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Implementation of CAR-T technology into clinical practice: challenge for cell bank

Ewa Bembnista

Stem Cell Bank, Department of Hematology and Bone Marrow Transplantation, Poznan University of Medical Sciences, Poznań, Poland

Abstract

This publication is based on experience gained during the accreditation and certification process of the first Polish center (Department of Hematology and Bone Marrow Transplantation in Poznan University of Medical Sciences) to perform the procedure of treating a patient with chimeric antigen receptor T therapy.

It focuses on the functioning of the quality assurance system in the cell bank both on a general and a detailed level, concerning in particular the processing of the autologous lymphocyte product by the cell bank, i.e. its preparation for further processing steps by the manufacturer of the marketing authorization: advanced therapy medicinal product/advanced therapy investigational medicinal product (MA-ATMP/ATIMP). It also provides practical guidelines to help other cell banks to successfully meet national requirements expressed by the accreditation of the Ministry of Health for the processing and release for circulation of autologous lymphocyte product and the certification pathway of the MA-ATMP/ATIMP manufacturer (companies: Kite/Gilead, Novartis and Janssen).

Key words: chimeric antigen receptor, CAR-T, advanced cellular therapy

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Introduction

Research on chimeric antigen receptor, primarily targeting T cells (CAR-T), has led to a revolution in the treatment of patients with relapsed/refractory B-cell hematological malignancies. The first CAR-T drug used worldwide was tisagenlecleucel (Kymriah®, Novartis). Treatment of patients with diffuse large B-cell lymphoma with this therapy resulted in an overall response rate of 52% and over 80% for patients with B-cell acute lymphoblastic leukemia and non-Hodgkin lymphoma. In a study using axicarbtagene ciloleucel (Yescarta®, Kite/Gilead), an overall response was achieved in 82% of patients [1].

The Department of Hematology and Bone Marrow Transplantation at Poznan University of Medical Sciences has over 30 years of experience in the field of cellular therapies. From the early 1990s until 2019, hematopoietic stem cell transplantation procedures and related activities (e.g. donor lymphocyte infusions) were the main focus of this therapy. For all of these highly specialized procedures, each cell product was processed by a single processing unit. Since 2012, this unit has been accredited by Poland's Ministry of Health as a Stem Cell Bank (SCB). It prepares cells for various cell therapies according to a quality assurance system (QAS). Since 2012, the SCB has worked under the ISO 9001 quality management system certification. The procedures for processing hematopoietic cells and donor lymphocytes at the SCB are overwhelmingly derived from practical experience based on activities within the Hematology Diagnostic Laboratory.

Over the past 30 years, the profile of cell therapy, in terms of stem cell transplantation performed by Transplant

Address for correspondence: Ewa Bembnista, Stem Cell Bank, Department of Hematology and Bone Marrow Transpantation, Poznań University of Medical Sciences, Szamarzewskiego 84, 60–569 Poznań, phone +48 61 854 95 98, fax +48 61 854 95 60, e-mail: ewa.bembnista@skpp.edu.pl

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Centers (TC) around the world and by the Department of Hematology and Bone Marrow Transplantation of Poznan University of Medical Sciences has changed. At the same time, the range of procedures performed in SCBs has expanded. Initially, only a few preparations were made, mainly of autologous hematopoietic cells. Currently, most of the procedures in our TC are allogeneic stem cell transplants from unrelated, haploidentical and family donors. The specimens prepared at SCB allow us to treat over 130 patients per year at our center. Additionally, approximately 30 donor lymphocyte infusions are performed annually at the TC in Poznan. This activity is extended to the collection, processing and distribution of cells for over 180 patients treated at other TCs.

In 2019, the first collection of autologous lymphocytes for CAR-T therapy was performed in our unit [2]. SCB now has experience of processing and releasing 13 autologous lymphocyte products. For all of the above cell therapy activities performed at our and other TCs for hematopoietic stem cell transplantation procedures, donor lymphocyte infusions, and CAR-T therapy, more than 800 cell products are prepared annually at SCB.

