Lymphocyte collection for cellular therapy

Sérgio Luiz Parente Filho[1*], Suzanna Araújo Tavares Barbosa[2], Naliele Cristina Maia de Castro[2], Araci Sakashita[2]

1. Hospital Universitário Walter Cantídio – Fortaleza (CE), Brazil.
2. Hospital Israelita Albert Einstein – São Paulo (SP), Brazil.

***Corresponding author:** sergioparente_@hotmail.com

Section editor: Fernando Barroso Duarte

Received: Jun. 03, 2026 • **Accepted:** Jul. 13, 2026

ABSTRACT

Introduction: Leukapheresis is the starting point of cellular therapies such as chimeric antigen receptor T cells (CAR-T cells) and donor lymphocyte infusion. **Methods**: After a review of the literature, we provided guidance on how to evaluate candidates for leukapheresis and how to optimize the safety and efficacy of the procedure. **Results**: Clinical and laboratory assessment is warranted prior to leukapheresis. T cell fitness is impacted by previous treatments, ageing, and tumor burden. The most common adverse reaction is hypocalcemia. **Conclusion**: Leukapheresis is a safe and effective procedure. Technical and clinical aspects must be observed to preserve T cell fitness, ensure manufacturing success, and guarantee patient safety and clinical efficacy. The current challenge is to standardize guidelines for pre-leukapheresis requirements and collection yield improvement.

Keywords: Leukapheresis; CAR-T cell; DLI; Lymphocyte collection.

INTRODUCTION

Chimeric antigen receptor T cells (CAR-T cells) therapy represents a paradigm shift in the treatment of relapsed or refractory (R/R) hematologic malignancies, yielding unprecedented responses for patients, especially those who suffer from multiple myeloma, acute lymphoblastic leukemia and non-Hodgkin's B cell lymphomas.[1-3] Donor lymphocyte infusion (DLI) has long been used to treat or prevent relapses from certain malignancies after allogeneic hematopoietic stem cell transplant (HCT).[4]

Apheresis is the in-line separation of blood components by specific machines, enabling the exchange or collection of a selected part of the blood for therapeutic purposes and returning the other blood components to the patient/donor. Leukapheresis is the process by which the individual's peripheral blood mononuclear cells (MNCs) are collected, providing T cells, which will be subsequently modified to target malignant cells in the context of CAR-T cell therapy. However, it has been more frequently employed to collect autologous or allogeneic hematopoietic stem cells or allogeneic donor lymphocytes.

As most current CAR-T cell products utilize autologous cells, the number and quality – or "fitness" – of these T cells are primary determinants of manufacturing success and subsequent clinical efficacy. Indeed, there is convincing evidence that a better leukapheresis yield improves response and even survival.[5] Therefore, leukapheresis is the starting point of a successful CAR-T cell treatment. On the other hand, T cell fitness is not a concern for DLI.

The fundamentals of T-cell fitness

T-cell fitness is yet to be comprehensively defined, but it reflects the ability of T cells to proliferate, mount a CAR-mediated effector immune response that eliminates malignant cells, and differentiate into long-lived memory cells, providing durable responses.[6] This fitness is cumulatively compromised by advanced age, tumor burden, and heavy pretreatment.

- Ageing: natural thymic involution reduces the output of naïve T cells, leading to a repertoire enriched with differentiated, less proliferative subsets.
- Malignancy: chronic inflammation (e.g., high IL-6 levels in myeloma or chronic lymphocytic leukemia) drives T-cell exhaustion and decreased CD4:CD8 ratio.
- Prior treatment: conventional therapies, particularly autologous haematopoietic cell transplant (auto-HCT) and alkylating agents like bendamustine, cause profound lymphopenia and metabolic remodeling that can permanently impair T-cell proliferative capacity. Moreover, bispecific monoclonal antibodies may also lead to lymphopenia and T cell dysfunction.[6,7]

Taken together, these findings support that leukapheresis for CAR-T cell therapy should preferentially occur earlier in the disease course, and attention must be paid to the choices of treatments to control the disease either before or after leukapheresis. In this regard, two important definitions must be mentioned:

Holding therapy (HT)

