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NK cell expansion using scale up bioreactors for cell therapy

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ABSTRACT

NK cells are powerful effector cells for adoptive immunotherapy of cancers. As part of the innate immune response, NK cells respond to anything they perceive as “non-self”, including malignant cells. NK cells provide an anticancer response in an antigen independent manner, allowing NK cells to be a potential “off the shelf” allogeneic therapeutic product. They have the potential to be safer, less expensive, and more effective than current engineered T-cell therapies. One of the key challenges faced by the cell and gene therapy industry is the ability to expand and process NK cells using regulation-compliant reagents and closed manufacturing systems. Gibco™ CTS™ NK-Xpander™ Medium expands functional primary human NK cells without the need for feeder cells. Up to 1 billion NK cells may be required per treatment with multiple doses required. Effective scale up expansion of NK cells will be critical for the success of these therapies. NK cell expansion with CTS™ NK-Xpander™ Medium in G-rex®, and HyPerforma™ Glass Bioreactors yields up to 10 billion functional NK cells within 2 weeks from qualified donors.

The Gibco™ CTS™ Rotea™ Counterflow Centrifugation System is a closed cell processing system developed for small-batch cell therapy manufacturing, reducing risk and hands-on time during the manufacturing process. Human NK cells expanded in CTS™ NK-Xpander™ Medium were loaded into the CTS™ Rotea™ system to form a stabilized bed for subsequent washing in CTS™ DPBS. Recovery was ~90% with high viability and maintenance of cellular phenotype and function.

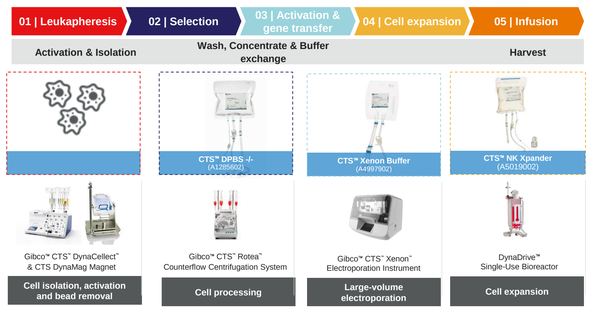
Together, Gibco™ CTS™ NK-Xpander™ Medium and the CTS™ Rotea™ System enable the expansion and processing of clinically relevant numbers of functionally viable NK cells, filling a need for translational researchers to run phase I and II clinical trials.

INTRODUCTION

The goal of this research was to develop a feeder free NK cell expansion medium that fulfills the needs of customers interested in NK cell therapies.

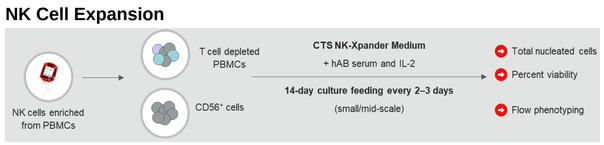
Enriched NK cells from PBMCs can be expanded with Gibco™ CTS ™ NK-Xpander ™ Medium supplemented with 5% hAB serum and IL-2. Expanded NK cells can be cryopreserved or prepared for infusion into patients, allowing for an “off the shelf” allogeneic therapeutic product.

MATERIALS AND METHODS



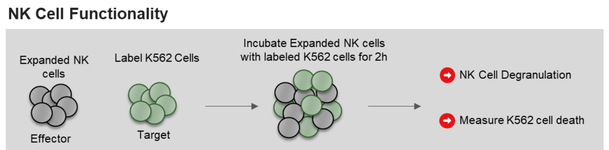
Thermo Fisher offers many solutions for cell therapy manufacturing. The DynaCellect System, the CTS Rotea system allows for efficient cell processing and the CTS Xenon offers a solution for closed large-volume electroporation. These instruments with CTS NK-Xpander and the HyPerforma bioreactors allow for the isolation, selection, gene modification and expansion of NK cells for therapy.

