

# Expanding T cell-based therapies with human platelet lysate

## Summary

Expansion of T cells ex vivo holds great promise in regenerative medicine for treating cancer and autoimmune diseases. Despite the great therapeutic value of culturing T cells, today much of the T cell culture for research and therapeutic development is performed using cell culture media supplemented with fetal bovine serum (FBS) or human AB serum.

Over the last several years, human platelet lysate (HPL) has emerged as a successful alternative to effectively culture primary human lymphocytes. HPL is a cost-effective, xeno-free serum substitute that enables rapid cell expansion, without the extreme variation in composition, quality, and performance that comes with using FBS. HPL is produced from therapeutic-grade platelets and is proven to be an effective, consistent, and safe alternative to FBS in T cell applications.

This research spotlight reviews a recent poster presented by Mill Creek Life Sciences demonstrating PLTGold® Human Platelet Lysate as the gold standard cell culture supplement for the expansion of T cells in vitro. This clinically compatible serum substitute is critical for researchers developing cell-based therapies, including T cell and CAR-T therapies. PLTGold HPL efficiently promotes T cell expansion, generating higher cell yields and lower cell exhaustion rates, which ultimately helps bioprocessing and manufacturing workflows to enable highly proliferative, healthy, and safe cells for therapeutic use.

## The Spotlight

**Research area:** Cell therapy, T cell isolation, T cell culture, T cell expansion, cell expansion, gene editing, CAR-T, CRISPR, clinical cell culture

**Cell type(s):** T cells, CAR-T cells

**Experiment purpose:** Expansion of T cells using human platelet lysate shows unprecedented levels of growth and expansion, improving the performance of cultures for cell-based products.

## Experiment Overview

- **HPL vs. FBS T cell culture evaluation**
- **T cells and 3D suspension using HPL supplemented media**
- **Characterization of T cells using HPL**

## Product Highlights

### PLTGold® Human Platelet Lysate

PLTGold is a next generation, growth factor rich, xeno-free, heparin-free supplement that is a superior alternative to FBS or human AB serum. PLTGold is available in research and clinical grade formats.

### PLTGold® Human Platelet Lysate, Gamma Irradiated

PLTGold-GI is a clinical grade pathogen reduced product that has undergone gamma irradiation at a dose of 25-38 kGy. Ideal for therapeutic research and clinical trials.

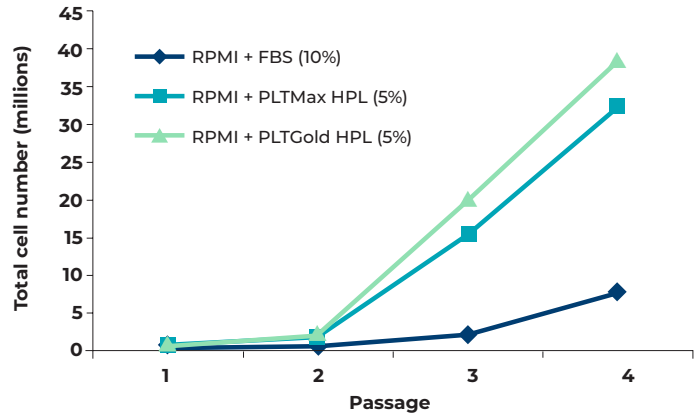
- Superior alternative to FBS
- Produced from clinical-grade platelets
- Available in 27 mL, 100 mL, 500 mL, and 1000 mL bottles
- Drug Master File with the FDA available

## Results

### Human platelet lysate outperforms fetal bovine serum

Culturing T cells supplemented with either PLTMax or PLTGold HPL exceeded the performance of cultures grown using media supplemented with FBS. As observed with many cell types in addition to T cells, media supplemented with HPL shows faster cell kinetics, higher overall cell yields, and better cell morphology. (Figure 1)

HPL provides enhanced T cell culture expansion over FBS with reduced supplementation requirements. FBS is generally supplemented at 10% concentrations (vol/vol) in T cell culture media, whereas PLTMax and PLTGold HPL are ideally more effective at supporting cell growth over time when used at only 5% or less in culture media.