Is the treatment used to control the disease before leukapheresis is performed. Considering that untreated disease progression is often fatal and a high tumor burden may impact T cell fitness, HT is crucial, but should cause as little T cell toxicity as possible. Avoiding drugs that are strongly associated with impaired T cell fitness (such as bendamustine, fludarabine, alkylating agents, and bispecific monoclonal antibodies) is recommended. On the other hand, other drugs (e.g., lenalidomide and Bruton's tyrosine kinase inhibitors) might improve T cell fitness and are reasonable options if clinically indicated. Depending on which HT is given, it is necessary to observe different washout periods before proceeding to leukapheresis (Table 1).[7-9]

Table 1. Recommended washout periods prior to leukapheresis.

Type of treatment	Washout period	Comments
Short-acting cytotoxic drugs (e.g., hydroxyurea)	3 days	Qayed et al.,[8] EHA/EBMT[9]
Short-acting growth factors	5 days	Qayed et al.[8]
Nilotinib	5 days	Qayed et al.[8]
Systemic corticosteroids	1 week	Qayed et al.,[8] EHA/EBMT[9] allow 3 days if ALC > 200/mm^3
Intrathecal chemotherapy	1 week	Qayed et al.,[8] EHA/EBMT[9]
Lenalidomide	1 week	Qayed et al.[8]
Long-acting growth factors	2 weeks	Qayed et al.[8]
Systemic chemotherapy	2 weeks	Qayed et al.[8]
Combinations of proteasome inhibitors, immunomodulatory drugs, corticosteroids, and/or single-target monoclonal antibodies	2 weeks	IMWG[7]
Imatinib, dasatinib and ponatinib	2 weeks	Qayed et al.[8]
Blinatumomab	2 weeks	Qayed et al.[8]
High-dose chemotherapy	3-4 weeks	EHA/EBMT[9] recovery from cytopenias is required.
Teclistamab, elranatamab, talquetamab	At least 4 weeks	IMWG[7]
DLI	4 weeks	Qayed et al.,[8] EHA/EBMT[9]
Pegylated asparaginase	4 weeks	Qayed et al.[8]
Allogeneic HCT	At least 1 month, GvHD-free and off immunosuppression	EHA/EBMT[9]
Clofarabine	8 weeks	Qayed et al.[8]
Bendamustine	At least 12 weeks	Qayed et al.[8]
Fludarabine	At least 12 weeks	Qayed et al.[8]
Alemtuzumab	6 months	Qayed et al.[8]
Antithymocyte globulin	6 months	Qayed et al.[8]

EBMT: European Bone Marrow Transplant Association; IMWG; International Myeloma Working Group. Source: Elaborated by the authors.

Bridging therapy (BT)

Refers to the treatment administered between leukapheresis and CAR-T cell infusion (i.e., "vein-to-vein" time). Since the manufacturing process may take a long time, BT is often needed. Drug choice is more liberal for BT, since T cells have already been harvested.[7]

Clinical readiness, laboratory evaluation, and patient optimization

There is no uniform criterion to determine when a patient is able to proceed to leukapheresis, even in clinical trials.[10,11] However, there have been initiatives to make recommendations on this matter.[9,10] We herein present some general guidance, but it should not replace compliance with the manufacturer's recommendations, as well as with local and international regulations. For a safe and successful leukapheresis, patients are expected to present:

- Evidence of bone marrow regeneration after HT;
- Hemoglobin > 8 g/dL or hematocrit > 24%;
- Platelets > 20.000/mm^3 (some references recommend > 30.000/mm^3);
- Absence of active infection;
- Absence of active graft-versus-host disease (in case of previous allogeneic hematopoietic stem cell transplant);
- Absence of significant electrolyte disturbances (especially calcium, magnesium, and potassium);
- Clinical and hemodynamic stability;
- Adequate venous access.

