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DIPLOID PROGENITOR CELL ACTIVE PHARMACEUTICAL INGREDIENTS

Diploide progenitor cellulae pharmaceutical activa substantiis

This general monograph constitutes a technical norm for the preparation and the control of primary diploid progenitor cells for therapeutic use. The contents of this general monograph do not exclude or supersede the use of alternative processes and methods for the preparation and control of the cellular active substance, as agreed upon with the competent Health Authority.

DEFINITION

Fibroblastic progenitor cell (FPC) active pharmaceutical ingredients (APIs) are pharmaceutical active substances composed exclusively or in part by cultured mammalian primary diploid progenitor cells (i.e., viable or non-viable cells) of fibroblastic nature at defined population doubling levels or *in vitro* passage levels, which are conditioned appropriately with the use of excipients in view of long-term storage (e.g., cryopreservation medium, cryo- and lyo-protective solutions). Primary FPCs are non-modified and non-genetically manipulated eukaryotic cellular organisms, isolated from qualified donors, appropriately maintained and expanded *in vitro*, and stably stored for extended time periods. Primary FPCs adhere and proliferate *in vitro* in monolayer culture under appropriate growth-inducive conditions. As primary diploid cells, FPCs possess an extensive but finite *in vitro* cell type lifespan, at the end of which they naturally reach senescence and cease to proliferate. Primary FPCs are pre-terminally differentiated tissue-specific cells, which are incapable of self-renewal. Diploid cells (e.g., WI-38 and MRC-5 cell types) such as primary FPCs have been safely used as biotechnological vaccine substrates since the 1960s.

Primary FPC APIs are intended for use as an active substance in advanced therapy medicinal products (ATMP), combined advanced therapy medicinal products (cATMP), or medical devices (MD) for *in vivo* human or veterinary therapeutic use. Therein, the mechanism of action of the API may be principal or ancillary. Primary FPCs are intended for homologous or non-homologous use in appropriate preparations in tissue engineering and in regenerative medicine, for the management of diverse acute and chronic cutaneous tissue wounds (e.g., burns, ulcers) or of musculoskeletal (e.g., bone, tendon, or cartilage lesions) affections. Therapeutic approaches using primary FPC APIs may be allogeneic or xenogeneic.

Primary FPC APIs are available as individual cryopreserved vials of cells or as individual vials of cell lyophilizates (i.e., freeze-dried powder). The APIs are intended to be initiated from liquid nitrogen cryopreservation storage and used as viable cellular active components of therapeutic products or to be reconstituted from dry form in appropriate vehicles for use as devitalized cellular active components of therapeutic products. The APIs are intended to be appropriately extemporaneously reconstituted and formulated, with combination to appropriate materials or excipients if necessary, by qualified personnel within an appropriate infrastructure and quality system. Final therapeutic product dosage forms may comprise creams, ointments, liquids, or other preparations for cutaneous application, preparations for inhalation, local injection (e.g., powders for injection), or parenteral administration. The excipients used to formulate the final product must neither hinder the therapeutic activity of the APIs during the intended validity and application period, nor react with the APIs to form toxic by-products.

Each FPC API contains one defined fibroblastic primary diploid cell type (e.g., originally isolated from donated skin, cartilage, tendon, muscle, bone, lung tissue, etc.), which may be used alone or in combinations, in homologous or in non-homologous therapeutic applications. Considered API materials cumulatively comply with Ph. Eur. general monographs 2034 "*Substances for pharmaceutical use*", 2619 "*Pharmaceutical preparations*", and 1483 "*Products with risk of transmitting agents of animal spongiform encephalopathies*".

This general monograph does not apply to the use of cultured primary FPCs as cell substrates or as biotechnological hosts for use in vaccine product or molecularly defined biological product manufacture. It does not necessarily entirely apply to APIs and therapeutic products for veterinary use. In addition to the specified requirements of the present general monograph, specific requirements pertaining to API production, qualification testing, characterization testing, safety testing, and product release testing may be included in individual specific monographs, such as cell and tissue monographs defined by the EDQM "Guide to the quality and safety of tissues and cells for human application". The present general monograph generally refers to Regulation EC 1394/2007 on advanced therapy medicinal products, Directive 2001/83/EC on the Community code relating to medicinal products for human use, the EMA Guideline "Human cell-based medicinal products" (EMEA/CHMP/410869/2006), Commission Directive 2003/94/EC laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use, and the EC "Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products" (GMP ATMP vol. 4 - Part IV). The objective of the present general monograph is to standardize the sourcing, production, and testing of specific FPC APIs, to obtain qualified active substances whose critical quality attributes are specified, and to guarantee their quality, safety, and efficacy for application in human or in veterinary medicine.

