Clinically relevant T cells expanded using the WAVE Bioreactor 2/10 system

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Clinically relevant T cells expanded using the WAVE Bioreactor™ 2/10 system

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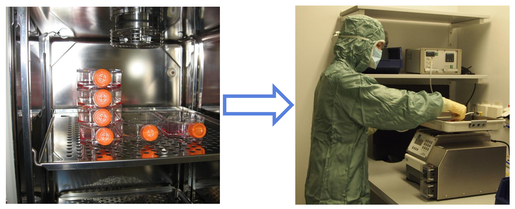
Introduction

Lymphocytes, expanded for clinical use, often consist of a small selected starting population, which requires multiple rounds of replication to achieve therapeutic doses. By using perfusion culture with the WAVE Bioreactor 2/10 System, high cell density cultures which are sufficient for therapeutic doses, can be generated. The Cellbag™ bioreactors, used together with the WAVE system, are functionally closed, single-use bioreactors that are delivered pre-sterilized and suitable for cGMP production. Perfusion is automatically maintained by the WAVE system, which removes metabolites through an internal filter while supplying the culture with nutrients.[1]

The handling of only one culture using the WAVE system, compared to having to manipulate multiple T-flasks or static bag cultures, simplifies the handling and sampling process. Samples can be withdrawn from the Cellbag Bioreactor through the sample clave port, by connecting e.g. a Luer syringe, thus keeping the system functionally closed. Cells produced for use in clinical trials often need to meet specific release criteria, which can include showing that the product contains low levels of endotoxin and is sterile.

Rolf Kiessling and his team are currently running a phase I clinical trial to evaluate safety, feasibility and immunologic response of adoptive T cell transfer with or without dendritic cell vaccination in patients with metastatic melanoma (ClinicalTrials.gov identifier: NCT01946373). A protocol for *in vitro* expansion of tumor infiltrating lymphocytes (TILs) using the WAVE 2/10 system has been developed and cells expanded with this protocol have been shown to meet the release criteria defined in the clinical trial and can be used for adoptive T cell transfer.



Material and Methods

TILs from skin or lymph node metastasis expanded in 24-well plates in the GMP-facility at CancerCenterKarolinska (CCK), Solna. TILs were cultured in CellGro™ serum free medium (CellGenix) supplemented with 2% autologous serum and 6000 IU/ml IL-2 (Proleukin™) for two weeks, to create a TIL-pool. Those cells were subsequently expanded further for 7 days in T75 flasks according to a Rapid Expansion Protocol REP2.

Following expansion in T75 flasks, cells were transferred into a 2 L perfusion Cellbag bioreactor on a WAVE 2/10 system, at the GMP-facility at Vecura, Huddinge. Expansion in the bioreactor was initiated at approximately 450 mL culture medium, consisting of CellGro medium supplemented with 2% autologous serum and 300 IU/ml IL-2, with a cell density of 0.5×10^6 cells/ml. Initially, cell density was maintained at $0.5\text{-}1\times10^6$ cells/ml by addition of fresh culture medium until the full working volume of 1 L had been reached. Subsequently, perfusion was started and adjusted to maintain glucose levels above 2 mM. The cells were harvested after 8 days of expansion in the Cellbag bioreactor, an overview of the expansion process is shown figure 1. Cell density and metabolite levels were analyzed at days 8, 10, 12 and 13. Phenotypic analysis of cells (including CD3, CD4, CD8, and CD45) as well as markers associated with melanoma cells (HMB45, DT101, BC199) was performed by flow cytometry at days 13 and 15. Samples for endotoxin and sterility testing was taken on day 8 and 15.

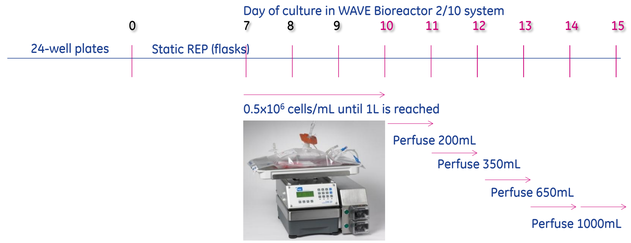


Fig 1. Overview of the expansion process in WAVE Bioreactor 2/10 system. Days indicated in black correspond to static or fed batch culture and numbers in lilac to perfusion. Cells were inoculated in a 2 L perfusion Cellbag bioreactor at a density of 0.5×10^6 cells/ml. Media was added to maintain the cell density until the final culture volume of 1 L had been reached. Subsequently, perfusion was initiated on day 10, at rates indicated in the figure. Perfusion rates imply the amount of media exchanged in the Cellbag bioreactor in ml/24 h.

