Synapse-localized γ-chain Cytokine Presentation Enables Single-dose, Soluble Cytokine-free T cell Expansion in Plate and G-Rex Bioreactor Formats With Reduced Manufacturing Complexity and Cytokine Dosing

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Main Conclusion

Integrated cytokines in this formulation support expansion with reduced metabolic demand while favoring memory associated T cell phenotypes.

Background

Soluble γ-chain cytokines are essential for T cell expansion in adoptive cell therapy manufacturing.

Repeated soluble cytokine dosing adds reagent use, handling, and manufacturing complexity.

sbPCI™ emulates immune synapse architecture and enables presentation of γ-chain cytokines at defined density and organization

Rationale

Intentional integration of cytokines, activation signals and costimulatory signals can improve culture quality by balancing T cell expansion with memory-associated, cytotoxically armed differentiation.

Methods

T cells (Gulf Coast Blood) were expanded with multiple sbPCI configurations (see table) or a Nanomatrix industry standard in G-Rex 6M bioreactor.

Integration and activation efficiency was evaluated with pSTAT5 and PI (CytoflexS); cell counts and viability were measured via NC-200. Results were used to determine which sbPCI conditions were carried forward.

Expansion over 7 days included control wells with soluble cytokines added at Day 0; **test sbPCI cultures received no cytokine after Day 0.**

Expansion, memory, and exhaustion were measured using flow (CytoFlexS).

Data are mean ±SD from 3 independent normal donors.

sbPCI Configuration	sbPCI : T cell
αCD3/αCD28	1:1
αCD3/αCD28/IL-2/IL-15	1:1
αCD3/αCD28/αCD137	1:1
αCD3/αCD28/αCD137/IL-2/IL-15	1:1
IL-2 (4 pmol)	1:1, 0.5:1, 0.25:1
IL-15 (4 pmol)	1:1, 0.5:1, 0.25:1

Results: Precise Control of Cytokine Number and Geometry

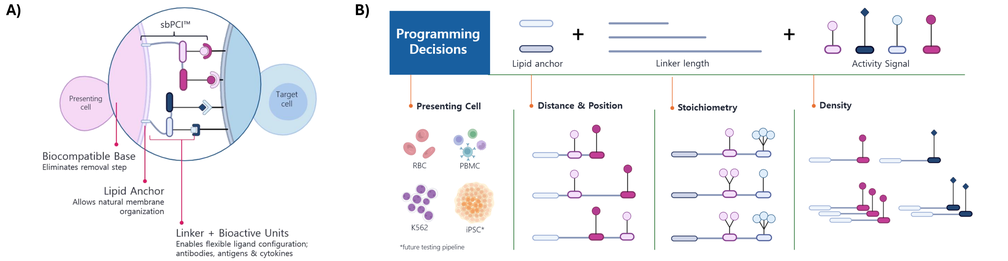


Figure 1. Assembly and characterization of programmable cellular interfaces (sbPCI). (A) Schematic of the sbPCI architecture, consisting of a biocompatible cell surface, lipid anchor, programmable linker and modular bioactive ligands that enable controlled presentation of molecules to direct immune synapse signaling. **B)** sbPCI enables quantitative control of ligand number and spatial organization to direct cell-to-cell signaling.

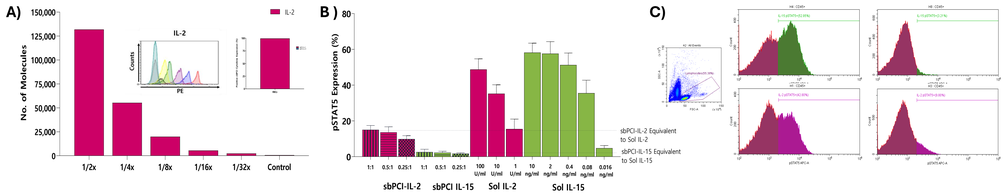


Figure 2. Defined sbPCI cytokine presentation enabled quantitative control of STAT5 signaling. A) Measured number of molecules showed that titrated IL-2 quantitatively integrated into the RBC surface; insets show dose distribution and RBC modification efficiency. **B)** sbPCI IL-2 and IL-15 were functionally titrated (as shown by STAT5 phosphorylation) relative to soluble cytokine controls; 4 pmol surface-presented cytokine selected for subsequent G-Rex evaluation. **C)** Representative flow cytometry plots demonstrate STAT5 phosphorylation was induced by sbPCI presented IL-15 (top) and IL-2 (bottom).

