Modular Lipid Bilayer Platform Enables Antigen-Specific vs. Polyclonal CD19 CAR-T cell Expansion with Distinct Phenotypic and Functional Outcomes

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Main Conclusion

A single programmable RBC platform supports feeder-free T-cell activation and antigen-specific CAR T-cell expansion, with quantitatively controlled antigen presentation.

Background

Current CAR T-cell manufacturing often uses polyclonal activation, which can generate heterogeneous cell products.

Antigen-specific activation enriches CAR$^+$ cells but depends on target-specific engineered feeder lines.

A programmable interface can control ligand identity, density, and geometry to drive antigen-specific CAR activation.

Methods

sbPCI were integrated on red blood cells (RBCs, Gulf Coast Blood) on demand (<4 h) without feeder cells. Antibodies, ligands, and antigens were attached as shown in table below (Biolegend and Acro Biosystems).

sbPCI	sbPCI : T cell	Day Added
αCD3/αCD28	1:2	0 and/or 3
CD19/αCD28/αCD137	1:1 (CAR$^+$)	3

Activated T cells (n=4) were electroporated with TcBuster (transposase) / CD19 CAR-T2A-eGFP-T2A-DHFR on Day 2 ((BioTechne transposon, Lonza 4D Nucleofector).

Cultures were grown in either T75 flasks or 6M G-Rex (ScaleReady). All cultures were supplemented with antigen specific sbPCI, soluble IL-7 and IL-15 at 10ng/ml on Day 3. Additional supplementation and stimulation occurred as show in Figure 2.

Expansion and phenotype were evaluated by NC-200 and flow cytometry.

Results

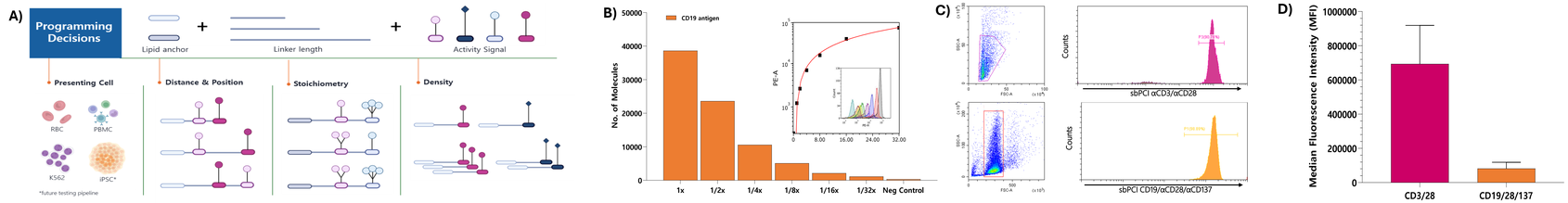


Figure 1. Dose titration of sbPCI CD19 on red blood cells enables quantifiable antigen presentation. (A) sbPCI design parameters enable quantitative and spatial organization. **(B)** RBCs dose titrated with sbPCI-CD19ag (1x to 1/32x). Show dose-dependent control, enabling testing of CAR response at low, medium, or high antigen levels. **(C)** Flow cytometry of RBCs labeled with sbPCI αCD3/αCD28 (top, pink) or sbPCI CD19/αCD28/αCD137 (bottom, orange). **(D)** RBC labeled with sbPCI αCD3/αCD28 or sbPCI CD19/αCD28/αCD137, MFI Mean ± SD.



Figure 2. CAR T-cell manufacturing workflow in flasks (14 days) and G-Rex (10 days). T cells were activated on Day 0 with NanoMatrix or sbPCI αCD3/αCD28, electroporated on Day 2, and stimulated on Day 3 with sbPCI αCD3/αCD28 (in initial test workflows only) or CD19/αCD28/αCD137 (1:1 sbPCI:CAR$^+$). Flask and G-Rex were then treated based on standard workflows for each. CAR expression, phenotype, and growth were measured throughout.

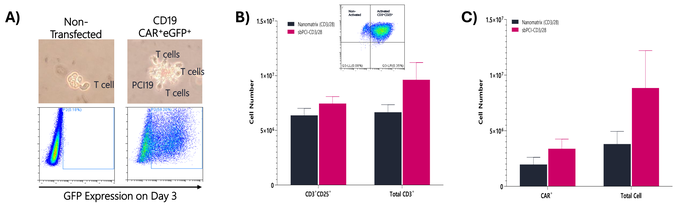


Figure 3. sbPCI pre-activation increases T cell number at electroporation and improves CAR$^+$ yield. (A) Brightfield images show clustering of T cells around sbPCI αCD3/αCD28 (left) or sbPCI CD19/αCD28/Acd137 (right). Corresponding flow plots of eGFP expression on Day 3. **(B)** CD3$^+$CD25$^+$ and total CD3$^+$ cell numbers on Day 2 after activation increased with sbPCI αCD3/αCD28 compared to Nanomatrix (αCD3/αCD28.) **(C)** sbPCI αCD3/αCD28 pre-activation increased CAR$^+$ and total cell numbers 24 hours after electroporation compared with NanoMatrix pre-activation. Mean ± SD, n = 2.

