nature protocols
Recipes for Researchers

Production of Chimeric Antigen Receptor T cells

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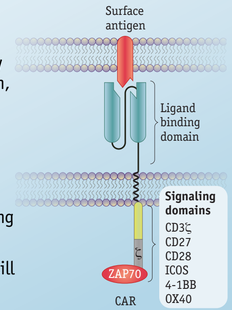
T cells engineered to express Chimeric Antigen Receptors (CARs) induce high rates of clinical responses in patients with relapsed/refractory hematologic malignancies, and have demonstrated early indications of clinical activity in solid tumors. The manufacture of CAR T cell therapies presents significant and unique challenges. The manufacturing typically begins with autologous cells and ends with an expanded, modified and viable therapy to be re-infused to patients. Collection and enrichment of adequate starting T cell populations is often difficult in heavily pre-treated populations. *Ex vivo* modification, activation, and expansion require sophisticated equipment and expertise. Manufacture of these products according to current Good Manufacturing Practices (cGMP) must operate within the bounds of a robust quality management system. Finally, handling of a formulated product must maintain product stability and chain of custody.

What is a CAR?

- Chimeric Antigen Receptors are designed with domains derived from different origins, typically including an extracellular ligand binding domain, a transmembrane domain, and intracellular signaling domains.
- The extracellular ligand binding domain confers target specificity. The intracellular signaling domains drive CAR T cell effector functions. For durable T cell activation, co-stimulatory signaling is also required.
- CARs allow the expressing T cell to effectively kill target tumor cells[1,2] and ideally to persist and provide ongoing immune surveillance.



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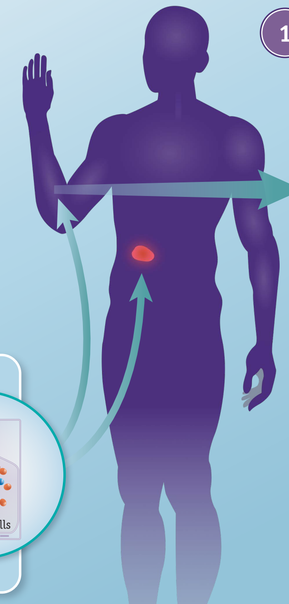
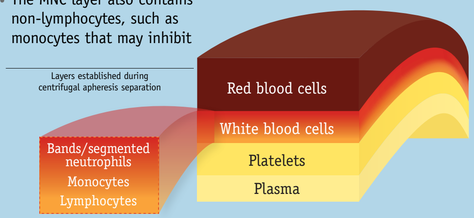


1 Apheresis collection

For most patients, leukapheresis is an efficient centrifugation-based method for collecting large numbers of MNCs, including T cells. T cell yields vary significantly based on patient, disease and collection factors. Particularly in patients with advanced malignancy who have an extensive treatment history, collection of T cells sufficient for the CAR T cell manufacturing cycle may be difficult.

Points to consider

- MNC collection requires consistent blood flow through the instrument of about 50-100 mL/min. Peripheral access in patients with advanced malignancy is challenging. Inconsistent access, leading to intermittent decreases in flow rates, can generate low purity products. Placement of a central venous catheter maintains more consistent blood flow; however, such access is associated with additional risk to the patient (e.g. infection, traumatic placement).
- Patients treated with cytotoxic therapy often have low peripheral lymphocyte counts and therefore fewer lymphocytes will be collected.
- Contaminants such as red blood cells and granulocytes may be found to varying degrees in MNC collections.
- The MNC layer also contains non-lymphocytes, such as monocytes that may inhibit CAR T cell growth in culture and may contain circulating tumor cells. Further enrichment can reduce these non-lymphocyte MNCs, however, these techniques may also decrease T cell yield.
- MNC collection and cryopreservation procedures may vary from site to site. Standardization and acceptance criteria are necessary when starting material is received from multiple sites.