PRODUCTION

MANUFACTURING PROCESS

Specified manufacturing process workflows and parameters are to be applied in conjunction with applicable requirements set forth in ICH Q7 "Note for guidance on good manufacturing practice for active pharmaceutical ingredients" (CPMP/ICH/4106/00) and ICH Q5D "Note for guidance on quality of biotechnological products: Derivation and characterisation of cell substrates used for production of biotechnological/biological products" (CPMP/ICH/294/95).

Primary FPC APIs are obtained by appropriate *in vitro* cell isolation (i.e., enzymatic or mechanical cell dissociation) from donated pre-natal tissues, preferably within the regulated workflow of a cell transplantation program. Primary FPCs are appropriately maintained and expanded *in vitro* using a defined cell banking system, as defined in Ph. Eur. general chapter 5.2.1. "*Terminology used in monographs on biological products*", which is designed and validated to ensure cellular starting material sustainability, homogeneity, and consistency in the quality of produced cellular API batches. Primary FPC API batch definition may be specified in individual monographs. Following *in vitro* culture, primary FPC APIs are cryopreserved in homogenous lots (i.e., single operation for filling of equal volumes in individual containers) until eventual use in finished therapeutic product reconstitution activities or may be further processed by appropriate methods (e.g., lyophilization) for cellular devitalization and stabilization, while maintaining the biological properties and the activity of the original FPCs. Cryopreserved cell stocks form cell banks of various tiers, for which specific population doubling levels or *in vitro* passage levels

are specified as determinants of *in vitro* cell age. For each cell bank lot, all the containers are treated identically during storage and, once removed from storage, are not returned to the stored cell stock. Cell banks are appropriately characterized and tested at different stages (i.e., including cells at or beyond the maximum passage level used for production) using defined testing schemes based on specific *ad hoc* risk assessments, to ensure the quality and safety of processed cellular materials, within the wider applicable control strategy. Primary FPC APIs are sourced and manufactured in strictly defined conditions using validated processes and qualified items, to minimize the inherent presence or the extraneous incorporation of adventitious contaminants or cross-contamination with alternate cell types or cell lines. No process of terminal sterilization is considered for viable FPC APIs, whereas appropriate methods may be applied to the lyophilized versions thereof, while maintaining the biological properties and the activity of FPC APIs. Where possible and indicated in the *ad hoc* manufacturing workflows, raw materials and reagents of animal origin should be substituted by defined synthetic equivalents, after appropriate qualification thereof and comparative validation tests. Applicable elements are to be applied appropriately from the position paper "*Re-establishment of working seeds and working cell banks using TSE compliant materials*" (EMEA/22314/02), the "*Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products*" (EMA/CHMP/BWP/814397/2011), and the document "*Creutzfeldt-Jakob disease and advanced therapy medicinal products*" (CHMP/CAT/BWP/353632/2010).

For each individual primary FPC API, the manufacturing process (i.e., including all the equipment, materials, processes, controls, specifications, and criteria) must be exhaustively described in a cell type master file and in an active substance master file. Appropriate qualification and standard validation steps are implemented to confirm the adequacy of the FPC API and of the related processes for the considered application and should allow to confirm with a sufficient confidence level that the safety standards and the quality criteria of the manufactured APIs have been met.

The cellular API manufacturing processes described herein have been validated at industrial scales in GMP settings, with the demonstration that produced cellular APIs fulfil the safety and quality standards for human therapeutic use. Described processes correspond to the current state-of-the-art, have been published in peer-reviewed scientific literature, and have been implemented in clinical use for over a decade in University Hospital settings and in multiple clinical trials.

***De novo* primary FPC isolation and cell type establishment.** An *ad hoc* progenitor cell transplantation program is devised, to ensure traceable and safe processing of the biological tissue from a qualified donor. Specifically, the requirements set forth by Directive 2004/23/EC on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells, by Directive 2006/17/EC as regards certain technical requirements for the donation, procurement and testing of human tissues and cells, by Commission Directive 2006/86/EC as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells, and by the EDQM "*Guide to the quality and safety of tissues and cells for human application*" must be followed. Generally, quality parameters aimed at the definition of standard acceptance criteria must be properly established. Preliminarily qualified donors (i.e., appropriate medical screening and health questionnaire administration) are screen tested for specified pathogens, using the appropriate specific and sensitive analytical methods. Specified pathogens included in the donor serological screen comprise, at minimum, cytomegalovirus (CMV), human immunodeficiency viruses (HIV-1, HIV-2), hepatitis B virus (HBV), hepatitis C virus (HCV), human T-cell lymphotropic viruses (HTLV-1, HTLV-2), S-West Nile virus, Zika virus, *Treponema pallidum*, and *Toxoplasma gondii*. Donors are tested at the time of the donation and are retested after an appropriate period, to exclude seroconversion for the specified pathogens. The donated tissue is extensively examined by a qualified pathologist, to exclude any macroscopic and microscopic anatomical or physiological abnormalities in the main structures and systems. Then, specific tissue biopsies are appropriately isolated, dissected, and procured for *in vitro* primary progenitor cell culture initiation. Therein, an appropriate aseptic method for *in vitro* cell isolation (i.e., enzymatic or mechanical cell dissociation) is applied, for the initiation of adherent primary FPC cultures. Usually, Dulbecco's modified Eagle medium (DMEM) supplemented with 10% *v/v* fetal bovine serum (FBS) is used as the cell proliferation medium, which is initially exchanged every 48 hours after the initiation of *in vitro* culture.

