Version 10/2021/LALA

SKIN FIBROBLAST PROGENITOR CELLS

Cutis fibroblast progenitor cellulae

This specific monograph refers to the corresponding general monograph "Diploid progenitor cell active pharmaceutical ingredients".

DEFINITION

Primary skin fibroblast progenitor cells (SFPC) are active pharmaceutical ingredients (API) composed exclusively or in part by mammalian cultured diploid progenitor cells at defined population doubling levels or *in vitro* passage levels, primarily isolated from pre-natal skin tissue, which are conditioned appropriately with the use of excipients in view of long-term storage. Primary SFPCs 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 SFPCs adhere and proliferate *in vitro* in monolayer culture under appropriate growth-inducive conditions and possess an extensive but finite *in vitro* lifespan. Primary SFPCs are pre-terminally differentiated tissue-specific cells, which are incapable of self-renewal.

Primary SFPC APIs are intended for use as an active substance in appropriate therapeutic preparations in tissue engineering and in regenerative medicine, for homologous management of diverse acute and chronic cutaneous tissue affections (e.g., burns, ulcers, scars) or alternative non-homologous uses. Primary SFPC APIs are available as individual cryopreserved cell vials or as cell lyophilizates to be appropriately reconstituted for finished therapeutic product formulation.

Provisions of the corresponding general monograph "Diploid progenitor cell active pharmaceutical ingredients" apply.

PRODUCTION

MANUFACTURING PROCESS

Primary SFPC APIs are obtained following appropriate in vitro cell isolation from donated pre-natal skin tissues and appropriate serial in vitro expansions using a defined cell banking system. Following in vitro culture, the APIs are cryopreserved until eventual use for finished therapeutic product reconstitution or may be further processed by appropriate methods (e.g., lyophilization) for cellular devitalization and stabilization. An API batch is defined as a homogenous WCB lot or as a lyophilizate lot produced on a specific day in a single operation.

***De novo* primary SFPC isolation and cell type establishment.** Qualified pre-natal skin samples are made available for processing and *in vitro* primary cell culture initiation. Therein, an appropriate aseptic method for *in vitro* cell isolation (i.e., enzymatic or mechanical cell dissociation) is applied.

Primary SFPC parental cell banks (PCB). Following *in vitro* cell isolation from skin tissues and initiation of preliminary *in vitro* expansion, primary cultures are further appropriately maintained, harvested, and cryopreserved to form the PCB. Individual SFPC vials contain 10^6 to 10^7 cells.

Primary SFPC pilot cell banking for API qualification and manufacture optimization. *Provisions of the corresponding general monograph "Diploid progenitor cell active pharmaceutical ingredients" apply.*

Primary SFPC master cell banks (MCB). PCB vials are used to generate MCB lots in the optimized manufacturing conditions established during the pilot cell banking campaign. Appropriate characterization and release testing is performed on MCB materials.

Primary SFPC working cell banks (WCB). MCB vials are used to generate WCB lots in the optimized manufacturing conditions established during the pilot cell banking campaign. Appropriate characterization and release testing is performed on MCB materials.

Primary SFPC end of production cell banks (EOPCB). WCB vials are used to generate EOPCB lots in the optimized manufacturing conditions established during the pilot cell banking campaign. Appropriate characterization and safety testing is performed on EOPCB materials.

Primary SFPC APIs. The SFPC APIs may be defined as cell vials of the respective and appropriate tiers of the produced WCB, or such materials may be initiated and appropriately expanded or reconstituted for lyophilization. APIs are constituted by cryopreserved cell vials or refrigerated cell lyophilizate ampoules each containing 10^6 to 10^7 cells at the defined *in vitro* passage level for clinical use.

TESTS

Provisions of the corresponding general monograph "Diploid progenitor cell active pharmaceutical ingredients" apply with regard to the differential testing of the various cell bank tiers. Where specific criteria apply to SFPC material testing, they are mentioned hereafter.

PRIMARY SFPC CELL BANKS

Cell enumeration. Total cell counts must be within 100% ± 25% of the specified value upon cell initiation. Viable cell counts must be ≥ 80%.

Cell morphology. The morphology of the cells is fibroblastic, with elongated and spindle-shaped cell bodies. Cell morphology is consistent throughout manufacturing.

