A guide to the collection of T-cells by apheresis for ATMP manufacturing-recommendations of the GoCART coalition apheresis working group

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EBMT

PERSPECTIVE

A guide to the collection of T-cells by apheresis for ATMP manufacturing—recommendations of the GoCART coalition apheresis working group

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Autologous chimeric antigen receptor-modified T-cells (CAR-T) provide meaningful benefit for otherwise refractory malignancies. As clinical indications for CAR-T cells are expanding, hospitals hitherto not active in the field of immune effector cell therapy will need to build capacity and expertise. The GoCART Coalition seeks to disseminate knowledge and skills to facilitate the introduction of CAR-T cells and to standardize management and documentation of CAR-T cell recipients, in order to optimize outcomes and to be able to benchmark clinical results against other centers. Apheresis generates the starting material for CAR-T cell manufacturing. This guide provides some initial suggestions for patient's apheresis readiness and performance to collect starting material and should thus facilitate the implementation of a CAR-T-starting material apheresis facility. It cannot replace, of course, the extensive training needed to perform qualitative apheresis collections in compliance with national and international regulations and assess their cellular composition and biological safety.

Bone Marrow Transplantation (2023) 58:742–748; https://doi.org/10.1038/s41409-023-01957-x

INTRODUCTION

T-cells equipped with a chimeric antigen receptor (CAR), i.e. a synthetic antigen receptor consisting of an antibody-derived domain for tumor antigen recognition and short-circuited T-cell receptor-co-stimulatory receptor kinase domains, recognize and specifically kill cells expressing the cognate surface antigen, and promote CAR-T expansion [1–3]. Convincing evidence of efficacy of this innovative treatment modality especially for a number of B-cell dyscrasias [4–7] drive both expansion of access in the established conditions including application in an earlier stage of the disease, and expansion of novel indications with the same but also additional antigens. All currently available CAR-T products leverage on autologous T-cells as starting material. It should be obvious that, except in allo-transplanted patients [8], the T-cells are as old as the patient, and have been subjected to as much chemotherapy as the patient has. In other words, their quality as immune effector cells may be limited, and indeed a body of evidence supporting this notion has accumulated [9]. In addition to chronic damage incurred from the repeated insult of chemotherapy on the immune system, many anti-cancer medicines cause reversible functional impairment of T-cells that can be alleviated by adhering to certain wash-out periods. Indeed, after disease control [10], best possible observation of wash-out periods would seem the most critical factor for the success of CAR-T therapy which the treating medical team may be able to influence. Direct evidence of negative effects of certain medicines on outcome after CAR-T therapy has mostly not been provided, in fact, the less stringent observation of stopping rules after approval, e.g. in real life, has not obviously negatively affected outcomes [8, 11, 12]. Instead, the available recommendations are largely based on general observations of the strength and duration of the medicines' effects on cellular immunity. In general, a wash-out period of five half-lives should suffice. For some medicines (e.g. bendamustine, T-cell lytic antibodies), however, the immunosuppressive effect can long outlast their pharmacokinetics [13, 14]. Data on the use and sequencing of therapeutic antibodies targeting the same antigen are inconclusive [15, 16]. For a list of recommendations for stopping rules for certain frequently used medicines, the reader is directed towards more detailed work, both by clinical expert consortia and by CAR-T marketing authorization holders (MAH) [17]. A more extensive one is currently being developed by the GoCART consortium in the hope of providing a reference document. Apheresis leverages on time-tested, robust and very safe technologies which are similarly used to extract platelets for transfusion or mobilized peripheral blood stem cells (PBSCs) for transplantation from donors or patients. Certain differences between patients scheduled for apheresis of starting material for CAR-T production, PBSC patients

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Received: 25 February 2023 Revised: 3 March 2023 Accepted: 13 March 2023
Published online: 6 April 2023

or donors and platelet donors exist which may necessitate special accommodations including the often very low concentrations of target cells and relatively poorer health status of patients slated for CAR-T therapy. Suggestions for assessment of patient suitability and feasibility of apheresis targets are provided in this manuscript. For management of the underlying disease during CAR-T manufacturing (i.e. bridging therapy), lymphodepletion, CAR-T cell therapy itself, forbidden concomitant medicines, CAR-T therapy's adverse events and their management, aftercare and long-term management, we refer to other work, especially [17–21].

