Expansion of Different Types of Therapeutic Cells Using Xenogeneic Free and Gamma Irradiated Human Platelet Lysate.

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Introduction

Mill Creek Life Sciences' 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. However, over the past few decades, the incidence of emerging infectious diseases has increased. Even though transmission of Covid-19 and other potential emergent pathogens via blood transfusion has not been documented, it remains a concern. For that reason, we validated a process to produce gamma irradiated human platelet lysate for use in clinical applications.

We previously demonstrated the high efficacy of our platelet lysates for the *in vitro* expansion of clinically relevant numbers of different types of stem/stromal cells as well as endothelial cells. Here, we also present the results obtained from the expansion of T cells using our gamma irradiated hPLs, which showed unprecedented levels of product quality and efficiency. T cells cultured with PLTGold®-GI (gamma irradiated) in 2D systems, as well as in a vertical wheel bioreactor, show an improved cell yield and cellular phenotype in comparison to other products available in the market. We believe, based on these preliminary studies, that our products will be a game changer for the growing market of T cell and CAR-T cell based therapies bioprocessing and manufacturing.

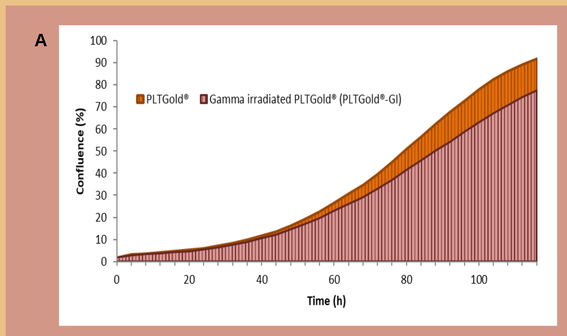
Results

I. Biological characterization of gamma irradiated hPL:

Previously, we evaluated the effects of gamma irradiation on the performance of our products by comparing the growth kinetics of adipose-derived mesenchymal stromal cells (MSCs) cultured with media supplemented with different lots of the non irradiated products, PLTGold® and PLTMax®, *vs* the gamma irradiated PLTGold®-GI and PLTMax®-GI. As expected after gamma irradiation of a biologic product, there are some differences between non-irradiated and irradiated products, with higher doubling times and lower percentages of confluence at day 5 of culture for the irradiated products *vs* the non-irradiated products ($p<0.0001$ for PLTGold® *vs* PLTGold®-GI and $p=0.0041$ for PLTMax® vs PLTMax®-GI). However, we only observed a 6-16% reduction in product potency (Figure 1 A) as compared to a 20% reduction observed by competitor products available on the market.

Average doubling times obtained with both our non-irradiated and irradiated platelet lysates are lower than those obtained by competitors with their equivalent products. Our gamma irradiated products lowered doubling times, offering a higher cell yield in a smaller period of time and therefore being more cost effective (Figure 1B).

A viral clearance study performed on our gamma irradiated products showed a pathogen reduction that was equivalent or better than for competitor gamma irradiated hPLs (Figure 1C).



C

Model Virus	MCLS	Competitor 1	Competitor 2
Human Immunodeficiency Virus (HIV)	≥5.3 (HIV)	2.9 (HIV)	N/A
Pseudorabies Virus (PRV) or equivalent DNA virus enveloped	≥6.2 (PRV)	≥5.4 (PRV)	≥4.5 (HSV1)
Bovine Viral Diarrhea Virus (BVDV) or equivaent RNA virus enveloped	≥6.7 (BVDV)	≥5.1 (BVDV)	≥5.4 (BVDV)
Hepatitis A Virus (HAV) or equivalent RNA virus no enveloped	≥6.5 (HAV)	4.9 (EMCV)	≥4.4 (Reo-3)
Porcine Parvovirus (PPV) or equivalent DNA virus non enveloped	3.0 (PPV)	2.2 (PPV)	4.55 (MMV)

Figure 1. Growth of adipose -derived MSCs in medium supplemented with non-irradiated vs irradiated hPL. A) Growth curve of PLTGold® vs PLTGold® -GI. B) Differences in ultimate cell yield with different hPL products after starting with 10 million cells and using a 7-day growth period. Cell yields were calculated using internal data and published competitor cell doubling times. C) Comparison of results of viral clearance study compared to published competitor's gamma irradiated products. Values given are Log10 reduction factors. The higher the reduction, the more viral inactivation. EMCV: Encephalomyocarditis Virus; Reo-3: Mouse reovirus type 3; HSV1: Herpes Simplex virus type 1.

