cell culture

Optimal Serum-Free *Ex Vivo* Expansion and Activation of T Lymphocytes Using Stemline™ T Cell Expansion Medium

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- Developed for consistent and rigorous expansion of T cells in serum-free culture conditions
- Maintains proper CD4/CD8 ratio
- Expanded T cells display outstanding *ex vivo* and *in vivo* functionality

Introduction

T lymphocytes (T cells) are a part of the cellular arm of the immune response directed against virally infected cells and also cancerous cells. A variety of diseases such as HIV infection or even medical treatments like chemotherapy and radiation can produce an impaired immune system with diminished T cell number and function. A reconstitution of T cell function may be accomplished through adoptive T cell immunotherapy, which involves the *ex vivo* expansion of autologous T cells and subsequent infusion of the expanded cell population into a patient. Also, the infusion of allogeneic T cells as a "Donor Lymphocyte Infusion" (DLI) may be used to eliminate cancerous cells in relapsed leukemias, since they are able to selectively recognize tumor cells. However, the use of allogeneic T cells in such settings is often complicated by the induction of Graft versus Host Disease (GvHD), which may even be lethal. Gene therapy offers a solution to this problem. If a suicide gene can be reliably inserted into allogeneic donor T cells during *ex vivo* culture without impairing their *in vivo* function, these T cells could be aborted in the patient after elimination of the leukemic cells, and before the onset of GvHD.

To achieve sufficient cell numbers in culture, T cells are usually stimulated with anti-CD3 and anti-CD28 antibodies, and are expanded in a culture medium that contains Interleukin-2 and Fetal Bovine Serum (FBS). FBS often exhibits lot-to-lot variability and may potentially contain adventitious agents, viruses, or prions. Also, FBS cultured cells should only be administered to patients once, since subsequent administrations may cause "serum sickness"; the patient's immune system may be sensitized to bovine serum proteins, which can be caused by minute quantities of bovine protein carried on the surface of cultured cells, not removable even after repeated wash steps. Therefore, culturing of cells without FBS is preferable in clinical settings. For these reasons, we have developed a serum-free culture medium, Stemline T Cell Expansion Medium (Product Code S1694) for the optimal expansion of human T cells.

T Cell Expansion and Phenotype

When compared to another commercially available serum-free lymphocyte growth medium, Stemline Medium outperformed the competitor's medium by 55% (Figure 1) with viability greater than 95%. Important parameters to measure after T cell culture are cell phenotype and maintenance of the CD4 to CD8 ratio as observed in resting peripheral blood T cells. Phenotypic analysis by flow cytometry after 4 and 8 days of *ex vivo* expansion demonstrated that of the peripheral bloood mononuclear cells (PBMC) cultured in Stemline T Cell Expansion Medium, the predominant expanded cell type was CD3 positive, and that a proper $CD4^+/CD8^+$ ratio was maintained (compared to the input ratio).[1]

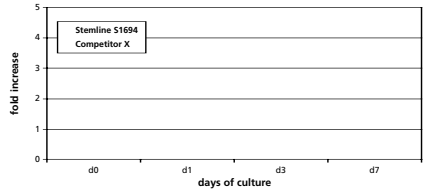

Figure 1. Comparison of T cell expansion in serum-free media. *Competitor X demonstrates 3.1-fold expansion while Stemline T Cell Expansion Medium demonstrates 4.8-fold expansion.*

Ex Vivo Functionality: ^{51}Chromium Release Assay

In addition to the parameters of T cell expansion and phenotype, we examined the functional state of the expanded T cell population in both *ex vivo* and *in vivo* functional assays. Figures 2A and 2B demonstrate that the T cell population expanded in Stemline Medium is highly functional and possesses cytolytic potential greater than T cells expanded in serum-containing medium (RPMI with 10% FBS).

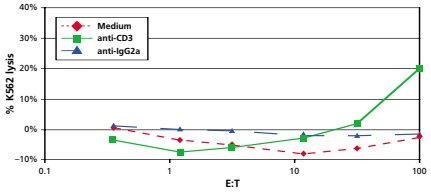

Figure 2A. Chromium Release Assay. *Cells expanded in RPMI with 10% FBS.*

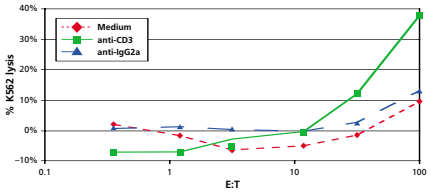

Figure 2B. Chromium Release Assay. *Cells expanded in Stemline T Cell Expansion Medium.*

In Vivo Functionality: GvHD Induction

Finally, expanded human T lymphocytes were injected into NOD/SCIDβ2M mice (n=12) as a test for *in vivo* functionality. After 4 days in culture, activated T cells were injected at an initial cell dose of 10^7 cells. Engraftment, perivascular infiltration, and lethal GvHD were observed by Day 15 in 100% of the mice[1] (Figures 3A and 3B). GvHD was verified in tissues by histopathological evaluation. Spleen and other organs were massively infiltrated with human lymphocytes.[2] Thus, T cells cultured in Stemline T Cell Expansion Medium display outstanding *in vivo* expansion and functionality.

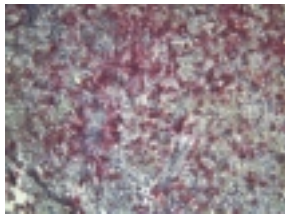

Figure 3A. Engraftment of human T cells in the NOD/SCID-β2M mouse spleen (200x mag, anti-human CD45 - Red).

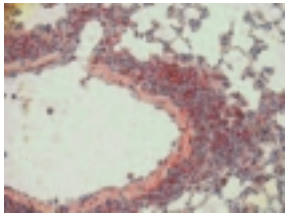

Figure 3B. Induction of Graft vs Host Disease in the NOD/SCID-β2M mouse lung (200x mag, anti-human CD45 - Red).

Summary

Developed for optimal cell growth under serum-free conditions, Stemline T Cell Expansion Medium outperforms both serum-containing and other commercially available serum-free media. T cells expanded in Stemline T Cell Expansion Medium exhibit rigorous and consistent growth kinetics, maintenance of the proper CD4/CD8 ratio, and both *ex vivo* and *in vivo* functionality. Overall, these characteristics of Stemline T Cell Expansion Medium make it ideal for the *ex vivo* expansion of T cells for use in human clinical studies.

Acknowledgements

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References

1. Bauer, G., Walker, J., Nervi, B., Ritchie, J., Hughes, J., Eades, B., Nolta, J.A., Devine, S., and DiPersio, J.F. A system for GMP expansion and transduction of human T cells with high functionality proven by consistent induction of GvHD in a mouse Xenotransplant Model. Annual Meeting of the American Society for Gene Therapy, Abstract #288, June 2, 2005.
2. Nervi, B., Rettig, M.P., Ritchey, J.K., Bauer, G., Walker, J., Herrbrich, P.E., Meyerrose, T.E., Bonyhadi, M., Nolta, J.A., and DiPersio, J.F. *Ex Vivo* Activation and Genetic Manipulation of Human T Cells Results in Consistent *In-Vivo* Expansion and Lethal GvHD in a Novel Murine Xenotransplant Model. Annual Meeting of the American Society for Gene Therapy, Abstract #810, June 4, 2005.

Ordering Information

Product	Description	Unit
S1694	Stemline™ T Cell Expansion Medium	1 L