Transfusion of platelets and/or packed red blood cells (PRBCs) is suitable to achieve the aforementioned thresholds. For small pediatric patients, priming of the extracorporeal circuit with a unit of PRBCs is recommended if the patient weighs less than 25 kg or if the extracorporeal volume exceeds 10 mL/kg or 15% of the individual's total blood volume (TBV), regardless of their hemoglobin level. If PRBCs are used either for circuit priming or transfusion prior to leukapheresis, they should be leukoreduced and irradiated to avoid contamination of the product with allogeneic T cells.[8]

Absolute lymphocyte count (ALC) and T cell (CD3+) count in peripheral blood before leukapheresis are strong predictors of adequate yield. An ALC > 200/mm^3 and CD3+ count > 150/mm^3 are generally recommended, but there is evidence of successful manufacturing with lower counts.[8,12] However, it is not clear if such a product will result in comparable clinical efficacy. Therefore, if the patient is still recovering from HT, and ALC is expected to increase, it is desirable to delay leukapheresis until it occurs.

Technical aspects of leukapheresis

The majority of current CAR-T manufacturers request Spectra Optia (Terumo BCT) or Amicus (Fresenius Kabi) as apheresis instruments to be used for MNC collection. The first one is probably the most widely utilized, since it has the advantage of enabling continuous MNC collection.[13] Support for COBE Spectra (Terumo BCT) has been discontinued with the advent of Spectra Optia. Please check which device is available at your institution and refer to the operator's manual.

Peripheral venous access is preferred to avoid the eventual complications of a central venous catheter (CVC) placement. A peripheral cannula of 16-18 gauge or 20 gauge is required for the inlet line for adults or pediatric patients, respectively. For the return line, a 20-gauge cannula is usually enough.[10] However, CVCs are often required, especially in pediatric patients or adults with poor venous status due to previous treatments. Patients must be assessed for the possibility of peripheral venous access by expert nurses beforehand, so that the placement of a CVC can be planned, if it is really necessary.[8]

Acid-citrate-dextrose solution A (ACD-A**)** is the standard anticoagulant for apheresis procedures. The initial recommended ACD-A:whole blood ratio is usually around 1:12-13, and the citrate infusion rate must not exceed 1.2 mL/min/L of TBV to avoid toxicity.[10] In our center, we do not add heparin to accelerate the procedure because it is generally not necessary and elevates the risk of bleeding, especially in thrombocytopenic patients.

Each CAR-T product manufacturer has its own cell collection target, which may be specified in terms of nucleated cells, lymphocyte count, or CD3+ cells. There is no standardization yet on pre-leukapheresis requirements and total whole blood volume (WBV) to be processed.[13] Current recommendations rely on expert opinion and are displayed in Table 2,[8] though it is still advisable to refer to the manufacturer's guidance.

Table 2. Number of TBVs to be processed according to ALC.

Pre-leukapheresis ALC	Number of TBVs to be processed
> 1.000/mm³	1.5
500-999/mm³	2.5
300-499/mm³	3.0
100-299/mm³	3.5-4.0
< 100/mm³	Consider a 2-day collection

Source: Adapted from Qayed et al.[8]

Once an apheresis unit develops experience in leukapheresis for CAR-T cell products, the decision on the WBV to be processed may be tailored by using the average collection efficiency (CE) from previous procedures, which is calculated as follows[13]:

$$\text{CE (\%)} = \frac{\text{Product cell count (CD3+, ALC, or MNC)} \times \text{Product volume}}{\text{Pre-apheresis cell count (CD3+, ALC, or MNC)} \times \text{TBV}} \times 100$$

$$\text{WBV to be processed} = \frac{\text{Target cell yield (CD3+, ALC, or MNC)}}{\text{CE} \times \text{Pre-apheresis cell count (CD3+, ALC, or MNC)}}$$

There is another formula using pre- and post-leukapheresis cell counts, which is more precise for measuring the real device efficiency, but for the aim of estimating blood volume to be processed, the formula mentioned above is sufficiently accurate.[8]. Another option is to use a calculator developed by British researchers,[14] which is available online at https://cd3yield.shinyapps.io/cd3yield/.

Prevention and management of adverse reactions

Most side effects of leukapheresis are related to ACD-A toxicity and are usually mild and easily reversible. Patients may present with perioral and extremity paresthesias – often referred to as numbness or tingling – as a result of citrate-induced electrolyte disturbances, notably hypocalcaemia. In more severe cases, especially if not treated promptly, symptoms might worsen to muscle cramps, tetany, QT interval prolongation, and arrhythmias. In addition, ACD-A may also cause alkalosis, which may lead to dizziness, nausea, hypokalemia, and a nonspecific complaint of feeling unwell.

