

Cell and Gene Therapy Definitions for Raw Materials, Starting Materials, Drug Substance and Drug Product

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The complexity of processes to manufacture cell and gene therapy medicinal products can cause confusion around definitions of raw materials, starting materials, process intermediates, drug substance and drug products. This document will discuss the definitions of the above process components based upon statements to be found in recent regulatory guidance documents. Examples from cell and gene therapy processes will be given.

Raw Materials

US FDA regulatory guidance, EU directive and ISO Standards are consistent about the definition of raw materials (also called ancillary materials in the US). The defining property of raw materials (ancillary materials) is that they are not intended to be present in the final product.

EU Directive 2001/83/EC: 'Materials used during the manufacture of active substance (e.g. culture media, growth factors) and that are not intended to form part of the active substance shall be considered as raw materials.'

ISO Standard ISO/TS 20399-1 Biotechnology – Ancillary materials present during the production of cellular therapeutic products: 'Material that comes into contact with the cell or tissue product during cell processing but is not intended to be part of the final product formulation.'

US FDA Guidance for Industry: CMC information for human gene therapy investigational new drug applications (January 2020): '... reagents (or ancillary materials) are those materials used for manufacturing (e.g., cell growth, differentiation, selection, purification, or other critical manufacturing steps) that are not intended to be part of the final product. Examples include foetal bovine serum, digestive enzymes (e.g., trypsin, collagenase, DNase/RNase, restriction endonucleases), growth factors, cytokines, monoclonal antibodies, antibody-coated beads, antibiotics, media, media components and detergents.'

This guidance also points out that these reagents can affect the safety, potency and purity of the final drug product by introducing adventitious agents or other impurities.

EMA Draft Guideline on Quality, non-clinical and clinical requirements for investigational advanced therapy products in clinical trials (Jan 2019): 'Raw materials are the reagents that are used during the manufacturing process but are not part of the final product. Examples include foetal bovine serum, trypsin, digestion enzymes (e.g., collagenase, DNase), growth factors, cytokines, monoclonal antibodies, antibiotics, resins, cell-separation devices, and media and media components.'

The qualification and testing of raw materials (ancillary materials) should be based on a quality risk management strategy. Details about the implementation of a qualification strategy can be found in European Pharmacopoeia 5.2.12 'Raw materials of biological origin for the production of cell-based and gene therapy medicinal products' and in US Pharmacopoeia < 1043 > Ancillary materials for cell, gene and tissue-engineered products, for information, qualification and risk classification.

Starting Materials

The term 'starting materials' has been defined for chemically synthesised pharmaceuticals and recombinant protein-based products in ICH Q11 Development and manufacture of drug substances (chemical entities and biotechnological/ biological entities). November 2012:

'A starting material should be a substance of defined chemical properties and structure. A starting material is incorporated as a significant structural fragment into the structure of the drug substance. "Significant structural fragment" in this context is intended to distinguish starting materials from reagents, solvents, or other raw materials. Commonly available chemicals used to create salts, esters or other simple derivatives should be considered reagents.'

In the case of cell and gene therapy products there are other critical process components (e.g. cell lines producing viral vectors) where the quality of the component can have a significant impact on the quality of the final drug product even though the component is not incorporated into the structure of the final drug product. Recent regulatory guidance indicates that these components should be treated as starting materials. They should be produced under an appropriate quality system and the quality confirmed using appropriate quality control assays.

These requirements are indicated in the following quotes from regulatory guidance documents:

EMA Draft Guideline on Quality, non-clinical and clinical requirements for investigational advanced therapy products in clinical trials (Jan 2019).

Gene therapy Products

'Viral vectors are starting materials when used to transduce cells and not remaining in the active (drug) substance. The same level of information that is needed for the vector as active (drug) substance should be provided in this situation. Genome editing tools used *ex vivo* to generate genetically modified cells are by analogy also considered as starting materials. For *in vitro*-transcribed (m)RNAs used as active substances, the linearized template plasmid DNA should be considered as a starting material.'

