Cellular Therapy and Transplantation (CTT). Vol. 10, No. 2, 2021
doi: 10.18620/ctt-1866-8836-2021-10-2-69-82
Submitted: 06 July 2021, accepted: 30 July 2021

Specific aspects of the quality control strategy for biomedical cell products containing genetically modified human cells

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Citation: Rachinskaya OA, Melnikova EV, Vodyakova MA, Merkulov VA. Specific aspects of the quality control strategy for biomedical cell products containing genetically modified human cells. Cell Ther Transplant 2021; 10(2): 69-82.

Summary

Regulatory authorities around the world have so far endorsed eleven products containing cells with a genome modified by a viral vector (VV) carrying the sequence of the target gene (*ex vivo* gene therapy cellular products, GTPs). These products (referred to as biomedical cell products, BMCPs, in accordance with Russian legislation) are intended for treatment of haemato-oncological diseases (using the technology of chimeric antigen receptors) and genetic hereditary diseases. Approval of production and marketing for such *ex vivo* GTPs is based on adequate and comprehensive assessment of their quality, which is challenging due to specific composition of these products that include both viable cells and viral genetic material. The aim of this study was to analyse international experience in quality assurance of *ex vivo* GTPs in order to identify specific aspects of their quality control strategy during development, production, and expert quality control as a part of the national authorisation procedures.

The data presented in public reports of the U.S. Food and Drug Administration (FDA) (Summary basis for regulatory action) and the European Medicines Agency (EMA) (Assessment reports) provided the basis for evaluation of quality control strategies used in the production and release control of approved *ex vivo* GTPs. The comparative analysis helped to identify specific features of quality control strategies used for these products upon control of raw and initial materials, creation and characterisation of cell banking systems, implementation of in-process and release controls, including development of specifications for active substances and final products, validation of the manufacturing process, stability studies during storage and transportation of active substances and final products. For instance, the use of VVs for transfection requires assessment of such quality attributes as insert integrity, quantitative titer, biological activity, purity and impurities (admixtures), bacterial endotoxins, biological burden, sterility, presence of potentially replication-competent viruses, etc. Transduction of viable cells, in its turn, requires quality control of medicinal products in terms of such quality attributes as viability and proportion of transduced cells (TCs), activity, purity, impurities, safety, and others. Due to specific nature of *ex vivo* GTP production which is a two-stage process (obtaining the vector at stage 1, and producing TCs and final product at stage 2) characteristic of all the products considered in the study, it seems reasonable to provide separate specifications for the active substance (viral vectors), and a general specification for the active substance (TCs) and the ready-to-use product. Test results for a number of parameters included into the final product specification could not be obtained at the stage of product inspection, but may be derived from earlier stages of production, or even during pre-clinical studies, upon validation of the manufacturing process as well as during the product development.

Keywords

Ex vivo gene therapy products, biomedical cell products, quality control, active substance, viral vector, transduced cells, specification, quality attributes.

Introduction

At present (as of May 2021), ten *ex vivo* GTPs are approved for medical use in the EU countries, the USA, and the Republic of Korea. Approval for commercial use of another product (Zalmoxis, MolMed S.p.A., Italy) was withdrawn by the European Medicines Agency in October 2019. These products are intended for treatment of immunological, haemato-oncological, haematopoietic, and joints disorders. Genetic modification of cells in all these products was carried out using the *ex vivo* gene transfer technique, which involves collection of cells from a patient or donor, their cultivation, modification, and transplantation to the patient (recipient). The oncohematology products based on modified autologous T-cells are obtained using the chimeric antigen receptor technology (CAR, chimeric antigen receptor, CAR-T technology).

Russian Federation has no experience in marketing authorisation of biomedical cell products containing genetically modified human cells (GMHC-based BMCPs), which are analogues of *ex vivo* obtained gene therapy products (GTPs), and, thus, no experience in their quality assessment in the frame of authorisation procedures. However, the development and studies on such products are underway [1-4].

Therefore, it is high time to address the problem of the absence of national regulatory and guidance documents establishing quality attributes and test methods for GMHC-based BMCPs (guidelines, collection of standards and quality specifications for BMCPs, i.e., some analogues to the Russian State Pharmacopoeia for medical drugs), which would regulate their quality control (QC). At present, the quality control of vector molecules for genetic modification could be based on "Recommendations for Organisation of Production, Quality Evaluation, Pre-clinical and Clinical Studies of Gene Therapy Medicinal Products" [5].

The aim of the study was to analyse international experience in quality assurance of *ex vivo* GTPs, in order to identify specific aspects of their quality control strategy during development, production, and expert quality control as a part of the national authorisation procedure.

Adopted *ex vivo* gene therapy cellular products

The analysis of data on quality control strategies used in the production and release control of authorised *ex vivo* GTPs, was performed based on the materials presented mainly in the public reports of the EU and the USA regulatory authorities for the following products:

1. The following products were developed for medical use in oncohematology using CAR-T cell technologies:

- KYMRIAH (tisagenlecleucel), Novartis (USA), for adult patients with relapsed or refractory (r/r) acute lymphoblastic leukemia (ALL) including diffuse large B-cell lymphoma (DLBCL) [6, 7];
- YESCARTA (axicabtagene ciloleucel), Kite Pharma, Inc. (USA), Gilead Sciences, Inc. (USA), for the treatment of adult patients with r/r large B-cell lymphoma after two or more lines of systemic therapy, including DLBCL, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma [8, 9];
- TECARTUS (brexucabtagene autoleucel), Kite Pharma, Inc. (USA), Gilead Sciences, Inc. (USA), for the treatment of r/r mantle cell lymphoma [10, 11];
- BREYANZI (lisocabtagene maraleucel), Juno Therapeutics, Inc. (USA), for the treatment of adult patients with r/r large B-cell lymphoma after at least two or more lines of systemic therapy, including DLBCL (including DLBCL arising from indolent lymphoma), high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B [12];
- ABECMA (idecaptagene vicleucel) Celgen Corporation, Bristol-Myers Squibb (USA), for the treatment of adult patients with r/r multiple myeloma after four or more prior lines of therapy including an immunomodulatory agent, a proteasome inhibitor, and an antiCD38 monoclonal antibody [13-15].