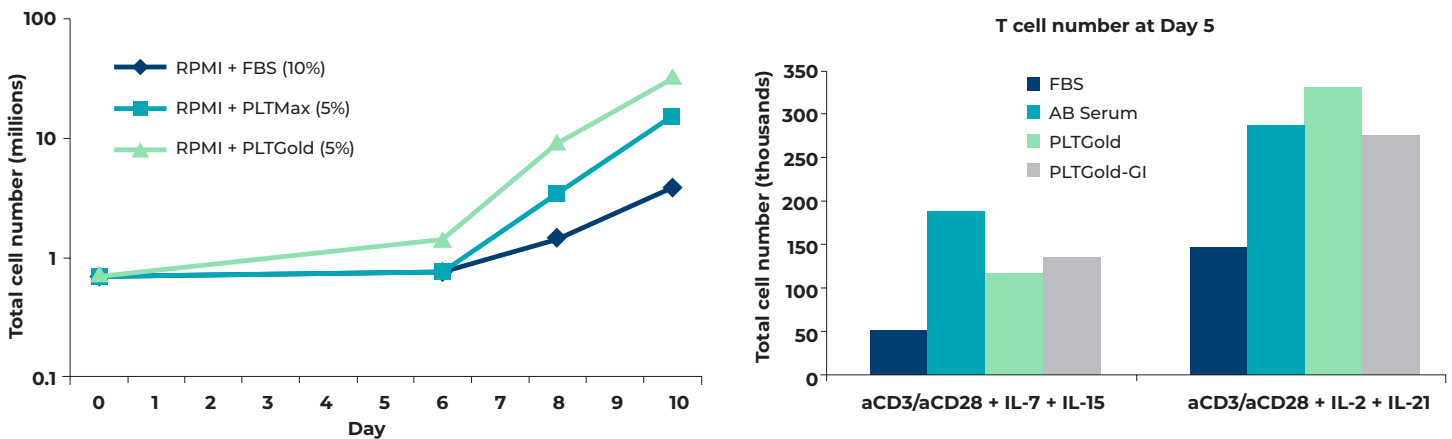
**Figure 1. Comparative media supplement conditions.**

Comparison of T cell expansion and cell yield over 4 passages with media supplemented with PLTMax and PLTGold hPL compared to standard FBS. Data shows improved cell proliferation and yield when HPL is used in the culture medium.

### Optimizing T cell culture conditions with human platelet lysate

CD3+ cells isolated from donor peripheral blood were expanded using a standard basal medium and supplemented with either FBS (10%), PLTMax (5%), or PLTGold (5%). The medium formulation for this experiment was RPMI 1640, IL-2 cytokine, and an anti-CD3/anti-CD28 antibody mixture, along with the variable FBS or HPL. Results showed that media supplemented with PLTGold provided the most efficient T cell expansion through 10 days of culture. (Figure 2)

The classic use of IL-2 alone was compared against other cytokine combinations: IL-7 and IL-15, and IL-2 and IL-21. Additionally, variable media supplements were added in each cytokine combination, including FBS, human AB serum, PLTGold HPL, and PLTGold HPL, Gamma Irradiated (PLTGold-GI). Results showed that PLTGold-GI efficiently expanded T cells with similar yields to the non-irradiated PLTGold. Additionally, the use of IL-2 and IL-21 seemed to provide a growth advantage compared to the other cytokines evaluated. The most optimal culture conditions contained RPMI 1640, 5% PLTGold-GI, IL-2, IL-21, and the anti-CD3/anti-CD28 antibody mixture. This media was used for the subsequent experiments and characterization assays. (Figure 2)



**Figure 2. Comparative T cell culture media conditions.** (Left) Comparison of T cell expansion using IL-2 supplemented RPMI 1640 medium with either FBS (10%), PLTMax (5%), or PLTGold (5%). Results showed that hPL outperforms FBS when supplemented at half the concentration. (Right) T cell counts at day 5 in culture evaluating two cytokine combinations (IL-7 and IL-15, IL-2 and IL-21) and supplementation strategies. The most optimal T cell culture medium tested was RPMI 1640 with 5% PLTGold, IL-2, IL-21, and an anti-CD3/anti-CD28 antibody mixture.

### T cell expansion in 3D suspension culture

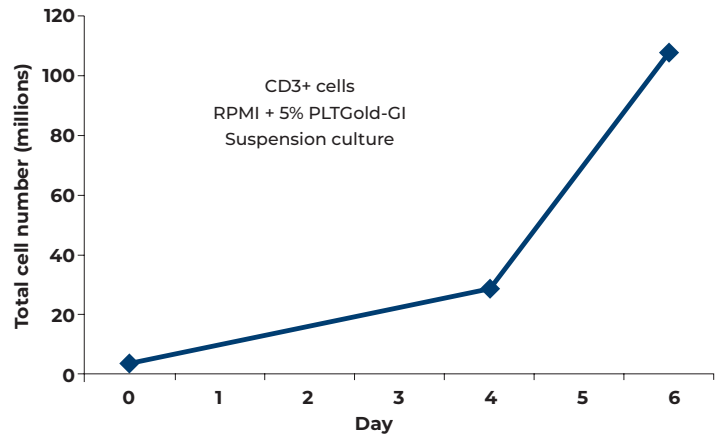
Peripheral blood CD3+ cells were expanded using the RPMI 1640 and PLTGold HPL, Gamma Irradiated medium was validated previously in a vertical wheel bioreactor from PBS Biotech Inc. Cell yield at day 6 in this 3D suspension culture (Figure 3) far exceeded the yield obtained in a 2D flask culture system in 10 days (Figure 2).