For primary FPC API manufacturing purposes, FBS sources must comply with the requirements of Ph. Eur. monograph 2262 "Bovine Serum", Ph. Eur. general chapter 5.2.8. "Minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products", and the EMA "Guideline on the use of bovine serum in the manufacture of human biological medicinal products" (CPMP/BWP/1793/02). All the substances of human or animal origin that are used in the API manufacturing processes must be free from extraneous and contaminating agents and must comply with Ph. Eur. general chapter 5.1.7. "Viral safety" and Ph. Eur. general chapter 5.2.12. "Raw materials for the production of cell-based and gene therapy medicinal products".

Usually, incubation of cell culture vessels in humidified incubators at 37°C and under 5% *v/v* CO_2 is appropriate for the induction of *in vitro* primary FPC adherent proliferation in cell monolayers. Regular microscopic assessment of cellular morphology and cell growth characteristics is performed and is appropriately recorded. Once preliminary cultures have attained the appropriate confluency, enzymatic cell dissociation is performed, and the primary cells are passaged into new sterile cell culture vessels using defined cell seeding densities.

For primary FPC API manufacturing purposes, trypsin must comply with the requirements of Ph. Eur. monograph 0694 "Trypsin". Unless of recombinant origin, the trypsin used for the preparation of cell cultures is tested by suitable methods and is shown to be sterile and free from mycoplasmas and viruses.

Usually, cell seeding densities of 1.5×10^3 to 6.0×10^3 cells/cm^2 are appropriate for the *in vitro* culture of primary FPCs. For the removal of dissociation reagents and of the rinsing medium during the cell passage procedure, harvested cell suspensions may be centrifuged at $280 \times g$ for 10 minutes before being resuspended in the appropriate proliferation medium for *in vitro* cell seeding.

For material and information traceability purposes, the following elements are recorded in the cell type master file, at minimum: donor ethnic and geographical origin, age, sex, general physiological condition; tissue or organ used; results of any tests for pathogens; the method used for in vitro cell culture initiation; cell culture methods; any other procedures used to establish the primary cell type, notably any process that might expose the primary cells to extraneous agents.

Primary FPC parental cell banks (PCB). Following *in vitro* cell isolation from donated tissues and initiation of preliminary *in vitro* expansion (i.e., primary cell culture), progenitor cell cultures are further appropriately incubated and maintained, as described hereabove. Once the cell cultures have attained an appropriate

confluency (i.e., usually 80%–100% confluency), enzymatic cell dissociation is performed, the cells are pooled, and are appropriately conditioned in individual vial lots for cryopreservation. Following controlled rate freezing (i.e., usually -1°C/minute rate of cooling), the constituted vial stocks are stored in the vapor or the liquid phase of liquid nitrogen storage tanks. Individual primary FPC vials typically contain 10^6 to 10^7 cells suspended in FBS and DMSO-based or equivalent synthetic cryopreservation medium. At this point, the obtained lot of cryopreserved cell vials is defined as the FPC parental cell bank (PCB).

Particular attention is paid to the systematic recording of all the available documents, material information, and data generated at the time of the parental cell bank establishment (e.g., materials and consumable certificates of analysis, freezing curves, storage logs), as the ad hoc cell type master file will potentially serve as a basis and as an integral part of the active substance master files relative to the cell type of interest.

Primary FPC pilot cell banking for API qualification and manufacture optimization. In order to first qualify the considered FPC type and then to optimize and to validate the *ad hoc* technical specifications applied in all the subsequent cell banking and manufacturing steps, a pilot cell banking campaign is carried out. Therein, the cells are expanded up to the appropriate passage level for the assessment of maximal *in vitro* lifespan and safety testing (i.e., usually at a passage level 1.5 times greater in value than the *in vitro* passage level considered for production and clinical use). Therein, karyotypic analysis is performed at low, intermediate, and high passage levels, to assess the cell type genetic stability. Then, intermediate passage level cells are used in technical optimization procedures, which comprise benchmarking, for the selection of the most appropriate materials and consumables (i.e., culture surface type and size, source of FBS) and of the cell culture parameters (i.e., cell seeding densities, culture medium volumes, total culture periods) for obtention of maximized harvest yields of cells retaining appropriate quality attributes. Finally, the optimized parameters and selected items are specified in the *ad hoc* technical specifications for the further manufacture of primary FPC master cell banks (MCB), working cell banks (WCB), and end of production cell banks (EOPCB).

