Expansion of T-cells using the Xuri Cell Expansion System W25 and WAVE Bioreactor 2/10 System

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Expansion of T-cells using the Xuri™ Cell Expansion System W25 and WAVE Bioreactor™ 2/10 System

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Introduction

Immunotherapeutics include drugs and biologics that render therapeutic benefit by harnessing the power of the immune system. The promise of immune-mediated therapies is to target specificity with a consequent reduction in off-therapeutic effects. Immunotherapeutic products can be classified broadly into (1) active immunotherapy (therapeutic vaccines), (2) adoptive cellular immunotherapy (transfer of immune cells, genetically modified T-cells or precursor cells) or (3) passive immunotherapy (antibody or receptor ligand administration). Recent scientific advances have led to clinical trials of both active and passive immunotherapeutic products that have the potential to convert life-ending diseases into manageable conditions. Of the three broad categories of immunotherapeutic products, adoptive cellular immunotherapy products are the most recent to show early signs of benefit and therapeutic value to the patient population. The successful manufacture of cellular immunotherapeutics requires a system that can minimise the risk of cell contamination, achieve high cell densities, allow careful control of the expansion protocol and is designed for use in a regulated environment. The Xuri Cell Expansion System W25 uses a functionally closed, single use bioreactor for working volumes up to 25 L. The system is based on the well known WAVE rocking technology that provides mixing and aeration to the culture. With the Xuri Cell Expansion System W25, the rocking mode is enhanced with features such as advanced sensors and intelligent control strategies. Data presented here demonstrate the equivalent yield and functional performance of cells expanded in the WAVE 2/10 and Xuri W25 systems using comparable culture protocols. The expanded T cells remain biologically functional and can be re-activated to produce high amounts of cytokines.

Materials and methods

Activation of T cells in static culture

Frozen human peripheral blood mononuclear cells (PBMCs) were thawed, washed twice and cultured in T225 flasks at 1 × 10^6 cells per ml in X-VIVO™ - 10 (Lonza) supplemented with 5% heat-inactivated human serum (PAA), 2 mM GlutaMAX™ (Life Technologies), 1% penicillin-streptomycin (Life Technologies) and 20 ng/ml of IL-2 (Peprotech). T cell expander™ CD3/CD28 beads (Life Technologies) were added to the culture at a ratio of 3:1 beads: CD3 + T cell. After 3days cells were counted and maintained at 0.5 × 10^6 cells per ml for an additional 2 days before transfer into Cellbags for culture on the WAVE Bioreactor 2/10 System and the Xuri Cell Expansion System W25 on day 5 of culture.

T cell culture in the Xuri Cell Expansion System W25 and WAVE Bioreactor 2/10

Once a minimal number of 5 × 10^8 cells were obtained in static culture, cells were transferred to two Cellbag-2L with perfusion filter. One Cellbag was loaded onto the Wave Bioreactor 2/10 and the other was loaded onto the Xuri W25. Both bioreactors were set at 37°C with a rock rate of 10 rpm and a rock angle of 6°. Cells were maintained at 0.5 × 10^6cells per ml by adding medium until the maximum volume of 1000mls for the Cellbag was reached. Once the cell concentration had reached a minimum of 2 × 10^6 cells/ml, perfusion commenced for the remainder of the expansion. Perfusion on the Wave 2/10 is run as semi-continuous with shot volumes of 50 mls. To replicate this method using the Xuri Cell Expansion System W25, a semi-continuous perfusion program was written into the method editor module of the Unicorn software (Figure 1).

Phenotypic analysis

The cells were immunophenotyped by flow cytometric analysis at days 0 and 10 of culture: 1 × 10^6 cells were stained with CD3-per CPCy5.5, CD4-PE, CD8-AlexaFluor488, CD28-APC and CD27-V450, or CD57-APC and CD62L-V450, and analysed on a FACS Fortessa flow cytometer using FACS Diva software, according to the manufacturer's instructions (reagents, instrument and software from BD Biosciences).

T cell reactivation and cytokine production

On day 10, cells were harvested from the Cellbag and reseeded in 2 ml of fresh T cell media at 1 × 10^6 cell per ml in a 24 well plate. The cells were reactivated with CD3/CD28 T cell Expander beads for 18 to 20 h. The supernatants were collected and measured for the production of 7 different cytokines (Millipore multiplex assays).

System setup and integration of subunits

Fig 1. Xuri Cell Expansion System W25 set up. The system is supplied with software, bioreactor unit and an environmental control unit. Xuri Cell Expansion System W25 can be used for the cultivation of human T cells at cell densities of > 1 × 10^7/ml, with high viabilities. Because Cellbag bioreactors are disposable, pre-sterilized and functionally closed, they are ideally suited for applications where the prevention of cross contamination is critical.

