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Transfusion and Laboratory Aspects of CAR-T Cell Therapy: A Pathologist's Perspective on T Lymphocyte Collection, Transport, and Infusion

Dr. Anubhuti Jain[1], Dr. Joyce Regi[2]

Clinical Assistant

Consultant & Head

Abstract: ***Chimeric Antigen Receptor T-cell (CAR-T) therapy has revolutionized treatment options for relapsed/refractory hematologic malignancies. This article describes the transfusion and laboratory processes associated with a CAR-T therapy case from the viewpoint of a pathologist. Our centre was responsible for the leukapheresis collection of autologous T lymphocytes using the COM. TEC system, with subsequent transport to a processing facility and infusion of the CAR-T product after it was processed. The study highlights the critical roles of cell collection protocols, quality assurance during transport, and laboratory coordination in ensuring a successful outcome.***

Keywords: CAR-T cell therapy, T lymphocyte collection, leukapheresis, transfusion medicine

1. Introduction

CAR-T cell therapy represents a paradigm shift in personalized cancer treatment, particularly for B-cell malignancies such as acute lymphoblastic leukemia and diffuse large B-cell lymphoma. It involves genetic modification of a patient's autologous T lymphocytes to express chimeric antigen receptors that target tumor-associated antigens, most commonly CD19.

CAR T cell therapy is a type of cancer immunotherapy treatment that uses immune cells called T cells that are genetically altered in a lab to enable them in locating in destroying cancer cells more effectively. Treatment can be very effective against some types of cancer, even when other treatments are not working. Currently, CAR T therapy is FDA-approved [1] to treat several types of haematological malignancies, including Leukaemia, Lymphoma and Multiple myeloma. Each T cell has a receptor that can recognize antigens (proteins or molecules that are recognizable by the immune system). When the immune system recognizes foreign or abnormal antigens, it can work to destroy them. Different types of cancer have different antigens. Each kind of CAR T cell therapy is made to fight a specific kind of cancer antigen. So, a CAR T cell therapy made for one type of cancer won't work against another type of cancer. Currently CAR-T cell therapy is approved effective for certain hematologic malignancies, specially. B-ALL, DLBCL, Mantel cell lymphoma, Multiple Myeloma.

Why only these? Reason being, target antigen availability-[2-5] These malignancies express unique surface antigens, CD19, B Cell Maturity Antigen (BCMA). These are high on tumour cells and low/absent on essential healthy tissues. Thereby reducing off target toxicity.

Solid organ malignancies are yet not in the reach of the therapy mainly because, they have poor T-Cell infiltration and they are mostly having suppressive microenvironments, making them difficult to reach.

The execution of CAR-T therapy requires seamless coordination between clinical, laboratory, and transfusion medicine services. As pathologists and transfusion medicine specialists, our responsibilities extend from patient preparation and T cell collection to ensuring regulatory compliance and monitoring post-infusion parameters.

This paper presents our experience managing the transfusion medicine aspects of a CAR-T therapy case, emphasizing the collection of T lymphocytes using the COM. TEC apheresis system, the logistics of cryopreserved product transport, and the eventual infusion of the therapeutic product.

Aim:
To detail the role of the pathology and transfusion medicine team in the collection, processing, and coordination of autologous CAR-T cell therapy, emphasizing procedural insights and operational challenges from a single patient case.

2. Materials and Methods

1) Patient Selection and Preparation:

A patient with relapsed Mixed Phenotype Acute Leukaemia was deemed eligible for CAR-T cell therapy after failing standard chemotherapy. After consent and pre-therapy assessment, the case was coordinated with the central CAR-T processing facility. The patient's peripheral blood counts were optimized to meet apheresis eligibility criteria, including a minimum absolute lymphocyte count of >2 x 10^9/L.

2) T Lymphocyte Collection:

Leukapheresis was performed using the COM. TEC (Fresenius Kabi) apheresis system, designed for mononuclear cell (MNC) collection. Central venous access was used. The procedure lasted approximately 3 hours, and continuous monitoring of vital signs and cell counts was performed. Target MNC yield was based on the protocol provided by the CAR-T manufacturer (Absolute lymphocyte count >2 x 10^9). A volume of 85 ml was collected with absolute lymphocyte count of 18.22 x 10^9 cells. After the collection 1ml of 5000IU

heparin was injected in the product on laminar flow bench and mixed thoroughly. The patient tolerated the procedure well.

3) Sample Handling and Transport:

The collected T cells were transferred into validated cryogenic containers and shipped in dry ice containers with temperature monitors (maintained at +-80°C) to the processing centre. A chain-of-custody form was meticulously filled to document time of collection, freeze, dispatch, and receipt.

4) Quality Control and Communication:

The pathology department performed in-house quality checks to ensure:

- Adequate MNC yield and absolute lymphocyte count
- Absence of gross haemolysis or contamination
- Proper labelling and documentation

Coordination was maintained with the processing facility to confirm receipt and suitability of the product for CAR-T manufacturing.

5) CAR-T Product Infusion and Monitoring:

Twenty days post-collection, the genetically modified CAR-T cells were shipped back to our centre. The infusion was administered under sterile conditions in an intensive care setup. The transfusion medicine team ensured the product's identity, thawed it per manufacturer's instructions, and maintained aseptic technique during infusion.