Patients should be assessed for calcium, magnesium, and potassium levels prior to apheresis, and any electrolyte disturbance should be corrected. Prophylactic calcium replacement is warranted for all patients[15] and must be administered via the return line or a separate access, never through the inlet line. Magnesium replacement is not routinely needed and must not be diluted or infused in the same line with other electrolytes,

such as calcium and potassium. Nevertheless, if a patient still develops mild symptoms of citrate toxicity, some actions must be taken:

- Increase the rate of prophylactic calcium replacement and/or give slow push-doses;
- If symptoms recur or do not improve, decrease the inlet flow (aim for an ACD-A infusion rate of 0.8-1.0 instead of 1.2 mL/min/L of TBV);
- If symptoms worsen, the procedure should be paused, and the patient must be reassessed by a clinician to consider other interventions (electrocardiogram, laboratory tests, replacement of other electrolytes, definitively stopping the procedure, etc.).

Leukapheresis also causes a positive fluid balance, especially if a large WBV is processed. This is usually not a problem unless the patient has significant comorbidities, such as kidney disease or cardiac failure. Therefore, signs of volume overload must be actively searched for before and after the procedure, and administration of diuretics should be considered. If the patient is already oligoanuric on chronic renal replacement therapy, having dialysis scheduled after leukapheresis is appropriate.

Severe adverse reactions are exceedingly rare and occur in approximately 0-1.5% of cases.[13] They are mostly related to complications of central line placement, inadequate patient selection, or not observing general criteria of patient readiness (see "Clinical readiness, laboratory evaluation, and patient optimization" above).

CONCLUSIONS

Leukapheresis – the starting point of a successful CAR-T cell treatment – is a safe and effective procedure. Technical and clinical aspects must be observed to preserve T cell fitness, ensure manufacturing success, and guarantee patient safety and clinical efficacy. The current challenge is to standardize guidelines for pre-leukapheresis requirements and collection yield improvement.

CONFLICTS OF INTEREST

Nothing to declare.

AUTHOR CONTRIBUTIONS

Conceptualization: Parente Filho SL, Barbosa SAT, Castro NCM, Sakashita A. **Investigation:** Parente Filho SL, Barbosa SAT, Castro NCM, Sakashita A**. Methodology:** Parente Filho SL, Barbosa SAT, Castro NCM, Sakashita A. **Formal analysis:** not applicable. **Data curation:** Parente Filho SL, Castro NCM. **Project curation:** not applicable. **Project administration:** not applicable. **Funding acquisition:** not applicable. **Writing:** Parente Filho SL, Sakashita A. **Supervision:** not applicable. **Final approval:** Parente Filho SL.

DATA AVAILABILITY STATEMENT

All datasets were generated or analyzed in the current study.

FUNDING

This research did not receive any specific funding from public, commercial, or not-for-profit funding agencies.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE TOOLS

Notebook LM was used for brainstorming and to organize and format references. Human writing was maintained.

ACKNOWLEDGEMENTS

Not applicable.

