Manufacturing Processes for Engineered T-Cell Therapy – CAR-T

Why T-cells are important

• They recognize host vs. non-host cells to initiate an immune response
• They’re the primary immune system agents that attack diseased cells or infection
• They’re used in several cell-based therapies:
  • Transplantation of donor T-cells - a bone marrow transplant, in which a matched donor’s cells are used to replace the patient’s cells, is the most widely used approach—typically for diseases that originate in the immune system (e.g., leukemia)
  • Isolation and enrichment of a patient’s T-cells – an approach considered the safest because it enhances a patient’s own immune response to disease
  • CAR-T therapy – a more recent approach in which T-cells are removed, genetically engineered to express a synthetic receptor against specific cancer cells, then infused back into the patient’s bloodstream; this therapy is effective because many cancer cells “hide” from the immune system by appearing to be normal cells, thereby triggering no immune response

Examples of parameters collected during cell therapy production

<table>
<thead>
<tr>
<th>Data Collected</th>
<th>Why and When</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable Cell Count</td>
<td>Used throughout process to monitor cell health and impact of any cell manipulation events</td>
</tr>
<tr>
<td>Cell Size and Volume</td>
<td>Used as label-free method to monitor cell population during expansion, differentiation and/or transduction stages</td>
</tr>
<tr>
<td>Cell Count</td>
<td>General population monitor, particularly during expansion stages and when sample volume is manipulated (e.g., during concentration and packaging)</td>
</tr>
<tr>
<td>Aggregate Detection</td>
<td>Primarily a concern during packaging and prior to patient administration</td>
</tr>
<tr>
<td>Gene Expression</td>
<td>Looking for specific surface marker expression or reporter gene expression</td>
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<tr>
<td>Metabolic Indicators</td>
<td>General monitoring during expansion stages when cells are under continuous culture</td>
</tr>
<tr>
<td>Immunophenotyping</td>
<td>Critical to ensure correct cell types are isolated and enriched in therapy population; often used to define purity</td>
</tr>
</tbody>
</table>

Learn more about CAR-T Solutions at Beckman.com/home
CAR-T production workflow

T-cell Handling Workflow

- Leukapheresis
- Isolation of T-cells
- CAR genetic modification of the cell
- T-cell activation/expansion
- Selective cell enrichment
- Larger scale expansion
- Concentration of cells
- Optimization of cell conditions and packaging
- Administration

Generating the CAR Vector

- Cancer biopsy
- Biomarker identification
- CAR engineering
- Vector production
- T-cell transduction

Cryopreservation (Optional)

Vi-CELL MetaFLEX
Multisizer 4e
Vi-CELL XR
Elutriation Centrifuge Avanti J-26S XP
Vi-CELL XR
Multisizer 4e
CytoFlex
Multisizer 4e
Vi-CELL XR
High-Performance Centrifuge
Vi-CELL MetaFLEX
Multisizer 4e
CytoFlex
Leukapheresis, sample screening and viability monitoring

Multisizer 4e Coulter Analyzer
• Evaluate incoming samples
• Accurate cell volume and size analysis
• Rapid cell count

Avanti J-26S XP High Performance Centrifuge for Elutriation

The counterflow centrifugation elutriation system is a gentle, yet powerful technique for harvesting large populations of living cells, resulting in high viability rates.
• Collection and separation of cells using the JE-5.0 elutration rotor
• Select chamber flow rate and centrifugal speed based on cell size
• Uses counterflow to separate based on density and size
  • Centrifugal force versus flow pump
  • Pushes cells into flask for easy, high-yield collection

T-cell activation and population expansion

Cell volume is a critical indicator of the activation state of the T-cell.

Multisizer 4e Coulter Analyzer
• Monitor cell volume change after activation to determine cell state
• Monitor cell proliferation during expansion

Vi-CELL XR and MetaFLEX
• Viable cell count using trypan blue if required/desired
• Rapid metabolite analysis to monitor bioreactor health
T-cell enrichment and characterization

**Multisizer 4e Coulter Analyzer**
- Monitor cell volume change after transduction with CAR vector
- Monitor cell proliferation during expansion

**CytoFLEX Flow Cytometer**
- Determine gene expression level of CAR vector
- Measurement of cell surface markers to confirm the appropriate cell population

**Vi-CELL XR and MetaFLEX**
- Viable cell count using trypan blue if required/desired
- Rapid metabolite analysis to monitor bioreactor health

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Collection, concentration, packaging and QC of T-cells

**Multisizer 4e Coulter Analyzer**
- Evaluate incoming samples
- Accurate cell volume and size analysis
- Rapid cell count

**Avanti High-Performance Centrifuges**
Pelleting of cells from large harvest/culture
- 1L fixed-angle option with the Avanti JXN-26 and JLA-8.1000
- 2.25L swinging bucket option with the Avanti J-HC and JS-5.0

Packaging of cells into Harvest Liner System
- Minimize risk of contamination
- Higher yields
- Increase in workflow efficiency