2. For the treatment of genetic and other types of disorders:

- Strimvelis (autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with a retroviral vector that encodes for the human ADA cDNA sequence), GlaxoSmithKline plc (UK), Orchard Therapeutics (Netherlands), for the treatment of patients with severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID), for whom no suitable human leukocyte antigen (HLA)-matched related stem cell donor is available [16];
- Zynteglo (betibeglogene autotemcel), bluebird bio (Netherlands), for the treatment of transfusion-dependent β-thalassaemia [17];
- Libmeldy (autologous CD34+ cell encoding ARSA gene), Orchard Therapeutics (Netherlands), for the treatment of children with late infantile and early juvenile forms without clinical manifestations of the disease, as well as with early juvenile form and early clinical manifestations of metachromatic leukodystrophy caused by mutations in the arylsulfatase A (ARSA) gene [18];
- Zalmoxis (allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2)) MolMed S.p.A. (Italy), used as an add-on treatment in adults with a high risk of haematological malignancies, who have received a haematopoietic stem cell transplant (HSCT, a transplant of cells that can develop into different types of blood cells) from a partially matched donor (a so-called haploidentical transplant). The therapeutic effect of the product is aimed at restoring immunity, increasing the chances of successful transplantation, and maintaining a long-term anti-cancer effect [19];
- Skysona (elivaldogene autotemcel), Bluebird Bio (the Netherlands), for the treatment of early cerebral adrenoleukodystrophy: on May 20, 2021, the EMA Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion recommending the issuance of marketing authorisation for this product [20];

- INVOSSA (TissueGene-C) Kolon TissueGene, Inc. (the Republic of Korea) for the treatment of knee osteoarthritis [21].

Logical methods of system analysis and modeling were used in the study.

Quality control of cellular GTP: general issues

The production of *ex vivo* GTPs in accordance with the Good Manufacturing Practice (GMP) is significantly more difficult than the production of other biologicals, primarily due to the variability of the cells and the importance of the viral vector (VV) cell transduction step [22]. For example, up to 10% of manufacturing cycles of products based on the CAR-T technology usually do not result in the production of a high-quality product [23].

Currently, there are no uniform technical standards for the production of *ex vivo* GTPs. Since there are differences in the design of the insert, the way it is introduced into cells, and cell cultivation and purification technologies, the QC of such products should take into account the specific manufacturing process and its individual characteristics. Control of *ex vivo* GTPs should include an integrated approach to quality assessment – a range of tests to confirm the safety and efficacy of the product. The analysis of the FDA and EMA public reports on the authorised products helped to identify the following general areas of their quality control:

- control of raw and starting materials;
- in-process control;
- release control;
- validation of the manufacturing process;
- study of the stability of active substances and final products.

In addition, *ex vivo* GTPs have a specific feature – they combine attributes of both cell therapy products (transplantation of somatic cells into the human body), and those of gene therapy products (introduction of genetic material containing therapeutic genes into target cells using the vector). This combination of different features in *ex vivo* GTPs creates certain difficulties, typical for both cellular and gene therapy products, during production and quality assessment, and accounts for the use of requirements that are applied to both types of products.

The manufacturers distinguish two active substances: a vector and genetically modified cells, in the FDA and EMA public reports for *ex vivo* GTPs authorised in the USA and the EU.

Risks and strategies of vector quality control

All the products under consideration contain vectors (as an active substance) based on viruses from the retrovirus family (γ-retrovirus or lentivirus) encoding for a transgene: chimeric antigen receptor (KYMRIAH, YESCARTA, TECARTUS, BREYANZI, ABECMA), human adenosine deaminase gene (Strimvelis), β-globin (Zynteglo), arylsulfatase A (Libmeldy), human transmembrane peroxisome ALD (adrenoleukodystrophy protein) (Skysona), transforming growth factor β1 (TGF-β1) (INVOSSA), or low affinity nerve growth factor receptor (ΔLNGFR) with the herpes simplex virus thymidine kinase (HSV-TK) gene sequence (Zalmoxis) (Table 1).

It should be noted that the development and study of other ways of delivery of target genes into the cells of patients is underway, for example, such as the Sleeping Beauty transposon system or direct transfection of RNA (mRNA) [24-26], however, products based on these technologies are not currently authorised anywhere in the world.

The use of VV is the most efficient method for delivering genetic material to target cells, but it is associated with a number of difficulties in creating an efficacious and safe product:

- Restrictions on the size of the genetic material (inserted segment) that can be delivered to the target cell using the vector. Therefore, not every gene can be delivered [27, 28].

- Lacking efficiency of genetic modification of target cells, which affects the number of insert copies in cells, expression level of the encoded target gene, and integrity of the genetic construct [29].

- Potential for replication-competent viruses (RCV) formation and spontaneous virulence recovery (were observed for adenoviral, herpesvirus-based, and retroviral vectors) [30, 31] resulting from recombination with either wild-type or endogenous viruses. This mobilisation of viral nucleic acid may promote its horizontal transfer to non-target cells or transmission to healthy people.

Though both retroviral and lentiviral vectors used in these gene therapy products are designed to be replication-defective, and, moreover, a virus is often pseudotyped with envelope proteins of another virus in order to minimize the vector recombination risk and its evolution to a wild-type virus [32], there is a need to confirm the absence of RCV formation during manufacturing process. According to the FDA recommendations, the RCVs should be monitored in the vector active substance, cell product, and in the patients after administration of the product, using molecular biology methods and serological tests [33]. In addition, RCVs may be detected by S+/L- assays, as well as by marker rescue assays [34, 35].

- Probability of insertional mutagenesis (especially, for retroviral vectors that often integrate in the transcription start sites) [36] can lead to changes in proto-oncogene and suppressor gene activities, thus causing higher risk of carcinogenesis.