QAS in a cell bank

In Poland, the procedures for cell processing (steps such as collection, storage, quantitative and qualitative assessment, processing) and distribution/release for circulation are strictly regulated by the Transplantation Act (TA), which is based on European Union (EU) directives (European Parliament Directive 2004/23/EC, Commission Directive (EU) 2015/565 and Commission Directive (EU) 2015/566) [3]. According to the TA, all these procedures can only be performed by a cell bank. Accreditation by the Ministry of Health, preceded by a positive decision of the Transplantation Council and a positive opinion from the National Center for Tissue and Cell Banking (NCTCB) is required for each type of cells and for each process related to their processing by this entity. This requirement also applies to the individual stages of processing autologous lymphocyte preparations. A very important element of cell bank operations is the implementation and subsequent maintenance and improvement of QAS, i.e. the organizational structure of procedures and processes affecting the achievement and maintenance of high quality of cells processed in the cell bank. The QAS enables processes to be carried out in a reproducible and repeatable manner, thereby maintaining the traceability of cell product processing.

In the early 2000s, we witnessed another, perhaps breakthrough, milestone in Polish medicine. A patient's autologous lymphocytes were collected and then released for MA-ATMP production according to banking procedures. The cell product at each stage of MA-ATMP/ATIMP production carries the status 'for use by intended recipient only'. By genetically modifying the T-lymphocyte, these cells gain the ability to recognize and destroy cancer cells when administered to a patient. In other words, the cells obtained from the patient in the TC are properly prepared (including cryopreservation) and released for circulation by the cell bank to the MA-ATMP/ATIMP manufacturer. This product, as MA-ATMP or ATIMP, will then be returned to the hospital where it will be administered to the patient via the hospital pharmacy. The autologous lymphocyte product collected from the patient will undergo multiple processes at multiple sites. At each, it is important to follow QAS and maintain all processes to identify the product at each stage of preparation. The audit of Kite/Gilead, Novartis or Janssen concerns the control of compliance with both company requirements and those stated in EU regulations.

Our experience in the certification processes conducted by the above companies indicates that the QAS system in place at SCB, and its general elements such as e.g. training procedure, qualification of equipment and environments, and process validations, are common to the processing of hematopoietic cells for transplantation and autologous lymphocyte product to the company. Nevertheless, past experience with cellular therapy is not everything. All cell bank personnel involved in CAR-T procedures must complete the manufacturer's entire training program.

One of the most important points of cell bank certification is the procedure for reporting serious adverse events. According to this procedure, the audit analyzes the cell bank's experience with the adverse event and analyzes the corrective and preventive actions taken by the unit. Corrective actions allow the CB to minimize the consequences of serious adverse events. Preventive actions allow the CB to minimize the likelihood of a recurrence. The corrective and preventive actions need to be aligned with CAR-T therapy.

Implementation of new procedures in the unit's existing QAS requires its adaptation to the new requirements. The new procedures must be consistent with the unit's quality system. When creating a QAS for CAR-T procedures, there are some general and unique manufacturer requirements. One of these is the requirement for what is known as the 'four eyes' rule. According to this 'gold standard', all cell processing points must be performed by two people trained in banking procedures. It should be noted that this rule was already in place at SCB early on, even before the introduction of CAR-T therapy. Importantly, for most collaborators, each detailed step in the preparation of the product for release must be confirmed on the appropriate forms provided by the company, while also being documented in the bank's forms. An equally important criterion for the preparation of autologous lymphocyte product is to minimize the risk of cross-contamination. This involves the need to ensure working in a laminar flow chamber and cryopreservation of cells obtained from a single patient. Hence, given the bank's involvement in the processes of preparing cells for transplantation, especially

in the case of banks supplying large TCs with material for transplantation, proper equipment is extremely important. To a large extent, this requirement is ensured by the need to have dual, so-called critical, equipment which overlaps with the tools required for CAR-T procedures.

