a donor, and viral reactivation. Attempts should be made to determine the duration of persistence of the product and its activity. The potential for migration from the target site, ectopic tissue formation, or other abnormal cell activity should be addressed. There is a need for devising modules for tracking the physiological location and functionality of CTPs. This is an area where new preclinical analytical methods are being developed.

In general, the duration of monitoring for adverse events should be designed to cover the time during which the product might reasonably be thought to present safety concerns. The appropriate duration of follow-up depends on the results of preclinical studies, experience with related products, knowledge of the disease process, and other scientific information. For most CTPs, a year or more of follow-up is appropriate for each subject in early-phase trials. In other cases, additional long-term follow-up might be appropriate. Additional clinical guidance is available [43, 44].

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Table 1. Infections other than those included in mandatory donor screening tests that have been reported to occur in transplant patients

Tissue source	**Viruses known to cause malignant transformation of human cells**	**Organisms known to cause persistent infection of human cells and/or Infection in compromised recipients**
Reproductive cells: gamete, embryo	Human papillomavirus (HPV)	Human herpes viruses (notably HHV8)
Bone	-	Parvovirus B19, Brucella spp
Tendon and other cartilage structures	-	-
Adipose tissue	-	Adenovirus, T cruzi
Skin	HPV	Adeno-associated virus
Cornea and limbal tissue	Herpes simplex virus 1 (HSV-1)	Adenovirus, chlamydia
Upper respiratory tract: mouth buccal and gingival oral surfaces, teeth etc	Epstein-Barr virus (EBV)	EBV
Lower respiratory tract (trachea, bronchus and lung)	-	*Chlamydia pscittaci*, Pneumocystis spp
Liver (e.g. hepatocytes, Kupffer cells and reticular endothelial cells)	EBV, HSV	Hepatitis viruses A-E, adenovirus, parvovirus B19, Human herpes viruses 6 & 7.
Pancreatic islets	HSV	Enteroviruses, mumps, Varicella-Zoster virus
Spleen	-	Parvovirus B19, measles
Vasculature and placenta	-	Parvovirus B19, human herpes virus 7
Blood and bone marrow	EBV	Parvovirus B19, *Coxiella burneti*, Brucella spp
Brain and central nervous system	HSV	Varicella-Zoster, enteroviruses (echovirus, coxsackie A and B viruses), mumps, Brucella spp, measles