Expansion of human T cells using xenogeneic free and gamma irradiated human platelet lysate.

Vanesa Alonso-Camino, Jessica Vanderwielen, Sethey Ben, Beth Clarke, Jeff Nielsen, Lisa Carey, Kailee Schaeffer and William Mirsch.

Introduction

Mill Creek's human platelet lysate (hPL) is produced using expired human platelets obtained from accredited blood banks in the United States. These platelets were originally intended for use in patient transfusion. The safety of platelets used in transfusion is managed by the U.S. Food & Drug Administration (FDA), as well as the Association for the Advancement of Blood & Biotherapies (AABB). These organizations set standards, including testing for transmissible diseases. The Covid-19 pandemic has increased awareness of emerging infectious diseases, even though transmission of Covid-19 via blood transfusion has not been documented. For that reason, we developed a gamma irradiated version of our products, which offer an additional safety measure in the clinic.

Chimeric Antigen Receptor (CAR) expressing T-cells have demonstrated potent clinical efficacy in patients with hematological malignancies. In addition, there are several phase I clinical trials evaluating the use of CAR-T-cells for targeting of solid tumor-associated antigens. Some of the challenging issues found during production of CAR-T cells are the efficiency of T cell transduction to generate CAR-T cells, the expansion of T cells to clinically relevant numbers and the long-term survival in vivo of the therapeutic cells. The use of human platelet lysate has been demonstrated to improve these issues.

Our data from experiments performed using human CD3+ from donors suggests that our human platelet lysates offer an improved performance on T cell expansion versus serum derived products, as wells as other platelet lysates on the market. PLTGold® and PLTGold®-GI efficiently promote T cell expansion, with higher cell yields and lower cell exhaustion rate. Additionally, our hPLs are suitable for suspension culture of T cells, potentially facilitating the large-scale expansion of allogeneic CAR-T cells.

Results I

I. Expansion of T cells using PLTGold® and PLTGold®-GI

Peripheral blood CD3+ cells from donors were expanded using RPMI supplemented with FBS, PLTMax® or PLTGold® and the classic combination of IL-2 (Sigma-Aldrich) plus anti-CD3+/anti-CD28+ antibody mixture (Immunocult™, STEMCELL™ Technologies). Fetal bovine serum (FBS) was used at 10% supplementation as per classic protocols of T cell expansion using FBS. Our hPLs were used at 5%, as it is a standard supplementation across most of the cell types previously grown in our laboratory. As seen in Figure 1A, PLTGold® showed the most efficient T cell expansion thorough the 10 days of culture.

Additionally, we compared the use of IL-2 to other classic cytokine combinations, as well as the use of human AB Serum and PLTGold®-GI vs FBS and PLTGold®. As seen in figure 1B, PLTGold®-GI proved to efficiently expand T cells with similar yields to those obtained with non irradiated products. The use of IL-2 and IL-21 seemed to provide a growth advantage with respect to the use of other cytokine combinations. Therefore, this combination was used for further analysis.

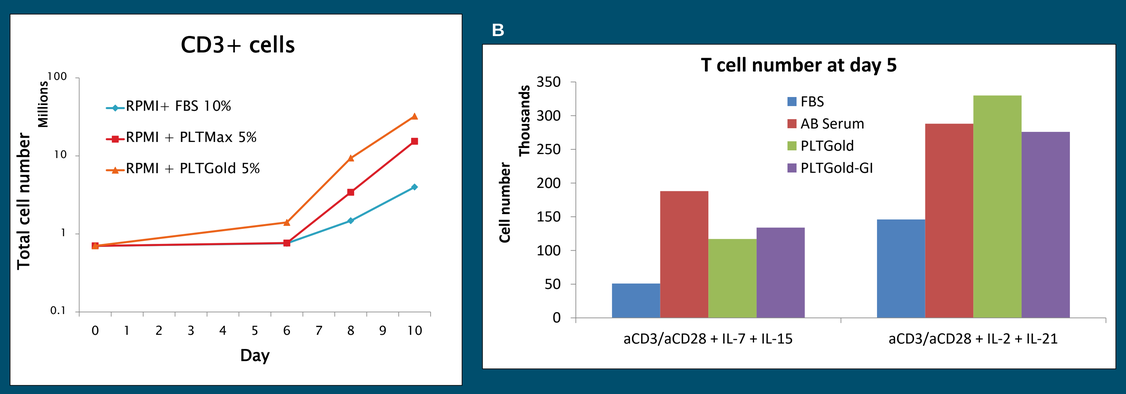

Figure 1. Expansion of donor peripheral blood CD3+ T cells using hPL. A) Growth of T cells in RPMI medium supplemented with Immunocult, IL-2 and IL-21 and either FBS, PLTMax® or PLTGold® for 10 days. B) T cell number at day 5 using different combinations of cytokines and supplementation with FBS, AB Serum, PLTGold® or PLTGold®-GI.

