Risk reduction in adoptive cellular immunotherapy workflows with the Xuri Cellbag Bioreactor

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Risk Reduction in adoptive cellular immunotherapy workflows with the Xuri™ Cellbag™ Bioreactor

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Introduction

The successful manufacture of cellular immunotherapies requires systems that can maximize process standardization, minimize the risk of cell contamination, achieve high cell densities, and are designed for use in a regulated environment.
Based on user experience the Cellbags have been redesigned into Xuri Cellbag Bioreactors. This was to address the immunotherapy workflow and the cell therapy manufacturing environment and mitigate some risks associated with the set up of a culture.

This work evaluates how the improvements implemented on Xuri Cellbag Bioreactors affect process setup and T cell expansion efficiency on both WAVE Bioreactor 2/10 System, Xuri W5 and Xuri W25 Cell Expansion Systems.

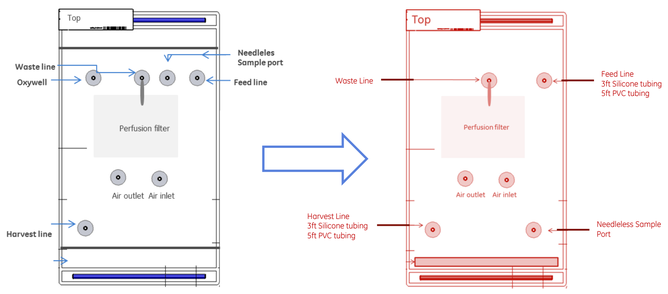

Figure 1. Shows a schematic of the original Cellbag and the Xuri Cellbag. Bioreactor.

Simplification of set up – summary

To aid the cell manufacturing set up and use of the Cellbag Bioreactors, the following list of changes have been incorporated into the Xuri Cellbag Bioreactors, which have been modified to contain the necessary tubing pre-attached so that once unpacked, it is ready to be loaded onto the bioreactor to begin your cell expansion (Fig 1)

Addition of labels to the fluid lines – minimize risk of error during setup.

Addition of harvest line and dip tube – reduced setup time and operator error, maintain closed system and reduce risk of sample loss.

Platinum cured silicone tubing – pre attached peristaltic pump grade tubing reduces setup time and minimizes contamination risk.

PVC tubing for sterile welding – pre attached weldable tubing allows the operator to maintain a closed environment again minimizing the contamination risk.

Removal of redundant features – reducing the ports to 4 per Cellbag – reduce risk of operator error and promotes automation.

Figure 2. Shows label design for the individual fluid lines on the 2 L and 10 L Xuri Cellbags.

Cellbag Workflow

Seven steps removed from the setup process – Reducing time, minimizing error and possible contamination.

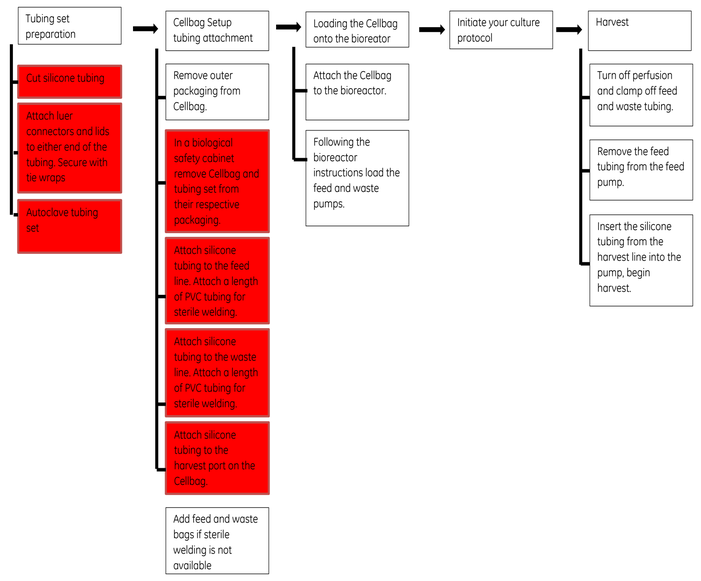

Figure 3. Shows the workflow for configuring the existing and redesigned Xuri Cellbag Bioreactor. Simplification of the Cellbag set up is shown by the reduced number of steps highlighted in red required for configuration of the redesigned Xuri Cellbag Bioreactor.