APHERESIS READINESS

Apheresis normally is a safe and well-tolerated procedure even at the extremes of age [22, 23], and rarely medical concerns will preclude apheresis readiness if an indication for CAR-T therapy has been established by the clinical team responsible for CAR-T treatment (CAR-T team). Certain risk factors for inferior outcomes of leukocyte collections by apheresis and CAR-T therapy should be documented and weighted, including, but not limited to, underlying disease status and disease activity, prior treatments (e.g. allogeneic haematopoietic progenitor cell transplantation with graft versus host disease (GvHD), prior CAR-T therapy, immunotherapy targeting the same antigen as the CAR-T therapy), Eastern Cooperative Oncology Group (ECOG) performance status, laboratory test results, and organ function. The CAR-T team should designate an experienced clinician or clinical pharmacist to assess and evaluate past and concurrent medications for their possibility to interfere with T-cell or CAR-T cell function. For reasons discussed above, leukocyte apheresis should be delayed for as long as the patient status, especially disease control, safely permits. Some evidence of bone marrow regeneration, a minimum of 150–200 T-cells/µl (ideally more), a hemoglobin level ≥80 g/l (i.e. hematocrit ≥24%) and a platelet count ≥20.000/µl are recommended before apheresis is initiated [17] using transfusion support to achieve the latter two parameters if necessary.

Venous status is evaluated in the process of the pre-apheresis work-up.

Apheresis should not be performed during an active infection, with a view to both procedure tolerability and product safety. Since apheresis at the end increases the intravascular volume, causes (pseudo)hypocalcemia, hypokalemia, hypothermia and, depending on the blood separator used, reduces platelet counts by as much as one third of the baseline value [24, 25] patients at risk for clinical complications from any of these idiosyncrasies should be identified during apheresis work-up and monitored accordingly [26].

VENOUS ACCESS AND ANTICOAGULATION

Depending on the requested cell dose the starting material should contain and the patients mononuclear (MNC) or T-cell count in the peripheral blood, more or less whole blood needs to be processed during the apheresis procedure. In most cases a peripheral venous cannula of 16 to 18 gauge (preferred in adults) and 20 gauge (preferred in paediatric patients), will suffice as inlet line, and an 18 to 20 gauge (for adults) and 20 to 22 gauge (for paediatric patients) cannula for blood return. Ultrasound can help to identify access sites where an obviously suitable vein can otherwise not be located. Rarely a central venous catheter (CVC) will be required. When an indwelling CVC or apheresis port is available and venous access is limited, these can be also used as return lines. However, the risk of bacterial product contamination caused by CVCs that have been inserted already for a longer time has to be considered. If low MNC and/or T-cell counts predict the need for large apheresis volumes and therefore rapid inlet flow rates are required, large bore lines are helpful. In all cases, strictly aseptic technique must be used during line placement and during handling including connecting to the apheresis disposable.

During apheresis, an anticoagulation fluid (typically acid-citrate-dextrose type A, ACD-A) is added to whole blood typically at an ACD-A-to-blood ratio of 12:1. The ACD-A rate should not exceed 1.2 ml/l*min of total blood volume of the patient [27]. After processing one time patient's total blood volume, ACD-A ratio can be reduced to 1:13 or higher, although then the tubing set/collection port must be observed for platelet aggregation. A higher ACD-A-to-blood ratio may reduce citrate toxicity or allow increasing the inlet flow rate.

APHERESIS MATHEMATICS – "APHERITHMETIC"

Use of mathematical formulas instructs apheresis planning, guides apheresis performance and facilitates benchmarking [28].

We recommend performing certain calculations during apheresis planning: It has proven useful to calculate the achievable process volume for a given patient based on gender, height, weight, and patient's total blood volume. The required process volume to achieve the target cell dose of typically $>1–4 \times 10^9$ T-cells (may differ for different MAH and specific products) or $5–10 \times 10^9$ MNCs is calculated based on the patient's T-cell or MNC concentration in the peripheral blood [29, 30]. Moreover, apheresis performance, i.e. collection efficiency (CE; CE2 is sufficient, although CE1 is the more precise measure[1]), [27, 28] should be continuously tracked by the apheresis facilities to assess process stability. If monitoring of CE2 identifies changes in performance, this should trigger a root cause investigation and could identify potential training needs.