NK Cell Expansion



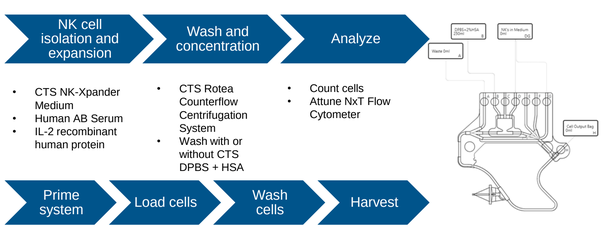
NK cells are CTS NK-Xpander Medium supplemented with 5% hAB serum and 500 U/mL IL-2. NK cells are isolated by either negative selection or T cell depletion.

NK Cell Functionality



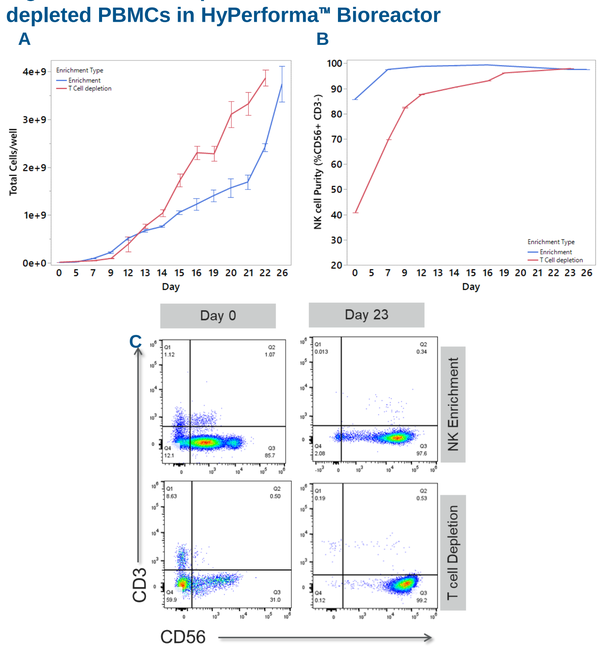
NK cells expanded in CTS NK-Xpander are coincubated with K562 target cells labeled with the Invitrogen Cell Trace CFSE Proliferation Kit for 2 hours. The ratios of NK cells to K562 cells were 0.625:1, 1.25:1, 2.5:1, and 5:1. Following incubation, degranulation was assessed by the expression of CD107a on CD56+ NK cells. NK cell cytotoxicity was assessed by measuring K562 cell death on the Attune NxT Flow Cytometer.

Workflow and CTS Rotea Single-Use Kit configuration for NK cell washing and concentration.



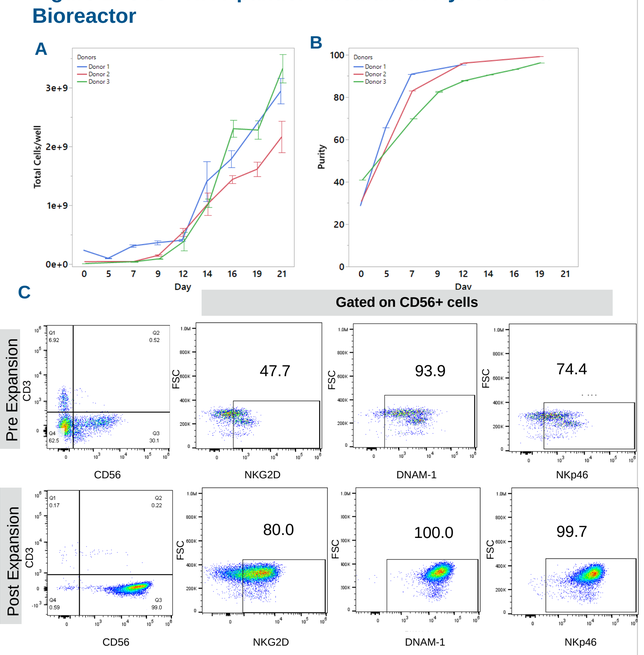
RESULTS

Figure 1: NK cell expansion from enriched or T cell depleted PBMCs in HyPerforma™ Bioreactor



