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Establishment of Relevant Cell Line (2)

- It is necessary to demonstrate the maximal number of passages within which the cells remain stable.
- In some cases, it may be important to discuss the possibility of tumorigenicity and malignant transformation of an established cell line and to investigate such a possibility using an appropriate animal model, where necessary.

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Establishment of Cell Banks (1)

- When a cell bank is established at any stage during manufacture of hCTPs, describe
  1) The rationale for preparing the cell banks;
  2) The methods used to prepare the cell banks;
  3) The characteristics of the cell banks; and
  4) The storage, maintenance, control methods, and
  5) Renewal methods as well as any other processes and tests performed.
- Explain the appropriateness of each.

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Establishment of Critical Intermediate Cell Lines(1)

- It should be noted that in some cases, the establishment of a cell line (intermediate cell line) as an intermediate product may be important for the stable manufacture of a safe final product and for scientific validity of the procedure. When such a measure is chosen, explain its advantages and appropriateness.
- If a cell line that exhibits a different phenotype is established in stages, describe the methods (e.g., methods for induction of differentiation, isolation, culturing, and cell line establishment of the target cells as well as the media, culture conditions, culture duration, and the yield at each stage) until establishment of each respective cell line, and explain their appropriateness to the extent possible.

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Establishment of Critical Intermediate Cell Lines(2)

- To maintain stability and consistency of the quality of the intermediate cell lines, identify CQA of the cells and set acceptance criteria.
  (**CQA**: e.g., cell purity, morphological features, phenotype-specific markers, karyotype, cell growth properties, and multi/pluripotency)
- It is important to demonstrate the maximal number of passages or of cell divisions within which the cells can proliferate while maintaining their quality in terms of the criteria specified.

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Processing of Cells (1)

- Processing of cells includes any processing of cells, such as 1)-7) by means of 1)-4) with the aim of preparing desired cell products to treat a patient or to repair or regenerate a tissue.
  - 1) Propagation, 2) Reprogramming, 3)Direct reprogramming, and/or 4) Induction of differentiation of cells,5) Production of a cell line, 6) Cell activation, or altering a biological characteristic as well as 7) Combination with an NCC
  - 1) Cultivation, 2) Chemical, physical, and/or biochemical treatment(s), 3) Genetic engineering and/or 4) their combination, with the aim of preparing desired cell products to treat a patient or to repair or regenerate a tissue.

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Processing of Cells (2)

- It is necessary to describe all important and relevant information concerning the cell processing employed. Provide individual technical details and explain the reason for using the said processing to obtain the target product from the mfg. perspective.
- So-called minimal manipulations (described later) are not considered "processing."

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Minimal Manipulations & Manufacture

- "Minimal manipulations" are defined as isolation of a tissue, homogenization of a tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, washing, sterilization by gamma-irradiation or other methods, freezing, thawing, and other procedures that do not change the original properties of the cells or tissues.
- "Manufacture" : Actions undertaken up until the final product is released to market. This includes: Processing of Cells and Minimal Manipulations.

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Preparation of Desired Cell Products

- For preparation of desired cell products, describe the methods via which cells that serve as an active ingredient in the final product were prepared directly from a starting cell line or via an intermediate cell line derived from the starting cells.
- The methods to be described include any processing, isolation, and culture of the desired cells, and the media, culture conditions, culture period, and yields of the desired cells at each step.
- Describe to the extent possible the appropriateness of each method.

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Formulation: Preparation of final product

The form and packaging of the final product shall ensure the quality of the final product.

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Characterization and Understanding of Specific Profiles of Cells at Critical Stages (1)

- Characterization and understanding of specific profiles of cells at critical stages (e.g., starting, bank, intermediate, and final stage) are essential.
- The content and extent of characterization of cells in question depend on each intended purpose, stage, quantitative limit on the sample, and reasonably available and applicable testing methods, and do not necessarily require the most stringent and extensive procedures.
- It is necessary to explain the appropriateness of the approach used.

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Characterization and Understanding of Specific Profiles of Cells at Critical Stages (2)

Examples of Cell Characteristics:

1)Morphological characteristics, 2)Growth characteristics, 3)Biochemical markers, 4) Immunological markers, 5) Specific substances produced, 6)HLA typing(*allogenic*), 7)other suitably chosen and appropriate Genotypic or Phenotypic indicators/markers,8) Clinically useful stemness (*stem cells*), 9)Karyotype,10)DNA fingerprinting, 11)Pluripotency (*iPS cells, ES cells*), 12) Differentiation potency, 13) Specific biological function;

Examples of Quality Attributes:

1) Contamination by non-target cells (Cell purity),2) Cell viability, 3)Absence of unintended changes in cells cultured for duration beyond the proposed culture period, 4) Stability

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Verification of a mfg. process and constancy of manufacture as well as process control(1)

- Describe in detail the mfg. method for minimal manipulation of cells/tissues and preparation of a characterized cell substrate that served as a raw material through the establishment of cell lines, cell banks, and/or critical intermediate cell products (if any), differentiated cells, and the final product.
- Describe the technical details of the process and necessary process control and product quality control.

