Case Study – Improving the Expansion of Patients’ T Cells
Overcoming challenges associated with poor starting material
Outline

• Patient material can be limited due to past medical history and treatments
• T cell phenotype is very indicative of efficacy of therapy
• Serum-free media can aid in deeper understanding of cell phenotype and lead to streamlined process development
CAR-T Therapy Limitations

Global pain points

**CAR-T Manufacturing Challenges**

1. “The biggest challenge is that every patient’s cell is somewhat different... Need to adjust the process accordingly”
   - Customer concerns in China

2. “The challenges start with patient material... when you design processes with healthy material, it is fine... [patients] sometimes have multiple treatments already.”
   - Customer concerns in Europe

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**Potential ways to improve manufacturing from inherently variable starting material**

- Improving transfection/transduction efficiency and cell viability
- Early identification of markers in patient cells
CD45 isoform expression depends on the stage of T-cell maturation, activation, and differentiation.\textsuperscript{2} Naïve T-cells express the isoform CD45RA and the isoform CD45RO is primarily found on primed/memory T-cells.\textsuperscript{2}

“CD45RO\textsuperscript{+} T cell infiltration was significantly associated with improved overall survival (OS) and disease-free survival (DFS)”\textsuperscript{1}
Using the Right Materials from the Start

Robust expansion of T cells needed for improved clinical outcomes

- CFSE represents an extremely valuable fluorescent dye for immunological studies, allowing lymphocyte proliferation, migration and positioning to be simultaneously monitored.

- Around 8 cell divisions can be identified before the CFSE fluorescence is too low to be distinguished above the autofluorescence background.

Figure 1: T-cell proliferation of PBMCs cultured for 4 days in X-VIVO® Medium, AIM V™, TexMACS™, or OpTimizer™ Media in the presence of IL-2 and activated by CD3/CD28 beads. Proliferation is illustrated as CFSE fold change to cells + media only control (represented as 1.0) as measured by flow cytometry. Data is summarized as an average of 3 donors.
Selecting the Right Cell for Expansion is Key for Effective Therapies

Medium used for expansion is critical for process optimization

- When starting with patient material that has been compromised from previous treatments, expansion of memory T cells may help streamline CAR-T manufacturing processes.

- The more details about what makes each therapy effective is greatly important to improve success rates and hopes of moving to allogeneic platforms.

Figure 2: Differentiation of PBMCs (T-Cells) assessed via phenotyping via flow cytometry after 4 days of culture in X-VIVO® Medium, AIM-V™, TexMACS™, or OptiMizer™ Media. Phenotyping at Days 0 and 4 is displayed for CD45RA and CD45RO subtypes as a percentage of the total T-cell population. Data is summarized as an average of 3 donors.
Critical Components of a Successful CAR-T Therapy
Viability, transduction efficiency, and large cell numbers

“T cells naturally experience a dip in viability early in culture which is why most processes have to go beyond 7-10 days. Getting the critical cell number for each therapy is mandatory”

- CAR-T Process Development Scientist

Figure 3: Differentiation of PBMCs (T-Cells) assessed via phenotyping via flow cytometry after 4 days of culture in X-VIVO® Medium, AIM-V™, TexMACS™, or OptiMizer™ Media. Phenotyping at Days 0 and 4 is displayed for CD45RA and CD45RO subtypes as a percentage of the total T-cell population as viability on day 4. Data is summarized as an average of 3 donors.
Market Pressure

Growing number of CAR-T therapies with only a few being truly novel

Regulatory standards increasing globally amid supply chain challenges

Raw material costs, manufacturer costs, and patient costs under more scrutiny than ever

CAR-T therapies that have reduced costs, less variability, and have been regulatory approved, are key to accelerating speed to market even when starting with previously treated patient material.
Inherently variable starting material complexities with many CAR-T therapies

- Patients that undergo CAR-T therapies have likely had radiation or chemotherapy treatments previously. This may lead their cells to be immunocompromised, stressed, and overall unhealthy.

- Scientists have found that looking at specific cell surface markers that may help select the subset of cells that provide the most efficacy for their therapy.

- T cell surface markers CD45RA and CD45RO have become increasingly important in determining the proliferative capability of a T cell population.

- This study shows that in serum-free media, you can select for these markers and expand an appropriate number of cells to be used for CAR-T therapy.

- Out of the standard serum-free media on the market, X-VIVO® Medium has been shown to perform better than the competition even with short proliferation cycles and smaller initial cell counts.
References

Characteristic CD45RA/CD45RO maturation pattern by flow cytometry associated with the CD45 C77G polymorphism. Elizabeth L. Courville, Monica G. Lawrence First published: 08 February 2021
https://doi.org/10.1002/cyto.b.21993


Interviews of KOLs and cell therapy development experts
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