Enabling a Healthier World

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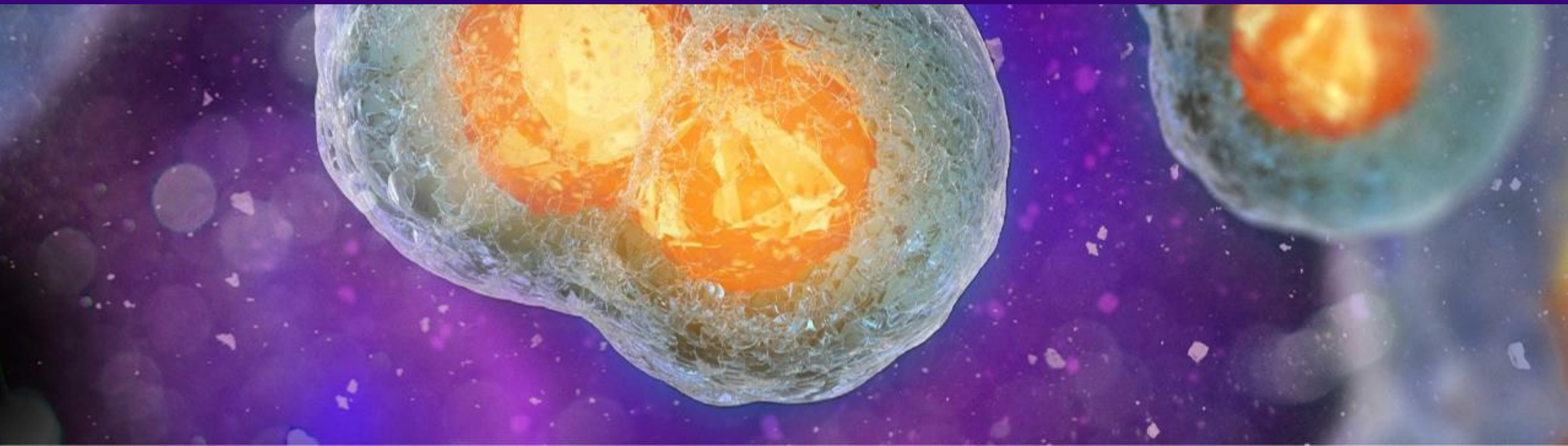
Case Study – Improving the Expansion of Patients' T Cells

Overcoming challenges associated with poor starting material

Public



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

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CAR-T Manufacturing Challenges

01

"The biggest challenge is that every patient's cell is somewhat different... Need to adjust the process accordingly"

-Customer concerns in China[3]

02

"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[3]

Potential ways to improve manufacturing from inherently variable starting material

Improving transfection/transduction efficiency and cell viability

Early identification of markers in patient cells

Selecting the Right Phenotype

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CD45 isoform expression depends on the stage of T-cell maturation, activation, and differentiation.[2]

Naïve T-cells express the isoform CD45RA and the isoform CD45RO is primarily found on primed/memory T-cells.[2]

"CD45RO$^+$ T cell infiltration was significantly associated with improved overall survival (OS) and disease-free survival (DFS)"[1]

Using the Right Materials from the Start

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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