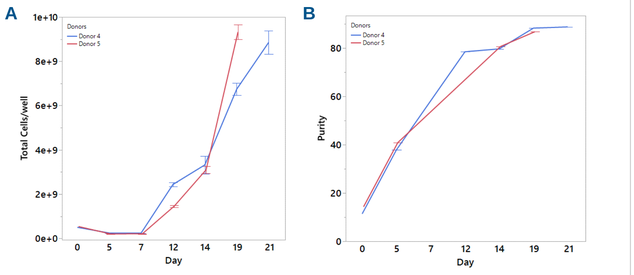
Enriched NK cells (0.5e6 cells/mL) (A) and T cell depleted PBMCs (8e6 cells/mL) (B) were seeded in 100mL G-Rex and transferred into 3L bioreactors at day 12. NK cells were maintained with fresh complete NK-Xpander Medium supplemented with 5% hAB serum and 500U/mL IL-2 and expanded to >2 Billion cells in 23 days. Enriched NK cells and T cell depleted PBMCs reach > 97% pure NK cells (CD56+, CD3-) by day 23 with minimal T cell contamination (<1% CD3+) (C).

Figure 2: CTS™ NK-Xpander™ Medium in HyPerforma™ Bioreactor



NK cells cultured in CTS NK-Xpander Medium and 3L HyPerforma™ Bioreactor expanded to over 2 Billion NK cells by Day 21 in 3 donors tested (A) and are >90% CD56+ CD3− (B). T cell depleted cells were seeded in G-Rex and transferred into 3L bioreactors at day 12-13. NK cells were maintained with fresh complete NK-Xpander Medium supplemented with 5% hAB serum and 500U/mL IL-2. NK Cells maintained activation marker expression including NKG2D, DNAM-1, and NKp46 after expansion (C).

Figure 3: CTS™ NK-Xpander™ Medium in 5L G-Rex® vessel

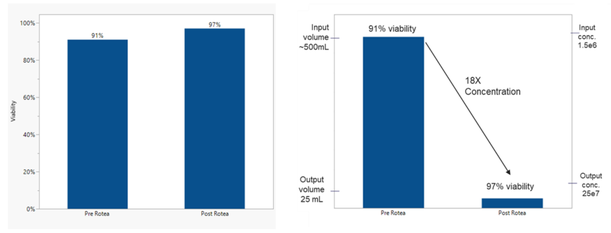


NK cells cultured in CTS NK-Xpander Medium and 5L G-Rex expanded to over 8 Billion NK cells by Day 19 in two donors tested (A) and are >85% CD56+ CD3− (B). Enriched NK cells were seeded in 1L G-Rex and transferred into 5L at day 14. NK cells were maintained with fresh complete NK-Xpander Medium supplemented with 500U/mL IL-2. NK Cells maintained activation marker expression including NKG2D, DNAM-1, and NKp46 after expansion.

Table 1: Wash and concentration runs of expanded human NK cells with CTS ™ Rotea ™ System with input and output recovery