- Potential hazards from residual contaminant viruses, e.g., contaminating adenoviruses, may be present, e.g., if adeno-associated viruses are used in gene constructs, as well as the possibility of reactivation of latent viruses (for example, herpes simplex virus, Epstein-Barr virus, and cytomegalovirus) can lead to the production of infection-active virus [37].

- Difficulties in scaling-up production associated, e.g., with the need to obtain stable packaging cell lines [38].

Table 1. Active substance (viral vectors) used for different CAR-T cell products

Product name	Vector type	Encoded gene	Vector manufacturer
KYMRIAH	The CTL019 (murine) HIV-1 lentivirus vector (approximately 85 % sequence has been removed) – replication-defective, recombinant self-inactivating	anti-CD19 CAR expressed under the control of EF-1α promoter	OxfordBioMedica, UK
YESCARTA	γ- Retroviral vector PG 13-CD19-H3 – replication-defective, non-self-inactivating, encompasses the 5'LTR with promoter for transgene expression	anti-CD19 CAR expressed under the control of 5'LTR MSCV	- packaging cell (National Cancer Institute, Bethesda, Maryland, USA); - retroviral vector (Kite Pharma, Inc., USA)
TECARTUS			
BREYANZI	Lentivirus vector, replication-defective, self-inactivating	anti-CD19 CAR and a truncated EGFRt	not available
ABECMA	Lentivirus vector	anti-BCMA02 CAR	not available
Strimvelis	γ- Retroviral vector, replication-defective, based on MoMLV	the human ADA cDNA	MolMed S.p.A., Italy
Zynteglo	Lentivirus vector, replication-defective, self-inactivating, based on HIV-1, pseudotyped with vesicular stomatitis virus glycoprotein G	The human β^{A-T87Q}-globin gene with a single modification at codon 87 under the transcriptional control of the erythroid specific human β-globin promoter and erythroid specific enhancer elements (DNase I hypersensitive sites HS2, HS3, and HS4) of the β-globin locus control region	Bluebird bio (Netherlands) B.V.
Libmeldy		1 or more copies cDNA sequence of ARSA	Orchard Therapeutics, Netherlands
Zalmoxis	γ- Retroviral vector, replication-defective	ΔLNGFR gene and HSV-TK suicide gene	MolMed S.p.A., Italy
Skysona	not available	human ALDP	Bluebird bio (Netherlands) B.V.
INVOSSA	γ- Retroviral vector	TGF-β1	not available

Notations: *HIV-1 – human immunodeficiency virus type-1; EF-1α – human elongation factor 1α; LTR – Long terminal repeat; MSCV – murine stem cell virus; EGFRt – epidermal growth factor receptor; MoMLV – Moloney murine leukaemia virus; ADA – adenosine deaminase; ARSA – arylsulfatase A; ΔLNGFR – Low Affinity Nerve Growth Factor Receptor; HSV-TK – Herpes Simplex virus Thymidine Kinase; ALDP – adrenoleukodystrophy protein; TGF-β1 – transforming growth factor β1*

All these risks were taken into account in the manufacturing procedures of the recently authorised *ex vivo* GTPs. The QC strategy for active substances has the following specific aspects:

1) Control of raw and starting materials. The basic materials used in VV production are viral particles, packaging plasmids, lines of bacterial and packaging eukaryotic cells, culture media, sera, etc. All the reagents and materials must be certified and approved for use in manufacture of products for medical use. In addition, raw materials of animal origin should be at low risk for transmissible spongiform encephalopathy (TSE). Therefore, it is necessary to create a standardized quality management system for the production materials, including assessment of risks associated with their use, audits of suppliers, and incoming control [39].

2) Cell banking systems. The multistage production of VV requires a complete cell banking system including a master bank and a working bank of bacterial cells containing packaging plasmids and those with target gene, as well as a packaging eukaryotic cell strain(s). In addition, the manufacture of cell products transduced with a vector encoding a distinct gene sequence (Strimvelis, Zynteglo, Zalmoxis), includes establishment of post-production cell banks (or end-of-production cell banks) containing cells at the limit of *in vitro* cell age. According to the guidance document ICH Q5A and the general monograph 5.2.3 of the European Pharmacopoeia, the cell bank testing program should include studies of post-thawing viability, stability of proliferative activity, titer, transduction efficiency, integrity and consistency of the vector and insert, sterility, bacterial endotoxins, mycoplasma, as well as RCV, cell genetic stability studies, and other tests [40-42].

Some EMA public reports highlight the need for release control of plasmids (packaging and carrying the target gene), which should be carried out according to the rules set out

in the general monograph 5.14 of the European Pharmacopoeia [43]. For instance, release control of plasmids during production of lentiviral vector for Zynteglo, included tests for purity, strength, identity, safety, and stability. Checking the plasmids during production of a lentiviral vector for Libmeldy included identity, host cell DNA, insert integrity, sterility, and bacterial endotoxins.

3) In-process control is required at clearly defined critical production stages, with established limits of test parameters, test methods, and acceptance criteria. E.g., the in-process control during production of the VV active substance for KYMRIAH included such quality control parameters as identity (integrity) of the genetic insert, number of viral particles, biological activity of the vector (transduction efficiency, infectivity rate, transgene expression level), purity and impurities, bacterial endotoxins, biological burden, sterility (contaminating infectious agents), RCV, tested in accordance with requirements of the General Monograph 5.14 from the European Pharmacopoeia [43].

One should note that the determination of process-related (host cell DNA and proteins, BSA, etc.) and product-related impurities (RCV, p30 protein, viral aggregates, etc.) is an important component of in-process control, and it is described for a number of *ex vivo* GTPs.

4) Release control. The main goal of release control is to ensure compliance of the manufactured product with the product file requirements on all the established quality characteristics. Quality attributes, test methods, and the expected value ranges are provided in the product specifications. Due to the fact that the production of a ready-to-use *ex vivo* GTP is a two-stage process, the manufacturer draws up a specification not only for the final product, but also for its active substance (viral vector). The only exception is Zalmoxis, whose vector specification is not included into the public report, and the main quality attributes are tested during in-process control.