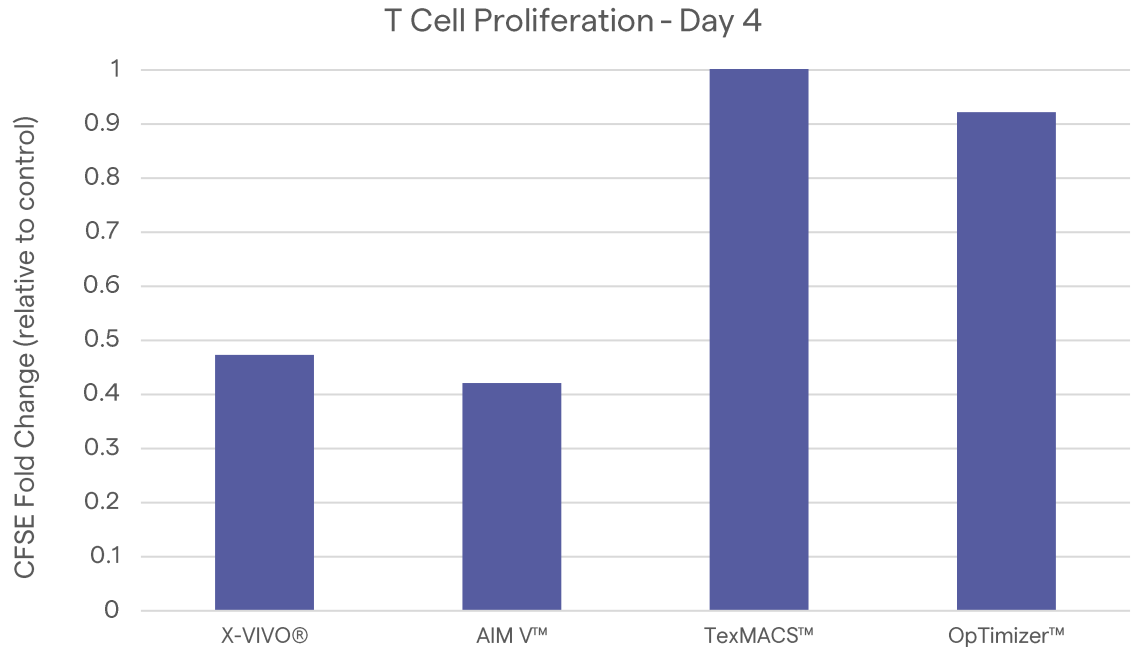

Figure 1: T-cell proliferation of PBMCs cultured for 4 days in X-VIVO® Medium, AIM V™, TexMACS™, or OpTimizer™ Media in the presence of IL-2 and activated by CD3/CD28 beads. Proliferation is illustrated as CFSE fold change to cells + media only control (represented as 1.0) as measured by flow cytometry.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

Selecting the Right Cell for Expansion is Key for Effective Therapies

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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 gravely important to improve success rates and hopes of moving to allogeneic platforms

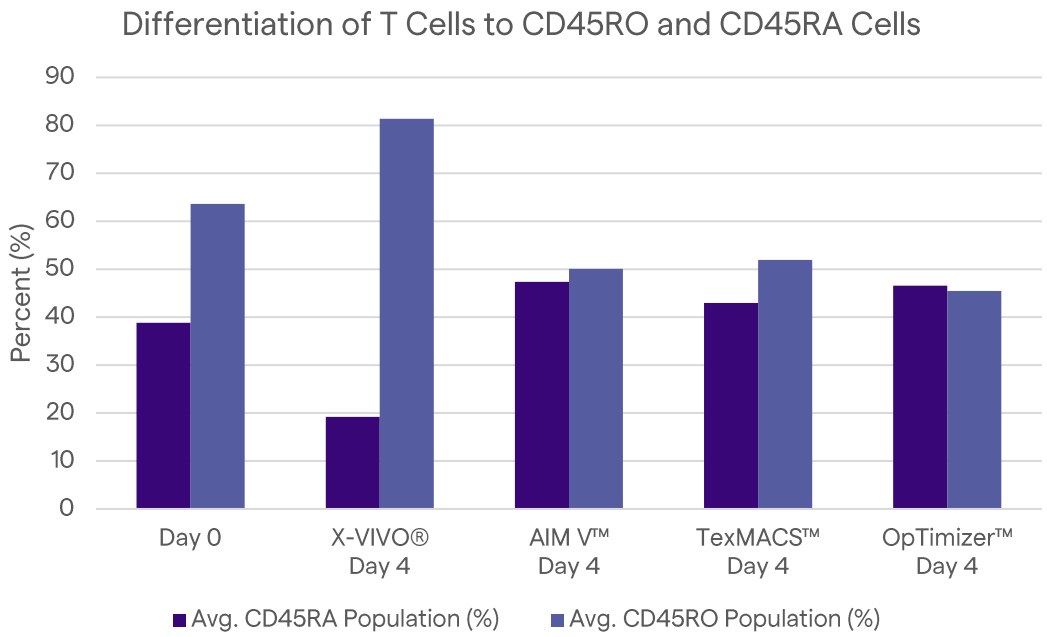

Figure 2: Differentiation of PBMCs (T-Cells) assessed via phenotyping via flow cytometry after 4 days of culture in X-VIVO® Medium, AIM-V™, TexMACS™, or OpTimizer™ Media. Phenotyping at Days 0 and 4 is displayed for CD45RA and CD45RO subtypes as a percentage of the total T-cell population. Data is summarized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

Critical Components of a Successful CAR-T Therapy

Viability, transduction efficiency, and large cell numbers

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"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

High viability from the beginning of expansion further aids in expansion from poor starting material

