Taming Donor Variability: Quantitative Surface Programming of Activators and Co-stimulatory Molecules Collapses Donor Response Expansion Phenotype Heterogeneity Across Immunologically- and Age-Diverse Donors

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Main Conclusion

High-throughput screening of sbPCI configurations reveals that cytokine incorporation collapses donor response heterogeneity from three qualitative expansion phenotypes to a single responsive profile, as visualized by PhenoGraph analysis.

Background

- Donor variability remains a major contributor to autologous T cell manufacturing failures, driven by differences in starting material fitness associated with donor age and immunologic status.
- Commercial activation reagents apply fixed, uniform stimulation that do not adapt to donor-specific immune states, limiting adaptability for outlier donors and requiring workflow modifications to compensate.
- Immune synapse architecture, specifically the density, ratio, and spatial organization of activating and co-stimulatory ligands, is a powerful but underexplored lever for controlling the quality of T cell activation outcomes.

Rationale

Programmable control of co-stimulatory molecule and cytokine density at the immune synapse, combined with high-throughput screening and PhenoGraph visualization, may identify configurations that reduce donor response heterogeneity.

Methods

- PBMCs isolated from healthy donors were stratified into young (≤35 years) and older (≥65 years) cohorts, with each group comprising at least 3 male and 3 female donors.
- sbPCI were generated same-day and programmed to vary the surface density, relative ratios, and spatial organization of anti-CD3/CD28/CD137 antibodies as well as integrated IL-2 (25 U/mL), IL-7 (5 ng/mL), and IL-15 (5 ng/mL) on RBCs.
- PBMCs were activated for 7 days in 96-well plates with sbPCI supplemented by either soluble cytokines or cytokines directly incorporated.
- Samples were analyzed for fold expansion; CD4:CD8 ratio, memory, activation, exhaustion, and cytotoxicity were also assessed (data not shown).

Results

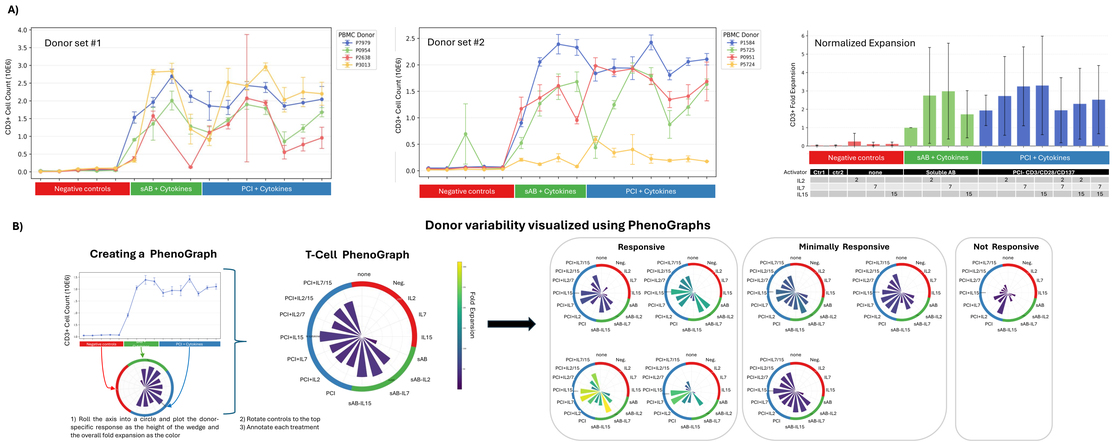


Figure 2. Soluble cytokine supplementation produces heterogeneous donor expansion profiles. PBMCs from 8 healthy donors (2 sets, n = 4 each) were activated with sbPCI (CD3/CD28/CD137) or sAB with soluble IL-2, IL-7, IL-15, or combinations for 7 days. **(A)** Line plots show CD3⁺ fold expansion per donor with visible inter-donor spread across conditions. **(B)** PhenoGraphs reveal three qualitative response patterns: Responsive, Minimally Responsive, and Not Responsive, which are defined by visual clustering, not statistical classification.

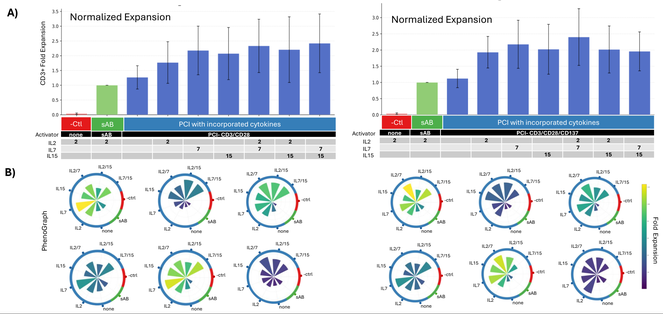


Figure 3. Cytokine incorporation into sbPCI activation surfaces collapses donor response heterogeneity to a single expansion phenotype. **(A)** PBMCs from 6 healthy donors were activated with sbPCI containing anti-CD3/CD28 (**left panels**) or anti-CD3/CD28/CD137 (**right panels**) with cytokines (IL-2, IL-7, IL-15, and combinations) incorporated into the sbPCI surface rather than added as soluble supplements. **A)** Bar plots show fold expansion normalized to sAB; across most conditions, all 6 donors exceeded the sAB baseline, particularly in the CD3/CD28/CD137 configuration. **(B)** PhenoGraphs show a single 'Responsive' profile across all donors, in contrast to the three heterogeneous patterns observed with soluble delivery (**Figure 2**), with CD137 co-stimulation further contributing to this convergence. Statistical and quantitative confirmation is needed.

Technology

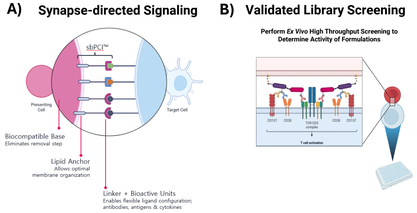


Figure 1. Programmable cellular interfaces enable quantitative control of immune synapse signaling. **A)** sbPCI are generated using modular design comprising membrane organizers, spatial organizers, and immunomodulatory biomolecules, enabling defined presentation architectures. **B)** Formulations are evaluated using *ex vivo* high-throughput screening to identify synapse configurations that produce desired signaling and functional outcomes.

Conclusion

- Soluble cytokine supplementation produces three qualitatively distinct donor expansion phenotypes (Responsive, Minimally Responsive, Not Responsive), highlighting the challenge of donor variability under fixed stimulation strategies.
- Cytokine incorporation into sbPCI collapses this heterogeneity: all donors display a single "Responsive" PhenoGraph profile, with CD137 co-stimulation further contributing to this convergence. Statistical and quantitative confirmation is needed.
- sbPCI enables rapid, high-throughput screening of immune synapse configurations, with PhenoGraph providing a practical tool to visualize and categorize donor-specific expansion behavior.
- sbPCI positions immune synapse engineering as a screenable lever to de-risk autologous T cell manufacturing.

Future Directions

- Expand cohort to disease-associated immune dysfunction and extend to CAR/TCR-redirected formats
- Scale into closed-system bioreactors
- Validate with clinical manufacturing partners