Table 2 summarises the main quality attributes given in the EU and USA public reports for VV active substances of authorised *ex vivo* GTPs.

It should be noted that the *identity* attribute means the integrity of the insert, assessed using PCR technique and further sequencing of the PCR products; the *biological activity* stands for titer, number of viral particles (determined, for example, by ELISA method), the ratio of the total number of particles to the number of infectious particles, transduction efficiency, infectivity rate, and transgene expression level [32]. The public report for Libmeldy states that transduction efficiency is determined using a reference cell line. The *safety* attribute also includes several parameters: sterility, mycoplasma, contaminating pathogens, and potential for RCV formation, and the *purity* attribute includes tests for residual host cell proteins and DNA, reagent impurities (antibiotics, BSA, cryoprotectants, etc.) [32].

5) Validation. To obtain a VV active substance of reproducible quality, the process validation should cover the following aspects:

- Small-scale preparation of the product, followed by scaling up the process across several production cycles. Validation of Zalmoxis manufacturing process was developed in a stepwise approach, which consisted of small-scale studies to investigate growth rates, metabolism and productivity of the cells, followed by validation of full-scale manufacture at the level of cell expansion, bioreactor inoculum and growth phase, production and harvest of viral particles, filtration and filling of the active substance.
- Manufacturing process validation can be performed using material from healthy donors.
- Whenever possible, a VV reference standard (manufacturer's reference standard) is used, which must be qualified, characterized and tested for stability.
- Ensuring that the manufacturing process is carried out under aseptic conditions, since sterilisation cannot be performed for the final active substance.
- Ensuring that all the process-related impurities are removed.
- Validation of all non-pharmacopeial techniques and verification of all pharmacopeial test methods used for the release and in-process controls, in accordance with appropriate regulatory requirements [44-47].

Table 2. Quality attributes in viral vector release specifications (examples)

Product name	Quality attributes
KYMRIAH	Identity, quantity of viral particles, biological activity, purity/impurities, bacterial endotoxins, bioburden, sterility and adventitious agents tests, RCL test
YESCARTA	Appearance, identity, titer, potency and safety (bacterial endotoxins, mycoplasma, sterility), purity/impurities (host cell protein and DNA, BSA, and p30 protein)
TECARTUS	Contaminating infectious agents and RCR tests during release control; Titer and purity/impurities during in-process control
BREYANZI	Appearance, purity/impurities, sterility, bacterial endotoxins during release testing; testing of mycoplasma during in-process control
Strimvelis	Vector and provirus stability, integration site analysis during release testing (some quality attributes are hidden by the manufacturer)
Zynteglo	Appearance, identity, potency, safety, purity/impurities, RCL testing
Libmeldy	Potency, purity/impurities, identity, safety, pH, osmolality

Abbreviations: *RCL, replication-competent lentivirus; RCR, replication-competent retrovirus; BSA, bovine serum albumin*

6) Stability studies. VV stability assessment consists of long-term studies during storage under recommended conditions (commonly at -60 to -90 °C for 3 years) and under stress conditions (accelerated ageing test with storage at ambient temperature), which are usually carried out for development batches.

Risks and strategy for quality control of transduced cells

Production of the active substance represented by transduced cells (TCs) consists in various manipulations performed with initial donor material to obtain a sufficient amount of target cells with specific properties essential to achieve the desired therapeutic effect.

All the products authorised for CAR-T therapy in the EU and USA (as of May 2021) are produced on the basis of autologous T cells obtained from peripheral blood, using leukapheresis. T cells isolated from leukapheresis material are also used for the production of Zalmoxis, but in this case, they are obtained from allogeneic product (haploidentical donor cells). Another product, INVOSSA, registered in the Republic of Korea, is based on allogeneic cells (primary and irradiated chondrocytes isolated from the surgical material of the people with polydactyly). Strimvelis, Zynteglo, and Libmeldy contain CD34+ haematopoietic stem cells obtained from BM or peripheral blood (apheresis fraction). Data on the composition of active substance in transduced cells of the authorized *ex vivo* GTPs, as well as the key manipulations performed during the manufacturing process included in the FDA and EMA public reports, are listed in Table 3.

There are certain risks associated with clinical use of viable human cells in *ex vivo* produced GTPs:

- Risk of infection. Contamination of the final cell product with infectious agents (viruses, other microorganisms including mycoplasmas, prions) can occur as a result of disturbed production process and non-compliance with GMP rules for reasons related to the staff and environment, and when using reagents of inadequate quality. Moreover, the donor of cell material may be infected. In the latter case, the risk is significant only for allogeneic products [48]. It should be noted that all traditional decontamination methods (thermal, chemical, radiation treatments, filtration, cultivation with antibiotics) are not applicable here, or they will affect cell viability.
- Risk of malignant cell transformation and tumorigenicity. Long-term cultivation, as well as significant manipulations (for example, VV transduction) can lead to immortalization and malignant transformation of cells. The tumorigenicity risk is considered to be higher when undifferentiated cells, especially pluripotent stem cells, are used [49].
- Risk of immunogenicity (relevant only for allogeneic products). The GMHCs may be represented by different somatic cells (blood cells, myoblasts, epithelial cells, etc.) which should be transplanted with respect to histocompatibility and immunogenicity [37].