The above data can be used to calculate the total amount of blood which can be processed during an apheresis session and can predict the feasibility of achieving the target dose. A formula predicting the process volume required to achieve a given target cell dose was recently introduced by O'Reilly et al. [31] and should be useful in all patients where a process volume of at least 7 litres can be achieved. The formula being rather complicated, the web-application the authors created to perform the calculation is linked here (https://cd3yield.shinyapps.io/cd3yield/) [32]. For smaller patients, estimation of the number of T-cells which can be collected in one session is supported by the observation that during T-cell apheresis peripheral blood T-cell concentration does not meaningfully change and that a well-performed apheresis in a CAR-T patient will collect at least 40% of all T-cells presented in the cell separator [33]. Formulas for calculation of the mentioned items are provided in Fig. 1.

APHERESIS TECHNOLOGY AND APHERESIS PERFORMANCE

All available leukapheresis technologies are suitable for CAR-T starting material collection. Personnel must be specifically trained for the locally used apheresis devices. The three most commonly used technologies are briefly introduced, which should not be understood as a specific endorsement of these technologies by the Consortium. The anticoagulant ACD-A binds calcium, potentially resulting in (pseudo)hypocalcemia and alkalosis which is known to induce hypokalemia [26, 34]. Calcium can be administered prophylactically to avoid citrate induced hypocalcemia and will also attenuate hypokalemia [35]. Monitoring of electrolytes before and after apheresis is advisable. Hypothermia is

[1]Collection efficiency (CE) is the fraction of the circulating cells that is extracted during apheresis and takes into consideration the cell concentration in the peripheral blood, processed blood volume and number of collected cells. CE1 uses the mean of pre- and post- target cell concentrations in the peripheral blood whereas CE2 uses only the pre-apheresis concentration of target cells.

a Calculating feasible process volume:

$$\textit{Maximum process volume} = \textit{total blood volume (liters)} * 1.2\ (\textit{ml per liter of blood of ACDA}) * 13\ (\textit{AC: blood ratio}) * 300\ \textit{min}$$

b Calculating collection efficiency „pre“ (CE2):

$$CE2 = \frac{\textit{Target cells in apheresis bag}}{\textit{Target cells (per liter)in peripheral blood} * \textit{processed blood volume}} * 100\%$$

c Calculating required process volume (O'Reilly formula)[31,32]:

$$\textit{Target process blood volume (l)} = [LN(\textit{target cell yield } (*10^9)) - 0.41 - LN(\textit{pre-count target cell concentration in blood } (*10^9/l)) * 0.86 - \textit{Hct pre-apheresis (l/l)} * 1.3\ ] / 0.009$$

d Calculating required process volume (40% formula):

$$\textit{Target process blood volume (l)} = \frac{\textit{Desired cell dose}}{\textit{CE2\%} * \textit{target cells (per liter)in blood}}$$

Fig. 1 Apherithmetic describes the formulas used in a leukapheresis procedure. a Displays the calculation of the feasible process volume when using 300 min as maximum apheresis duration and a whole blood to ACD-A ratio of 13:1. The whole blood to ACD-A ratio can be meaningfully reduced if heparin can be added for anti-coagulation. AC anticoagulation fluid, ACDA acid citrate dextrose A. **b** Displays the calculation of Collection Efficiency "pre" (CE2). A typical mean CE2 for MNCs and T-cells in not G-CSF-stimulated patients has to be expected in the low 60 s. CE collection efficiency, MNC mononuclear cells, G-CSF granulocyte-colony stimulating factor. **c** Displays the formula to calculate required process blood volume (O'Reilly formula) [31, 32]. The minimal process volume is always 7 litres. LN natural logarithm. **d** Displays a formula to calculate the required process blood volume by using an estimated CE2 of 40% (i.e. 40% formula). For this formula the local 10th percentile for CE2 should be used or 40% if local data are not yet available. CE collection efficiency.

a frequent side effect and may disturb blood flow through peripheral lines; at least potentially hypothermia could also interfere with primary hemostasis in this often already thrombocytopenic patient cohort. Therefore, warming blankets or blood warmers should be available to prevent hypothermia. A recent complete blood count, preferably taken the day of the collection procedure but at least within 24 h before start of apheresis, is needed for the set-up of the apheresis device. During apheresis, patients should be monitored clinically including pulse oxymetry, intermittent non-invasive blood pressure, and ECG if indicated. Monitoring of pH is typically not necessary during apheresis. Process volumes for CAR-T aphereses are typically small, less than 5 litres in the mean [27], so that despite the often relatively poorer health of the patients, CAR-T aphereses are not often associated with complications.

