Point-of-Care Production of CAR-T Cells

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CAR-T cells for clinical application are classified as advanced therapy medicinal products (ATMPs), and their manufacture is subject to laws and regulations governed by the European Medicines Agency (EMA) and by federal and regional authorities. CAR-T cells must be manufactured to achieve good manufacturing practice (GMP) compliance and are defined as potent products manufactured safely according to standardized methods under closely controlled, reproducible, and auditable conditions. BioPharma supplies the vast majority of CAR-T products for patients, but some academic centres have developed point-of-care cGMP CAR-T manufacturing capability, striving to uphold the same stringency of product quality while improving patient access to CAR-T cells and streamlining the costs of therapy. Point-of-care CAR-T manufacturing can only be performed in facilities with the appropriate regulatory approvals in place.

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GMP Vector Production

Retroviral and lentiviral vectors are the most common gene delivery methods used in CAR-T manufacture. Viral vectors are considered an intermediate reagent by regulatory agencies, but in manufacturing, adherence to cGMP conditions is recommended.

Vector manufacture is conducted in grade A laminar flow cabinets in grade B clean rooms, commonly using HEK293T packaging cell lines derived from a master cell bank (MCB), assuming the appropriate licencing agreements with Rockfeller University are in place. Quality control of the MCB is outlined in Table 8.1 (Perpiñá et al. 2020).

Nonviral techniques for gene transduction or gene-editing are under investigation in preclinical and early clinical trials (Prommersberger et al. 2021).

The vector manufacturing process takes 10 to 14 days and is outlined here. Packaging cells are expanded in flasks and transferred into cell culture chambers followed by plasmid transfection using polyethylenimine. Fixed quantities of plasmids encoding CAR, viral envelope, and gagpol are required. Following transfection, supernatants containing the secreted vector are harvested, clarified using 0.45-mm membranes, concentrated prior to diafiltration and cryopreservation in aliquots, and stored at -80°C until use. Quality measures are outlined in Table 8.2 (Castellà et al. 2019).

Table 8.1 Quality control for the HEK293T master cell bank

Parameter	Method	Acceptance criteria
Appearance	Visual inspection	Presence of adherent cells with thin extensions
Sterility	Microbial growth	Sterile
Mycoplasma	PCR	Absent
Adventitious viruses	PCR	Absent
Karyotype	G-band staining	Informative
Cell viability (%) after thawing	Neubauer cell counting with trypan blue exclusion	>70%

Table 8.2 Quality control for GMP-grade virus production

Parameter	Method	Acceptance criteria
Appearance	Visual inspection	Yellowish liquid solution
Viral titre	Limiting dilution	$>3.75 \times 10^7$ TU/mL
Sterility	Microbial growth	Sterile
Mycoplasma	PCR	Absent
Identity	PCR	Positive
Replication-competent lentivirus	Real-time PCR	Absent

Manufacturing CAR-T Cells

CAR-T cell manufacturing is conducted over approximately 8–12 days in an approved cGMP clean room facility in a closed or functionally closed system to reduce the risk of product contamination (Roddie et al. 2019; Schubert et al. 2019; Castellá et al. 2020).

Starting material for CAR-T cells includes CD3+ T cells derived from nonmobilized leukapheresis (see Chap. 6). Mandated leukapheresis requirements of academic manufacturers for total nucleated cells (TNCs) and CD3+ T cells must be defined; as an illustration, the Uni. Heidelberg HD-CAR-1 protocol (EudraCT No. 2016-004808-60) requires 20×10^8 TNCs and 10×10^8 CD3+ T cells, similar to Novartis requirements for the manufacture of tisagenlecleucel. Leukapheresis material may be cryopreserved prior to manufacture, but in a bid to shorten the manufacturing process, there is a trend towards using fresh leukapheresis material. CAR-T manufacture is a stepwise process outlined in Table 8.3:

Upon completion of manufacturing, CAR-T products must comply with quality control/end-product specifications stipulated in the certificate of analysis. Parameters may vary, but CAR-T products are usually characterized for release according to