Such pilot cell banking optimization phases are to be carried out by each manufacturer, in view of establishing dedicated FPC MCBs for the appropriate and specific products or production purposes.

Primary FPC master cell banks (MCB). PCB vials are used in appropriate recovery procedures (i.e., rapid thawing in a 37°C water-bath) and the cells are seeded for appropriate *in vitro* culture expansion in the optimized manufacturing conditions established during the pilot cell banking campaign. After one or two serial *in vitro* expansions, which may be separated by cryopreservation phases, the cells are harvested and are cryopreserved as described hereabove to form a homogenous FPC MCB. Appropriate characterization and release testing is performed on MCB materials (i.e., using vials from the start, middle, and end of the produced batch), in order to assess GMP compliance thereof.

Sub-tiering of the MCBs may be indicated, in order to augment the potential overall yield of the MCBs, for sustainable provision of starting materials covering the whole lifecycle of specific products. In the case of sub-tiering, the multiple in vitro cell expansions are performed serially, with or without cryopreservation of the intermediate (i.e., lower MCB tier) cell stock.

Primary FPC working cell banks (WCB). MCB vials are used in appropriate recovery procedures (i.e., rapid thawing in a 37°C water-bath) and the cells are seeded for appropriate *in vitro* culture expansion in the optimized manufacturing conditions established during the pilot cell banking campaign. After one or multiple serial *in vitro* expansions, which may be separated by cryopreservation phases, the cells are harvested and are cryopreserved as described hereabove to form a homogenous FPC WCB. Appropriate characterization and release testing is performed on MCB materials (i.e., using vials from the start, middle, and end of the produced batch), in order to assess GMP compliance thereof.

Sub-tiering of the WCBs may be indicated, in order to augment the potential overall yield of the WCBs, for sustainable provision of starting materials and API materials covering the whole lifecycle of specific products. In the case of sub-tiering, the multiple in vitro cell expansions are performed serially, usually with cryopreservation of the intermediate (i.e., lower WCB tier) cell stock.

Primary FPC end of production cell banks (EOPCB). WCB vials are used in appropriate recovery procedures (i.e., rapid thawing in a 37°C water-bath) and the cells are seeded for appropriate *in vitro* culture expansion in the optimized manufacturing conditions established during the pilot cell banking campaign. After multiple serial *in vitro* expansions, which may be separated by cryopreservation phases, the cells are harvested and are cryopreserved as described hereabove to form a homogenous FPC EOPCB. Appropriate characterization and safety testing is performed on EOPCB materials (i.e., using vials from the start, middle, and end of the produced batch), in order to assess GMP compliance thereof.

Cell type safety testing and qualification testing are performed on EOPCB materials or cells derived therefrom, wherein cell type genetic stability is assessed, and appropriate assays are performed to exclude cell type tumorigenicity.

Primary FPC APIs. The APIs may be defined as cryopreserved vials of the respective and appropriate tiers of the produced FPC WCBs, or such materials may be initiated and appropriately expanded or reconstituted for lyophilization processing. Therefore, appropriate cryo- and lyo-protectant excipients are combined with the cell suspensions in adequate proportions, before conditioning in adapted ampoules and lyophilization thereof. FPC APIs are usually constituted by cryopreserved cell vials or refrigerated cell lyophilizate ampoules each containing 10^6 to 10^7 cells at the defined passage level qualified for clinical use. Primary containers or conditioning units for APIs are usually CE-marked devices.

If justified, alternative manufacturing methods may be designed, validated, and used for the manufacture of primary FPC banks, provided that the critical safety and quality attributes of the obtained cellular APIs can be demonstrated as being equivalent. Therefore, the general principles of ICH Q5E "Biotechnological/biological products subject to changes in their manufacturing process: Comparability of biotechnological/biological products" (CPMP/ICH/5721/03) may be followed. If justified, an appropriate terminal sterilization method (i.e., one that does not compromise API function, quality, and safety) may be used on the lyophilized form of the APIs, if aseptic material processing alone does not guarantee an acceptable level of safety and quality assurance.

QUALITY SYSTEM AND PRODUCTION ENVIRONMENT

The original tissue biopsy procurement and all the subsequent API manufacturing steps should be carried out under an appropriate quality system, usually a pharmaceutical quality system with appropriately implemented quality risk management procedures, as described in ICH Q9 on quality risk management (EMA/CHMP/ICH/24235/2006) and in ICH Q10 on pharmaceutical quality systems (EMA/CHMP/ICH/214732/2007).

