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Road to clinical implementation of CAR-T technology in Poznań

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Abstract
The objective of this paper is to present the process of the national and international accreditation leading to the establishment of the first certified chimeric antigen receptor T (CAR-T) Cell Unit in Poland on the basis of the Department of Hematology and Bone Marrow Transplantation in Poznan University of Medical Sciences and first successful CAR-T therapy in Poland. During 12 months from the initial decision to establish the CAR-T Cell Unit to the application of CAR-T cell treatment in the first patient, the center had to undergo the multidisciplinary external and internal training, as well as the adaptation of multiple procedures within the Transplant Unit and Stem Cell Bank. In order to get accreditation for the implementation of CAR-T cell therapy, an initial training of the team involved in handling cellular products and patient care was organized and updated as a continuous process. The Department fulfilled the site-selection international criteria. The first patient diagnosed for refractory/relapsed DLBCL was qualified, and finally CAR-T cells were administered with successful clinical outcome.

Keywords:
chimeric antigen receptor, CAR-T, axicabtagene ciloleucel, tisagenlecleucel, diffused large B-cell lymphoma

Introduction

New insights into genetic characteristics of the cancer cell and progress in biotechnology have initiated the development of the chimeric antigen receptor T (CAR-T) cell therapy. Clinical studies are underway for multiple B-cell malignancies, including B-cell acute lymphoblastic leukemia, Hodgkin and non-Hodgkin lymphomas, chronic lymphocytic leukemia, acute myeloid leukemia, and multiple myeloma. However, the most advance is seen in B-cell malignancies. Results from clinical trials have resulted in approval by European Medicines Agency in August 2018 of two first in class CAR-T cells, namely, tisagenlecleucel (Kymriah, Novartis) and axicabtagene ciloleucel (Yescarta, Gilead/Kite) [1, 2, 3]. CAR-T cells that are used in the two approved products and in the most advanced clinical trials are directed against CD19 which is expressed on the surface of the vast majority of B cells, including B-cell malignancies.

CAR-T therapy involves *ex vivo* genetic manipulation of patient's own T lymphocytes to create recombinant receptor with cell activating functions. After reinfusion, CAR-T cells penetrate tissues, amplify, and eliminate target neoplastic cells. In approved indications, the treatment leads to durable responses of up to 70%; however, unique profile of toxicities requiring specific therapy may be observed. Centers willing to administer CAR-T cell therapy should have an experience that guarantees safety of treated patients and be able to go through high-quality accreditation procedures.

The objective of this paper is to present process of national and international accreditation leading to the establishment of the first certified CAR-T Cell Unit in Poland on the basis of the Department of Hematology and Bone Marrow Transplantation in Poznan University of Medical Sciences and first successful therapy with CAR-T cells in Poland.

Material and Methods

Legal background

Presented data are based on the experience and knowledge from accreditation process for CAR-T cell therapy organized by Gilead/Kite, Novartis and Janssen in our center, European guidelines, Poltransplant and National Centre for Tissue and Cell Banking guidelines, and Polish law [4].

Center and patients

Process of arrangements of the center to the accreditation and implementation of CAR-T cell therapy was based on the following activities:

1. Initial education of the team involved in handling cellular products and patient care
2. Site selection
3. Patient's qualification
4. CAR-T cells administration
5. Management of CAR-T cells toxicity and patient safety.

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Initial education of team involved in handling cellular products and patient care

Highly specialized CAR-T technology requires cooperation of the multidisciplinary team consisting of laboratory team handling with cellular products, clinical team including physicians, and nurses from different departments who are all responsible for patient care. Initial education is necessary and it is based on both the international experience and the local hospital principles of the management of the patient.

Site selection

The intense immune activation results in severe adverse reactions which need to be managed appropriately to allow successful and safe clinical use of CAR-T cells. A 24/7 on-call service must be provided by a highly specialized team consisting of experienced physicians trained in allogeneic stem cell transplantation and intensive care. Relevant experience is associated with carrying out of at least 120 allogeneic transplantations over the past 3 years. The transplant team is complemented by neurologists and radiologists specifically trained for CAR-T cell patient care. To minimize the risk associated with CAR-T cells treatment, there must be an immediate access to four doses of tocilizumab for each patient as cytokine release syndrome (CRS) management medication prior to treating patients.
Each site that is qualified for CAR-T cells treatment must have established procedures:

1. Facilitating identification, monitoring, and treatment of CRS and serious neurological adverse reactions.
2. Ensuring that adverse reactions are adequately and appropriately reported.
3. Ensuring that detailed instructions about the storage and thawing procedure are provided.