II. 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 2A, 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 2B, 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 respect to the use of other cytokine combinations. Therefore, this combination was used for further analysis.

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 (Figure 2C) exceeded the cell yield obtained in a 2D system (flask) in 10 days (Figure 2A). Cells grown in the vertical wheel bioreactor remain mainly in a single cell suspension for the duration of the culture (Figure 2E), in comparison to the classic T cell culture look (Figure 2D), 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 may explain the higher cell yield in less time.

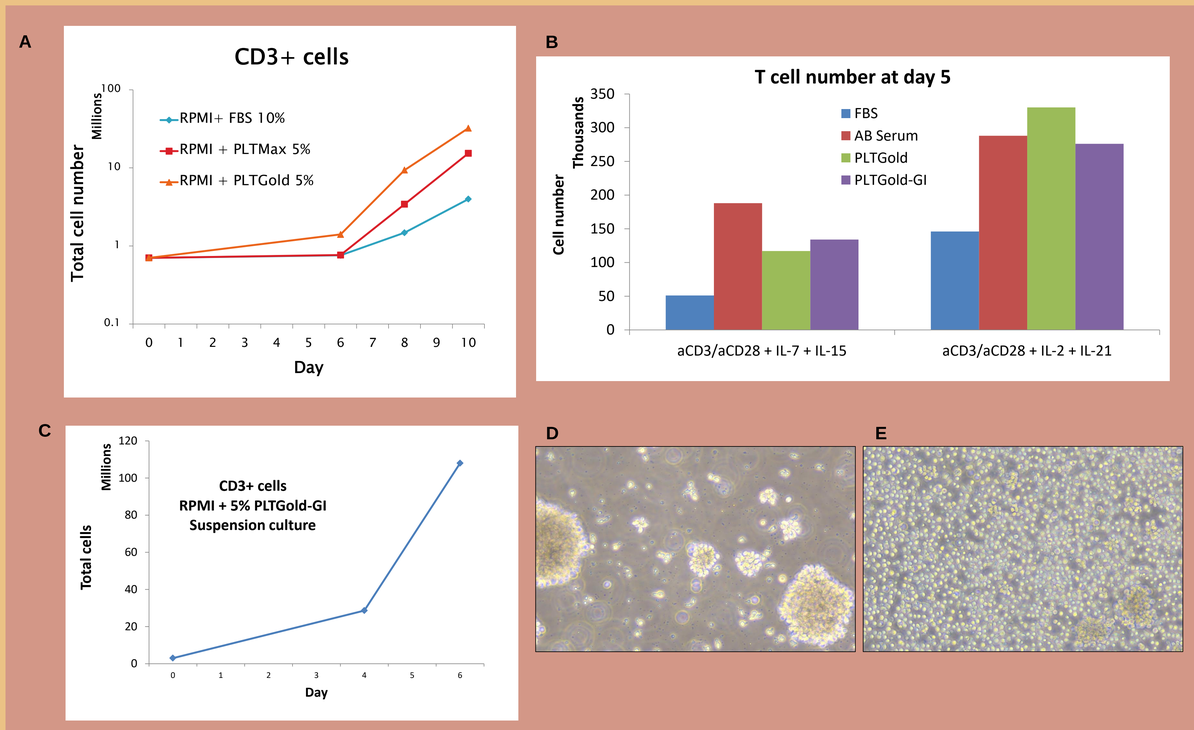


Figure 2. Expansion of donor peripheral blood CD3+ T cells using hPL. A) Growth of T cells in RPMI medium supplemented with Immunocult, IL-2 and IL-21 and either FBS, PLTMax® or PLTGold® for 10 days. B) T cell number at day 5 using different combinations of cytokines and supplementation with FBS, AB Serum, PLTGold® or PLTGold®-GI. C) T cell expansion in a vertical wheel bioreactor using RPMI medium supplemented with Immunocult, IL-2, IL-21 and PLTGold®-GI for 10 days D) Appearance of T cell culture in a 2D system (flask). E) Appearance of T cell culture when using a vertical wheel bioreactor.