Tissue procurement is carried out in an appropriate environment under conditions minimizing the risk of the contamination of

starting materials by adventitious agents. The manufacturing steps including open container manipulations are performed in a dedicated environment, for which the air quality is specified, validated, and monitored. Therein, and in the absence of microbiological contaminant removal or inactivation steps (e.g., terminal sterilization), the manufacturing environment must conform to GMP Grade A as defined in Directive 2003/94/EC (i.e., with particle enumeration and microbial colony counts), with a background environment conforming at least to Grade B, if the primary manufacturing environment is not isolated.

A minimal biosafety level (i.e., BSL-1) is sufficient for the manufacture of primary FPC banks and of cellular APIs, after qualification of the diploid progenitor cell type of interest in terms of biological safety.

EQUIPMENT AND MATERIALS

All the equipment and materials used in the manufacturing of the APIs are specifically designed and appropriately maintained to suit the intended purpose and to minimize any hazard to patients and to manufacturing personnel. All critical equipment and devices are identified, qualified, regularly inspected, and preventively maintained appropriately. Key and critical process parameters in relation with the equipment and materials must be defined, along with acceptance margins and criteria, and are included in general and in specific risk analyses. In the case where the equipment, devices, or materials are determined to affect the critical processing or storage parameters (e.g., temperature, air pressure, particle counts, and microbial contamination levels), they must be appropriately identified and subjected to appropriate monitoring, including alerts, alarms, and corrective actions if necessary, to detect any malfunctions or defects and to ensure that the critical parameters are always maintained within acceptable limits. Any equipment purposed with a critical measuring function is calibrated against a traceable standard, if available. Maintenance, servicing, cleaning, disinfection, and sanitation of all the critical equipment are performed regularly and recorded.

The technical specifications for API manufacturing necessarily list all the critical materials (i.e., starting materials, raw materials, ancillary materials, conditioning materials), reagents, and consumables (i.e., contact-process consumables, non-contact-process consumables), along with their respective specifications. If applicable, the critical materials, reagents, and consumables must meet compendial requirements and/or documented specifications and the requirements of Regulation 2017/745 on medical devices and of Regulation 2017/746 on *in vitro* diagnostic medical devices. Materials, reagents, and consumables exempt of animal-sourced components should be used where possible, provided that they are appropriately comparatively qualified and validated against historical specifications and data.

Specifically, raw materials included in the manufacturing processes are produced in appropriate qualified facilities under a recognized quality management system and are qualified. This aspect may be important for the insurance of API critical quality attribute consistency (i.e., quality, safety, and efficacy). The use of antibiotics is avoided wherever possible during production. Control of raw material change or use of new raw material batches must be considered from a production perspective in light of Ph. Eur. general chapter 5.2.12. "Raw materials for the production of cell-based and gene therapy medicinal products".

RISK ASSESSMENTS

Appropriate multifactorial risk assessments are to be carried out, specifically concerning raw materials used in the manufacture of APIs, to guarantee maximal quality, safety, and efficacy of the medicinal products or of the medical devices containing such APIs. In general, risk assessments are to be carried out following Ph. Eur. general chapter 5.2.12. for raw materials, and an appropriate HACCP approach may be applied to the manufacturing system as a whole. Risk analyses may be the basis of the working risk management plan and should be used to justify the product development and evaluation plans. Therefore, risk analysis and risk minimization activities govern the whole product lifecycle, from the product development to the post-market risk management (e.g., pharmacovigilance plan).

TESTS

Various test categories are applied throughout API manufacturing, to ensure consistent quality and safety of the produced cellular APIs. Distinct categories of tests must be implemented, comprising general qualification-related testing (e.g., full tumorigenicity testing for cell type safety qualification) and routine quality control testing (e.g., routine microbiological quality testing) at appropriate points of the manufacturing process. Validated tests are performed to confirm the cell type identity and purity, the specific and general microbiological quality, cell type safety, and the cell type biological parameters. Appropriate testing strategies are implemented following the general and specific risk analyses performed for critical processes. Specifically, viral biosafety testing schemes are devised following the requirements of ICH Q5A R1 "Quality of biotechnological products: Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin" (CPMP/ICH/295/95) and general biosafety testing is performed according to or based on Ph. Eur. general chapter 5.2.3. "Cell substrates for production of vaccines for human use". If the tissue procurement and the initial cell culture initiation steps have not been performed directly within a GMP-accredited tissue procurement establishment, a quarantine screen may be warranted for PCB materials before further admission to manufacturing facilities. All tests are performed under appropriate quality systems and standards (e.g., ISO 17025, GLP conditions), following ICH Q2 R1 "Validation of analytical procedures: Text and methodology" (CPMP/ICH/381/95).

PRIMARY FPC PARENTAL CELL BANKS

Cell enumeration. Nucleated cell count determination, as well as relative cellular viability are determined upon cell initiation according to Ph. Eur. method 2.7.29. "*Nucleated cell count and viability*". Total and viable cell counts must comply with specifications of individual API monographs.

Cell morphology. The morphology of the proliferating cells is adequately described and is documented by qualified and experienced personnel.

