Characterization of human T cells and NK cells expanded with human platelet lysate for Chimeric Antigen Receptor based therapies.

Vanesa Alonso-Camino and William Mirsch.

Mill Creek Life Sciences, Inc. Rochester, Minnesota, USA

Introduction

- T cells and NK cells expressing chimeric antigen receptors (CAR) have demonstrated potent clinical efficacy in patients with hematological malignancies. One of the issues to overcome in the treatment of solid tumors using CARs is the lack of a process that generates large amounts of CAR-T cells, reduces cell exhaustion and differentiation, and improves long term survival after infusion into patients.
- Previously, Torres Chavez et al. demonstrated in a series of in vitro and in vivo experiments, performed in both hematologic and solid tumor models, the profound qualitative impact on CAR-T cell performance after using human platelet lysate (hPL) as a cell growth supplement. T cells expanded with their hPL showed a good proliferative capacity and enhanced long-term in vivo persistence compared to Fetal Bovine Serum (FBS) or human AB Serum (ABS), resulting in superior anti-tumor effects. Additionally, this group demonstrated that the transduction of T cells to generate the CAR-T cells was significantly improved by the use of hPL.
- 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 United States record for blood safety is well established, with extremely low rates of disease transmission, making the platelet units used for hPL manufacture low risk. 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 gamma irradiated version of our products, which offer an additional safety measure in the clinic.
- Previously, we have shown that the use of our platelet lysates has the potential of significantly impacting T cell manufacture by improving the quality and potency of the end product.
- In this study, we compare the phenotypic profile of different subsets of peripheral blood lymphocytes cultured using our different hPL products versus serum derived products. Additionally we establish a protocol for large scale suspension culture, which could be used for expansion of allogeneic CAR cells.

Results I

I. Expansion of PBLs and NKs using PLTGold®

Peripheral blood lymphocytes (PBLs) from donors were isolated (Figure 1A) and expanded using RPMI supplemented at 5% with human AB Serum or our human platelet lysate PLTGold® and the classic combination of IL-2 (Sigma-Aldrich) plus anti-CD3+/anti-CD28+ (Immunocult™, STEMCELL™ Technologies) (Figure 1C). NKs from the same donors were isolated using an immunodensity negative selection cocktail (RossetteSep ™ Human NK Cell Enrichment Cocktail, STEMCELL™ Technologies) (Figure 1B) and expanded using RPMI supplemented at 5% with human AB Serum or PLTGold® and the IL-2 IL-15 combination (Sigma-Aldrich). As seen in Figure 1C and D, PLTGold® produced higher cell yields for both PBLs and NKs thorough the duration of the culture.