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Verification of mfg. process and constancy of manufacture as well as process control(2)

- Verify, to the extent possible, the validity of the mfg. method and the technology employed in order to maintain constancy of manufacture and thereby consistency of the quality of the product from the mfg perspective.
- Note that quality, safety, and consistency are ensured by mutual complementary measures throughout the mfg.
- Note that the measures be rational and that they serve the intended purpose.

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Quality control of final products according to product aspects and process aspects

The overall quality control strategy of cell-based products includes:

1) Specifications *(a set of acceptance criteria and analytical procedures)* for the final products
2) Quality control of raw materials
3) Verification of the validity of the mfg. process
4) Maintenance of consistency, and
5) Proper quality control of intermediate products if any

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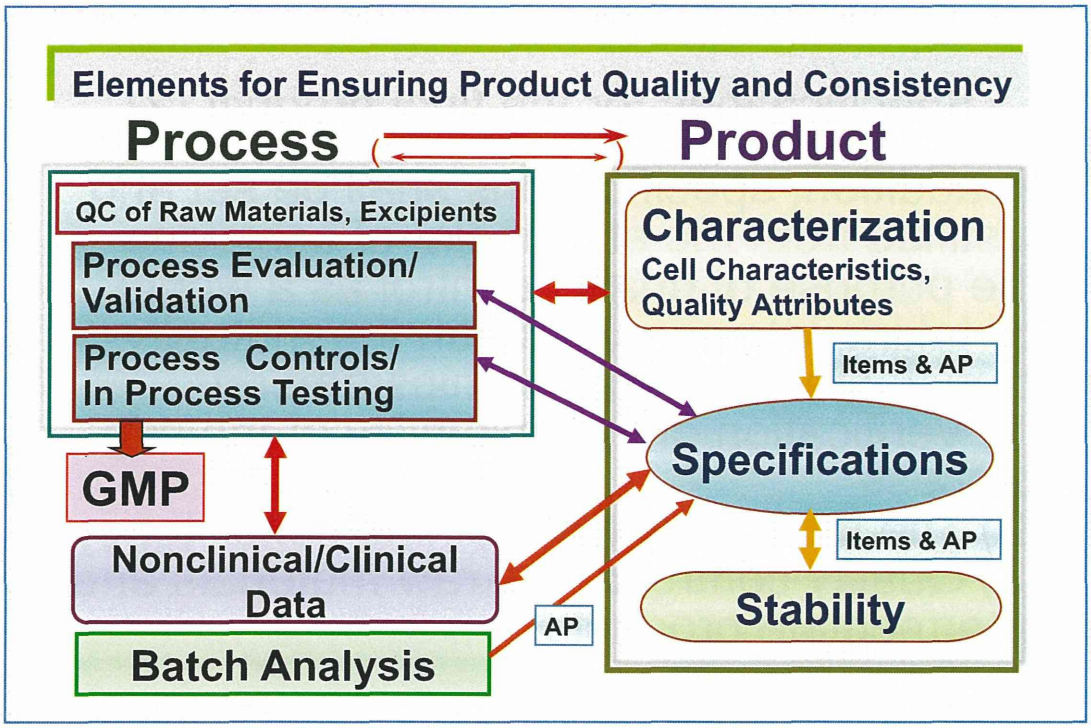

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Specifications for the final product (1)

Specifications will differ among final products, depending upon the type and properties of the desired cells and tissues, mfg methods, intended clinical use, the mode of administration of each product, stability, and test methods available. These differences shall be taken into consideration when setting the acceptance criteria and test procedures.

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Specifications for the final product (2)

In addition, specifications shall be set and justified from the standpoint of achieving the purpose of quality control as a whole, by taking into consideration the mutually complementary relationships among

1) Verification of the suitability of the mfg. process
2) The method of maintaining consistency
3) Quality control of the raw materials and intermediate products.

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Specifications for the final product (3)

- The purpose of the assessment at the initiation of clinical trials is to confirm that the product in question is unlikely to pose significant Q/S problems during investigational clinical trials.
- Therefore, it is possible to set provisional specifications with allowance for some variation on the basis of measurements performed on a few test specimens, as long as one can be certain of the relationship between the results of clinical tests and the quality attributes after the clinical trials.
- However, testing for sterility and the absence of mycoplasma is essential.
- It should be noted that the quality control strategy including specification should be enriched and developed along with the progress of clinical trials.

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Specifications for the final product (4)

When setting specifications for an individual final product, it may be necessary to refer to the quality control parameters and tests shown below. It should be noted that they are just examples, and it is necessary to provide the rationale for these specifications.

- The Cell number and cell viability
- Tests of Identity
- Tests of Purity
- Tests for cell-derived undesirable physiologically active substances
- Tests for process-related impurities
- Sterility tests and tests for mycoplasma
- Endotoxin tests
- Virus tests
- Specific biological activity tests
- Potency tests
- Mechanical compatibility tests

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Product stability

- Taking into consideration the storage and distribution periods and the storage form, test the cell viability, (potency) and other characteristics of hCTPs, and/or critical intermediate products to establish storage methods and an expiration date. Explain their appropriateness.
- When product storage and use involves freezing and thawing, confirm that the freezing and thawing processes do not affect the stability or acceptance criteria of the product.
- Where necessary and possible, it is recommended to conduct stability studies on the products whose mfg. period or storage period exceeds normal periods in order to confirm to the extent possible the limits of stability. This does not apply if a product will be used immediately after its production.