Isoenzyme testing. Appropriate isoenzyme testing is performed, to exclude misidentification or cross-contamination of the original manufactured cell type.

DNA fingerprinting. The manufactured cell type is appropriately and specifically identified by DNA fingerprinting.

Surface marker profiling. For confirmation of cell type identity and cell population purity, appropriate cell surface markers (e.g., clusters of differentiation) are analysed by flow cytometry according to Ph. Eur. method 2.7.24. "*Flow cytometry*" and possibly using Ph. Eur. method 2.7.23. "*Numeration of CD34/CD45$^+$ cells in haematopoietic products*".

Immunohistochemical testing. Appropriate and specific molecular markers are analysed using appropriate methods, for the exclusion of cell population impurity or of cell type misidentification.

Stability. Suitable cell viability maintenance in the intended storage conditions is validated.

Sterility testing. Test-materials comply with the test for sterility, following Ph. Eur. method 2.6.1. "*Sterility*" and ICH Q5D. Testing is carried out using, for each testing medium, 10 mL of supernatant

from cell cultures. The test is carried out on 1% of the containers, with a minimum of 2 containers.

Alternatively, use may be made of Ph. Eur. method 2.6.27. "Microbiological examination of cell-based preparations" or Ph. Eur. method 5.1.6. "Alternative methods for control of microbiological quality".

Mycoplasma testing. The test-materials comply with the test for mycoplasmas following Ph. Eur. method 2.6.7. "*Mycoplasmas*". The test is carried out with a minimum of 1 container.

Bacterial endotoxins. The test-materials comply with the test for bacterial endotoxins following Ph. Eur. method 2.6.14. "*Bacterial endotoxins*". The endotoxin concentration in the tested API sample should remain below the endotoxin threshold specified in individual monographs.

***In vitro* assays for the presence of adventitious viral contaminants.** Presence of viral contaminants and related effects (i.e., hemadsorption, haemagglutination, cytopathic effect) are investigated in extended *in vitro* assays (i.e., incorporating day 14 blind passage) in six-well plates using detector cell lines. Viable cells (i.e., at least 10^7 cells) or the equivalent cell lysate, in their culture supernatant, are either co-cultivated (i.e., for viable cells) or inoculated (i.e., for cell lysate) onto detector cells. Choice of detector cell lines is based on the US FDA "*Points to Consider"* (1993) and EC CPMP "*Notes for Guidance*", and generally comprise MRC-5 cells, Vero cells, and HeLa cells, wherein positive viral controls are included appropriately.

Karyology analysis. A minimum of 200 cells in metaphase are examined for exact chromosome count and for the frequency of hyperploidy, hypoploidy, polyploidy, breaks, and structural abnormalities.

RT-PCR for the presence of human viruses. Appropriate testing, following Ph. Eur. method 2.6.21. "*Nucleic acid amplification techniques*", is performed for specified viral pathogens. Specified pathogens comprise KI polyomavirus (KIPyV), Wu Polyomavirus (WUPyV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency viruses (HIV-1, HIV-2), human T-cell lymphotropic viruses (HTLV-1, HTLV-2), human herpes viruses (HHV-6, HHV-7, HHV-8), Epstein-Barr virus (EBV), human cytomegalovirus (hCMV), B19 parvovirus, human papillomavirus (HPV), human polyomavirus (HuPyV), human bocavirus (HBoV), West Nile virus, Zika virus, and simian virus 40 (SV40).

Quantification of reverse transcriptase activity by ultracentrifugation and quantitative fluorescent product enhanced reverse transcriptase (QFPERT) assay. Appropriate testing, following Ph. Eur. method 2.6.21. "*Nucleic acid amplification techniques*", is performed for the exclusion of reverse transcriptase activity.

Characterization and release testing is performed on quarantined PCB materials or on materials derived therefrom, if necessary and justified. Where appropriate, sampling is performed at the start, middle, and end of the cell bank lot.

PRIMARY FPC MASTER CELL BANKS

Cell enumeration. See PCB section.

Cell morphology. See PCB section.

Isoenzyme testing. See PCB section.

DNA fingerprinting. See PCB section.

Surface marker profiling. See PCB section.

Immunohistochemical testing. See PCB section.

Growth parameters. Cell growth parameters such as population doubling values (PDV) and population doubling times (PDT) are appropriately determined.

Stability. See PCB section.

Sterility testing. See PCB section.

Mycoplasma testing. See PCB section.

Mycobacteria testing. If the cells are susceptible to infection with *Mycobacterium tuberculosis* or other species, the test-materials comply within assays performed following Ph. Eur. method 2.6.2. "*Mycobacteria*".

Mycoplasma testing. See PCB section.

Bacterial endotoxins. See PCB section.

***In vitro* assay for the presence of adventitious viral contaminants.** See PCB section.

Karyology analysis. See PCB section.