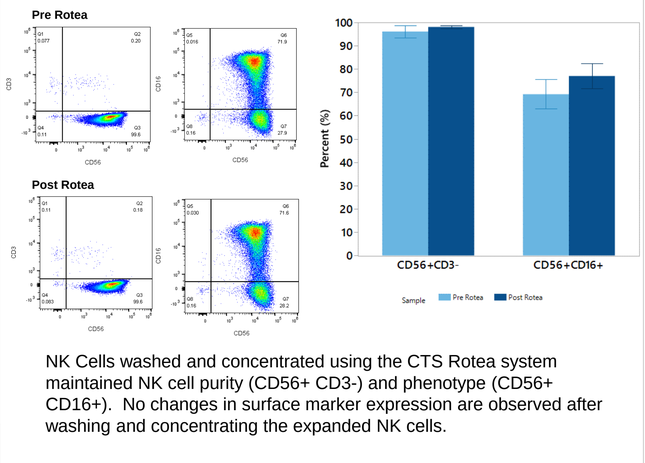
Run	Loading cell density (cells/mL)	Total number of cells loaded	Volume Processed	Harvest Volume	Total cell recovery	Recovery (%)	Viability (%)	Total run time (min)
Run 1	1.54e6	6.82e8	442mL	25mL	6.27e8	91.9	97.0	22.2
Run 2	2.42e6	1.21e9	500mL	50mL	1.11e9	91.5	96.7	20.9
Run 3	3.38e6	3.04e9	900mL	50mL	2.79e9	91.9	97.7	19.5
Run 4	2.75e6	1.28e9	464mL	50mL	1.03e9	80.6	99.7	30.3

Figure 4: Wash and concentration of expanded human NK cells with the CTS ™ Rotea ™ System



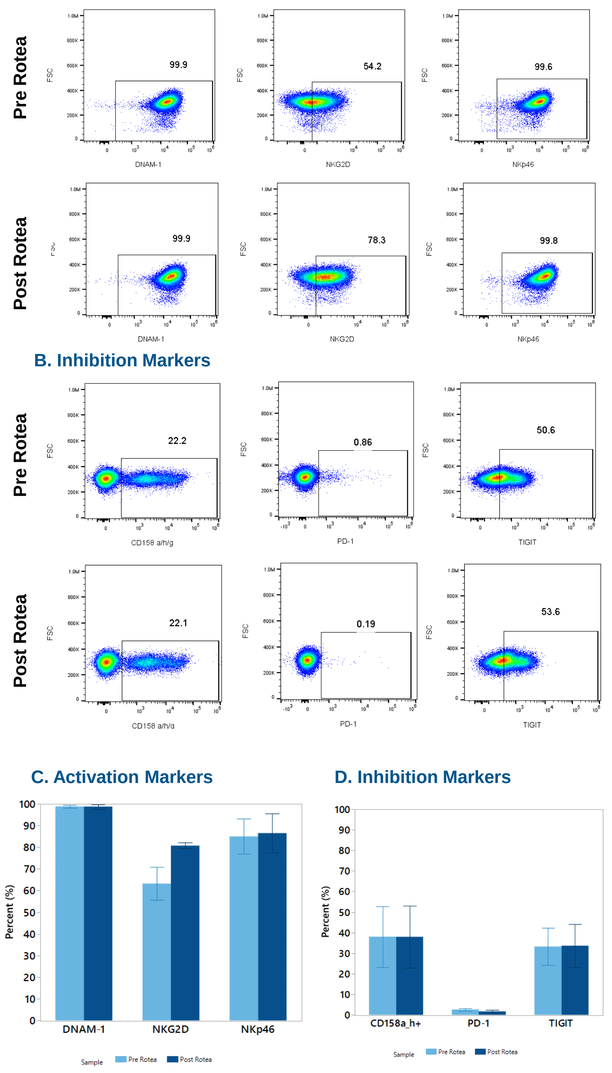
Gentle processing with the CTS Rotea system enables >90% cell recovery while maintaining cell viability through NK cell washing and concentration. The Rotea was used to concentrate ~0.6 Billion NK cells in a starting volume of 450mL down to 25mL achieving 92% recovery while maintaining viability of 97%.