'Complexing materials (a substance used to form a complex with DNA which facilitates transfer of that DNA into a cell e.g. calcium phosphate, lipids or proteins.) for formulating the drug substance are considered as starting materials and have to be qualified for their intended purpose. The level of information to be provided will depend on nature of the complexing material and resulting drug substance.'

'In the case of gene therapy *ex vivo* (i.e. genetically modified cells), the active substance is composed of the modified cells. The unmodified cells, the viral or non-viral vectors and any other nucleic acid and/or protein used in the genetic modification of the cells are considered starting material. The requirements for the gene/vector component should additionally be taken into consideration. In the case of *ex vivo* use, viral vectors, plasmids, recombinant proteins and recombinant mRNA, the components to produce them (e.g. plasmids, cells) are also considered starting materials. In this case, the principles of GMP, as provided in the General Principles in the Guidelines for GMP for ATMP, should be applied from the cells bank systems used to produce the starting materials, when applicable.'

Cell Therapy Products

'Donated cellular material (cells or tissues) from single or multiple donors, once processed. These may be:

A single primary cell isolate or cell suspensions containing various naturally occurring cell types used directly for the cell based medicinal product;

Primary cells cultured for a few passages before being cryopreserved as cell stocks;

Cells based on a well-defined cell bank system consisting of a master cell bank and a working cell bank.'

'Additional substances (e.g. scaffolds, matrices, devices, biomaterials, biomolecules and/or other components) when combined as an integral part with the manipulated cells are considered part of the active substance and are therefore considered as starting materials, even if not of biological origin. Information on relevant manufacturing and control and viral safety aspect of these additional substances need to be provided.'

PIC/S GMP Annex 2A Manufacture of ATMPs for human use. (2019):

'For products consisting of viral vectors, the starting materials are the components from which the viral vector is obtained, i.e. the master virus seed, or the plasmids used to transfect the packaging cells and the MCB of the packaging cell line. '

'For genome editing approaches, the starting materials shall be, as appropriate, the vector (viral or non-viral vector) carrying the DNA sequences encoding the modifying enzyme, the mRNA expressing the modifying enzyme, the modifying enzyme itself, the genetic sequence for modification of the cell genome (e.g. a regulatory guide RNA) or a ribonucleoprotein (e.g. Cas9 protein pre-complexed with gRNA), the repair template (e.g. linear DNA fragment or a plasmid), and the components to produce them. When vectors, mRNA or proteins are used, the principles of GMP shall apply from the bank system used to produce these materials onwards.'

'For genetically modified cells, the starting materials are the components used to obtain the genetically modified cells, i.e. the starting materials to manufacture the vector and the human or animal cell preparations.'

US FDA Guidance

US FDA guidance documents do not give such a clear definition of 'starting materials.' *US FDA Guidance for Industry: CMC information for human gene therapy investigational new drug applications (January 2020)* in the section 'Control of Materials' does require a manufacturer to list all materials used in manufacturing and describe the quality and control of these materials. This listing includes raw materials and equipment, ancillary materials and components such as cells and cell and virus banking systems. The guidance then describes in detail the expectations for mammalian cells, bacterial cells used to prepare plasmids and viral vector banks. DNA plasmids used for transient transfection to produce AAV or lentiviral vectors are described as 'intermediates.'

In clinical trial and license applications, using the Common Technical Document format, the listing of materials is included in the Drug Substance section (3.2.S.2.3). This can lead to confusion on the definition of these materials. By indicating that some critical process components should be treated as starting materials, the regulatory expectation would be that these components should be produced using a phase appropriate GMP quality system and characterised using assays defined in a risk management strategy.