RT-PCR for the presence of human viruses. See PCB section.

Quantification of reverse transcriptase activity by ultracentrifugation and quantitative fluorescent product enhanced reverse transcriptase (QFPERT) assay. See PCB section.

Tests for viruses using broad molecular methods. Broad molecular methods may be used, where justified, either as alternatives to *in vivo* tests and specific NAT or as supplements to *in vitro* culture tests, based on an appropriate risk assessment.

TEM examination of cell cultures. The MCB (i.e., a minimum of 200 cell profiles) is examined by transmission electron microscopy (TEM) for the exclusion of the presence of extraneous agents (i.e., viruses, virus-like particles, mycoplasma, yeasts, fungi, and bacteria).

Characterization testing is performed on MCB materials or on materials derived therefrom, as appropriate and justified. Release testing is performed on quarantined MCB materials. Where appropriate, sampling is performed at the start, middle, and end of the cell bank lot.

PRIMARY FPC WORKING CELL BANKS

Cell enumeration. See PCB section.

Cell morphology. See PCB section.

Isoenzyme testing. See PCB section.

DNA fingerprinting. See PCB section.

Surface marker profiling. See PCB section.

Immunohistochemical testing. See MCB section.

Growth parameters. See MCB section.

Stability. See PCB section.

Sterility testing. See PCB section.

Mycoplasma testing. See PCB section.

Mycobacteria testing. See MCB section.

Bacterial endotoxins. See PCB section.

***In vitro* assay for the presence of adventitious viral contaminants.** See PCB section.

***In vivo* assays using suckling mice, adult mice, guinea pigs, and embryonated eggs.** Test-materials comply with applicable assays described in Ph. Eur. general chapter 5.2.3.

Karyology analysis. See PCB section.

RT-PCR for the presence of human viruses. See PCB section.

Quantification of reverse transcriptase activity by ultracentrifugation and quantitative fluorescent product enhanced reverse transcriptase (QFPERT) assay. See PCB section.

Tests for viruses using broad molecular methods. See MCB section.

TEM examination of cell cultures. See MCB section.

Characterization testing is performed on WCB materials or on materials derived therefrom, as appropriate and justified. Release testing is performed on quarantined WCB materials. Where appropriate, sampling is performed at the start, middle, and end of the cell bank lot.

PRIMARY FPC END OF PRODUCTION CELL BANKS

Cell enumeration. See PCB section.

Cell morphology. See PCB section.

Isoenzyme testing. See PCB section.

DNA fingerprinting. See PCB section.

Surface marker profiling. See PCB section.

Immunohistochemical testing. See PCB section.

Growth parameters. See MCB section.

Sterility testing. See PCB section.

Mycoplasma testing. See PCB section.

Mycobacteria testing. See MCB section.

Bacterial endotoxins. See PCB section.

***In vitro* assay for the presence of adventitious viral contaminants.** See PCB section.

***In vivo* assays using suckling mice, adult mice, guinea pigs, and embryonated eggs.** See WCB section.

Karyology analysis. See PCB section.

RT-PCR for the presence of human viruses. See PCB section.

Quantification of reverse transcriptase activity by ultracentrifugation and quantitative fluorescent product enhanced reverse transcriptase (QFPERT) assay. See PCB section.

Tests for viruses using broad molecular methods. See MCB section.

TEM examination of cell cultures. See MCB section.

***In vivo* tumorigenicity.** Test-items comply with requirements on tumorigenicity of Ph. Eur. general chapter 5.2.3.

Characterization and safety testing are performed on representative EOPCB materials or on materials derived therefrom. Where appropriate, sampling is performed at the start, middle, and end of the cell bank lot.

PRIMARY FPC ACTIVE PHARMACEUTICAL INGREDIENTS

The critical quality attributes and quality specifications are determined for the APIs. The specifications and acceptance criteria are specific to individual APIs or to finished products and are defined following ICH Q6B "Note for Guidance on Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products" (CPMP/ICH/365/96).

For the cryopreserved form of primary FPC APIs, refer to FPC WCB testing sections and only to the following "Activity assay". For the lyophilized form of primary FPC APIs, refer only to the following sections.

Descriptive analysis. Batch descriptive analysis is performed and is recorded. The results must be homogenous and consistent with historical data.

Sterility testing. See PCB section.

Mycoplasma testing. See PCB section.

Particle presence. Adventitious particle presence is excluded based on Ph. Eur. method 2.9.20. "*Particulate contamination: Visible particles*".

Uniformity of mass. Unit mass distribution is within the specified limits, following Ph. Eur. method 2.9.5. "*Uniformity of mass of single-dose preparations*".

Cell enumeration. See PCB section. Cellular devitalization is confirmed.

Total protein content. The total protein content is within the specified limits, following Ph. Eur. method 2.5.33. "*Total protein*".

