Production of Chimeric Antigen Receptor T Cells

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 T cells, an expanded, modified, and viable therapy to be 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 the current Good Manufacturing Practices (cGMP) standards 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 target tumor cells1 and ideally to persist and provide ongoing immune surveillance.

Apheresis collection
For most patients, leukopheresis is an efficient centrifugation-based method for collecting large numbers of MNCs, including T cells. T cell yields are 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
• PRC collection requires consistent blood flow through the instrument of at least 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, this approach 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 PRC collections.
• The PRC 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-lymphocytes; however, these techniques may also decrease T cell yield.
• PRC collection and cryopreservation procedures may vary from site to site. Standardization and acceptance criteria are necessary when starting material is received from multiple sites.

Intratumoral/tissue injections
When the final cell product is manufactured, it is often administered all become links which can include systemic chemotherapy administration and intratumoral/tissue injections. Which can include systemic chemotherapy administration and intratumoral/tissue injections.

Activation & ex vivo expansion
T cells can be polytetramethyl-stimulated using an off-the-shelf aAPC system consisting of anti-CD3/anti-CD28 immunomagnetic beads2 or a commercially available lentivector-based genetically modified CAR2 (lCARs) that can be armed with an array of non-targeting ligands to induce robust proliferation. Closed culture systems reduce the risk of contamination and facilitate efficient media exchange to promote optimal or viral 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 generate clinical protocol-specific doses according to cGMP.

Enrichment
T cell enrichment from MNC collection can occur via a variety of methods. Densely populated (high panel) can efficiently remove non-PMNC contamination such as granulocytes and red blood cells. Methods that separate based on both cell size and density like those previously used to isolate lymphocytes from monocyte fractions. Antibody-based conjugates (bottom panel) can isolate pure T cell subsets with high specificity via magnetic cell separation.

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

Quality assessment
The Quality Assurance unit through the Quality Management (panel below) assesses conformance control, bioactivity, and documentation and is maintained for all processes and approvals or the products for release. Cell therapy accrediting organizations such as FACT or AABB3 provide standards consistent with regulations outlined in Title 21 CFR, Parts 210 and 211 as governed by OMB of the FDA4. Release testing for clinical trials to ensure the identity, purity, 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 cells5), granulocytes, bacteria and fungal testing, and mycoplasma testing.

Points to consider
• Rapid, reliable release assays coordinated with formulation, packaging and shipping logistics reduce the time needed to deliver the cell product back to the patient.
• Validation and implementation of processes to ensure appropriate control of batch numbering and identity is needed for each clinical site.

Gene modification
Robust CAR gene delivery can be achieved either of option protocol (left panel) or viral vectors (right panel) from murine-derived retroviral elements.

Points to consider
• Viral vector integration is associated with a theoretical risk of insertional oncogenesis, this has not been observed in primary T cells.6
• Retroviral vector stocks must be tested to ensure the absence of replication-competent retroviruses.
• Restriction enzyme expression via dephosphorylation of mRNAs avoids the theoretical risk of insertional oncogenesis, but leads to transient expression.
• All methods of gene modification are associated with some degree of off-target cytotoxicity that may lead to substantial cell loss during manufacture.