The vast majority of collections of starting material for CAR-T cells manufacturing are performed with the Spectra Optia® or Amicus™ devices. A high-end comparison of the technologies is provided in Table 1.

Amicus™ (Fresenius Kabi, Bad Homburg, Germany) is a well-established, very efficient leukapheresis device that operates largely automatically [36–38]. The extracorporeal volume of the tubing system is 163 ml. Relatively slow blood flow rates limit total process volume to 4.8 l/h, which in the context of CAR-T aphereses will only ever be an issue for patients with extremely low T-cell counts. Platelet contamination of the apheresis product is meaningfully lower compared with products collected with other devices [38]. The required patient data to enter in the software are: height, weight, sex and some blood count parameters. Normally collections are performed by dual-needle access but single-needle operation is also possible, albeit significantly reducing achievable process volumes. Procedure specific settings can be adapted during the apheresis to optimize the collected product (Table 1). The device automatically collects the desired cells within the tubing system, then intermittently deposits them in the product bag.

Spectra Optia® cMNC (Terumo BCT, Lakewood, CO, USA) is a continuously collecting apheresis system with an extracorporeal volume of 253 ml [39, 40]. Using the cMNC platform, product volume is primarily a function of the duration of the procedure. The default setting of the collection flow rate is 1.0 ml/min but can be reduced if inlet flow or target cell concentration are low. As little as 0.5 ml/min can be collected if the collection line is observed for clotting. Collections are guided by the color of the collection line proximal to the tubing set's cassette. An apparent hematocrit of ≤2% (i.e. light salmon color) in the collection line provides products ideally balancing purity and CE. This is typically achieved at a collection preference of 35–40, a collection preference of 40 being a typical starting value. Collection preference is a unit-less measure of the position within the buffy coat where leukocytes are collected [41]. Correct positioning of the interphase and the apparent hematocrit in the collection line are monitored throughout the apheresis duration, and the collection preference is adjusted as necessary.

Spectra Optia® MNC (Terumo BCT) platform, the immediate predecessor technology of cMNC [25], is an alternative very efficient technology especially useful for patients with small target cell populations and where low apheresis product volumes are desirable [42]. The MNC procedure is an automated procedure with a small extracorporeal volume of 147 ml.

Minimal documentation of the apheresis process includes patient name and date of birth, unique product number, a copy of the final product label, apheresis device with identification number, LOT numbers and expiration dates of consumables as well as consumed fluid volumes, date, start and end time of the apheresis, type of venous access, involved personnel, and any observations during the apheresis.

QUALITY CONTROLS

Apheresis products should be subjected to a minimum of quality controls (QC) to ascertain their conformity with pre-defined product-relevant specifications. Indeed, in those countries where apheresis collection is considered a pharmaceutical manufacturing process, apheresis must conform to Good Manufacturing Practice

Table 1. Shows the characteristics of the most commonly used apheresis platforms for collection of unstimulated leukocytes as starting material for further CAR-T cell manufacturing.