Methods and Materials

Activation of T cells

Cryopreserved human peripheral blood mononuclear cells (PBMCs) were thawed, washed twice and cultured in T225 flasks at 1E06 cells per ml in X-VIVO™ - 10 (Lonza) supplemented with 5% heat-inactivated human serum (TCS), 2 mM GlutaMAX™ (Life Technologies), 1% penicillin/streptomycin (Life Technologies) and 20 ng/ml of IL-2 (Peprotech). Cell expander™ CD3/CD28 beads (Life Technologies) were added to the culture at a ratio of 3:1 beads: CD3+ T cells. After 3 days incubation, cells were counted and maintained at 0.5E06 cells per ml for an additional 2 days. On day 5 of the culture, the cells were transferred into the Xuri Cellbag Bioreactor for culture on the WAVE Bioreactor 2/10 System and the Xuri Cell Expansion Systems W5 and W25.

T cell culture on the Xuri Cell Expansion System W5 and W25 and WAVE Bioreactor 2/10 System.

Once a minimal number of 5E08 cells were obtained in static culture, cells were transferred to 2 L Xuri Cellbag Bioreactors with perfusion filter. Cellbags were loaded onto the WAVE Bioreactor 2/10 System, Xuri W5 and Xuri W25 Systems. All bioreactors were set at 37°C with a rock rate of 10 rpm and a rock angle of 6°. Cells were maintained at 0.5E06 cells per ml and cultures fed batch until a maximum volume of 1000 mls was reached. Once the cell concentration had reached 2E06 cells/ml in the 1 L culture, perfusion was commenced for the remainder of the expansion. Perfusion on the WAVE 2/10 and Xuri W5 Systems was run as semi-continuous perfusion with shot volumes of 50 mls. Continuous perfusion was used on the Xuri W25 system.

Phenotypic analysis

The cells were immunophenotyped by flow cytometric analysis at days 0 and 10 of culture: 1E06 cells were stained with CD3-per CPCy5.5, CD4-PE, CD8-AlexaFluor488, CD28-APC and CD27-V450, or CD57-APC and CD62L-V450, and analyzed on a FACS Fortessa flow cytometer using FACS Diva software, according to the manufacturer's instructions (reagents, instrument and software from BD Biosciences).

Results

Cell Growth and Perfusion

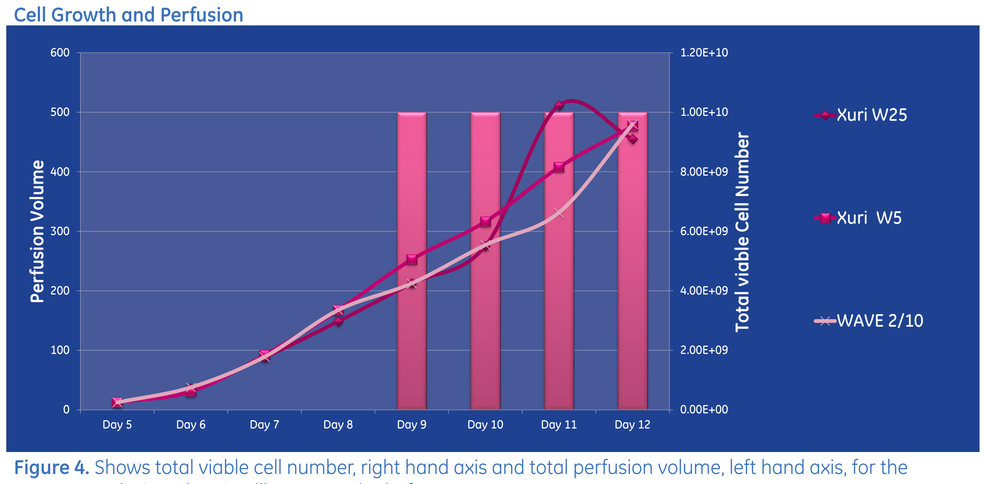

Figure 4. Shows total viable cell number, right hand axis and total perfusion volume, left hand axis, for the redesigned Xuri Cellbag on each platform.