Site must have access to the apheresis center which is capable to collect autologous lymphocytes and ship for further processing into dedicated laboratories. Hospital must also have an access to appropriate facility that provides storage adequate to the manufacturer's expectations (vapor phase of liquid nitrogen below 150°C). Since the CAR-T is formally the drug, storage and thawing must be under the supervision of a trained pharmacist. At every step, identification of the product (both autologous lymphocytes and the final CAR-T) with the patient is a crucial point of the whole process and needs to be performed independently by two staff members called "four eyes principle."
An additional but important element of the site selection is the availability of patients, which aims at the optimal, cost-effective use of expensive and complicated procedures such as CAR-T cells.

Patient's qualification

The administration of CAR-T cells is a complex and costly endeavor involving cell manufacture, shipping of apheresis products, and management of novel and severe adverse reactions. The use of CAR-T cells demands not only highly qualified multidisciplinary team but also an optimal patient selection.
For Yescarta the indications are diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma, high-grade B-cell lymphoma, transformed follicular lymphoma after two or more lines of systemic therapy. Indications for Kymriah include DLBCL, high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma. Separate population for Kymriah are pediatric and young adults up to 25 years of age with B-cell ALL that is refractory, in relapse posttransplant or in second or later relapse.
Patients receiving Yescarta or Kymriah should be deemed by their physician to have adequate organ function and performance status to tolerate the therapy. It is recommended, but not mandatory, to have tumors that are positive for the CAR-T target (CD19). Patients should have an adequate number of T cells for collection. The threshold for an absolute lymphocyte count varies from the manufacturers, but it is usually above 0.1 G/l.
Patients with an active central nervous system (CNS) disorder or inadequate renal, hepatic, pulmonary, or cardiac function are likely to be more vulnerable to the consequences of the adverse reactions and require special attention. CAR-T cells should not be administered to patients with an active infection or inflammatory disease until this condition has been resolved. HBV reactivation, in some cases resulting in fulminant hepatitis, can occur in patients treated with drugs directed against B cells. Screening for hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) should be performed in accordance with clinical guidelines before collection of cells for manufacturing of CAR-T.
The experience of use of CAR-T cells in patients with primary or secondary CNS lymphoma is limited and thus the risk/benefit has not been established in this population. The pregnancy status of women of child-bearing potential must be verified before starting the treatment. There are no available data in pregnant women. No reproductive and developmental toxicity animal studies have been conducted; thus, it is difficult to assess whether it can cause fetal harm when administered to a pregnant woman. Formally, CNS involvement and pregnancy are contraindications for CAR-T cell therapy [5].

CAR-T cells administration

CAR-T products must be administered in a qualified clinical setting and supervised by a physician experienced in the treatment of hematological malignancies and cellular therapy. The availability of Yescarta or Kymriah must be confirmed prior to lympho-depleting regimen. Lympho-depleting chemotherapy is recommended during 2 weeks prior to the administration of CAR-T cells. The most common regimen used is fludarabine-cyclophosphamide; however bendamustin might be considered in selected patients (for Kymriah) [5].
Prior to the infusion of CAR-T cells, the availability of tocilizumab and emergency equipment must be confirmed. For premedication, paracetamol by oral and diphenhydramine by oral or intravenous administration is recommended. Corticosteroids should be avoided as it may interfere with the activity of CAR-T cells. After patient and product verification, CAR-T cells are being thawed in a sterile bag at 37°C in a water bath (or by dry thaw method) within 3–5 minutes. Once thawed, the product is stable for 3 hours in a room temperature. Both Yescarta and Kymriah must be administered via intravenous

infusion through central venous catheter. Infusion that takes up to 30 minutes is performed via no leuko-depleting filter tubing filled with 0.9% NaCl.

