Production of T cells for adoptive cellular immunotherapy using the Xuri Cell Expansion System W5

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Production of T cells for Adoptive Cellular Immunotherapy using the Xuri™ Cell Expansion System W5

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Introduction

Adoptive cellular immunotherapy products are the most recent to show signs of benefit and therapeutic value to the patient population, and there is an emerging need to develop effective manufacturing processes. The Xuri Cellbag™ bioreactor in combination with the Xuri Cell Expansion Systems (Xuri W25 and Xuri W5) provides a functionally closed, single use bioreactor that requires fewer operator intervention steps, a reduction in the risk of contamination and control of the cell expansion conditions to maximise cell yield. To ensure the successful transition of autologous therapies to the clinic it will be essential that the technology chosen for the production of therapeutic cell preparations offers consistent performance for all patient samples. Data presented here will highlight that at a macro level, systems may appear equivalent or similar; however, it is at the micro level where differences can manifest. We will show how Gas permeable static bioreactors and stirred tank bioreactors (STR) that lack control of the cellular environment during the expansion phase may influence the level of a key surface marker, CD62L.

CD62L is commonly found on the cell surface of naïve T cells and binds to ligands present on endothelial cells. CD62L has a role in lymphocyte trafficking through the blood barrier and facilitating entry into a secondary lymphoid organs.

Materials and Methods

Activation of T cells in static culture

Frozen human peripheral blood mononuclear cells (PBMCs) were thawed and expanded in T-flasks at $1x10^6$ cells per ml in X-VIVO™ - 10 (Lonza)/5% heat-inactivated human serum (PAA or TCS CS-100HI), 2mM GlutaMAX ™ (Life Technologies), 1% penicillin-streptomycin (Life T echnologies)/20ng/ml of IL-2 (Peprotech). T cell expander™ CD3/CD28 beads (Life Technologies) were added at a ratio of 3:1 beads:$CD3^+$ positive cells. After 3 days incubation cultures were diluted and maintained at $0.5x10^6$ cells per ml for an additional 2 days before transfer into either the Cellbag bioreactor or alternative bioreactors.

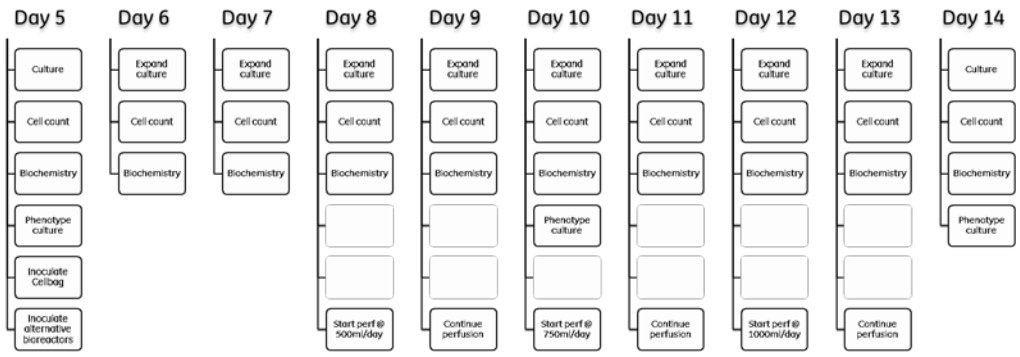
Expansion of T cells

On Day 5 of expansion, cells were transferred to either; 2L Xuri Perfusion Cellbag disposable bioreactor, 1L gas permeable static bioreactor or 500ml stirred tank bioreactor at $0.5x10^6$ cells per ml . Xuri Cellbag bioreactors were placed on the Xuri W5 set at 37ºC /5% CO_2 with a rock rate of 10 rpm and a rock angle of 6º. Cells were maintained at $0.5x10^6$ cells per ml until a maximum working volume of 1L was obtained. Semi-continuous perfusion, with shot volumes of 50ml, was initiated when the cultures reached a cell density of $2x10^6$ cells/ml, and maintained for the remainder of the expansion.
Alternative 0.5L stir tank and 1L gas permeable bioreactors were incubated at 37ºC/5% CO_2, replenished with fresh medium every 48-72 hours according to the manufacturer's instructions. On feed days, cells were collected from the waste medium and returned to the culture to avoid losses.

Phenotypic analysis

Cellular phenotype was performed by flow cytometric analysis at day 10 of culture. Briefly, $1x10^6$ cells were labelled with CD3-perCP-Cy5.5, CD4-PE, CD8-Alexa Fluor488, CD28-APC and CD27-V450, or CD57-APC and CD62L-V450, and analysed on a BD LSRFortessa™ flow cytometer.

Workflow



Results

	Culture Volume	Cell Yield x 10^{10}	Fold increase	Cell Viability (%)	CD3_CD4_CD62L (%)	CD3_CD8_CD62L (%)
Xuri W5 Donor 1	1L	1.64	3.28	97.7	83	71
Gas Permeable Bioreactor Donor 1	1L	0.5	1	91.3	76	64
Xuri W5 Donor 2	1L	1.25	1.78	97.7	99	99
Gas Permeable Bioreactor Donor 2	1L	0.7	1	94.4	96	97
Xuri W5 Donor 3	1L	1.60	2	96.3	91	95
STR Bioreactor Donor 3	0.5L	0.4	1	95.6	78	87
Xuri W5 Donor 4	1L	1.2	1.58	95.5	90	94
STR Bioreactor Donor 4	0.5L	0.38	1	96.8	59	80

Table 1. Summarises the typical data collected from the expansion for T cells. For all donors the Xuri W5 produced higher yields (when corrected for volume differences in the stirred tank systems). Using the % positive as an indicator of phenotype suggests that the systems are very similar for CD3/CD4/CD62L or CD3/CD8/CD62L expression.

