PROJECT CASE STUDY:

IMMUNE CELL EXPANSIONS

T Cell Expansions with Custom Flow Analysis and Patient-Matched Primary T Cells

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BACKGROUND:

The client in this case study is a Senior Scientist supporting CAR-T cell discovery efforts for a global pharmaceutical company. Discovery Life Sciences has been a human biologics partner to this client and company for over 8 years providing many different biospecimens and services. During a discussion on a related project, the client asked if we could collect and isolate T cells from healthy donors and expand them via cell culture. Their goal was to create a sample pool of patient-matched primary and expanded T cells to be used in multiple CAR-T cell research and development projects across 3 research groups within the company.

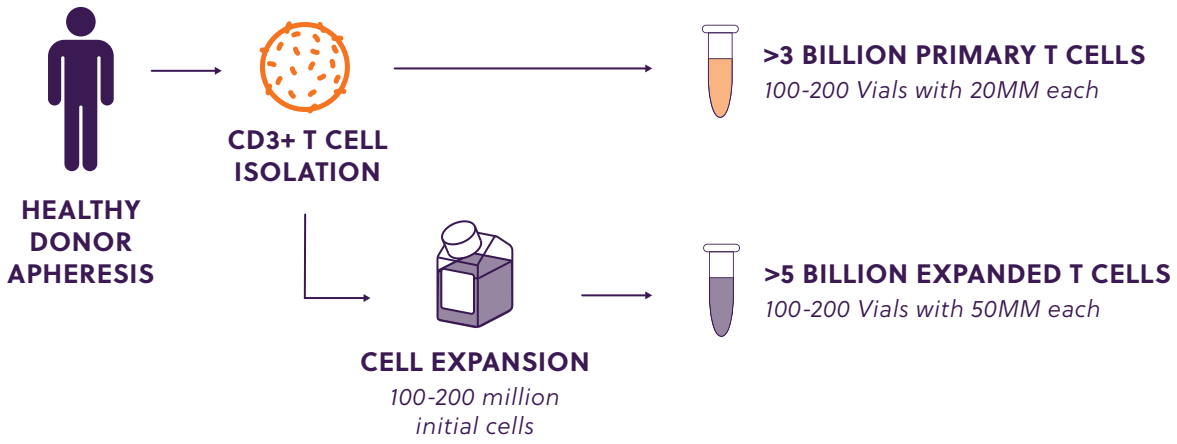
The name of the client and their company could not be disclosed for this case study due to the sensitive nature of their research.

REQUEST:

For this study, the client requested >3 billion primary T cells isolated per patient from apheresis collections from 4 healthy donors with NGS HLA typing. 100 - 200 million primary T cells would then be expanded via cell culture to generate an additional >5 billion expanded T cells.

STUDY PARAMETERS:

- 4 recallable healthy apheresis donors
- 100-200 frozen vials of primary T cells per patient, 20mm
- 100-200 frozen vials of expanded T cells per patient, 50mm
- Inclusion criteria: No steroids or ibuprofen at the time of draw and no vaccines within the last 6 weeks
- NGS characterization for patient HLA typing
- Flow characterization across multiple timepoints (including timepoint 0) for T cell activation markers including: cell count, cell size, CD3, CD4, CD8, CD45RO, CD25, CD27, CD69, HLA-DR, PD1, and 2 proprietary antibodies.



CONSULTATION:

In conjunction with the client, our scientific team evaluated multiple expansion protocols. The original protocols that the client suggested did not allow for the expansions to meet the high cell counts requested. Our experience with large scale immune cell isolations and cell culture ultimately lead to the selection of an alternative protocol that optimized the expansions to meet the goals of this project.

Also, our team suggested that time course data, consisting of cell counts and flow cytometry analysis of cell surface marker expression, be collected throughout the process to ensure that the T cells behaved as expected and to identify when the rest phase of the culture should be performed. The client was excited about this and then asked to include positive controls from internal cell lines and two proprietary antibodies of interest in the flow monitoring. These additions would provide the client with valuable data for ongoing internal projects.

Shawn Fahl, PhD adding suspended T cells and media to the cell culture flasks to initiate expansion.

FULFILLMENT:

Four healthy donor patients were selected, and T cell isolations were generated via apheresis and negative immunomagnetic selections. Both purity and viability met out quality standards when evaluated by flow cytometry, as is the standard practice for all cell isolations in our laboratory. The T cell expansions began the same day as collection, and the 100-200 million isolated T cells were cultured for 15-19 days to reach the desired cell counts.

Data was collected at intervals of 2-3 days throughout the expansions and included both time point 0 and the rest period. The data points collected included cell counts, cell size, and 9 cell receptor markers via flow cytometry. The data from time point 0 serves a dual role as reference for the both primary cells, as well as the baseline for the expanded cell sets.

The client received reports on this data on a weekly basis and at the completion of each expansion to evaluate T cell activation and expansion throughout the process. The raw flow data files were also provided to the client so that they could conduct their own analysis of receptor surface expression on different T cell subsets for their internal projects.

Our logistics team coordinated the delivery of these cell samples across three research groups during project completion. Overall the client was pleased with the outcome of this project, including the quality, purity, and viability of both the primary and expanded T cells.

SUCCESS:

Thanks to an ongoing relationship with Discovery Life Sciences, the client was able to proactively inquire about an upcoming need and receive consultation in the design and execution of a study that provided multiple research groups with the T cells samples needed to further their CAR-T cell research. Since the completion of this project, the client has recalled the 4 donors from this study and selected more healthy donors to continue providing primary T cells in support of their ongoing research studies.

This study was successful because the client was:

- Provided scientific consultations to optimize project deliverables
- Engaged in a direct and collaborative approach with our scientists that lead to the inclusion of additional data points
- Assured that the cells had high purity and post-thaw cell viability
- Delivered the quantities of patient-matched samples needed to conduct their R&D across multiple research groups