Water content. Water content in the API before reconstitution is within the specified limits, following Ph. Eur. method 2.5.12. "*Water: Semi-micro determination*" or Ph. Eur. method 2.5.32. "*Water: Micro determination*". The maximum residual water content is 5.0% *m/m*, unless otherwise specified in the specific monographs.

Solubility. Lyophilized APIs dissolve completely in the prescribed volume of reconstitution solvent within a specified time, at a specified temperature.

Osmolality. Reconstituted API osmolality is within the specified limits, following Ph. Eur. method 2.2.35. "*Osmolality*".

pH value. The pH value of the reconstituted API is within the specified limits, following Ph. Eur. method 2.2.3. "*Potentiometric determination of pH*".

Activity assay. Where relevant, the API biological activity is determined by a suitable and specific assay. Results comply with historical data.

Bacterial endotoxins. The test-materials comply with the test for bacterial endotoxins following Ph. Eur. method 2.6.14. "*Bacterial endotoxins*" or Ph. Eur. method 2.6.8. "*Pyrogens*". The endotoxin concentration in the API should remain below the endotoxin threshold specified in the individual monograph. The endotoxin limit depends on the final therapeutic product formulation and its route of administration.

The maximal product dose administered by the intended administration route should not contain sufficient endotoxins to cause a toxic reaction. The Ph. Eur. method 5.1.10. "Guidelines for using the test for bacterial endotoxins" is followed.

Impurities. Process-related impurities should be identified and quantified. Appropriate acceptance criteria should be defined.

STORAGE

Primary FPC APIs should be stored under the conditions specified in the individual monographs, under conditions of conservation without any significant alteration of their intended final characteristics. Generally, cryopreserved cellular materials are stored in the vapor or the liquid phase of liquid nitrogen (i.e., under -140°C) and lyophilized cellular APIs are stored at refrigerated temperatures (i.e., 2°C to 8°C). The adopted storage conditions enable the stable storage of the APIs and are validated.

SHELF LIFE

The API shelf life may be determined following ICH Q5C "Stability testing of biotechnological/biological products" (CPMP/ICH/138/95).

The shelf life is the time period during which the quality and, where relevant, the water content of the API remain within the limit thresholds specified in the individual monographs. The shelf life is the period during which the quality, safety, and efficacy of the API are demonstrated as being maintained.

LABELLING

The API label states the following:

● The identity and the quantity of FPCs within the API unit.

● A reference number and a batch number.

● *For multi-dose units*: The number of doses within the API unit.

● *For lyophilized units*: The name, composition, and volume of the reconstituting liquid to be added; the period of time within which the preparation is to be used after reconstitution.

● A list of API components.

● The API storage conditions.

● The API production date.

● The API expiration date.

● Instructions for reporting serious adverse reactions and/or events.

● Instructions on how to dispose of unused (expired) products.

● Information and contact details of the API manufacturer.

SURVEILLANCE

The clinical use of primary FPC APIs must be surveyed in the framework of an appropriate pharmacovigilance plan, including reporting of possible adverse events and reactions associated with their use. Establishment of a centralized reporting system and a dedicated register are warranted.

Surveillance is performed in accordance with requirements of Directive 2004/23/EC, Directives 2006/17/EC and 2006/86/EC, and of EMEA/CHMP/96268/2005.

ABBREVIATIONS

API	active pharmaceutical ingredient
ATMP	advanced therapy medicinal product
cATMP	combined advanced therapy medicinal product
CMV	cytomegalovirus
DMEM	Dulbecco's modified Eagle medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
FBS	fetal bovine serum
FPC	fibroblastic progenitor cells
EBV	Epstein-Barr virus
EC	European Commission
EDQM	European Directorate for the Quality of Medicines and Healthcare
EMA	European Medicines Agency
EOPCB	end of production cell bank
FDA	US Food and Drug Administration
GLP	good laboratory practices
GMP	good manufacturing practices
HACCP	hazard analysis critical control point
HAV	hepatitis A virus
HBoV	human bocavirus
HBV	hepatitis B virus
hCMV	human cytomegalovirus
HCV	hepatitis C virus
HHV	human herpes virus
HIV	human immunodeficiency virus
HPV	human papillomavirus
HTLV	human T-cell lymphotropic virus
HuPyV	human polyomavirus
ICH	international council for harmonization
KIPyV	KI polyomavirus
MCB	master cell bank
MD	medical device
NAT	nucleic acid amplification techniques
PCB	parental cell bank
PDT	population doubling time
PDV	population doubling value
Ph. Eur.	European pharmacopoeia
QFPERT	quantification of reverse transcriptase activity by ultracentrifugation and quantitative fluorescent product enhanced reverse transcriptase
RT-PCR	real-time polymerase chain reaction
SV40	simian 40 virus
TEM	transmission electron microscopy
US	United States of America
WCB	working cell bank
WUPyV	Wu polyomavirus