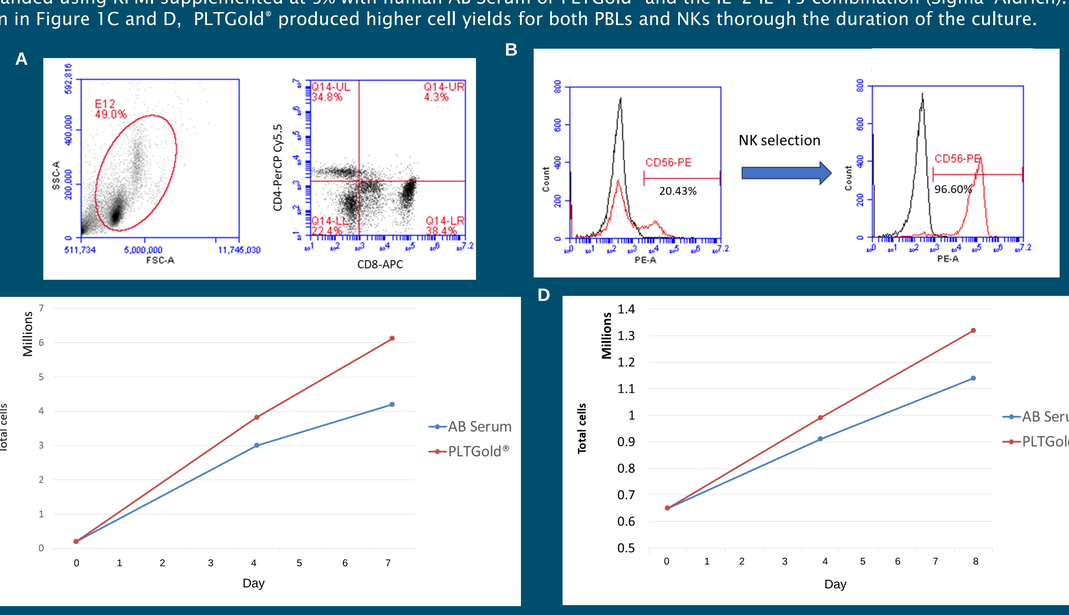

Figure 1. Expansion of donor PBLs and NKs using hPL. A) Characterization of T cell populations in the isolated PBLs. B) Enrichment of NKs from peripheral blood lymphocytes. C) Expansion of PBLs in RPMI medium supplemented with Immunocult, IL-2 and either human AB Serum or PLTGold® for 7 days. D) Expansion of NKs in RPMI medium supplemented with, IL-2, IL-15 and either human AB Serum or PLTGold® for 8 days.

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

T cells expanded with human AB Serum and PLTGold® 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® showed an increase in CD8+ cells and dual positive CD4+CD8+ cells (Figure 2A). Additionally, culture of T cells with PLTGold® led to a decrease on CCR7+ and CD45RA+ found in naïve cells, therefore indicating an increase on effector memory cells (Figure 2B) that were primed but not chronically activated (data not shown).

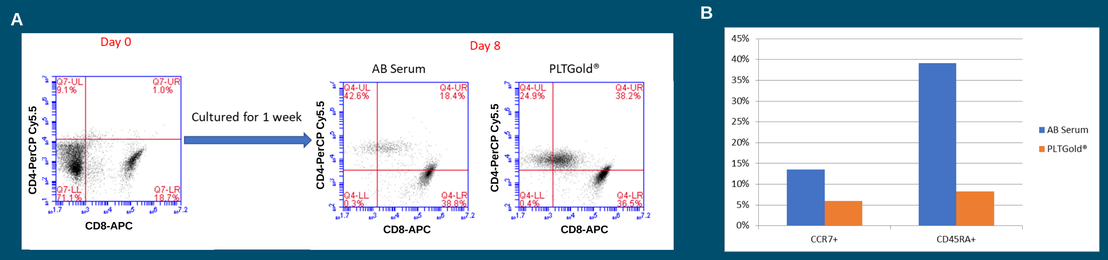

Figure 2. Phenotypic characterization of T cells expanded using PLTGold® A) Analysis by flow cytometry of CD4+ and CD8+ populations. Histograms show the distribution of the expression and co-expression of CD4 and/or CD8 in the CD3+ population. B) Percentage of Naïve *vs* Memory/effector T cell cells when using either AB Serum or PLTGold®.

Results II

III. Phenotypic characterization of NK cells grown with media supplemented with PLTGold®

Even in cases where donors had low NK count and the NK purification was suboptimal (Figure 3A), expansion of selected NK cells for a week using PLTGold® resulted in pure NK populations where almost 100% of the cells were CD3-CD56+ (Figure 3B). CD16 levels also increased for NKs of all donors after expansion with PLTGold® (Figure 3B).