Results

TILs for clinical use were successfully expanded with the WAVE 2/10 system at the GMP facility at the Karolinska University Hospital. Table 1 summarizes the total cell yield and fold expansion.

Total cell yield	14.3E9
Viability	98%
Fold expansion static REP (day 0-7)	7.5×
Fold expansion WAVE REP (day 7-15)	64×
Total expansion in REP (day 0- 15)	477×

Table 1. Results from TIL expansion using the WAVE Bioreactor 2/10 system.

In process, release QC analyzes and environmental monitoring were performed, resulting cell numbers, metabolite levels and phenotype are presented in figures 2, 3 and 4. Cell growth and viability recovered rapidly after non-optimal conditions during transportation between the GMP facilities, see figure 2. The semi-continuous perfusion supplied the cells with sufficient nutrients and prevented growth-inhibiting accumulation of metabolites, see figure 4. Cells in exponential growth, of which the majority was $CD3^+CD8^+$ cells (see figure 3), were harvested and washed after 8 days of expansion in the Cellbag. Prior to product release, microbial growth and endotoxin levels were tested and the results were within the acceptable range.

This culture provided proof of concept; expansion of TILs, using the WAVE Bioreactor 2/10 system, can successfully achieve requirements for sterility, phenotype and purity of the end product.

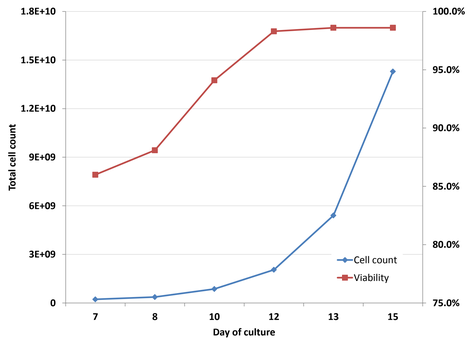


Fig 2. Cell numbers and viability (%) during the expansion. Total cell numbers doubled around 64 times during the culture. The cells were harvested when still in an exponential growth phase. Viability increased and leveled out on ≥98%. Cell count was determined by hand using hemocytometer and the viability measured by NucleoCounter 200 ™ .

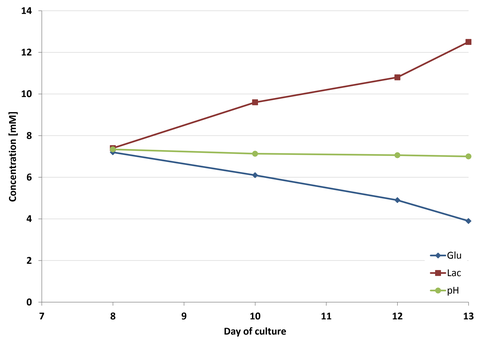


Fig3. Metabolite concentrations and pH. Glucose and lactate concentration (mM) and pH was monitored off-line, using cobas b 123 POC system ™ (Roche Diagnostics). pH was initially 7,3 and decreased by time to 7,0, probably due to the slightly increasing lactate level. Glucose declined to some extent as the cells grew, but stayed above the minimum level.

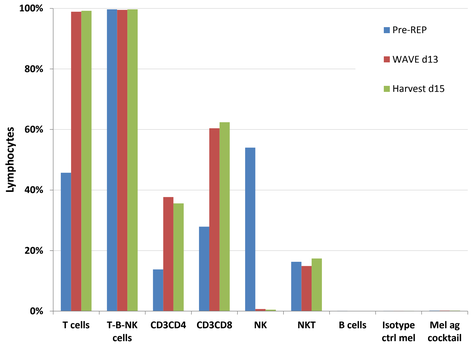


Fig 4. Phenotype data. Approximately 62% of the expanded lymphocytes were $CD3+CD8+$-cells, and 36% were $CD3+CD4+$-cells. After total 15 days culture. There were very few NK- and B cells in the final product. No melanoma cell markers was observed.

Conclusions

- Tumor infiltrating lymphocytes were successfully expanded, meeting the requirements for sterility, phenotype and purity set up for a clinical trial.
- 477× fold expansion was achieved for the whole rapid expansion protocol
- Perfusion enables a steady supply of nutrients and removal of metabolites

References

Application note: Perfusion culture of T lymphocytes in the WAVE Bioreactor System 2/10 (software version 2.61). 28-9650-52, edition AC (2011).

Besser MJ, Shapira-Frommer R, Treves AJ, Zippel D, Itzhaki O, Hershkovitz L, et al. Clinical responses in a phase II study using adoptive transfer of short-term cultured tumor infiltration lymphocytes in metastatic melanoma patients. Clinical cancer research : an official journal of the American Association for Cancer Research 2010; 16:2646-55.

The data presented was obtained during 2013.