II. Phenotypic characterization of T cells grown with media supplemented with PLTGold®-GI

T cells expanded with human AB Serum and PLTGold®-GI were analyzed by flow cytometry to determine the distribution of different T cell populations after culture, as well as the expression of activation and inhibition markers. T cells grown with PLTGold®-GI showed an increase in CD4+ and CD8+ cells and a decrease in dual positives in comparison to cells grown with human AB serum (Figure 2A). Additionally, culture of T cells with PLTGold®-GI led to an increase on activation and memory markers (Figure 2B) and decrease on inhibitory markers like CTL4 (Figure 2C).

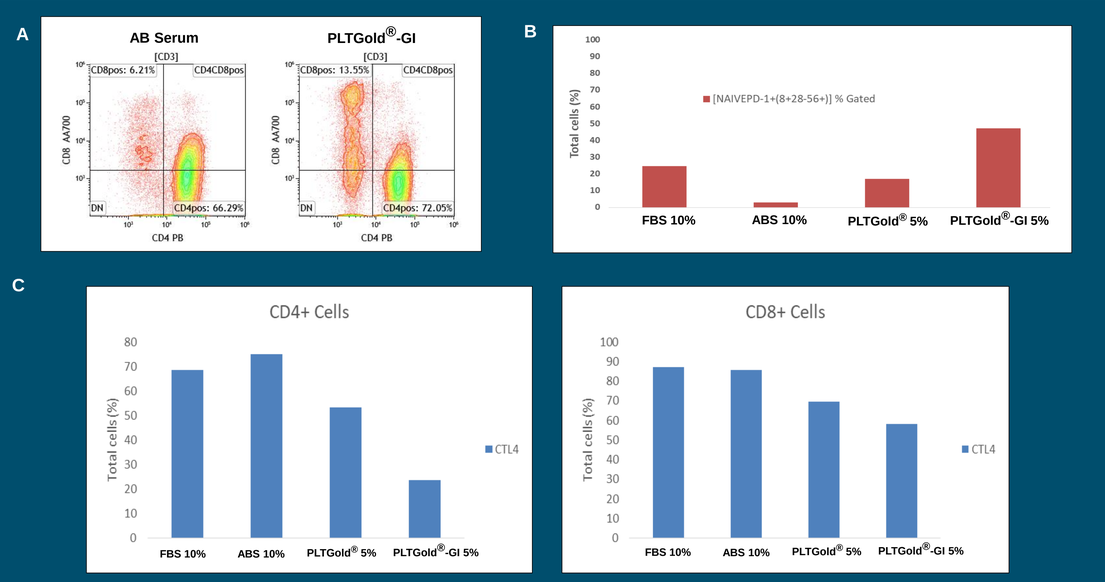

Figure 2. Phenotypic characterization of T cells expanded using PLTGold®-GI. A) Analysis by flow cytometry of CD4+ and CD8+ populations. Histograms show the distribution of the expression and co-expression of CD4 and/or CD8 in the CD3+ population. B) Memory/effector CD8+ cell percentage when using either FBS, AB Serum (ABS), PLTGold® or PLTGold®-GI. C) Analysis by flow cytometry of the expression of CTL4 in the CD4+ and CD8+ populations.

Results II

III. Lot to lot consistency of PLTGold®-GI and comparison of PLTGold®-GI to competitor hPLs

Peripheral blood CD3+ cells from donors were expanded using RPMI supplemented with different lots of PLTGold®-GI, and the Immunocult, IL-2 and IL-21 mixture. As seen in Figure 3 A, PLTGold®-GI showed a minimal lot to lot variation, which eliminates the need for lot pre-testing before purchase for T cell expansion.

PLTGold®-GI also demonstrated a significantly faster and higher cell growth during the 5 days of culture that has been standardized to expand CAR-T cells when compared to competitor heparin-free hPLs, all of them fibrinogen-depleted. (Figure 3 B).