Table 3. Obtaining and production of the cells transduced by active substance (examples)

Product name	Active substance	Source of obtaining cells	Basic in-process manipulations
Autologous products			
KYMRIAH	T cells	Peripheral blood	Apheresis collection from a patient, lymphocyte enrichment, leukapheresis material cryopreservation/thawing, T cell expansion, stimulation with anti-CD3/anti-CD28, transduction, final product: cryopreservation
YESCARTA			Apheresis collection from a patient, T cell enrichment and activation, retroviral transduction and T cell expansion
TECARTUS			
BREYANZI			T cells are activated with CD3 and some factor (information is hidden), lentiviral transduction, T cell expansion, final product: cryopreservation
ABECMA			PBMC are cultured in the presence of IL-2, anti-CD3 and anti-CD28 (stimulation of T cell proliferation), lentiviral transduction, cell expansion, final product: cryopreservation
Strimvelis	CD34+ HSC	BM	CD34+ cells isolation from BM and retroviral transduction
Zynteglo,		Peripheral blood	CD34+ cells isolation and enrichment, stimulation, lentiviral transduction and washing
Libmeldy	CD34+ HSC	BM or peripheral blood	CD34+ cells isolation and enrichment, stimulation, lentiviral transduction and cell harvest
Allogeneic products			
Zalmoxis	T cells	Peripheral blood	T cells stimulation and retroviral transduction
INVOSSA	chondrocytes	surgical material	not available

Abbreviations: *HSC, haematopoietic stem cells; BM, bone marrow; PBMC, peripheral blood mononuclear cells*

The use of vectors for target gene transfer may also cause immunogenicity due to expression of target proteins which may be new to the patient's body (this is relevant, e.g., for treatment of some genetic diseases) [37].

- Variable characteristics of a final active substance in TC. Production of a standardized TC active substance is practically impossible (TCs being a complex biological object with a large number of diverse biological characteristics), largely depending on the quality of the donor raw material, age and physiological state of the donor, time interval between the collection of biomaterial and manufacturing the final product, presence of infectious agents, technical conditions of the manufacturing process. Such quality metrics of the final cell substance, as cell viability, number of cells per dose, phenotypic characteristics, number of genetically modified cells, etc. may vary greatly from patient to patient. Therefore, it is advisable to indicate the value ranges for the quality attributes of products during in-process and release control.

- Limited shelf-life, and inability to carry out long-term storage. This problem is relevant both for the final product, donor raw materials, and active cell substance, and, in most cases, it is solved by manufacturers by cryopreservation of the product at certain production stages. When the production of TC with active substance is interrupted by cryopreservation of cultured cells, the need for a cell banking system and certification of cell lines may be considered. Transportation of the final product can also be carried out in the vapor phase of liquid nitrogen (at a temperature not exceeding -120 °C), which allows to separate the product manufacturing and its clinical use, in terms of time and/or location.

The limited shelf-life of *ex vivo* GTPs (without cryopreservation) makes it preferable to use fast analytic methods for the quality assessment, often presuming non-pharmacopeial techniques that require additional validation. Thus, the classical sterility test may be replaced by tests based on detection of carbon dioxide [50], or ATP bioluminescence [51]; nucleic acid amplification technique (NAT) [52] may be used to test mycoplasma contamination; while quantitative PCR may be used to detect RCVs [53]. Cryopreservation of active substance can also be included in the production process in order to make it possible to carry out all conventional studies (tests for sterility, mycoplasma, RCV, and additional tests for viral infectious agents) before the product is administered to a patient [32].

- Limited volume of the TC active substance and the final product. Nowadays, GMHC-based preparations are mainly used in clinical practice as autologous products, however, the volume of biopsy material may be limited (especially when the cells are isolated from BM), in addition, the number of target cells in patients with the disease may be insufficient to obtain a clinically effective dose of the product, the number of passages during cell cultivation is also limited, and only a part of the obtained cells are successfully transformed by the VV.

All this creates difficulties for obtaining a sufficient amount of active substance and the final product for the desired therapeutic effect, and the material required for comprehensive QC of the product may also be insufficient [54]. In this situation, it is considered acceptable to perform quality assessment using a limited number of quality parameters (a simplified release control procedure), and the missing data should be appropriately supplemented by in-process testing and an extended production process validation program [32].

The general aspects of the TC active substance QC in authorised products are similar to those of the VV active substance, but have some specific issues associated with origin of the cells:

1) **Control of raw and initial materials**. Particular attention is paid to the quality of biological materials used for apheresis (for example, cell culture media) which come into contact with the product. All these materials must have certificates of analysis and meet the GMP acceptance criteria [55]. Raw materials of animal and human origin and the vector preparations are tested for the presence of microbiological and viral contamination, and assessed for the risk of TSE transmission, which makes it possible to omit testing of the final product for the presence of contaminating viral agents. It should be noted that, in some cases, manufacturers try to avoid raw materials of animal or human origin in the manufacturing process. The requirements for QC of materials are similar to those used in the production of VV active substances. Preference is given to disposable materials, and appropriate tests are performed for potentially extractable and leachable substances from the materials contacting with the product.

The strategy for the control of donor material in the EU includes testing of donors and performing collection of donor material in accordance with Directive 2006/17/EU [56], and, in the case of BM or peripheral blood cells, also in accordance with Directive 2002/98/EU [57]. The donor material used in Libmeldy production had additional acceptance criteria in terms of visual appearance and cell counts in suspension. KYMRIAH manufacturers additionally provided data on variability of starting materials from the patients with ALL and DLBCL.

In the USA, all cell or tissue donors are subject to assessment of donor eligibility based on screening donors for infectious agents and diseases, in accordance with Subpart C of the Donor Eligibility of the Code of Federal Regulations [58] and guidance for industry "Eligibility determination for donors of human cells, tissues, and cellular and tissue-based products (HCT/Ps)" [59]. However, the requirements of these documents have limitations in case of obtaining material for autologous use. In the case of autologous products intended for patients infected with HIV types 1 and 2, hepatitis B, C, or other human viral infections, the manufacturer must state the acceptable viral load and methods for its determination in the final product. Cell apheresis procedure can be performed only by certified personnel at the licensed institutions.

2) **Cell banking systems**. Most of the authorised *ex vivo* GTPs (except of INVOSSA) contain autologous cells, or cells from haploidentical donors, which does not allow for

harvesting cellular substance at the amounts that often do not exceed the required therapeutic dose. This fact and the continuous procedure of obtaining final product from the TC active substance, explain the absence of a human cell banking system. However, in the case of products for allogeneic use, the arrangement of a certified cell bank with full characteristics of the banked cell lines is required, in accordance with ICH Q5D [60].

