Providing the Starting Material to the Manufacturer of an Approved and Commercially Available Autologous CAR-T Cell Treatment

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Introduction

CAR-T cell manufacturing starts from a collection of mononuclear cells (MNCs, although specifically only T lymphocytes will be used for the preparation) from the patient using apheresis. Although several initiatives are working on the development of allogeneic CAR-T cells, currently only CAR-T therapies of autologous origin are approved in the European Union. The present chapter only discusses already or soon-to-be marketed autologous CAR-T cells and excludes investigational CAR-T cells or rare CAR-T cells approved in the context of hospital exemption, such as the ARI-001 product (Ortiz-Maldonado et al. 2021); on this topic, please refer to Chap. 3.

Institutions aspiring to be CAR-T centres must generate sufficient apheresis capacity to ensure immediate access to apheresis slots; apheresis capacity must grow in synchrony with the CAR-T program (Tables 6.1, 6.2, 6.3, 6.4, and 6.5).

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Table 6.1 Before apheresis collection

Apheresis units might require a visit during the screening of patients for CAR-T therapies

In the visit the following aspects should be evaluated (Yakoub-Agha et al. 2020):

- Discontinue drugs that affect the number and functionality of circulating T lymphocytes (e.g., steroids, immune-suppressors, and chemotherapy) as long as possible before apheresis. Minimal stopping rules are defined in institutional guidelines and manufacturing authorization holder (MAH) instructions. Steroids are usually stopped at least 3–7 days before apheresis.
 - Evaluate for systemic infection, particularly in patients with an intravenous central line. Bacteremia is a relative contraindication for MNC apheresis due to the risk of contamination of the product. Note, however, that a contaminated blood product—although definitely not ideal—is not always rejected by the manufacturer because the manufacturing process may plan for the addition of antibiotics.
 - Assess venous access, ideally including an ultrasound evaluation if upper arm peripheral veins are not adequate by palpation (Gopalasingam et al. 2017) before deciding that a central line catheter must be placed. Blood can be collected from most adult patients via peripheral access sites. Central catheters are most often needed in low-weight children. Indwelling catheters are suitable (Jarisch et al. 2020).
 - Evaluate whether any form of sedation is necessary (mostly for the paediatric population) through a joint evaluation between the paediatricians and apheresis medical director or practitioner.
 - Review complete blood count (CBC) and differential (absolute lymphocyte count) results to calculate target process volume.
 - Check the MCV to rule out the presence of beta thalassemia minor. Microcytosis leads to abnormal sedimentation behaviour, necessitating modification of apheresis settings (Constantinou et al. 2017).
 - Note height and weight and calculate total blood volume. In particular, in low-weight children, consider the need for priming of the apheresis tubing set with irradiated red blood cell concentrate (which needs to be pre-ordered so as not to delay initiation of apheresis).
 - If the patient is transfused with cellular components in the days prior to apheresis collection, they must be gamma irradiated.
 - Note concurrent medications, especially anti-hypertensives. Angiotensin-converting enzyme inhibitors and beta-blockers increase the risk of hypotension reactions during apheresis.
 - Evaluate electrolyte levels: potassium, (ionized) calcium, and magnesium levels drop during apheresis and thus can become critically low if already abnormal before apheresis (Stenzinger and Bonig 2018).
 - Negative infectious markers of HIV, HBV, and HCV (in some countries also HTLV-1 and syphilis) must be available within 30 days of collection, in compliance with applicable laws and regulations for autologous cell products. MAH will require that results be available upon shipment of the starting material to the manufacturing site.
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Table 6.2 Designing apheresis collection

<div><div>– Select the apheresis platform suitable for the collection. Depending on the platform selected, the characteristics of the product collected might vary significantly. For instance, if an Amicus separator (Fresenius-Kabi, Bad Homburg, Germany) is used, the platelet content is very low in comparison to Spectra Optia (Terumo BCT, Lakewood, CO, USA) (Cid et al. 2019), see Table 6.3.</div><div>– Check if the total blood volume of the patient requires priming of the apheresis separator.</div><div>– Check lymphocyte count to define the blood volume to be processed, see Table 6.4 for apheresis targets of different manufacturers.</div><div>– Consider prophylactic administration of calcium and magnesium during the collection depending on the volume to be processed (Sørensen et al. 2013).</div><div>– Evaluate the haemoglobin and platelet count, consider transfusion prior to or after apheresis depending on circumstances. A haemoglobin level of 7 g/dL, better yet 8 g/dL, prior to apheresis facilitates establishment of interphase during apheresis. Spectra Optia will reduce platelet count by approximately 11% per total blood volume processed.</div><div>– Evaluate the potassium level and consider supplementation.</div></div>

Table 6.3 Characteristics of the apheresis platforms typically used for collection

	Amicus	Spectra optia	
Manufacturer	Fresenius-Kabi (Bad Homburg, Germany)	TerumoBCT, Lakewood, Co, USA	
Software kit		Continuous MNC (CMNC)	MNC
Flow type	Discontinuous	Continuous	Dual-stage separation
Flow rate	10–80 (85) mL/min ^a	10–142 mL/min	10–125 mL/min
Operation	Automatic	Semi-automatic	Semi-automatic
Mononuclear cell collection	Elutriation	Aspiration	Aspiration followed by elutriation
Platelets	Returned to the donor	Collected	Partially returned to donor

^aDepending on the leukocyte count

Table 6.4 Collection requirements of different manufacturers of CAR-T therapies

Product	Axicabtagene ciloleucel	Brexucabtagene autoleucel	Tisagenlecleucel	Lisocabtagene maraleucel	Idecabtagene vicleucel	Ciltacabtagene autoleucel
Registered name	Yescarta®	Tecartus®	Kymriah®	Breyanzi®	Abecma®	
Manufacturer	Gilead	Gilead	Novartis	Juno-Celgene- BMS	BlueBird Bio-Celgene- BMS	Legend Therapeutics- Janssen
Antigen recognized	CD19	CD19	CD19	CD19	BCMA	BCMA
Target cell dose during apheresis ^a	5–10 × 10 ⁹ MNCs		1–4 × 10 ⁹ CD3 ⁺ cells ≥ 2 × 10 ⁹ TNCs ≥ 3% CD3 ⁺ of TNCs (rounding rules apply)			
Target volume to be processed ^a	12–15 L		6–10 L			

^aManufacturer’s recommendation
MNCs mononuclear cells, TNCs total nucleated cells

Table 6.5 Interim storage, cryopreservation, and logistics

- Interim storage at 4–8 °C
- Tisagenlecleucel: Adjust concentration to 10⁸/mL (0.5–2 × 10⁸); freeze as soon as feasible but no later than 24 h after collection, with DMSO as a cryoprotectant in a controlled-rate freezer; store in LN₂ (institutional SOP to be validated by the manufacturer prior to start of operations).
- All other CAR-T cells: Ship as soon as possible at 4–8 °C, following the manufacturers' instructions and requirements.
- MAH to arrange pick-up, with temperature-controlled shipping containers of the appropriate temperature.
- Scheduling, documentation of pick-up, hand-over, and tracking supported by MAH-supplied specific web tools.

Key Points

- Prepare for apheresis by assessing the clinical and biological condition of the patient.
- Discontinue treatments that can lower immune effector cell numbers and functions.
- Tailor apheresis parameters to the patient condition.
- Tailor apheresis parameters to suit the manufacturer's needs and requirements.
- Tightly coordinate with the Cell Processing Facility to ensure smooth shipment to the manufacturing site, in compliance with the manufacturer's needs and requirements.

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