Patient safety issues and management of CAR-T toxicity

Administration of CAR-T cells is associated with the risk of immunological (cytokine release syndrome (CRS)), neurological (immune effector cell-associated neurotoxicity syndrome (ICANS)), and hematological (lymphopenia, neutropenia) complications, leading to the risk of metabolic (tumor lysis syndrome) and infectious complications including septic shock and risk of patient transfer to intensive care unit. According to international guidelines, during process of CAR-T cell therapy, patient should be continuously monitored for these complications [4, 5]. Antimicrobial prophylaxis is mandatory.

Results

Initial education of team involved in handling cellular products and patient care

At the end of 2018, just after registration of the first in class CAR-T cell products by the European Medicines Agency (EMA), the CAR-T multidisciplinary team, including physicians, nurses, and coordinators from the Bone Marrow Transplant Unit, the Stem Cell Bank, the Intensive Care Unit, consultant neurologists, and Hospital Pharmacy, was established in our hospital. The process of internal and external training was started. Additionally, Departments of Pathology, Radiology, and Microbiology were informed and educated regarding the principles of the planned therapy.

Site selection

The Poznan CAR-T Cell Unit rose in 2019 as a part of the Transplant Unit, founded in 1990. So far we did perform over 2000 stem cell transplants, including approximately 960 allogeneic ones (130 stem cell transplants per year including 70 allogeneic ones). Transplant physicians, qualified nurses, transplant coordinators do work together with multidisciplinary team of specialists including neurologists, cardiologists, pneumologists, dermatologists, surgeons, intensivists, and others. The Apheresis Unit, which is a part of the Transplant Unit, does perform over 300 procedures yearly. The Unit is accredited by Ministry of Health for both allogeneic and autologous stem cell and lymphocyte collection. After cell collection the apheresis product is received by Stem Cell Bank, accredited by Ministry of Health. The qualified staff, with many years banking experience, prepares 700 cellular products per year. The Stem Cell Bank is a perfectly organized structure cooperating with central, hematological (especially flow cytometry), and microbiological laboratories. Our bank cooperates with other national and international transplant centers. The activity of Stem Cell Bank according to national (composed on EU) requirements is confirmed by Ministry of Health accreditation. It works in accordance with ISO 9001:2015 standard. For CAR-T program quality assurance system, arising from requirements related to hematopoietic cell transplantation was implemented.

Patient's qualification

In our center, the qualification of patient is performed by the transplant team during transplant qualifications. The transplant team includes eight physicians who are experienced in cellular therapy (average experience >10 years). Qualification form includes information regarding diagnosis with histopathology/cytology results, disease stage, previous treatment, performance status according to World Health Organization (WHO), risk of the procedure measured by Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI). Final qualification is made based on the additional tests such as complete blood count, biochemistry, infectious diseases markers, cardiology assessment, magnetic resonance imaging (MRI) of CNS, positron emission tomography–computed tomography (PET-CT) scan. Patients who are not accepted for CAR-T cell therapy are advised to other treatment, including stem cell transplantation or clinical trials.

According to the standard guidelines, the patient qualified for CAR-T therapy, after reassessment (disease staging; infectious disease markers are valid for 30 days), undergoes leukapheresis. After verification with "four eyes principle", the apheresis product is under care of the Stem Cell Bank which is responsible for the assessment of the product, processing depending on the product (deep freezing for Kymriah), and shipping of the product to the manufacturer. The reception of the commercial CAR-T products (Yescarta or Kymriah) is provided by Hospital Pharmacy, which handle the products over to the Stem Cell Bank for storage, final packing, and transportation before administration. An agreement between Pharmacy and Stem Cell Bank confirms the responsibility of the pharmacist for the product identification, storage, and appropriate thawing.

CAR-T cells administration

In our hospital, CAR-T therapy takes place in the Transplant Unit under supervision of the transplant physicians and qualified nurses. Before the start of the lymphodepleting chemotherapy detailed reassessment is performed, including disease staging, comorbidities, infection/inflammatory status (with infectious diseases markers), and cardiac assessment. As manufacturing of the CAR-T product takes up to 4 weeks, the patient may require bridging therapy which is also provided in our center.