3) **In-process control**. One of the most important aspects of in-process control when obtaining a TC active substance is the microbiological control strategy which ensures all operations with the cell material to be performed under aseptic conditions, excluding the hazards of product contamination from operator or environment. In addition, the traceability of the cell product from the receipt of donor material to the administration of TC to the patient is important and is achieved by labeling and barcoding the containers with biological material.

Consistency of the manufacturing process of the TC active substance is maintained by determining the acceptance criteria for the quality attributes of intermediate products at the critical production stages. The most common quality attributes of the product presented in the public reports include visual appearance (including colour), cell viability, number of contaminating cells, mycoplasma, sterility, bacterial endotoxins.

4) **Release control**. At the end of the manufacturing process, the active substance undergoes testing and evaluation which include determination of TC numbers, specific activity, phenotypic features and safety. The main characteristics of TC active substances included as components in *ex vivo* GTPs authorised in the USA and EU, are given in Table 4. It should be noted that separate cell substance specifications are not provided for the most products, and cell quality attributes are listed in the final product specifications. There are two exceptions: (1) specification for Strimvelis cell substance presumes efficacy analysis based on functional tests (not detailed), determination of the vector copy number, and cell transduction efficiency; (2) separate specification is also provided for Zalmoxis active substance, but it includes a limited number of parameters due to continuous process of obtaining final product from the TC active substance. The publicly available data include only viability, sterility, and bacterial endotoxins. It is also emphasized that there is no such quality attribute as *total number of cells*, because the dose calculation is performed on a case-by-case basis for each patient.

The characterisation data were not provided for ABECMA cellular active substance, and all tests and analyses were carried out for the cryopreserved final product.

5) **Validation**. To obtain a TC active substance at a reproducible quality, validation of the production process should consider some specific aspects and cover the following areas:

- The manufacturing process validation should be carried out for several batches with different donor material compositions. Variability of the donor raw material should be assessed (for example, different content of B cells in the leukapheresis product).
- Validation of the manufacturing process may be performed without reference standards, due to variability of the donor raw material, target cell enrichment procedure, the presence/absence of intermediate stages of cell cryopreservation, specificity of the product storage and transportation systems, and the state of the final product (either fresh, or cryopreserved cells).
- The manufacturing process may be evaluated using cell material obtained from healthy donors.
- Similar to validation of VV preparation process, the manufacturing process validation for the cellular active substance should confirm compliance with aseptic conditions.
- It is necessary to ensure removal of process-related impurities, including admixtures arising during vector manufacturing (for example, host cell DNA and proteins).
- It is necessary to validate the transportation stages of initial donor material and the final product to the locations of its clinical usage.

Table 4. Qualitative and quantitative characteristics of the active substance – transduced cells (examples)

Product name	Characteristic
KYMRIAH	Number of viable T cells; proportion of CAR-positive cells; T cell subsets were described: the CD4:CD8 ratio, including naive T cells, central memory and memory effector cells; single cell phenotyping to obtain data on the proteome and activation status of the cells
YESCARTA	The mechanism of action and potency: studies on integration of the CAR gene, CAR expression, antigen recognition and engagement. Activation and release of cytokines, killing of target cells, cell composition and T cell phenotypes, multiplicity of infection
TECARTUS	The mechanism of action and potency: analysis of CAR sequence integration into the host cell genome, CAR expression, antigen recognition and engagement, T cell activation and proliferation, production of cytokines and chemokines, killing of target cells and CAR-T cell composition and phenotype
BREYANZI	The presence of cellular impurities and non-viable cells; purity; number of transduced T cells; viability, and others (not disclosed)
Strimvelis	Identity, purity and impurities, potency (comparability studies and additional tests that are not disclosed)
Zynteglo	Efficiency: vector copy number in transduced cells, and others (not disclosed)
Libmeldy	Immunophenotyping, clonogenic potential, impurities (residual host cell DNA), presence of replication-competent lentivirus, integration site analysis, vector copy number in transduced cells

6) Stability studies. Due to the continuous process of obtaining final product from the active substance (TC), and lack of necessity to store the active substance (TC) for a longer period than the time required for quality and release controls, the stability studies were carried out for the final product in the majority of cases. In the case of Strimvelis, the active substance stability tests were carried out for six hours under aseptic conditions at ambient temperature. The public reports on YESCARTA provide additional stability data for cryopreserved peripheral blood mononuclear cells.

Specification features for the final *ex vivo* obtained GTPs

The production of final *ex vivo* prepared GTPs consists of several stages, including enrichment of prepared TCs, creation of the final cell suspension, packaging, and storage of the end product (with potential transportation). The main characteristic feature of the production of final products containing viable cells is variability of cell counts and volume of cell suspension, depending on clinical indications for the given product and the patient's body weight. Therefore, manufacture of standardized product batches of reproducible composition seems to be an impossible and impractical task.

Another characteristic feature of most GMHC-based final products is inability to provide long-term storage of packaged products before their clinical use. To increase the shelf life and enable transportation of products to end-users, the manufacturers use cryopreservation of the packaged final products in the vapor phase of liquid nitrogen at a temperature not exceeding -120 °C.

Thus, the presence of additional chemical reagents in the final product (for example, dimethyl sulfoxide, dextran 40, CryoStor CS10, human albumin, and other substances), a short shelf life and, often, small volumes of the final product allow for testing only a limited number of quality attributes during release control. Some of the results included into summary specification of the final product are obtained at earlier stages of the production process during in-process control testing.

As already noted above, the manufacturing process of all currently authorised *ex vivo* GTPs is continuous in nature, i.e., the cell material is not stored after obtaining the active substance (TC), being immediately passed on for preparation of the final product. Thus, the submission of a specification is justified for the final product, and not for the active substance (TC). The only exception is absence of specification for Strimvelis final product, while all the product batches are tested for quality attributes, such as cell viability and cell concentration in the final product. The final product specifications for all other products authorised in EU and USA include similar sets of quality attributes presented in Table 5.