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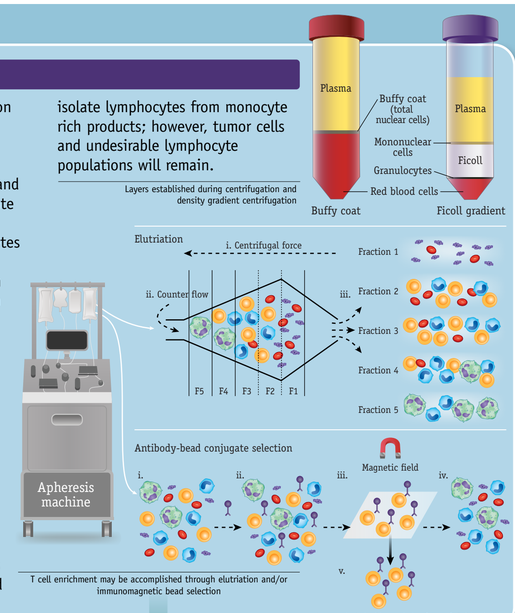
2 Enrichment

T cell enrichment from MNC collection can occur via a variety of methods. Density gradients (top panel) can efficiently remove non-MNC contaminants such as granulocytes and red blood cells. Methods that separate based on both cell size and density (middle panel) can isolate lymphocytes from monocyte fractions. Antibody-bead conjugates (bottom panel) can isolate pure T cell subsets with high specificity via magnetic separation.

Points to consider

- T cell yield and purity differs among collected products. Therefore, the optimal method(s) for T cell enrichment depends on analysis which may be unknown prior to receipt at the manufacturing facility.
- Ficoll density gradients are incapable of separating lymphocytes from monocytes and may require open systems. Systems that separate cell types by size and density are able to efficiently isolate lymphocytes from monocyte rich products; however, tumor cells and undesirable lymphocyte populations will remain.



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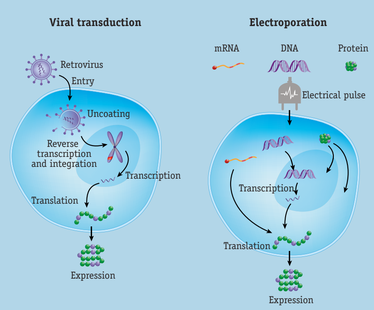
3 Gene modification

Robust CAR gene delivery can be achieved with electroporation (right panel) or viral vectors (left panel) from murine-derived retroviruses or lentiviruses[3-8].

Next generation CAR T cells will multiplex additional modifications, including through gene editing. ZFNs, TALENs, meganuclease-TALENs, and CRISPR RNA-guided nucleases specifically bind to unique DNA sequences based on amino acid sequence of the targeting region and direct cleavage and locus disruption[9,10].

Points to consider

- Although integration is associated with a theoretical risk of insertional oncogenesis, this has not been observed in primary T cells[11,12].
- Retroviral vector lots must be tested to ensure the absence of replication-competent retrovirus.
- Nonintegrative expression via electroporation of mRNA avoids the theoretical risk of insertional oncogenesis, but leads to transient expression.
- All methods of gene modification are associated with some degree of *ex vivo* cytotoxicity that may lead to substantial cell loss during manufacture.

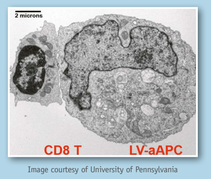


4 Activation & *ex vivo* expansion

T cells can be polyclonally stimulated using an off-the-shelf aAPC system consisting of anti-CD3/anti-CD28 immunomagnetic beads[13] or renewable cell-based lentivirally modified LV aAPCs[14] that can be armed with an array of costimulatory ligands to induce robust proliferation. Closed culture systems reduce the risk of contamination and facilitate efficient media exchange to promote optimal *ex vivo* expansion.

Points to consider

- Validation of critical raw materials used in manufacture is required to ensure consistency is achieved.
- Small-scale techniques conducted in preclinical laboratories require process validations for scale-up and development of standard operating procedures to produce clinical protocol-specified doses according to cGMP.