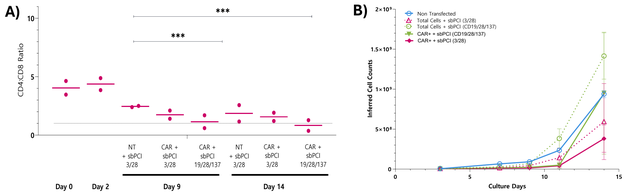


Figure 4. sbPCI configurations activate and expand electroporated CAR T cells in T75 Flasks. (A) CD4:CD8 ratio shifts toward CD8, most strongly with sbPCI CD19/αCD28/αCD137 (***p < 0.001). **(B)** Inferred cell numbers rose sharply after Day 11, and sbPCI CD19/αCD28/αCD137 gave the highest total cell count (~1.4x10^9 by Day 14) and more CAR$^+$ cells (~9x10^8) than sbPCI CD3/28 (~4x10^8). **(C)** CD25 expression was maintained on CAR$^+$ cells, whereas CD3$^+$CD25$^+$ cell numbers declined by Day 14 with sbPCI CD19/αCD28/αCD137, consistent with preferential antigen-driven activation of CAR$^+$ T cells. (*p < 0.05). Mean ± SD, n = 2.

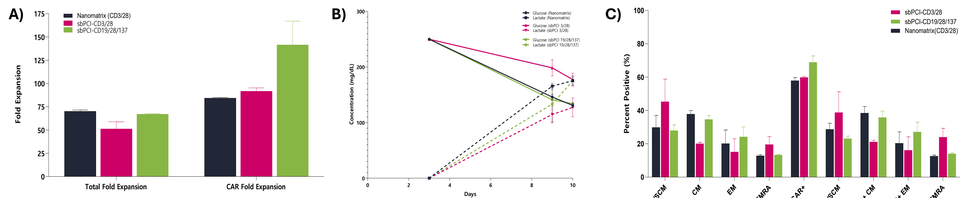


Figure 5. sbPCI is scalable for CAR T-cell manufacturing in 6M G-Rex. (A) sbPCI CD19/αCD28/αCD137 gives the highest CAR$^+$ fold expansion (~140-fold) after 10-day culture with both sbPCI producing increased CAR intensity per cell (geomean). **(B)** Metabolic demand highest with sbPCI CD19/αCD28/αCD137 and NanoMatrix, consistent with total cell expansion. **(C)** sbPCI αCD3/αCD28 enriched naive/SCM cells, including CAR$^+$ cells, and sbPCI CD19/αCD28/αCD137 yielded the highest CAR$^+$ frequency while sbPCI CD19/αCD28/αCD137 produced the most balanced CD4:CD8 ratio. Mean ± SD, n = 2 .

Rationale

Antigen-specific CAR$^+$ T cell expansion should not require a custom feeder line. Red blood cells that present antigen and co-stimulatory ligands could deliver those signals on demand.

Conclusions

Quantifiable antigen: sbPCI CD19 on RBCs can be titrated over a 32-fold range, giving a defined and measurable number of antigen molecules per cell.

Activation: sbPCI sbPCI αCD3/αCD28 produced more T cells at electroporation and more CAR$^+$ cells than NanoMatrix.

Antigen-specific expansion: In 6M G-Rex, sbPCI CD19/αCD28/αCD137 gave the highest CAR$^+$ fold expansion (about 140 versus 84 to 91 fold), with higher CAR$^+$ frequency and CAR$^+$ gMFI than NanoMatrix.

Phenotype: In flasks, sbPCI CD19/αCD28/αCD137 moved the CD4:CD8 ratio toward balance. In G-Rex, sbPCI αCD3/αCD28 enriched naive/SCM cells.

Workflow: sbPCI are made on demand in under 4 hours, require no feeder line, and scale to 6M G-Rex.

Pre-Transfection Activation: sbPCI αCD3/αCD28 activation produced more T cells at electroporation and more CAR$^+$ cells afterward than NanoMatrix.

Future Directions: Potency testing against CD19$^+$ targets, IL-2, IL-15, and/or IL-21 incorporated into sbPCI CD19/αCD28/αCD137 with bioreactor scale-up, and additional CAR targets.