Apheresis device	**Amicus™**	**Spectra Optia®**	**Spectra Optia®**
Apheresis platform	**MNC**	**cMNC**	**MNC**
Collection technique	In cycles with continuous blood flow. Volume of cycles determined by the peripheral blood WBC count. MNCs are isolated and concentrated by an elutriation process. and periodically transferred to the external collection bag while platelets are returned to the patient.	Continuously After establishment of the interface target cells are continuously collected in the collection bag. Interface can be adapted by changing the collection preference.	In cycles with continuous blood flow. Target cells are concentrated in a collection chamber until a RBC sensor recognizes a spill over of cells. The chamber is then flushed with plasma into the collection bag. A completely filled chamber can hold approximately 3×10^9 leukocytes
Extracorporeal volume	163 ml Red blood cell priming in pts. with low total blood volume (e.g. TBV < 900 ml)	253 ml Red blood cell priming in pts. with low total blood volume (e.g. TBV < 1500 ml)	147 ml Red blood cell priming in pts. with low total blood volume (e.g. TBV < 900 ml)
Inlet flow (considering ACD-A tolerance and quality of venous access)	Maximum flow rate according to the WBC count: 80 ml/min if WBC < 5.0×10^9/l 75 ml/min if WBC count between 5.0 and 10.0×10^9/l	As high as possible while maintaining a stable flow and stable interphase. Minimum possible: 5 ml/min Risk: with low inflow/constant collection flow, only approximately 10% of the processed volume may be collected.	As high as possible while maintaining a stable flow and stable interphase. Minimum possible: 10 ml/min.
Collection flow	n.a.	Default 1 ml/min (adjustable range from 0.5–10 ml/min) Consider <1.0 ml/min (0.8 to 0.5 ml/min) to avoid contamination with other non-target cells. Risk: low collection flow can cause aggregates in collection line.	n.a.
Product volume	Depends on number of cycles and plasma volume used for flushing the collection line.	Depends on procedure time and collection flow rate.	Depends on number of cycles and volume used for chamber flush (total product volume should be ≥20 ml).
Procedure specific settings	Default settings of offset volumes for MNC 2.3 ml and RBC 6.5 ml during MNC transfer. Possible optimization: • Platelets <300×10^9/L: Offset for MNC 1.5 ml; RBC 6.5 ml • Platelets ≥300×10^9/l Offset for MNC 2.3 ml; RBC 6.5 ml Product volume dependent on number of cycles.	Collection preference (CP): CP is set and adjusted by the operator to achieve a light salmon colour in the collect line (start with CP 40) Cologram: estimate a Hct <2% (between 1st and 2nd colour from the right).	Chamber flush and chase settings: Default volume 6 ml. Number of flushes depends on target cell count and blood volume processed
Anticoagulation	ACD-A 1:12 CAVE Heparin: only possible if accepted by the CAR-T manufacturer.	ACD-A initially 1:12 (in case of aggregates temporarily <1:12 possible) CAVE Heparin: only possible if accepted by the CAR-T manufacturer.	ACD-A initially 1:12 (in case of aggregates temporarily <1:12 possible) CAVE Heparin: only possible if accepted by the CAR-T manufacturer.
Additional plasma	In general, yes, to dilute the collected MNCs and reach at minimum 100 ml of product.	Depends on CAR-T manufacturer's requirements.	Depends on CAR-T manufacturer's requirements.
Procedure related specific considerations for centres with all three platforms	Lower platelet loss and lower platelet contamination of the collected product	Centres with both collection platforms of Spectra Optia®: Consider patient variables (TBV, target cell count, clinical situation) and experience of the operator.	Centres with both collection platforms Spectra Optia®: The MNC platform could be preferred in small (paediatric) patients, in patients with target cell population <1.000 cells/µl, and in patients where mathematically no more than 1 flush cycle/hour is expected.

Table 1. continued

Apheresis device	**Amicus™**	**Spectra Optia®**	**Spectra Optia®**
Apheresis platform	**MNC**	**cMNC**	**MNC**
Special considerations in patients with ALL/blastoid MCL	Indifferent to WBC within typical range (however not best suited for patients with >15.000–20.000 leukocytes/µl).	In patients with high leukocyte counts: Collect with the leukodepletion platform Risk: high blast-contamination in apheresis material can impair further manufacturing.	Not best suited for patients with >15.000–20.000 leukocytes/µl.
Special considerations blood counts	Haemoglobin ≥8 g/dl recommended, platelets ≥20.000/µl in adults (≥50.000/µl in children), ≥150–200/µl T-cells recommended for CAR-T apheresis according to EHA/EBMT guidelines [17]		
Advantage	Easy to use, low collection volume	Easy to use, large process volumes possible.	Low extracorporeal volume (paediatric patients), low collection volume.
Disadvantage	Maximum blood flow rate limited	Relatively high extracorporeal volume compared to other platforms Potentially high collection volume (depending on collection pump speed).	less total volume processed per time where frequent chamber flushes are indicated.

ACD-A acid citrate dextrose-A, *ALL* acute lymphoblastic leukemia, *CAR* chimeric antigen receptor, *EBMT* European Society for Blood and Marrow Transplantation, *EHA* European Hematology Association, *Hct* hematocrit, *MCL* mantle cell lymphoma, *TBV* total blood volume, *WBC* white blood cells.

(GMP), with all consequences, including that assays must be appropriately validated, controlled, and authorized, as well as specifications must be justified by the manufacturer and approved by the authorities. Irrespective the regulatory status, at a minimum, QC must include infectious disease marker testing by serology and/or nucleic acid testing (performed on a blood sample collected concurrently to the apheresis) according to the European Blood Directive 2002/98/EC [43] total WBC and T-cell content, and sterility. All tests methods have to be validated for the material and volume tested. Accordingly, access to appropriately qualified flow cytometry and microbiology laboratories must be established. In some countries, apheresis and QC laboratories will require GMP licenses.