III. 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 3 A). Additionally, culture of T cells with PLTGold®-GI led to an increase on activation/co-stimulatory molecules (Figure 3B and C) and decrease on inhibitory markers like CTL4 (Figure 3B). Finally, after culture with PLTGold®-GI, there was an increase on PD1, which was correlated with an increase of T cell activation (Figure 3C).

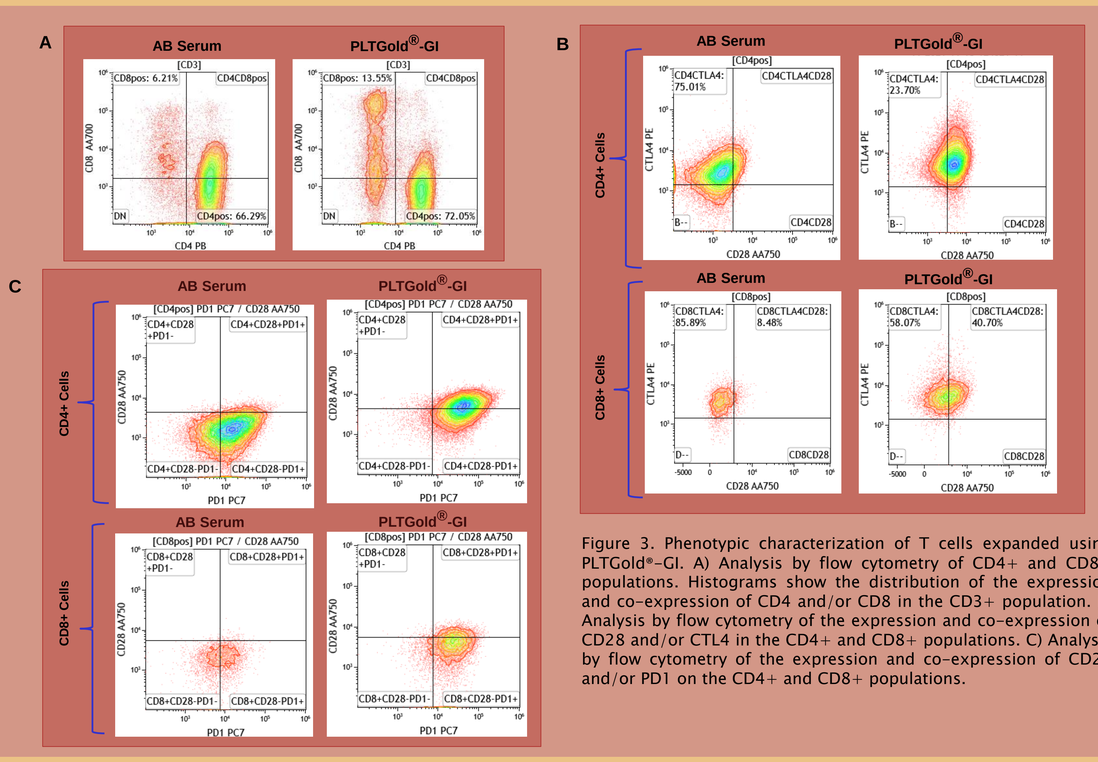


Figure 3. Phenotypic characterization of T cells expanded using PLTGold®-GI. A) Analysis by flow cytometry of CD4+ and CD8+ populations. Histograms show the distribution of the expression and co-expression of CD4 and/or CD8 in the CD3+ population. B) Analysis by flow cytometry of the expression and co-expression of CD28 and/or CTL4 in the CD4+ and CD8+ populations. C) Analysis by flow cytometry of the expression and co-expression of CD28 and/or PD1 on the CD4+ and CD8+ populations.

Conclusions

- Our 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 and stimulatory markers and lower levels of inhibitory markers.
- Since PLTGold®-GI produces higher cell yields in a shorter amount of time, consideration must be given to avoid T cell exhaustion, as could be indicated by the increase in PD1. In this regard, lower concentrations of PLTGold®-GI and shorter culture times may be used to expand T cells with outstanding cell yields using vertical wheel bioreactors for culture in suspension.

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