Receipt, coding, processing, quantitative and qualitative assessment, and release for circulation of autologous lymphocyte product for further processing

The draft QAS created for autologous lymphocyte preparations at the initial stage of the Ministry of Health accreditation process must include the manufacturer's requirements.

The main banking procedures for processing lymphocytes for CAR-T therapy are: product receipt, quantitative and qualitative assessment, coding, storage, cryopreservation, and release for circulation.

At SCB, an additional section of standard operating procedures dedicated to autologous lymphocyte product for CAR-T therapy has been implemented into the functioning quality system. The main procedure entitled: 'Storage, quantitative and qualitative assessment and release for circulation of autologous lymphocytes' is described in detail in the form of instructions. Due to differences in manufacturers' requirements for banking apheresis products, especially with regard to quantitative and qualitative product evaluation and release for circulation, very detailed instructions with the manufacturer's name were introduced. In addition, new forms were created for each autologous lymphocyte processing.

Receipt of autologous lymphocyte product

Procedures and requirements vary from company to company. There are several processing points for autologous lymphocyte products in the cell bank required by all of them. Upon receipt of the product, after the apheresis process, while adhering to the 'four eyes' rule, bank personnel validate the identity of the product. To meet traceability requirements, it is necessary to enter accurate data on the Receipt Form. Date, time, temperature and humidity values (actual, minimum and maximum) must be recorded at each stage of cell processing in the cell bank. All measuring devices at each stage of cell processing should allow for notification when the required limit range is exceeded. This function is crucial, especially for short- and long-term storage of autologous lymphocyte product at the correct temperature. All critical equipment and technical devices must be identified and qualified, regularly inspected and preventively maintained according to manufacturers' instructions.

The above solutions are critical to maintaining regulatory requirements. According to EU Directive 2006/86/EC: "Where equipment or materials affect critical processing or storage parameters, e.g. temperature (...), they must be identified and must be subject to appropriate monitoring, alarms and corrective action, as required, to detect malfunctions and defects and to ensure that critical parameters are always maintained within acceptable limits. All equipment with a critical measurement function must be calibrated against an identifiable standard, if available" [4].

Coding and processing of autologous lymphocyte product

The key to ensuring that autologous cell product can be identified at every stage, from collection through processing, evaluation and storage, to release into circulation, is proper coding of the product.

A single European Code (SEC) is assigned to all material donated to the cell bank to ensure proper donor identification and traceability of all donated material and to provide information on the main characteristics and properties of the cells. A label is required to identify the autologous lymphocyte product. The label should include the following elements: patient's name and date of birth, product SEC code, date and time of collection and expiration (including time zone), and number of bags. Contact information with the name of the cell bank (the entity responsible for release stage) and the manufacturer's information is also essential. Other essential elements of the label are: the statements "for use by intended recipient only", "cells for human use", "biohazard", "do not irradiate" and (for fresh product) "do not freeze".

Autologous lymphocyte product must be placed in a cell bank with policies in place to prevent mixing and cross-contamination with other cell therapy products. To prevent cross-contamination, only one patient's material can be processed at any one time in a chamber with laminar flow of sterile air.

Quantitative and qualitative assessment of autologous lymphocyte product

The quantitative and qualitative evaluation of autologous lymphocyte product varies among MA-ATMP/ATIMP manufacturers. According to the MA-ATMP manufacturer's requirements, the evaluation of nuclear and CD3+ cell counts, cell viability, and microbiological evaluation of the product need only be performed once. Knowing the number of nuclear cells in the product is particularly important for the cryopreservation process. It affects how the cells are processed (e.g. volume reduction) and the appropriate partitioning of the product.

Our cell bank asked the manufacturer (ATIMP) to perform a quantitative and qualitative assessment of the product. According to its procedures, product evaluation in the cell bank is not required. However, analysis of the product in the cell bank rationalizes the decision path for

the number of cell apheresis needed by the CAR-T manufacturer and is useful in organizing the TC work schedule. The aforementioned product qualification criteria are also necessary to meet the QAS requirements of the cell bank, i.e. traceability requirements.