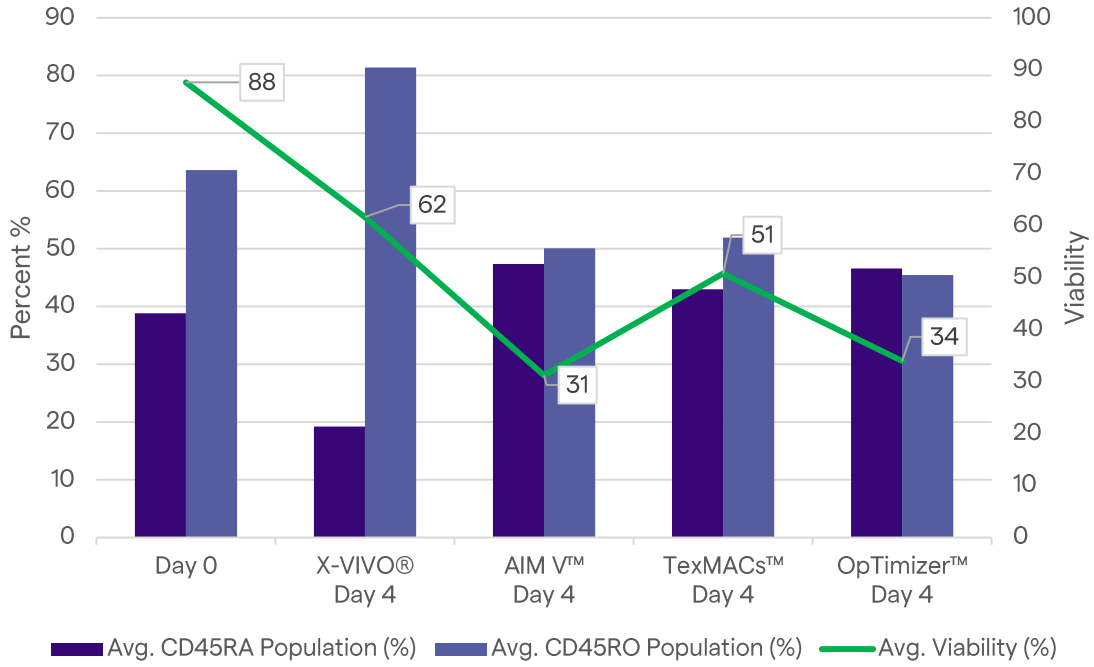

Figure 3: Differentiation of PBMCs (T-Cells) assessed via phenotyping via flow cytometry after 4 days of culture in X-VIVO® Medium, AIM-V™, TexMACS™, or OpTimizer™ Media. Phenotyping at Days 0 and 4 is displayed for CD45RA and CD45RO subtypes as a percentage of the total T-cell population as viability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

Market Pressure

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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 then 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.

Conclusion

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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 then the competition even with short proliferation cycles and smaller initial cell counts

References

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01

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

02

Lyons AB, Parish CR (May 1994). "Determination of lymphocyte division by flow cytometry". *Journal of Immunological Methods*. **171**(1): 131–7. doi:10.1016/0022-1759(94)90236-4. PMID 8176234.

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Interviews of KOLs and cell therapy development experts

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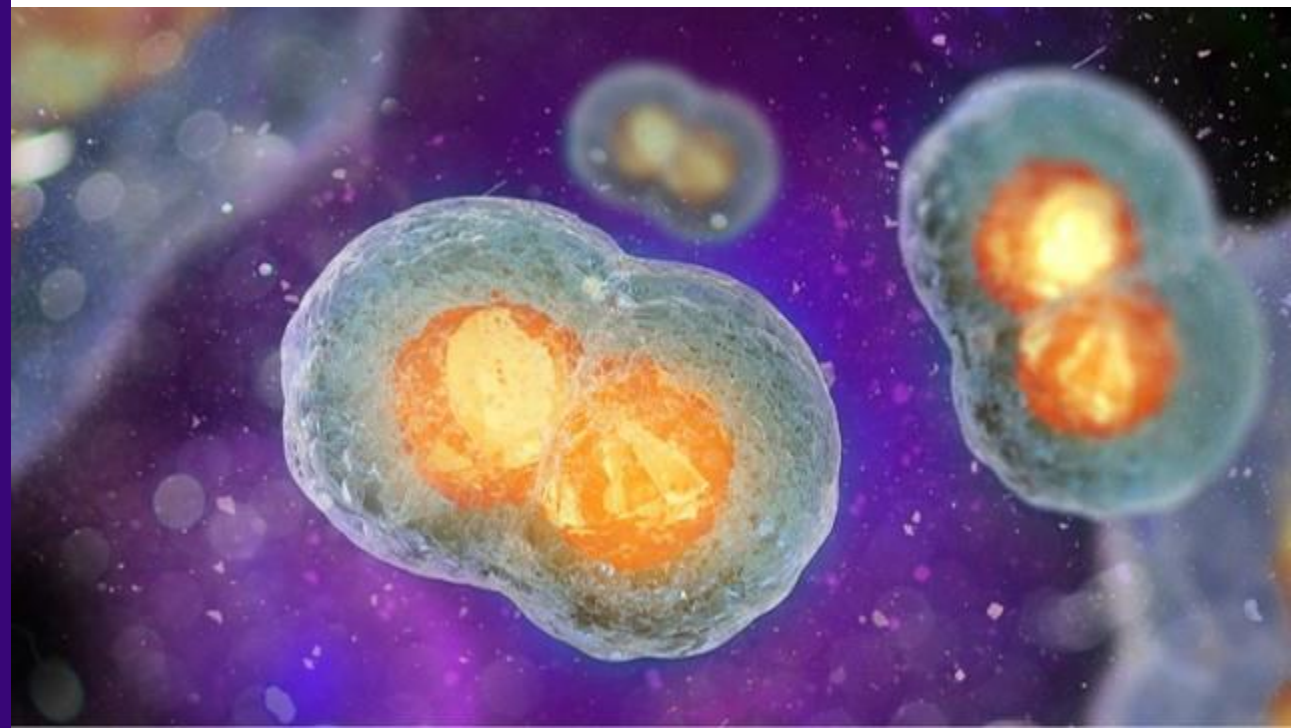

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