Table 8.3 CAR-T manufacturing methodology

	Potential methods	Timepoint
Step 1: T cell enrichment post-leukapheresis (<i>optional</i>)	Ficoll density gradient centrifugation; elutriation; immunomagnetic bead separation	Day 1
Step 2: T cell activation using synthetic antigen presenting technologies (CD3 +/- CD28) (<i>required</i>)	Soluble monoclonal antibodies; Para-magnetic anti-CD3/CD28 antibody coated beads; polymeric biodegradable CD3/28 incorporating nanomatrix (TransAct™)	Days 1, 2
Step 3: T cell stimulation (<i>required</i>)	IL-2, IL-7, and IL-15 in the culture medium (as per protocol) (Hoffmann et al. 2018; Gong et al. 2019)	From day 1 onwards
Step 4: Gene delivery/transduction with a retroviral or lentiviral CAR vector (<i>required</i>)	In some processes, retronectin or Vectofusin® is used to enhance transduction (<i>optional</i>)	Days 2, 3
Step 5: T cell expansion (<i>required</i>)	T-flasks, plates or culture bags; bioreactors, e.g., G-Rex™ flask (Wilson Wolf Manufacturing); Xuri WAVE™ Bioreactor (GE Life Systems); CliniMACS Prodigy™ (Miltenyi BioTec)	Days 3, 4 and onwards
Step 6: T cell harvest and cryopreservation (<i>required</i>)	The cryopreservation methodology often mirrors processes defined for haematopoietic cells. Methods include passive freezing (−80 °C freezer) and controlled-rate freezing	Day 8 onwards
Step 7: CAR-T cell quality assurance control and release testing	In-process and end of process controls are taken to ensure the product complies with release criteria specifications	Day 8 onwards

Table 8.4 Quality control of CAR-T cell biology and microbiology

Parameter	Method	Acceptance criteria
Appearance	Visual inspection	Cloudy liquid solution
CAR+ cells (%) ^a	Flow cytometry	>20%
CD3+ cells (%)	Flow cytometry	>70%
Cell viability (%)	Neubauer cell counting with trypan blue exclusion ^b	>70%
Sterility	Microbial growth E. Ph. 2.6.1	Sterile from bacteria/fungi
Mycoplasma	PCR ^c	Absent
Endotoxin	Chromogenic assay	<0.5 EU/mL
<i>Optional/R&D</i>		
CAR/CD45RA/CCR7 For detection of TE/ TEM/TEMRA/TCM/TN subpopulations	Flow cytometry	A high proportion of immature T cells is desirable for a long-lasting CAR-T cell effect in the patient
Cytotoxic potency	Cr-51 release assays in tumour CAR-T cell co-culture, assessed by flow cytometry	>40% killing at an effector/target ratio of 10:1 (or higher ratio) in a 4-h assay
Adventitious viruses	PCR	Absent
Number of transgene copies/cell	Real-time PCR (Kunz et al. 2019; Schubert et al. 2020)	<10 (range <7–15!) copies/cell ^d

^aAutomated cell counters, such as Luna™, are highly recommended

^bHighly specific detection reagents (e.g., the Miltenyi Detection Reagent™) are strongly advised to distinguish CAR-T cells from the negative fraction

^cEuropean standards stipulate PCR methodology, in contrast to US regulations, which require serology

^dDiffers between countries and products

immunophenotypic, functional, and sterility assessments (Table 8.4). An out-of-specification (OOS) product cannot be released in the usual way, and its clinical use is at the discretion of the treating physician in concert with the regulatory authorities, informed through an OOS report.

Summary and Key Points

- Point-of-care/decentralized CAR-T cell manufacturing has the potential to enhance patient access to CAR-T products.
- Limitations include the requirement for local cGMP facilities/trained staff and lack of standardization across multiple sites.
- Potential solutions include implementation of standardized, semiautomated manufacturing platforms, such as the Miltenyi CliniMACS Prodigy™, and the use of standardized release assays reported in a common format across manufacturing sites to enable the manufacture of consistent, high-quality products between patients.

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