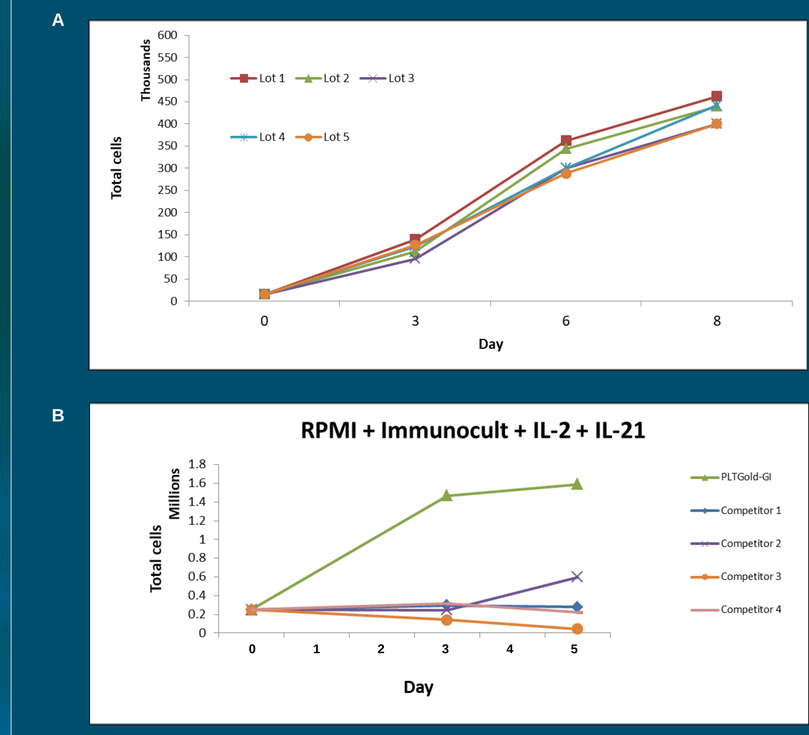

Figure 3. Characterization of performance of PLTGold®-GI. A) Lot to lot consistency of PLTGold®-GI for T cell growth. B) Expansion of pan CD3+ T cells using PLTGold®-GI in comparison to 4 competitor hPLs.

VI. Use of PLTGold®-GI for large scale suspension culture of T cells

Peripheral blood CD3+ cells from donors were expanded using RPMI supplemented with 5% PLTGold®-GI , IL-2, IL-21 (Sigma-Aldrich) and Immunocult™ (STEMCELL™ Technologies), using a vertical wheel bioreactor from PBS Biotech Inc. Cell yield at day 6 using this system for a suspension culture exceeded the cell yield obtained in a 2D system (flask) in 10 days (Figure 4A).

Cells grown in the vertical wheel bioreactor remain mainly in a single cell suspension for the duration of the culture (Figure 4C), in comparison to the classic T cell culture look (Figure 4B), where T cells grow in cell clumps. Growth of T cells in suspension in this type of bioreactor may resemble the natural environment of T cells better than a static 2D culture, which could explain the higher expansion efficiency of the suspension culture.

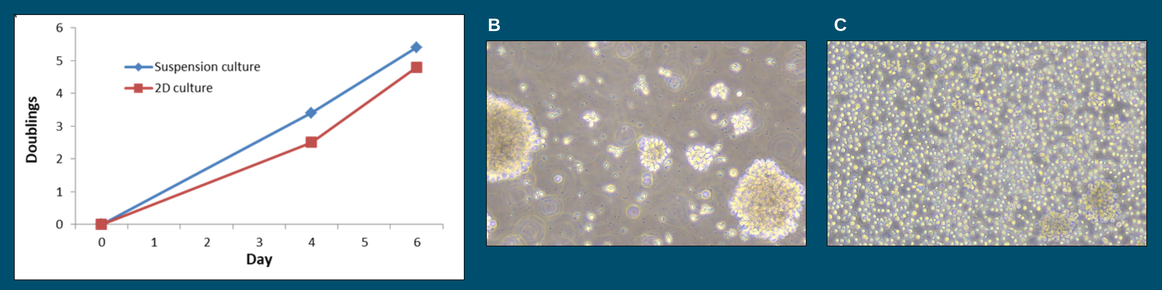

Figure 4. Expansion of pan CD3+ T cells using PLTGold®-GI in a 2D system vs a vertical wheel bioreactor. A) Doublings of T cell population in a flask *vs* a vertical wheel bioreactor, using RPMI medium supplemented with Immunocult, IL-2, IL-21 and 5% PLTGold®-GI for 6 days. B) Appearance of T cell culture in a 2D system (flask). C) Appearance of T cell culture when using a vertical wheel bioreactor.

Conclusions

- Mill Creek Life Sciences' gamma irradiated cGMP products offer an added safety measurement with a very low impact on cell growth.
- T cells expanded using media supplemented with PLTGold®-GI exhibited a high proliferative capacity.
- PLTGold®-GI produces an increase on CD4+ and CD8+ cells, potentially leading to the production of higher numbers of effective CAR-T cells.
- T cells expanded in the presence of PLTGold®-GI showed higher activation, stimulatory and memory markers and lower levels of inhibitory markers.
- PLTGold®-GI is a consistent and reliable T cell culture supplement with very low lot-to-lot variability and outstanding performance compared to other hPLs on the market.
- PLTGold®-GI allows for a very efficient large scale expansion of T cells in bioreactors

Alonso-Camino.Vanesa@millcreekls.com Mirsch.Bill@millcreekls.com