Release for circulation of autologous lymphocyte product

Another case of equal banking procedures for autologous lymphocyte product concerns the release process. Despite differences in manufacturers' procedures at the release stage, there is one single rule required by the TA. According to Article 37a(3) of the TA, any release of autologous lymphocyte product for a manufacturing facility located in another country requires NCTCB approval. Additionally, the cell bank must indicate the status of the product after cell manipulation (MA-ATMP or ATIMP) by submitting an application to the NCTCB. Much of the procedure for qualifying autologous lymphocytes for CAR-T therapy is based on our experience in processing hematopoietic stem cells for distribution to other cell banks. One of these procedures is cryopreservation and storage of a sample of the released product in the cell bank. Such a procedure can be very useful if, for example, discrepancies are found at different stages of product preparation and patient treatment.

Cryopreservation and storage of autologous lymphocyte product for further processing

Processing the cell therapy product according to the rules, and storing it under correct temperature conditions, allows the preservation of cell parameters even for many years. The experience of cell bank personnel who process autologous hematopoietic stem cells for transplantation provides the know-how of the cryopreservation process. This experience may be useful in the preparation of the CAR-T product. According to standard cell therapy guidelines, the cell therapy product should contain less than 2×10^8 nuclear cells/mL [5]. The final cell concentration affects the cell status expressed as cell viability after thawing the product. Too high a concentration of cells may cause the cells to clump together. On the other hand, too low a cell concentration may result in an inability to evaluate the product. To cryopreserve cells, it is necessary to prepare a cryoprotectant medium designed for cell preservation. This medium must contain appropriate protective proteins and DMSO at 10% final concentration [6]. It is important that the autologous lymphocyte preparation be cryopreserved in a bag and DMSO approved by the MA-ATMP manufacturer.

As a standard step in processing a cell therapy product with a cryopreservation step, it is necessary to collect several vials with a sample of autologous lymphocyte preparation, used to evaluate the product after the cryopreservation process. The vials must be frozen and stored with the cell product for CAR-T therapy. To avoid the risk of cross-contamination, only one patient's material and samples may be cryopreserved in the cryopreservation chamber at a time. It is essential that the cryopreservation of the material takes place in a programmed system in a chamber with automatic dispensing of liquid nitrogen vapor, according to the cryopreservation line. The cryopreservation line must be approved by the MA-ATMP manufacturer. The cryopreservation process must be monitored by the system. The duration of cryopreservation of the product must allow the product to reach temperatures below –80°C. Product so cryopreserved must be stored with the vials in liquid nitrogen auto-dispensing tanks, in a rack, in liquid nitrogen vapor phase. Cryopreserved cell therapy product must be stored in liquid nitrogen vapor for at least several hours before release to the MA-ATMP manufacturer. For safe storage of cryopreserved autologous lymphocyte products, liquid nitrogen tank mapping is critical. This qualification process helps to ensure the required temperature for the product throughout storage, even if the tank is opened, e.g. to remove another product.

As is evident in the QAS section of this manuscript, storage of the cryopreserved product must be constantly monitored, including an alarm system in the event of adverse temperature changes.

Summary

Cell bank experience is essential to the introduction of CAR-T therapy, but adaptation of QAS to conduct this therapy is indispensable. The tremendous success of implementing biotechnology solutions into clinical practice in the area of advanced cellular therapies, broadly defined, will likely result in the need to implement further procedures in cell bank practice.

Cell therapies are likely to go beyond the treatment of hematological diseases. Therefore, there will be a need to convert stem cell banks into cell therapy laboratories where material will be developed for a wide range of patients.

Authors' contributions

EB — sole author.

Conflicts of interest

None.

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Ethics

The work described in this article was performed in accordance with the World Medical Association Code of Ethics

(Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for experiments involving animals; Uniform requirements for manuscripts submitted to biomedical journals.

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