Pre-and post-infusion labs (CBC, CRP, ferritin, cytokine panel) were monitored closely.

3. Results

1) T Lymphocyte Collection: The leukapheresis procedure yielded 8.7 x 10^9 mononuclear cells with absolute lymphocyte count of 18.22 x 10^9. Lymphocytes comprised 83.3% of the MNC fraction. The procedure was well tolerated with no adverse events.
2) Transport and Processing: The product was successfully transported to the CAR-T manufacturing centre with continuous temperature monitoring. The processing facility confirmed receipt and acceptance of the product within 24 hours.
3) Infusion and Follow-up: CAR-T cells were infused 20 days post-collection. The patient tolerated the infusion well. Laboratory parameters showed a transient rise in inflammatory markers (CRP, ferritin), consistent with early immune activation.

4. Discussion

CAR-T cell therapy, although clinically administered by oncologists and immunologists, demands critical backend support from transfusion medicine and pathology services. The success of therapy hinges on precise collection, preservation, and coordination, areas where the transfusion pathologist plays a central role.

1) Importance of Pre-Apheresis Evaluation:
   Ensuring optimal hematologic parameters is crucial to maximize T cell yield and minimize the need for repeat procedures. Our case met all collection benchmarks in a single sitting, highlighting the value of pre-apheresis planning.
2) Technical Considerations in Apheresis:
   The COM. TEC apheresis system allows fine-tuning of collection parameters, facilitating high-purity mononuclear cell harvests. Operator training and adherence to protocol are vital to minimize contamination and maximize lymphocyte recovery.
3) Role in Logistics and Quality Assurance:
   Cold chain integrity is paramount during cryogenic transport. The use cryo-shippers, real-time GPS tracking, and validated temperature logs ensures product stability during inter-facility transit.
4) Post-Infusion Monitoring and Laboratory Correlation:
   Pathologists assist in evaluating response to CAR-T therapy via marrow morphology, immunophenotyping, and MRD testing. Inflammatory marker trends guide CRS grading and management. Knowledge of these kinetics aids in early recognition of complications like macrophage activation syndrome or neurotoxicity.
5) Challenges and Future Directions:
   - Turnaround Time: Twenty days between collection and infusion can be critical for rapidly progressing malignancies. In-house CAR-T manufacturing may reduce this lag in the future.
   - Standardization: Uniform guidelines for lymphocyte collection and handling are needed across institutions.
   - Safety: Transfusion-transmitted infections, although rare, must be vigilantly screened.

Our experience also underlines the need for institutional SOPs integrating haematology, apheresis, molecular pathology, and logistics teams. A multidisciplinary tumour board approach is advisable for optimal outcomes.

The successful implementation of CAR-T cell therapy in India marks a significant advancement in the treatment of relapsed and refractory hematological malignancies. While the therapy has revolutionized cancer care globally, its accessibility and affordability have remained substantial barriers in low-and middle-income countries. Recent Indian initiatives have aimed to overcome these challenges through indigenous development and clinical validation of CAR-T products.

A landmark effort was the development of NexCAR19 (actalycabtagene autoleucel), the first homegrown CAR-T cell product in India. In a multicenter study, Majumdar et al.6 reported an overall response rate of 73% in patients with relapsed or refractory CD19-positive B-cell malignancies, including diffuse large B-cell lymphoma and B-acute lymphoblastic leukemia, with manageable toxicity profiles and cytokine release syndrome in less than 15% of patients (Majumdar et al., 2023). This paved the way for regulatory approval by CDSCO and clinical rollout at a fraction of the international cost.

In another Phase I study conducted at Tata Memorial Hospital, Pendharkar et al.7 evaluated a humanized CD19 CAR-T cell product in adult patients with relapsed B-ALL and NHL. The study demonstrated safety, with no grade 3 or higher neurotoxicity, and early evidence of efficacy, including complete remission in a subset of patients

(Pendharkar et al., 2022). These findings underscore the feasibility of in-house CAR-T cell manufacturing and its clinical application in Indian centers with existing hematopoietic stem cell transplant infrastructure.

Furthermore, Kumar et al.8 reported on the scalability and manufacturing workflow of point-of-care CAR-T cell production, emphasizing the need for harmonization across centers and robust cryopreservation protocols to facilitate wider adoption (Kumar et al., 2023). Their work is critical in laying the groundwork for a nationwide CAR-T ecosystem under the National Cancer Grid.

These pioneering studies establish a strong foundation for the integration of CAR-T therapy into India's public and private oncology practice. Future directions include expanding CAR-T targets beyond CD19, exploring BCMA-directed CAR-T for multiple myeloma, and improving product durability through gene editing and dual-target approaches.

5. Conclusion

This case underscores the indispensable role of the pathology and transfusion medicine departments in CAR-T therapy. From lymphocyte collection using to product infusion, our team ensured rigorous quality standards and seamless coordination. With CAR-T therapy becoming increasingly available, pathology services must evolve to meet the demands of this complex, yet transformative, treatment.

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