REFERENCES

1. Neelapu SS, Jacobson CA, Ghobadi A, Locke FL, Miklos DB, Lekakis LJ, et al. Five-year follow-up of ZUMA-1 supports the curative potential of axicabtagene ciloleucel in refractory large B-cell lymphoma. Blood. 2023;141(19):2307-2315. https://doi.org/10.1182/blood.2022018893
2. San-Miguel J, Dhakal B, Yong K, Mateos MV, Landgren O, Lentzsch S, et al. Cilta-cel or standard care in lenalidomide-refractory multiple myeloma. N Engl J Med. 2023;389(4):335-347. https://doi.org/10.1056/NEJMoa2303379
3. Laetsch TW, Maude SL, Rives S, Hiramatsu H, Bader P, Baruchel A, et al. Three-year update of tisagenlecleucel in pediatric and young adult patients with relapsed/refractory acute lymphoblastic leukemia in the ELIANA trial. J Clin Oncol. 2023;41(9):1664-1669. https://doi.org/10.1200/JCO.22.00642
4. Pagliuca S, Schmid C, Santoro N, Potter V, Onida F, Snowden JA, et al. Donor lymphocyte infusion after allogeneic haematopoietic cell transplantation for haematological malignancies: basic considerations and best practice recommendations from the EBMT. Lancet Haematol. 2024;11(6):e448-e458. https://doi.org/10.1016/S2352-3026(24)00098-X
5. Wada F, Jo T, Arai Y, Nishida T, Saito S, Nakagawa M, et al. T-cell counts in peripheral blood at leukapheresis predict responses to subsequent CAR-T cell therapy. Sci Rep. 2022;12(1):18696. https://doi.org/10.1038/s41598-022-23589-9
6. Mehta PH, Fiorenza S, Koldej RM, Tognela A, Kueberuwa G, Mian A, et al. T cell fitness and autologous CAR T cell therapy in haematologic malignancy. Front Immunol. 2021;12:780442. https://doi.org/10.3389/fimmu.2021.780442
7. Costa LJ, Banerjee R, Mian H, Richardson PG, Rodriguez C, Kumar SK, et al. International myeloma working group immunotherapy committee recommendation on sequencing immunotherapy for treatment of multiple myeloma. Leukemia. 2025;39(3):543-554. https://doi.org/10.1038/s41375-024-02482-6
8. Qayed M, McGuirk JP, Myers GD, Bachanova V, Bishop MR, Turtle CJ, et al. Leukapheresis guidance and best practices for optimal chimeric antigen receptor T-cell manufacturing. Cytotherapy. 2022;24(9):869-878. https://doi.org/10.1016/j.jcyt.2022.05.003
9. Hayden PJ, Roddie C, Bader P, Basak GW, Bonig H, Bonini C, et al. Management of adults and children receiving CAR T-cell therapy: 2021 best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE) and the European Haematology Association (EHA). Ann Oncol. 2022;33(3):259-275. https://doi.org/10.1016/j.annonc.2021.12.003
10. Worel N, Holbro A, Vrielink H, Schmetzer HM, Bosman P, Hirscher V, et al. A guide to the collection of T cells by apheresis for ATMP manufacturing – recommendations of the GoCART coalition apheresis working group. Bone Marrow Transplant. 2023;58(7):742-748. https://doi.org/10.1038/s41409-023-01957-x
11. Thibodeaux SR, Aqui NA, Park YA, Tang Y, Lee DW, Bishop MR, et al. Lack of defined apheresis collection criteria in publicly available CAR-T cell clinical trial descriptions: comprehensive review of over 600 studies. J Clin Apher. 2022;37(3):223-236. https://doi.org/10.1002/jca.21964
12. Tuazon SA, Li A, Gooley T, Riddell SR, Maloney DG, Turtle CJ, et al. Factors affecting lymphocyte collection efficiency for the manufacture of chimeric antigen receptor T cells in adults with B-cell malignancies. Transfusion. 2019;59(5):1773-1780. https://doi.org/10.1111/trf.15178

13. Piñeyroa JA, Cid J, Lozano M. Get off on the right foot: how to plan an efficient leukocytapheresis to collect T cells for CAR T-cell manufacturing. Transfus Med Hemother. 2022;50(2):98-104. https://doi.org/10.1159/000528331

14. O'Reilly MA, Malhi A, Cheok KPL, Hwang WY, Lim SH, Ritchie DS, et al. A novel predictive algorithm to personalize autologous T-cell harvest for chimeric antigen receptor T-cell manufacture. Cytotherapy. 2023;25(3):323-329. https://doi.org/10.1016/j.jcyt.2022.10.012

15. Bolan CD, Cecco SA, Wesley RA, Hillyer CD, Corbin TM, Swerdlow PS, et al. Controlled study of citrate effects and response to i.v. calcium administration during allogeneic peripheral blood progenitor cell donation. Transfusion. 2002;42(7):935-946. https://doi.org/10.1046/j.1537-2995.2002.00151.x