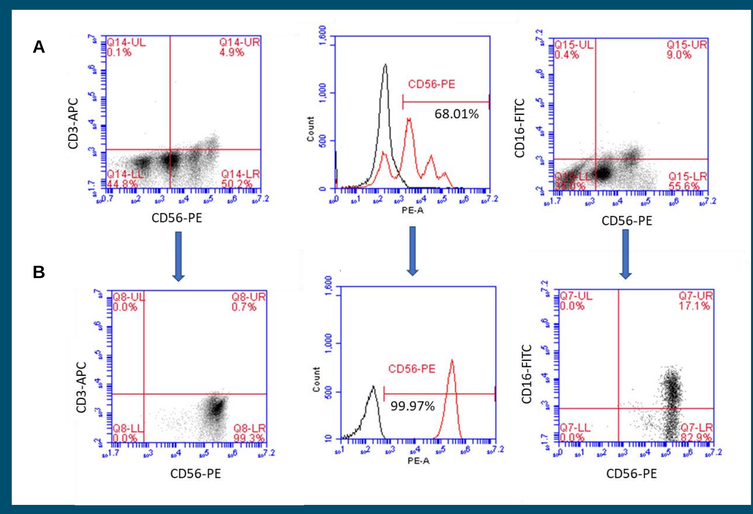

Figure 3. Characterization of NKs expanded with PLTGold®. A) Phenotype of NK cells isolated from a young donor with a suboptimal yield of 68% CD56+ cells. B) Phenotype of NK cells isolated with a suboptimal yield after expansion for a week using RPMI supplemented with 5% PLTGold®, IL-2 and IL-15.

IV. Use of PLTGold®-GI for culture of T cells in allogeneic therapies

Our hPLs are produced using expired human platelets that 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 United States record for blood safety is well established, with extremely low rates of disease transmission, making the platelet units used for hPL manufacture low risk. 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.

To determine if we can obtain large amounts of T cells for allogeneic CAR-T therapies, peripheral blood CD3+ cells from donors were expanded using RPMI supplemented with 5% of gamma irradiated PLTGold® (PLTGold®-GI), IL-2, IL-21 and Immunocult™ 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.

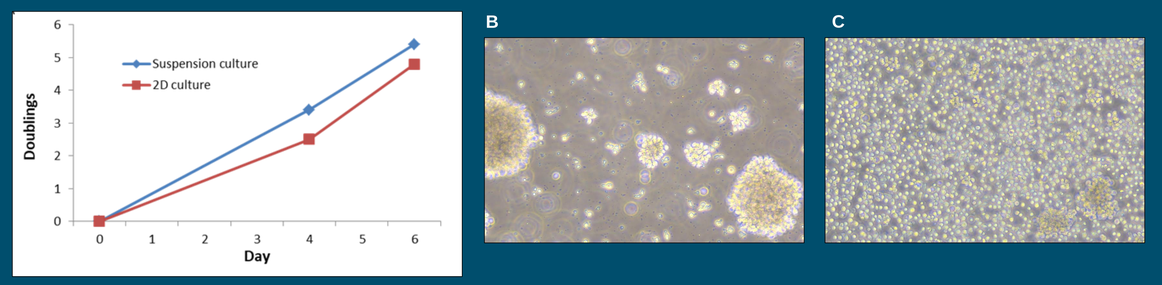

Figure 4. Expansion of pan CD3+ T cells using PLTGold®-GI in a 2D system vs a vertical wheel bioreactor. A) Doublings of T cell population in a flask *vs* a vertical wheel bioreactor, using RPMI medium supplemented with Immunocult, IL-2, IL-21 and 5% PLTGold®-GI for 6 days. B) Appearance of T cell culture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

Conclusions

- T cells and NK cells expanded using media supplemented with PLTGold® exhibited a high proliferative capacity.
- PLTGold® produces an increase In CD8+ cells, potentially leading to the production of higher numbers of cytotoxic CAR-T cells.
- T cells expanded in the presence of PLTGold® showed higher stimulatory and memory markers and lower levels of inhibitory markers.
- PLTGold® 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. The use of calcium chloride by competitors' products to precipitate fibrinogen in other hPLs, leaves residual precipitates that we previously demonstrated to be detrimental for T cell expansion. Our hPLs are not fibrinogen depleted and therefore, do not contain calcium chloride precipitates.
- Expansion of NK cells using PLTGold® resulted in high NK cell yields even with samples from donors that had lower percentages of NK populations and suboptimal NK cell isolation, therefore facilitating potential treatment of all patients in autologous CAR-NK therapies regardless of initial cell yields.
- PLTGold®-GI allows for a very efficient large scale expansion of T cells in bioreactors with an added safety measurement.

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