Interestingly, the T cells expanded in the vertical wheel bioreactor remained a single cell suspension for the duration of the culture, in comparison to the typical observations of clumps of T cells when cultured in 2D flasks. Culture of T cells in suspension in this type of bioreactor may resemble the natural environment of T cells more appropriately than a static T cell expansion system. This may also explain the higher cell yield generated in the suspension culture in less time than a flask system. (Figure 4)

### Phenotypic characterization of T cells

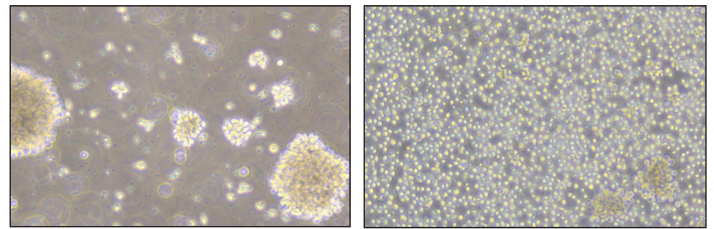
T cells expanded in culture medium supplemented with either human AB serum or PLTGold-GI were analyzed by flow cytometry to determine the distribution of different cell populations and the expression of activation and inhibition markers.

T cells cultured in HPL showed an increase in CD4+ and CD8+ cells and a decrease in dual positives when compared to cells grown with human AB serum (Figure 5). This increase in CD4+ and CD8+ T cells in the culture population could potentially lead to an increase in effective CAR-T cells. T cells cultured with PLTGold-GI led to an increase of activation/co-stimulatory molecules and a decrease of inhibitory markers, such as CTL4. After culture with PLTGold-GI, an increase in PD1 was observed, which correlates with an increase in T cell activation (Data not shown).

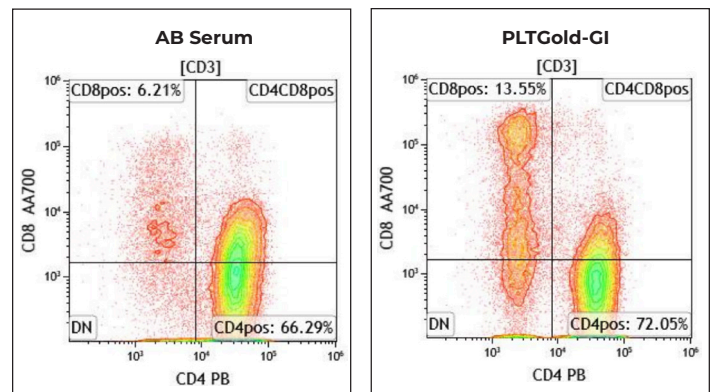


**Figure 3. Suspension culture enables rapid T cell expansion.**

T cell expansion in a vertical wheel bioreactor using the previously validated media formulation with 5% PLTGold-GI, yields exceedingly high cell numbers at only day 6 in culture.



**Figure 4. T cell culture observations with PLTGold-GI in both 2D and 3D conditions.** (Left) Typical observation of clumps of T cells when cultured in 2D flasks. (Right) T cells are maintained as single cell suspension when cultured in a vertical wheel bioreactor.



**Figure 5. Phenotypic analysis of T cell populations.** Flow cytometry analysis of CD4+ and C8+ cells in cultures expanded with either human AB serum or PLTGold-GI show the distribution of the expression and co-expression of CD4 and/or CD8 in the CD3+ populations.

### PLTGold outperforms competitor HPL

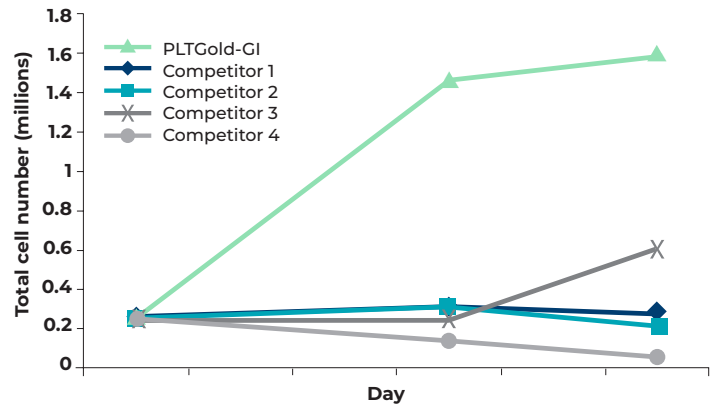
T cell cultures expanded with PLTGold-GI show an increase in CD4+ and CD8+ sub-populations, higher levels of activation and stimulatory markers, and lower levels of inhibitory markers. Additionally, PLTGold in both 2D flask culture and 3D vertical wheel bioreactor systems show an improved cell yield and cellular phenotype in comparison with other commercially available HPL on the market. (Figure 6)

### Conclusion

Today, several studies around the world are using HPL to expand T cells and to develop CAR-T based cancer therapies. Results in this latest poster show unprecedented levels of cell quality and culture efficiency when using PLTGold-GI HPL. With the high level of safety surrounding the need for pathogen-inactivated and gamma irradiated media supplements, PLTGold-GI sets the bar for an ideal culture supplement for T cell and CAR-T based therapy bioprocessing and manufacturing.

### References

1. Alonso-Camino et al. Expansion of different types of therapeutic cells using xenogenic free and gamma irradiated human platelet lysate. 2022. Mill Creek Life Sciences. Scientific poster.



**Figure 6. T cell expansion using PLTGold-GI is superior to competitor HPL.** Comparison between PLTGold-GI and other commercially available human platelet lysate solutions used as supplements for the expansion of T cells in vitro.

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