Further on, we shall consider some specific aspects of QC for authorised *ex vivo* GTPs.

External appearance: Includes visual analysis and determination of the cell suspension colour, presence of turbidity and foreign particles, performed at the stage of the final product packaging, or at the labeling stage.

Viability: Viability may be a separate quality attribute in the FDA public reports, or a part of the *activity* or *purity* and *impurities* attributes. The final product specifications contain the results of viability assessment obtained either at the final product stage, or during the manufacturing process. Test results are numerical values obtained by calculation.

Identity: The identity of *ex vivo* GTPs is often assessed by quantitative PCR (qPCR), which can be performed for the frozen packaged final product. For instance, YESCARTA release control at the packaging stage included identity test which assessed presence of the scFv heavy chain variable region, linker, and CD28 sequence.

Table 5. Quality attributes in the final product specifications for the EU and USA products

Quality attributes		KYMRIACH	YESCARTA	TECARTUS	BREYANZI	ABESMA	Zynteglo	Libmeldy
Appearance		+	+	+	+	+	+	not available
Viability		+	+	+	not available	+	not available	not available
Identity		+	+	+	+	+	+	+
Purity and impurities	Cellular admixtures (product-related)	–	–	–	–	–	+ (not detailed)	+ (not detailed)
	Process-related impurities	–	+	+	+	–		
Dose / Number of viable transduced cells *per* mL		+	+	+	+	+	+	not available
Biological activity / potency		+	+	+	+	+	+	+
Safety	Contaminating agents	–	–	–	+ (not detailed)	not available	–	–
	Sterility	+	+	+		+	+	+
	Mycoplasma	+	+	+		+	+	+
	Bacterial endotoxins	+	+	+		+	+	+
	Presence of replication-competent virus	+	+	–	–	not available	not available	not available

Purity and impurities: Specifications for the final product include two types of impurities: cellular (product-related) and process-related impurities. When assessing cellular impurities, the following parameters are often determined: the percentage of target cells, type and probable number of admixed cells, e.g. the percentage of viable CD19+ B cells transferred to the final product from leukapheresis material (erythrocytes, granulocytes, etc.), as well as dead cells.

NK cells may be present in a final CAR-T product, their quantification is carried out at the active substance (TC) stage, and this quality attribute is not included into the final product specification. This is due to the lack of influence of NK cells on the product's safety. Non-transduced T cells are also present in a CAR-T product. The residual content of non-target cells (NK, dendritic cells, monocytes) in the final product can be determined not only during the manufacturing process, but also at the stages of product development and pre-clinical studies.

Red blood cell counts in the final product may be omitted. A failure in the production process and an increase in the number of erythrocytes will become obvious due to a change in the colour of the final product, when assessing the *appearance* quality attribute.

An example of a test for process-related impurities in the final product is microscopic determination of residual beads conjugated to anti-CD3/CD28 antibodies. Potential residual content of antibiotics (e.g., gentamicin for YESCARTA and TECARTUS) and other ancillary materials and reagents should be considered.

If no tests are performed for the content of process-related impurities at the final product stage, the final product specification may include the evaluation data obtained during validation of the manufacturing process, with a referral that these impurities may be present in the final product under toxicological acceptance limits.

Dose: Typically, the dose required for achieving therapeutic effect in a patient is based on the patient's body weight, cell viability in the final product, and transgene expression level provided by the specification as a numerical estimated value.

Biological activity (potency): In the case of KYMRIAH, biological activity is assessed by determining transduction efficiency (percentage of TCs in the final product) by qPCR technique.

In the case of final CAR-T therapy products, the *activity* QC attribute is assessed by measuring CAR specific gene expression and cytokine secretion level using qPCR and flow cytometry, as well as interferon-γ production upon CAR-T cell interaction with CD19+ target cells.

Safety: Includes testing for sterility (usually at the stage of final product packaging), mycoplasma (usually at the last culturing stage before harvesting cells), bacterial endotoxins (usually for the final product), and replication-competent viruses. None of the analysed final GTP preparations were tested for contaminating viral agents, due to several factors: checking initial biomaterials of animal and human origin (including donor material), compliance with the GMP standards, use of disposable materials, in-process quality control during VV manufacturing, including the VV final active substance. Along with viral infectious agents, the potential risk of TSE transmission is also evaluated which is considered to be low, due to available analytic certificates, control of raw materials and reagents of animal origin, as well as usage of fetal bovine serum produced in countries with a favorable epidemiological situation.

Special attention should be paid to Zalmoxis specification for the final product, which does not contain the abovementioned quality attributes, and includes the following criteria: functionality, alloreactivity, immuno-phenotyping, potency, and cell sensitivity to ganciclovir. Ensurance of viral safety and prevention of TSE transmission are also achieved by incoming control of raw materials and reagents of animal origin. Potential risks of RCV and activation of endogenous retroviruses are also considered. However, the specifications do not include appropriate tests.

In addition to the final product specification, manufacturers must submit to the national regulatory authority the data on stability and integrity of the final product during its storage and transportation. Stability of the product is assessed when unfrozen, and upon storage in the liquid nitrogen vapour phase at the temperature not exceeding -120 °C. Other studies include assessment of potential leachable and extractable substances released from the packaging (silicon, zinc), which may contact with the product; assessment of their toxicity, as well as package integrity testing.

Conclusions

Due to complexity of *ex vivo* GTP manufacturing process, the limitations associated with removal of impurities (including non-transduced cells, or other types of cells), and inability to perform final product sterilisation, the in-process QC and validation of the production process are considered of great importance. During validation, the manufacturing process is repeatedly reproduced (often with cell material obtained from healthy donors), raw and initial materials are controlled, and critical parameters are established for in-process and release QC of active substances and final products. At the same time, verification of the product quality and production consistency covers a range of characteristics, i.e. molecular biological (genome integrity, identity and stability, vector copy number, transduction efficiency, RCV, risk of insertional mutagenesis); cellular (target cell identity and purity, cell growth characteristics, cell number, viability); immunological (immunophenotyping); microbiological tests (sterility, mycoplasma contamination, bacterial endotoxins), as well as control of manufacturing conditions (temperature, pH, media consumption).