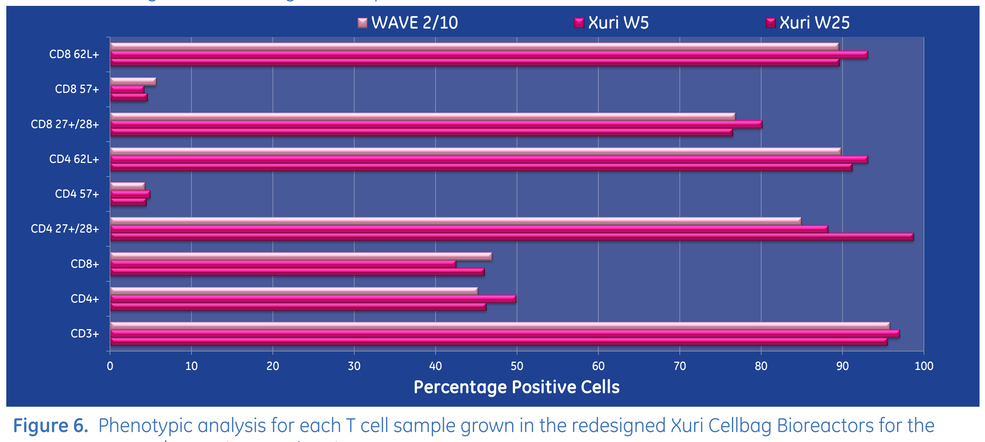

Figure 6. Phenotypic analysis for each T cell sample grown in the redesigned Xuri Cellbag Bioreactors for the WAVE 2/10, Xuri W5 and Xuri W25 Systems.

Cell Viability

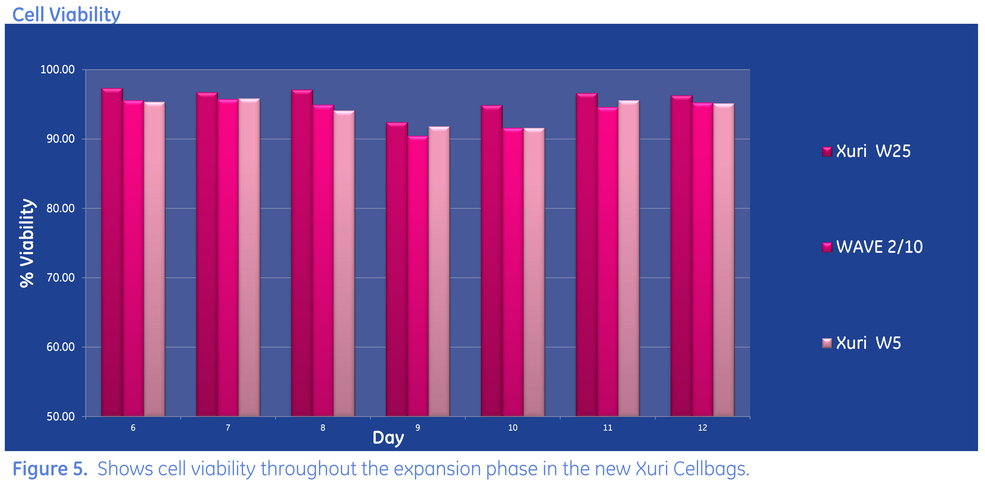

Figure 5. Shows cell viability throughout the expansion phase in the new Xuri Cellbags.

Conclusions

- Xuri Cellbag Bioreactors provide a reduction in the number of steps required to setup cultures for immunotherapy manufacturing.
- A detailed user manual in combination with labeling to the tube paths reduces the risk of operator set up error.
- There is equivalent cell number, viability and phenotype when T cells are grown in a Xuri Cellbag Bioreactor placed on either WAVE 2/10 System, Xuri W5 or Xuri W25.

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