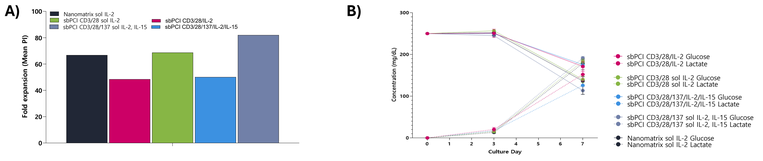


Figure 3. Single dose integrated cytokines support robust G-Rex 6M expansion without glucose limitation. A) All conditions achieved robust Day 7 T cell expansion consistent with reported G-Rex performance, although single dose sbPCI integrated cytokines produced lower fold expansion than repeated soluble cytokine and NanoMatrix controls. **B)** Higher expanding soluble cytokine cultures showed greater glucose consumption and lactate accumulation, while sbPCI cultures retained substantial glucose at Day 7, indicating that lower expansion was not associated with glucose depletion.

Results: Shift to More Desired Central Memory Phenotype

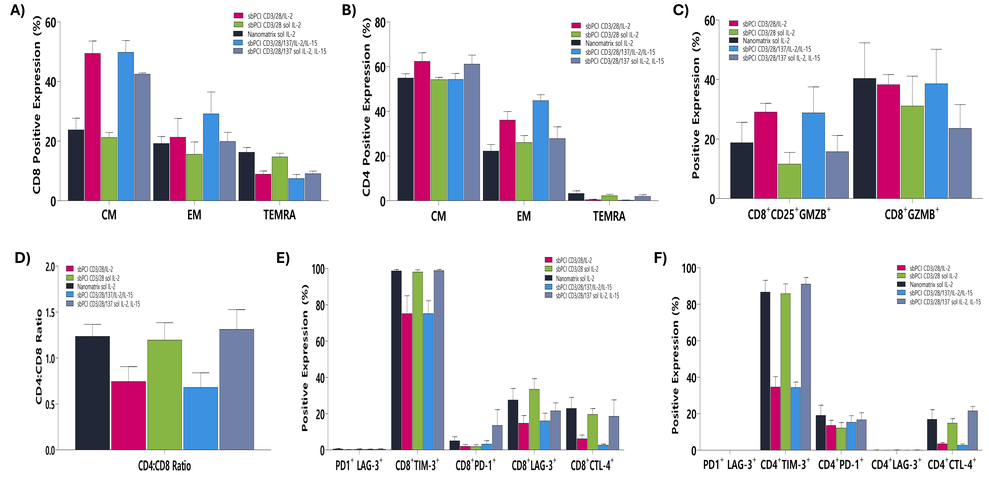


Figure 4. Integrated sbPCI cytokines shift Day 7 T cell phenotype toward memory associated, cytotoxic populations with lower inhibitory receptor expression. A, B) Integrated cytokines shifted memory subset distribution while limiting TEMRA differentiation, with a more pronounced phenotype shift in CD8 (A) than CD4 (B) T cells. **C)** sbPCI cytokine conditions maintained $CD8^+GZMB^+$ expression and increased the $CD25^+GZMB^+$ population relative to matched soluble cytokine conditions. **D)** Integrated cytokine conditions produced lower CD4:CD8 ratios, indicating greater relative CD8 representation. **E, F)** Integrated cytokine conditions showed lower Day 7 inhibitory receptor expression, most notably TIM-3 in both CD8 (**E**) and CD4 (**F**) T cells, while LAG-3 expression was largely restricted to CD8 T cells.

Conclusions

Synapse-localized cytokine presentation, through surface integration via sbPCI (a programmable immune-interface platform), supported effective T cell expansion in 6M G-Rex while favorably shifting differentiation toward memory-associated, cytotoxically armed phenotypes.

Quantitative cytokine presentation can be integrated with T cell activation and costimulatory signals to control immune synapse signaling.

The formulation of a single-dose integrated sbPCI cytokines produced lower expansion than repeatedly dosed soluble cytokines, but were associated with memory-enriched, cytotoxically armed phenotypes and lower inhibitory receptor expression.

Future Directions: Refine cytokine presentation geometry, molecule number and stoichiometry that optimize the balance between cell yield and phenotype, and evaluate persistence and function following extended culture and restimulation.