Cell Viability

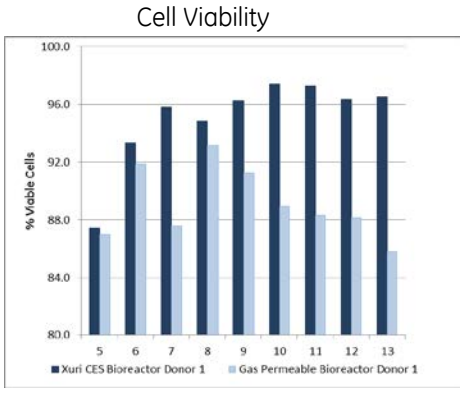


Figure 1. Shows an example of viability of the cell population during the expansion phase. Providing an optimal cellular environment ensures the final cell preparation retains a high degree of viability.

pH

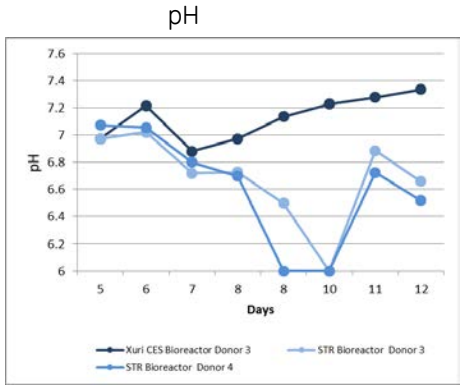


Figure 2. Shows an example of how the pH can vary during the expansion of T cells. At higher cell densities the stirred tank bioreactors , which lack perfusion features, are unable to maintain a suitable pH, resulting in the operator having to exchange medium to correct the pH.

pO2

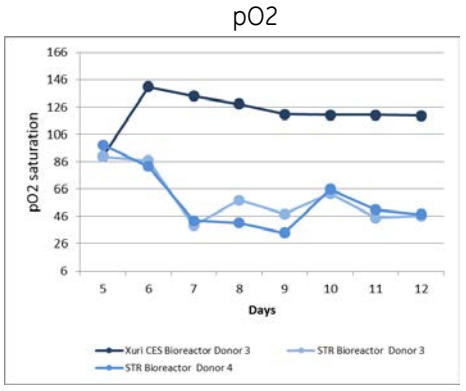


Figure 3. Shows an example of how the pO2 saturation can vary during the expansion of T cells. The lack of effective mixing in the STR bioreactor reduces the capability of the stirred tank system to maintain an acceptable level of dissolved oxygen.

CD62L expression

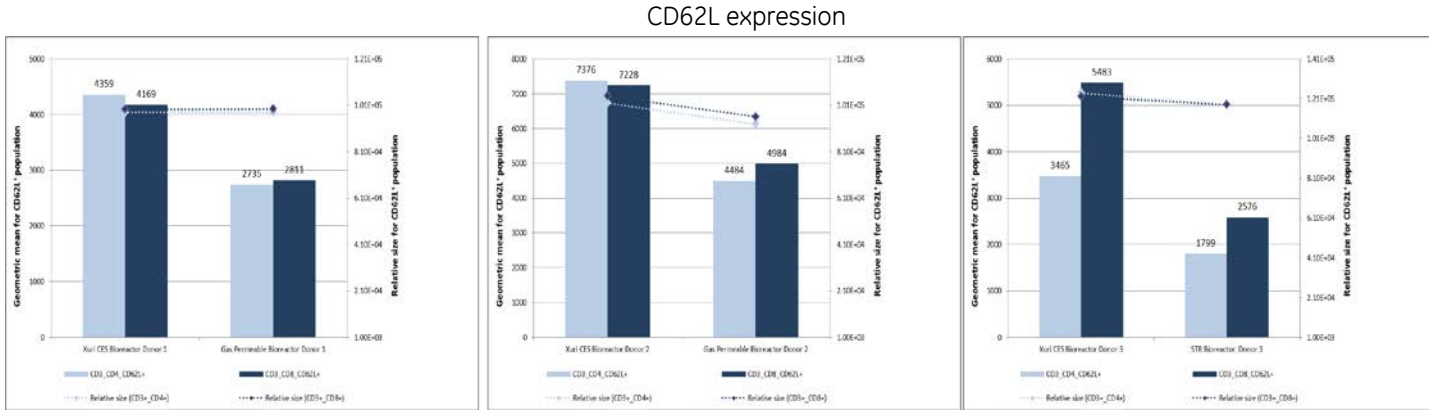


Figure 4. Shows how the expression of the key 'homing' receptor, CD62L (bars and left hand Y-axis), can vary between the alternative and Xuri W5 bioreactors for 3 different donors. Cell size is recorded on the right hand Y-axis and plotted as a dotted line illustrating the difference in object intensity is not a consequence of cell size.

In summary, the data shows equivalent or similar numbers of CD62L positive cells were produced for all bioreactors (Table 1). However, the CD62L populations produced in the Xuri W5 show a higher level of expression of the CD62L receptor.

Conclusion

- Enabling control of the cell culture environment during the expansion of T cells has the potential to provide a consistent outcome with respect to the cell health and phenotype.
- Automated perfusion based approaches increase cell yield, viability and reduce the risk from operator introduced contamination.
- The expression of CD62L on CD4 and CD8 positive cells appears higher on T cells cultivated in the Xuri W5.
- Xuri Cell Expansion System offers a simple, reliable and robust system for the expansion of T cells that may address some of the variation that comes with each patient sample.

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