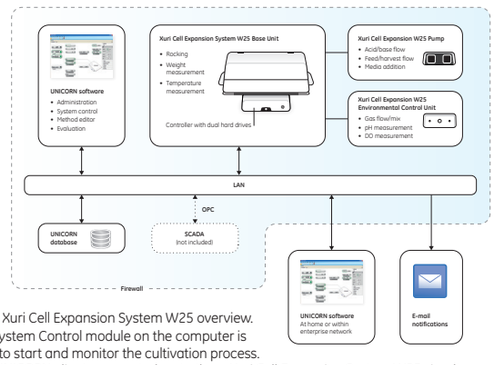
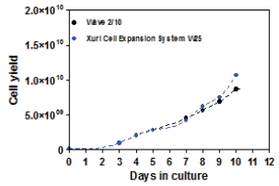
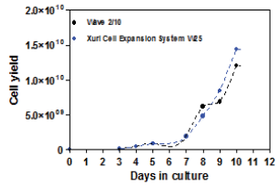


Fig 2. Xuri Cell Expansion System W25 overview. The System Control module on the computer is used to start and monitor the cultivation process. One UNICORN client can control up to three Xuri Cell Expansion System W25 simultaneously. SCADA = supervisory control and data acquisition. OPC = open platform communications. LAN = local area network.

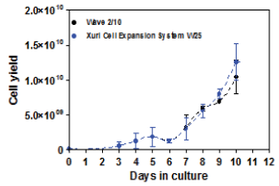
Results



(A) Donor 1 expansion.



(B) Donor 2 expansion.



(C) Combined data for Donors 1 and 2.

Fig 3. Comparison of T-cell expansion in Xuri Cell Expansion System W25 and WAVE 2/10. Donor cells from two individuals were expanded over a period of 10 days using the protocol described above. While the overall cell yields differ between individuals, the performance of the Xuri Cell Expansion System W25 is comparable to the WAVE 2/10.

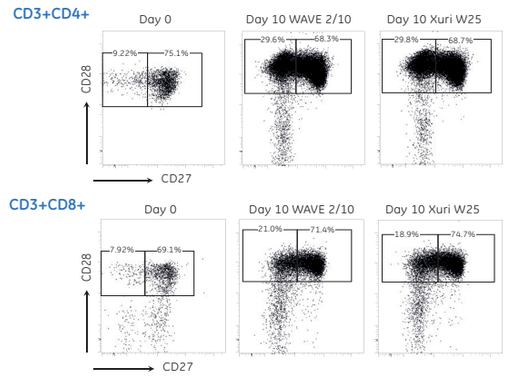


Fig 4. Differentiation state of expanded T cells using markers CD27 and CD28. Naïve and early activated cells are CD27+CD28+, effector T cells are CD27-CD28+ and late effectors or 'aged' T cells are CD27-CD28-. The expression of these two markers on cells grown on the WAVE 2/10 and Xuri W25 were equivalent and after 10 days of culture the majority of cells were in the early/intermediate stages of differentiation.

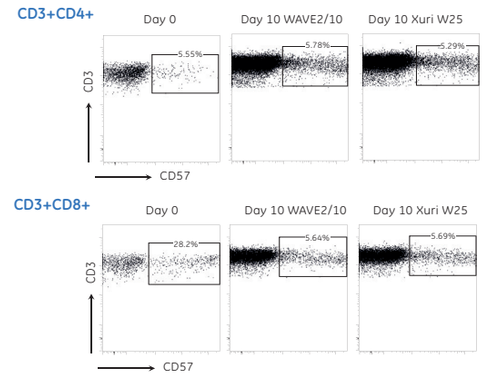


Fig 5. Continuous activation and proliferation can drive T cells to a state of senescence, which can be detected by the expression of the cell surface marker CD57. Analysis of CD57 expression showed that there was no difference between cells that have been grown on the WAVE 2/10 and Xuri W25 bioreactors, nor was there any accumulation of CD57+ T cells throughout the 10 day culture period.

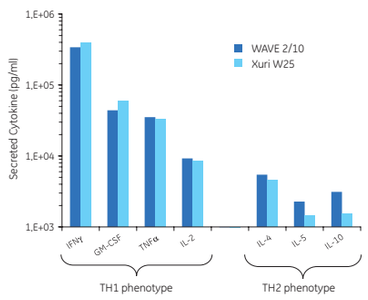


Fig 6. The secretion of both Th1 (IFNγ, GM-CSF, TNFα and IL-2) and Th2 (IL-4, IL-5 and IL-10) cytokines were analysed post re-stimulation after 10 days in culture. No significant difference was noted in the amounts of cytokine secreted from the cells expanded in WAVE 2/10 or Xuri W25 and it was noted that a Th1 cytokine profile was dominant.

Conclusions

- The cumulative fold expansion growth of peripheral blood T cells was 272-fold for WAVE 2/10 and 285-fold for Xuri W25 (Average of experiments A&B), with cell viability above 90% (results not shown).
- The expression of CD27 and CD28 on cells grown on the WAVE 2/10 and Xuri W25 was equivalent, and after 10 days of culture the majority of cells were in the early/intermediate stages of differentiation.
- Absence of the accumulation of CD57+ cells confirmed the culture had not reached a state of senescence throughout the 10 day culture period.
- Xuri Cell Expansion System W25 provides a convenient format for the generation of high density, viable and biologically functional T cells.

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