As based on experience of the foreign regulators' in quality assessment of authorised *ex vivo* GTPs, the following key aspects of the quality control strategy may be highlighted:

- Testing of donor material and control of raw materials of human origin for the presence of contaminating viral agents, as well as animal raw materials for the hazard of TSE transmission.

- Characterisation of bacterial, packaging, transduced (in the case of allogeneic products), and post-production cell banks with cell line certification based on the results of wide-scale testing.
- Detailed characterisation of the VV elements used for cell transduction, with description of testing techniques. Particular attention should be paid to evidence of low risk for insertional mutagenesis (especially for RVV), evolving RCV during the manufacturing process, and spontaneous recovery of viral virulence.
- Detailed characterisation of target cells confirming their activity and safety at the stage of donor material collection, cell culture, after the cell transduction procedure, as well as during the release control of final product.
- In view of the two-stage process of *ex vivo* GTP production, two specifications could be provided: (1) for the active substance (viral vector); (2) general specification for the active substance in transduced cells and the final product.
- Specification for the active substance (TC) and the final product includes the quantitative ratio of product-related admixtures (non-target cells, dead forms, and non-transduced cells) in the product, as well as description of potential admixtures arising during the VV production and observed in the final product.
- Ranges of values for critical quality attributes are established for in-process control and release specifications, due to inability to obtain final product with reproducible composition, and develop reference standards for the transduced cells and final product.
- The need for development of a reference standard for retroviral vector used in manufacture of transduced cells.
- The specifications include tests results not only from the release control, but also from earlier stages of in-process control, due to inability of obtaining final product at a volume sufficient for performing all the required tests. When justified, it is also possible to use potency and safety data obtained at the pre-clinical and development stages.
- Stability of the product may be confirmed by maintenance of quality attributes during long-term storage of the product in the liquid nitrogen vapour, and upon transportation.

The results of analysis of the *ex vivo* obtained GTP quality control strategy may be used by developers and manufacturers of BMCPs, as well as by experts performing quality control of medicinal products as a part of the national marketing authorisation procedure.

Acknowledgements

The study reported in this publication was carried out as part of a publicly funded research project No. 056-00005-21-00 and was supported by the Scientific Centre for Expert Evaluation of Medicinal Products (R&D public accounting No. 121021800098-4).

Conflict of interest

None declared.

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Особенности стратегии контроля качества биомедицинских клеточных продуктов, содержащих генетически модифицированные клетки человека

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Резюме

В настоящее время в мире существует опыт регистрации одиннадцати препаратов, содержащих клетки с геномом, модифицированным вирусным вектором (ВВ), несущим последовательность целевого гена (*ex vivo* генотерапевтические препараты, ГТП). Эти препараты (в соответствии с законодательством Российской Федерации – биомедицинские клеточные продукты, БМКП) предназначены для терапии онкогематологических (при использовании технологии химерных антигенных рецепторов) и наследственных заболеваний. При производстве и осуществлении процедуры регистрации подобных *ex vivo* ГТП встает вопрос об адекватной и всеобъемлющей оценке их качества, которая осложняется особым компонентным составом этих препаратов, включающих как жизнеспособные клетки, так и генетический материал вирусов. Поэтому целью данной работы стало изучение международного опыта обеспечения качества *ex vivo* ГТП для выявления особенностей стратегии их контроля качества при разработке, производстве, а также в экспертной оценке качества в рамках национальной процедуры государственной регистрации.

Основой исследования данных о контроле качества при производстве и выпуске зарегистрированных *ex vivo* ГТП в мире, послужили материалы, представленные в публичных докладах Управления по санитарному надзору за качеством пищевых продуктов и медикаментов (Summary basis for regulatory action в U.S. Food and Drug Administration, FDA) и Европейского агентства по лекарственным средствам (Assessment reports в European Medicines Agency, EMA). В результате анализа были выявлены особенности стратегии контроля качества этих препаратов на этапах осуществления входного контроля сырья и материалов, создания и характеризации системы банков клеток, осуществления внутрипроизводственного и выпускающего контролей, в том числе при составлении спецификаций на активные субстанции и готовый продукт, валидации процесса производства, исследованиях стабильности при хранении и транспортировке активных субстанций и готового продукта. Так использование для трансфекции клеток ВВ приводит к необходимости исследования таких показателей качества, как, например, целостность генетической вставки, инфекционный титр вируса, биологическая активность вектора, чистота и примеси, бактериальные эндотоксины, бионагрузка, стерильность, наличие репликационно-компетентного вируса и другие. Трансдукция жизнеспособных клеток, в свою очередь, делает необходимым осуществление контроля качества препаратов по таким показателям качества, как жизнеспособность и доля трансдуцированных клеток (ТК), активность, чистота, примеси и безопасность и другие. В связи с особенностями технологического процесса получения компонентов *ex vivo* ГТП (двустадийный процесс: первая стадия – получение вектора, вторая стадия – получение ТК и готового продукта), свойственных для всех исследованных в данной работе препаратов, обоснованным является предоставление отдельной спецификации на активную субстанцию – ВВ – и общей спецификации для активной субстанции – ТК – и готового продукта. Результаты тестирования по ряду показателей, включенных в спецификацию на готовый продукт, могут быть получены не на этапе выпускающего контроля, а на более ранних стадиях производства или даже при проведении доклинических исследований (ДКИ), валидации процесса производства, а также при разработке препарата.

Ключевые слова

Ex vivo генотерапевтические препараты, биомедицинские клеточные продукты, контроль качества, активная субстанция, вирусный вектор, трансдуцированные клетки, спецификация, показатели качества.