Isoenzyme testing. Isoenzyme test results are consistent throughout manufacturing.

DNA fingerprinting. Cell identity is consistent throughout manufacturing.

Surface marker profiling. SFPCs are generally positive for cell surface markers CD14, CD26, CD44, CD73, CD90, CD105, CD166, and HLA-ABC. SFPCs are generally negative for cell surface markers CD34, CD45, and HLA-DP/DQ/DR.

Immunohistochemical testing. SFPCs are negative for the nuclear marker p63.

Growth parameters. Cell growth parameters are consistent throughout manufacturing of clinically relevant passage level cells.

Stability. Viable cell counts upon initiation must be consistently ≥ 80% after the longest storage period included in the validity period.

Sterility testing. Test-materials are exempt of detectable specified and non-specified contaminants.

Mycobacteria testing. Test-materials are exempt of detectable mycobacterial contaminants.

Mycoplasma testing. Test-materials are exempt of detectable mycoplasma contaminants.

Bacterial endotoxins. The detectable endotoxin concentration remains below the appropriate threshold, as specified in Ph. Eur.

method 5.1.10. "*Guidelines for using the test for bacterial endotoxins*".

***In vitro* assays for the presence of adventitious viral contaminants.** Test-materials are exempt of detectable adventitious viral contaminants.

***In vivo* assays using suckling mice, adult mice, guinea pigs, and embryonated eggs.** Test-materials are exempt of detectable adventitious viral contaminants.

Karyology analysis. Test-materials are exempt of detectable genetic abnormalities.

RT-PCR for the presence of human viruses. Test-materials are exempt of detectable adventitious human viral contaminants.

Quantification of reverse transcriptase activity by ultracentrifugation and quantitative fluorescent product enhanced reverse transcriptase (QFPERT) assay. Test-materials are exempt of detectable retroviral activity.

TEM examination of cell cultures. Test-materials are exempt of detectable extraneous agents.

***In vivo* tumorigenicity.** Test-items comply with requirements of ICH Q5D and of Ph. Eur. general chapter 5.2.3. Test-materials are exempt of tumorigenicity potential in specified test-systems.

PRIMARY SFPC ACTIVE PHARMACEUTICAL INGREDIENTS

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

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

Sterility testing. Test-materials are exempt of detectable specified and non-specified contaminants.

Mycoplasma testing. Test-materials are exempt of detectable mycoplasma contaminants.

Bacterial endotoxins. The endotoxin concentration remains below the appropriate threshold, as specified in Ph. Eur. method 5.1.10. "*Guidelines for using the test for bacterial endotoxins*". The endotoxin concentration in the API remains below the specified threshold, depending on the final product formulation and its route of administration.

Particle presence. Adventitious and contaminating particle presence is excluded.

Uniformity of mass. Unit mass distribution is within 100% ± 10% of the mean unit mass.

Cell enumeration. Total cell counts must be within 100% ± 25% of the specified value. Cellular devitalization is confirmed by an appropriate method.

Total protein content. The total protein content is within the specified limits, which are consistent with historical data.

Water content. The maximum residual water content is 5.0% *m/m*.

Solubility. Lyophilized APIs dissolve completely in the prescribed volume of the appropriate reconstitution solvent within 90 seconds, at a specified temperature.

Osmolality. Reconstituted API osmolality is within the specified limits, depending on the administration route of the finished product.

pH value. The pH value of the reconstituted API is within the specified limits, depending on the administration route of the finished product.

Activity assay. Results comply with historical data of *in vitro* target cell stimulation (e.g., cell proliferation, cell migration).

Impurities. Impurity quantities remain within specified and acceptable margins.

STORAGE

Primary SFPC APIs in their cryopreserved form are stored in the vapor or liquid phase of liquid nitrogen (i.e., under -140°C) and lyophilized APIs are stored at refrigerated temperatures (i.e., 2°C to 8°C).

ABBREVIATIONS

API	active pharmaceutical ingredient
CD	cluster of differentiation
DNA	deoxyribonucleic acid
EOPCB	end of production cell bank
HLA	human leucocyte antigen
ICH	international council for harmonization
MCB	master cell bank
PCB	parental cell bank
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
SFPC	skin fibroblast progenitor cells
TEM	transmission electron microscopy
WCB	working cell bank