Administration of the CAR-T product is performed as described earlier. CAR-T cells are not administered to patients with an active infection or inflammatory disease until this condition has been resolved. Patient is monitored daily for the first 10 days following CAR-T infusion for signs and symptoms of potential complications, especially CRS, neurological events, or infections. After the first 10 days the patient can be discharged from the hospital; however the patient is instructed to remain within proximity to the hospital for at least 4 weeks following infusion. During the first 10 days, detailed assessment is performed daily which includes clinical, neurological (including neurocognitive testing), biochemistry, complete blood count, additional markers (ferritin, procalcitonin, C-reactive protein, troponin, NT-proBNP, immunoglobulins).

Outpatient management is focused on the treatment efficacy (PET-CT assessment) and potential complications (hypogammaglobulinemia,

prolonged neutropenia, infections, primary and secondary neoplasms).

First patient: treatment, safety, and management of CAR-T cell toxicity

The first patient, 24-year-old male with diffuse large B-cell lymphoma, not otherwise specified (DLBCL NOS) (refractory, early relapse after autologous stem cell transplantation), was qualified to therapy with axicabtagene ciloleucel. The patient was in overall good condition (ECOG1) without CNS involvement. Autologous T lymphocytes were obtained via apheresis on October 25, 2019 and underwent the genetic modification in dedicated laboratory in USA. Yescarta was shipped from the manufacturer to our Pharmacy/Stem Cell Bank in cryopreserved form. After leuko-depletion with cyclophosphamide and fludarabine the first in Poland CAR-T cell infusion was performed after thawing of the product on November 28, 2019 at the Transplant Unit. The infusion was well tolerated. Post-infusion period was complicated by leukopenia and neutropenia; however, the patient was afebrile and CRS/ICANS symptoms did not occur. The patient was discharged from the hospital at day +14 after infusion in good condition with improvement in disease in interim assessment.

Discussion

During 12 months from the initial decision of creation of the CAR-T Cell Unit to the application of CAR-T cell treatment to the first patient, our center had to undergo multidisciplinary external and internal training, as well as adaptation of multiple procedures within the Stem Cell Bank and Pharmacy.

In order to get accreditation for CAR-T cell therapy, an initial training of the team involved in handling cellular products and patient care was organized and updated as continuous process. The Department fulfilled the site-selection international criteria. The first patient diagnosed for refractory/relapsed DLBCL was qualified, and finally CAR-T cells were administered.

The three critical issues were as follows:

1. Creation and training of multidisciplinary team
2. Adaptation of multiple procedures to the new international requirements
3. The safety of patient during and after CAR-T cell infusion.

There is a number of complications seen after CAR-T cells therapy [5, 6]. The most common is CRS, observed in more than 90% patients; however, grades 3–4 are seen in about 11% patients with fatal events below 3%. The most typical symptoms include fever, hypoxia, hypotension, and organ toxicity. It is an early complication occurring usually in the first week after treatment, with median duration of 7 days. Neurological complications (ICANS) are diagnosed in up to 67% of patients, with grades 3–4 in 32% patients. Clinical picture include encephalopathy, headache, tremor, dizziness, aphasia, and seizures. Most of the neurological symptoms are transient and fully reverse with 3–4 weeks. The mortality with ICANS is currently <4%. Febrile neutropenia and infectious complications have rather mild to moderate course; however they are related to severity of CRS. Other toxicities observed after CAR-T cell therapy include tumor lysis syndrome, hypersensitivity reactions, prolonged neutropenia, hypogammaglobulinemia, and secondary malignancy. Management of the CAR-T toxicities is described elsewhere [4, 5, 6].

In conclusion, with the effort of dedicated personnel from multidisciplinary team, including handling of cellular products and taking care of patient, we were able to overcome existing issues and barriers.

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Authors' contributions

DD, LG – study design, manuscript writing. All authors – data collection, manuscript revision.

Conflict of interest

LG was a participant of Novartis and Gilead Advisory Board, and she received lecture fee and participated in the meetings organized by Gilead and Novartis.

DD was a participant of Janssen Advisory Board, and he received lecture fee and participated in the meetings organized by Janssen. He also received research grants from Janssen.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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