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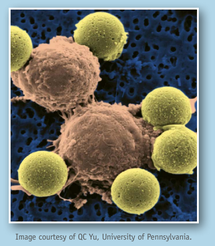

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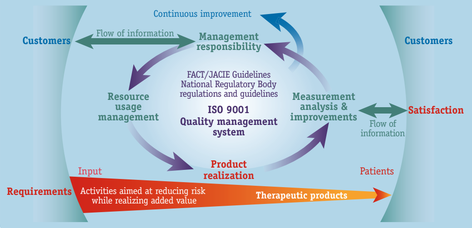
5 Quality assessment

The Quality Assurance unit through the Quality Management System (panel below) ensures continuous control, traceability, and documentation is maintained for all processes and approves or rejects the product for release. Cell therapy accrediting organizations such as FACT or JACIE[15,16] provide standards consistent with regulations outlined in Title 21 CFR, Parts 210 and 211 as governed by CBER of the FDA[17]. Release testing for clinical trials to ensure the identity, purity, sterility, safety, and potency is based on assays and specifications described in an FDA Investigational New Drug Application. Testing typically includes viability, immunophenotyping [including the percentage of CAR+ T cells], Gram stain, endotoxin, bacterial and fungal testing, and mycoplasma testing.

Points to consider

- Improved safety testing assays rapidly identifying product contaminants are necessary to reduce turnaround time to complete mandated safety testing to treat critically ill patients.
- While potency assays are not required to be validated until later phase trials prior to BLA submission, biomarker assays identifying cell, final product, tumor, or patient characteristics correlating with potency and clinical outcome may deepen understanding of mechanisms of action.



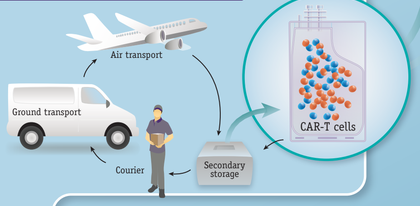
6 Formulation, delivery and administration

When the final cell product is formulated, samples are allocated for release testing, for infusible doses, and for archiving. One single dose may provide long term activity, as gene-modified T cells can still be detected more than a decade after initial infusion[18,19]. The clinical protocol specifies route of administration, which can include system intravenous infusion or intratumoral/tissue injections.

Cryopreservation, storage, shipping, transport, receipt, chain of custody, thaw and administration all become links in the logistics chain. Processes must be validated to maintain product integrity and stability.

Points to consider

- Rapid, reliable release assays coordinated with formulation, packaging and shipping logistics reduce the turnaround time needed to deliver the cell product back to the patient
- Validation and standardization of processes to ensure appropriate control of chain of custody and identity is needed for each clinical site.



STEMCELL Technologies

The process of manufacturing CAR T cells for cancer therapies or other applications may require the isolation of T cells or T cell subsets and the activation and expansion of T cells. STEMCELL Technologies provides fast, easy and column-free immunomagnetic separation platforms for the isolation of highly purified T cells. The isolated T cells are immediately ready for activation and expansion using optimized ImmunoCult™ T cell activation and expansion reagents.

ISOLATE. EasySep™ (www.EasySep.com) is a fast, easy and column-free immunomagnetic cell separation system for isolating highly purified immune cells in as little as 8 minutes. EasySep™ Release CD3 Positive Selection Kit (Catalog #17751) allows researchers to positively isolate T cells free of magnetic particles.

ACTIVATE AND EXPAND. ImmunoCult™ (www.ImmunoCult.com) is a collection of cell activators, expansion media and differentiation supplements. ImmunoCult™ Human T Cell Activators (Catalog #10970/10971) are designed for robust activation in the absence of magnetic beads, feeder cells or antigens. Once activated, T cells can be expanded in the serum- and xeno-free ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981).

For more information, please visit www.stemcell.com.

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Abbreviations

APCs: Antigen Presenting Cells; aAPCs: Artificial Antigen Presenting Cells; AV: Adenovector; BLA: Biologics Licensing Application; CAR: Chimeric Antigen Receptor; cGMP: current Good Manufacturing Practices; CBER: Center for Biologics Evaluation and Research; CFR: Code of Federal Regulations; DNA: Deoxyribonucleic Acid; FACT: Foundation for the Accreditation of Cellular Therapy; FDA: Food and Drug Administration; JACIE: Joint Accreditation Committee-ISCT & EBMT; LV: Lentiviral vector; RNA: Ribonucleic Acid; RV: Retroviral vector; TDN: Transduction; TFN: Transfection

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pathbio.med.upenn.edu/cvpf/site/

www.med.upenn.edu/cci/

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