LABELING, STORAGE AND PRODUCT HANDLING/ TRANSPORTATION

Application of a CAR-T product to a patient who is not the erstwhile T-cell donor is likely lethal. Therefore, the flawless vein-to-vein tracking from the patient to the apheresis unit, the courier, the CAR-T manufacturer, and back to the patient is critical ("chain of identity"). Prior to the apheresis procedure, the autologous donor needs to be identified by the apheresis team. The operator cross-checks the patient identification with the apheresis record and labels, and affixes a label containing all legally permitted identifiers, critically including a (numeric) unique identifier to the collection bag. Use of International Society for Blood Transfusion (ISBT)-128 labels [44] is recommended, but alternatives remain acceptable as long as they are demonstrably safe and contain the minimal defined data set.

Immediately after the end of collection, while the patient is still connected to the tubing set, the apheresis product is disconnected from the apheresis system by heat-sealing the collection tube, after once more confirming correct labeling of the apheresis bag. Sufficient tubing must be left with the bag (12–15 cm) for the establishment of several sterile connections, both for sampling by the apheresis site's QC laboratories and for manipulations by the CAR-T manufacturer. Sampling pouches available on the tubing set can alternatively be used for QC. The apheresis bag is either packed or stored according to the CAR-T product manufacturer's instructions, whether commercial or academic/in-house. Most CAR-T manufacturers have the apheresis product picked up within hours of the end of collection. Centers wishing to order these CAR-T products will thus need to contract a cell processing laboratory; transportation of the apheresis product to the laboratory must be organized between the partners. The contracting cell processing laboratories will be familiar with the process of cryopreservation, which is therefore not addressed here in any technical detail. Transportation of the apheresis product to the CAR-T manufacturing site, whether fresh or frozen, is organized by the MAH. Packing materials are typically supplied by the company-provided courier; the specific procedures by the MAH for pick-up request, detailed product packing instructions and accompanying paperwork must be observed, as the MAH have thus far defied the medical community's efforts of standardization.

REGULATORY CONSIDERATIONS

National regulations for leukapheresis collections and QC, especially where these will become starting materials for Advanced Therapy Medicinal Products (ATMPs) such as CAR-T cells, vary throughout Europe. Accordingly, a manufacturing authorization and GMP license may or may not be required, and a Qualified Person may or may not need to release the apheresis product to the CAR-T manufacturer. Irrespective the minimal regulatory requirements, the Joint Accreditation Committee of the International Society for Cellular Therapy and the European Group for Blood and Marrow Transplant (JACIE) requires from JACIE-accredited clinical centers quality assurance activities [45] which do not fall short of GMP guidelines by much. Centers newly embarking into the field of CAR-T therapy may consider integration into established stem cell transplant programs or partnering with established CAR-T starting material apheresis centers to which apheresis activities are outsourced. In the latter case, written contracts should precisely define each party's roles and responsibilities for patient eligibility assessment, apheresis performance and QC, and product handling towards the CAR-T manufacturer.

SUMMARY

Apheresis produces the critical starting material for CAR-T manufacturing. The timing, importantly including its timely execution, and quality of the apheresis relevantly affect CAR-T

quality and clinical outcomes. Centers entertaining the introduction of CAR-T therapies are advised to establish apheresis infrastructure, i.e. hire and train designated apheresis staff, acquire and qualify apheresis technology and product storage refrigerators and establish or contract QC laboratories. Unless integrated into a blood establishment which inherently has got pharmaceutical quality management systems, such systems must first be established. Integration into JACIE-accredited stem cell transplant programs can facilitate such efforts. Since most patients slated for CAR-T therapy cannot wait, it will be important to provision excess capacity, both in terms of personnel and equipment. Where establishment of these systems is not possible, outsourcing of apheresis activities to specialized centers, for instance to blood establishments, can support establishment of CAR-T therapy units.

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ACKNOWLEDGEMENTS

We thank Sofie Terwel and Yolanda Cabrerizo for organizing regular virtual meetings of the Apheresis Working Group of the GoCart project and supporting us with written summaries of these meetings.

AUTHOR CONTRIBUTIONS

NW and HB were responsible for writing the report, creating the figure and table, and reviewing and revising the report. AH, HV, CO, KLP, IBW, TR, and ML were responsible for writing, reviewing and revising the report.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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