Exploring Manufacturing Options for Engineered T-cell Therapies

International Society for Cell & Gene Therapy

Lab Practices Committee

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Introduction

- **T-cell immunotherapy has been growing exponentially over the past several years, with hundreds of new clinical trials being initiated every year for CAR and TCR therapies.**

- **Enabling world-wide availability of engineered T-cells in a robust, cost effective, and safe manner remains a significant challenge.**

- **Many options now exist for users to automate and optimize key process steps including**
  - **T-cell selection/purification**
  - **T-cell expansion**
  - **Viral transduction**
  - **Harvest/Fill finish**

Note: Materials and equipment listed in this presentation do not represent an exhaustive list. Due diligence is required from the user to ensure the appropriate material/equipment is used for their particular process.

Potential benefits to using automated closed-system technology

- Ability to maintain sterility using functionally closed disposables
- Reduce operator errors by relying on pre-programmed steps
- Increased consistency by reducing technician-to-technician variability
- Reduce hands-on time for technicians, which may reduce product cost
- Less skilled workforce required; potential cost savings in training staff

Cell Purification

1) **Counter flow centrifugation (Elutriation)**
   - Pros = quick; simple; good for separating out monocytes and granulocytes into discrete bags
   - Cons = not efficient in removing B/T cell blasts if present, which can inhibit the culture

2) **Density Gradient separated lymphocytes**
   - Pros = quick; simple; closed system automated device available (Sepax)
   - Cons = not efficient in removing B/T cell blasts if present, which can inhibit the culture

3) **Cell 'Enrichment' using CD3/CD28 Dynabeads**
   - Pros = used routinely; already part of the stimulation process in some cultures
   - Cons = monocytes (if present) engulf beads and are often carried into culture, suppressing T cell activation; requires additional Dynabead de-beading step mid-process on the Dynamag

4) **T-cell Positive Selection using antibodies directed toward T-cells**
   - Pros = high T-cell purity, automated closed system devices (CliniMACS Plus, Prodigy)
   - Cons = tends to be more costly option (beads, tubing set, instrument, maintenance)

5) **T-cell Selection using functionally closed flow cytometers**
   - Pros = ability to sort cells on multiple parameters at once (ex. CD45RA, CCR7, CD62L)
   - Cons = instrument cost and maintenance are high, achieving the number of cells for clinical culture may take a significant amount of time

Common examples:

Terumo Elutra

Cytiva Sepax

Thermo Dynamag

CliniMACS Plus

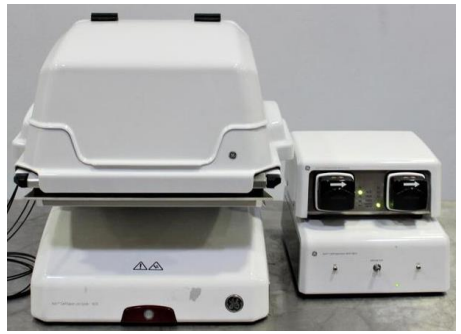
MacsQuant Tyto

Cell Expansion

1) **Cell Culture Bags**
   - Pros = simple and cheaper option; a number of suppliers available
   - Cons = may require extensive hands-on manipulation, with potential increased variability between operators

G-REX

Xuri



2) **Gas-permeable Rapid EXpansion (G-REX) bioreactors (Wilson Wolf)**
   - Pros = variety of sizes available to suit the cell numbers required, cells expand to high density due to optimal gas exchange
   - Cons = cells must be in exponential growth phase (i.e., activated outside G-Rex) requires some manual operations; requires dedicated incubator space

3) **Xuri W25 Cell Expansion System (Cytiva)**
   - Pros = rapid expansion perfusion system with ability to support large volumes (1L up to 20L); flexibility in feed and rock rates
   - Cons = high cost of instrument/disposables; high degree of flexibility may require significant process optimization; does not provide ability to select or enrich cells

Prodigy

4) **CliniMACS Prodigy (Miltenyi)**
   - Pros = automated closed system device, performs selections with high purity and transductions, very user-friendly programming and operation
   - Cons = high cost of instrument/disposables; less flexibility in programming

Cocoon

5) **Cocoon (Lonza)**
   - Pros = automated closed system device that performs transductions and newer versions allow for T-cell selections
   - Cons = high cost of instrument/disposables; less flexibility in programming

Viral Transduction

Spinoculation (or Spinfection)

- **Centrifuge Spinoculation**

  Provides a low-cost, simple method of enhancing viral transduction. However, if performed using bags, some bags are not designed for centrifugations at high speed and may tear/leak during the process. Bags must be qualified for use.

- **Closed System Spinoculation, SEPAX (Cytiva)**

  Closed-system approach that has demonstrated improved transduction efficiency of viral vectors (*Remley et al. 2021*). Cost of the instrument, tubing set, and spin program is significant compared to centrifuge spinoculation.

Transduction enhancers

- **Retronectin (Takara):** A recombinant human fibronectin that contains a heparin domain for binding viruses and a VLA-5 integrin cell binding domain that act together to co-localize cells and virus and enhance transduction efficiency. Available in GMP format. Best results when pre-coating flasks or bags prior to transduction.

- **Vectofusin-1 (Miltenyi):** A histidine rich amphipathic 26-amino acid peptide that acts by promoting the adhesion and fusion of viral and cellular membranes. Due to its solubility, it is easily transferable to closed system devices such as the Prodigy system and does not require pre-coating. Available in GMP format.

- **Lentiboost (Sirion):** A universal polaxamer-based, receptor-independent adjuvant that increases cell permeability which facilitates fusion of lentiviral particles with the cell membrane. Does not require pre-coating. Available in GMP format.

Harvest & Final Formulation

- **LOVO Cell processing system (Fresenius Kabi)**
  Features a spinning membrane technology that allows for automation of cell washing processes that can be useful at the beginning and end of the process. The LOVO can handle volumes up to 22L in a closed, sterile kit, and can concentrate the final product down to as little as 150 mL.

- **Sepax-C Pro & Sefia S-2000 (Cytiva)**
  Also features a spinning membrane in a sterile, closed-system disposable kit, but the addition of a temperature-controlled thermal mixer on the Sefia can be used for the addition of DMSO during final formulation (or staining reagents such as antibodies or beads for automating other parts of the cell manufacturing process).

- **Finia Fill Finish (Terumo)**
  Can perform the final formulation for cryopreservation of up to 3 product bags, chilling the cells and performing the DMSO mixing step in a temperature-controlled manner, potentially improving cell viability compared to manual mixing.

LOVO

Sepax

Sefia

Finia

Questions to consider when choosing a manufacturing platform

- What is the target dose? How scalable does the manufacturing process need to be?
- How close does the investigator want to replicate pre-clinical methods? Are they intending to compare to other clinical trials using a certain system?
- Does the manufacturing scheme require non-standard manipulations that would be difficult to perform on an automated system? Are there significant technical limitations?
- How quickly can staff be trained on the new process?
- How does cost factor into the decision-making?

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