From the above regulatory statements, the following components should be treated as starting materials:

- Viral vectors used for direct inoculation into humans (e.g. AAV vectors)
 - Bacterial cell bank used to produce plasmids
 - Plasmids used for transient transfection of producer cell lines
 - Producer cell lines that are transiently transfected.
 - Stably transfected producer cell lines
(In the case of stable producer cell lines, plasmids used for the transfection should be treated as starting materials)
- Genetically modified cells for direct inoculation into humans (e.g. CAR-T therapy with ex vivo transduced patient cells; engineered stem cells for cell therapy)
 - Bacterial cell bank used to produce plasmids
 - Plasmids used for transient transfection of producer cell lines (for lentivirus or AAV vector production)
 - Producer cell lines that are transiently transfected.
 - Stably transfected producer cell lines (In the case of stable producer cell lines, plasmids used for the transfection should be treated as starting materials)
 - Viral vector used for ex vivo transfection.
 - Purified autologous cells from patient.
- Cell therapy products
 - Cell isolates/cell suspensions; cell stocks made from primary cells cultured for a few passages; cells based on a well-defined cell bank system.
 - Scaffolds, matrices, devices, biomaterials, biomolecules and/or other components when combined as an integral part with the manipulated cells

Drug Substance

US FDA Draft Guidance for Industry: CMC information for human gene therapy investigational new drug applications (January 2020) defines Drug Substance as 'an active ingredient that is intended to furnish biological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease or to affect the structure or any function of the human body.'

In some regulatory documents the term 'active substance' is used instead of drug substance.

Regulatory dossiers (for IMPs and licensed products) requires the division into drug substance (DS) and drug product (DP) sections. For certain ATIMPs, the starting material, the active (drug) substance and the finished product can be closely related or nearly identical. However, the active substance and the final product should be identified, if possible (*EMA Draft Guideline on Quality, non-clinical and clinical requirements for investigational advanced therapy products in clinical trials (Jan 2019)*).

The active (drug) substance of a cell based medicinal product is composed of the manipulated or non-manipulated cells and/or tissues prior to filling into the final container for administration to the patient. The active substance of a gene therapy medicinal product based on gene transfer methods *in vivo* is composed of the recombinant nucleic acid and the viral or non-viral vector used to deliver it. In the case of gene therapy *ex vivo* (i.e. genetically modified cells), the active substance is composed of the modified cells.

For many pharmaceutical products guidance on the good manufacturing practice to produce active pharmaceutical ingredients can be found in ICH Q7 Good Manufacturing Practice for Active Pharmaceutical Ingredients. However, the scope of this guidance excludes all vaccine and gene therapy APIs.

Drug Product

This is the form of the drug substance formulated and filled into the final container to be used for administration. Drug substance may be formulated with excipients; diluted to final dose and filled into final containers. *CMC information for human gene therapy investigational new drug applications (Jan 2020)* defines Drug Product as the finished dosage form that contains Drug Substance, generally in association with one or more other ingredients (e.g. excipients).

Appendix: Examples of Raw Materials, Starting Materials, Drug Substance and Drug Product in the Manufacture of Gene Therapy Viral Vectors

AAV vector for *in vivo* administration

Raw Materials	Starting Materials*		Drug Substance	Drug Product
Tissue culture media Media supplements Bovine serum Components of buffers Transfection reagents Benzonase AAV affinity chromatography resin	Bacterial cell bank used to produce plasmids Plasmids for transfection HEK 293 producer cells	PRODUCTION & PURIFICATION	Purified AAV vector	Sterile filtered DS diluted to appropriate dose in endotoxin free buffer Excipient(s) Endotoxin free final container

Lentivirus vector transfected CAR-T cells

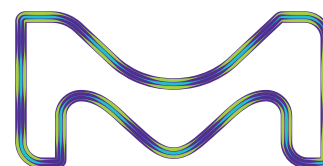
Raw Materials	Starting Materials*		Drug Substance	Drug Product
Tissue culture media Media supplements Bovine serum Components of buffers Transfection reagents Benzonase MAbs and beads used for patient cell selection	Bacterial cell bank used to produce plasmids Plasmids for transfection HEK 293 lentivirus producer cells Lentivirus vector T Lymphocytes	LENTIVIRUS TRANSFECTION OF PATIENT T CELLS	Transfected and expanded patient cells	DS diluted to appropriate dose Endotoxin free buffer Excipient(s) Endotoxin free final container

* Starting materials or treated as starting materials in that they are produced under GMP quality system, or equivalent, and are quality controlled.

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