Figure 5: Maintenance of NK cell purity



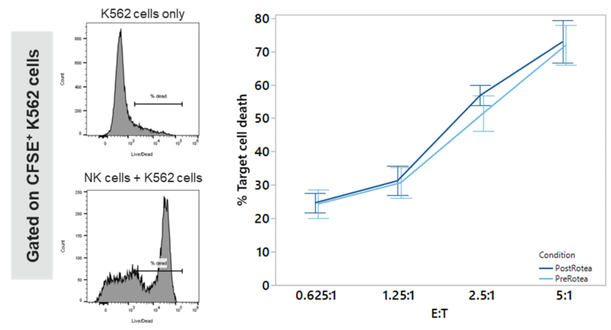
NK Cells washed and concentrated using the CTS Rotea system maintained NK cell purity (CD56+ CD3-) and phenotype (CD56+ CD16+). No changes in surface marker expression are observed after washing and concentrating the expanded NK cells.

Figure 6: Maintenance of NK cell phenotype



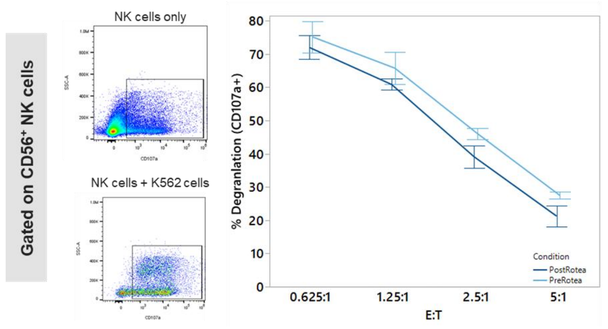
NK Cells washed and concentrated using the CTS Rotea system maintain NK cell activation marker expression: NKG2D, DNAM-1, and NKp46 (A,C) and inhibition surface marker expression: CD158, PD-1, and TIGIT (B,D). No changes in surface marker expression are observed after washing and concentrating the expanded NK cells.

Figure 7: Maintenance of NK cell cytotoxicity



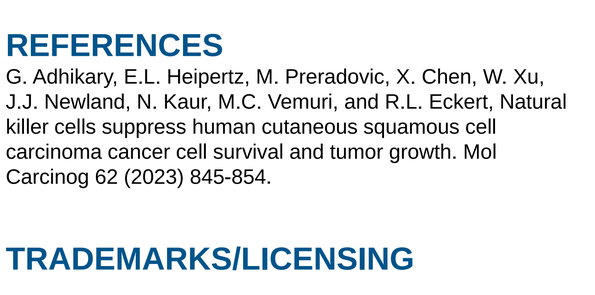
Cells washed and concentrated using the CTS Rotea system maintained cytolytic function and were able to kill K562 target cells in a dose dependent manner.

Figure 8: Maintenance of NK cell degranulation



Cells washed and concentrated using the CTS Rotea system maintained cytolytic function and were able to degranulate in a dose dependent manner.

Figure 9: Expanded NK cells reduce tumor burden in vivo.



Primary NK cells expanded in CTS NK-Xpander Medium reduce ADR-RES-Luc tumor burden in vivo with and without IL-2 combination treatment. NSG mice were injected with ADR-RES-Luc cells and beginning on day 7, 7.5 X 10^6 NK cells with or without IL-2 were injected i.p. per mouse twice a week, for three weeks. Reduced tumor burden was observed in mice treated with NK cells with or without IL-2.

CONCLUSIONS

We successfully developed a feeder-free NK Cell Expansion medium, CTS™ NK-Xpander™ Medium, that expands human primary enriched NK cells to clinically relevant levels. The expanded NK cells maintain their phenotype and functionality and able to kill over 50% of K562 cancer cells within 2 hours of co-incubation and can control tumor burden in vivo.

ACKNOWLEDGEMENTS

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REFERENCES

G. Adhikary, E.L. Heipertz, M. Preradovic, X. Chen, W. Xu, J.J. Newland, N. Kaur, M.C. Vemuri, and R.L. Eckert, Natural killer cells suppress human cutaneous squamous cell carcinoma cancer cell survival and tumor growth. Mol Carcinog 62 (2023) 845-854.

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