Rapid, Feeder-Free Expansion of NK Cells in Less Than 14 Days Using a Biocompatible Lipid Bilayer-Based Immunomodulating Expander in a Bioreactor System

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Main Conclusion

sbPCI-NK Expander enables feeder-free NK expansion from unfractionated PBMCs without NK-specific media. sbPCI-IL-21 activates STAT3 equivalently to soluble delivery yet produces distinct receptor and cytotoxic profiles, indicating that immune synapse architecture, not signal strength alone, is a tunable lever for NK manufacturing.

Background

- NK manufacturing faces a tradeoff between expansion performance (feeder-based) and process simplicity (feeder-free); neither offers programmable control of activation signaling.
- NK activating receptors are governed by distinct pathways: NKG2D is STAT3-dependent and IL-21-regulated (Zhu et al., Blood 2014); NKp46 signals through STAT3-independent, ITAM-based pathways.
- Current reagents deliver fixed stimulation that cannot independently modulate these axes, limiting control over NK phenotype and cytotoxic quality.

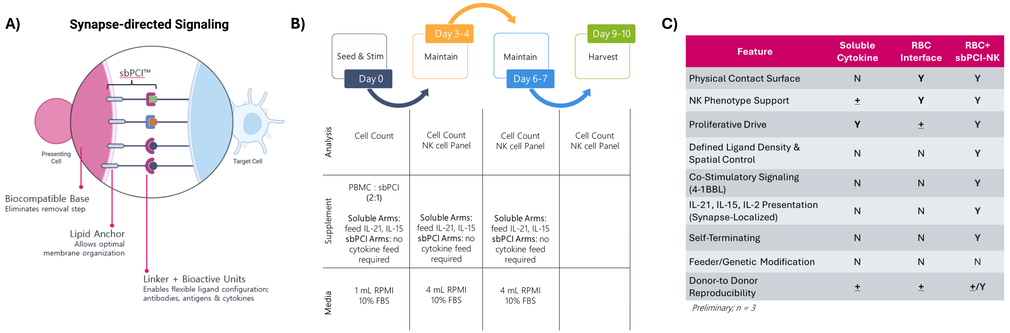
Rationale

Programmable control of cytokine density and spatial organization at the immune synapse may selectively tune NK receptor expression and cytotoxic arming without altering standard workflows.

Methods

- sbPCI-NK Expander displayed anti-CD137 with IL-15, IL-21, and/or IL-2 at defined spatial organization. The RBC-based interface self-terminates to limit carryover.
- PBMCs from healthy donors were screened in 96-well plates, then scaled into G-Rex bioreactors. NK cells were not isolated prior to culture in complete media + FBS. No NK cell-specific media were used.
- Controls received soluble cytokines 3×/week; sbPCI-cytokine groups received no exogenous soluble cytokines after Day 0.
- 96-well panel: CD3, CD45, CD56 (Days 3, 5). G-Rex panel: CD56, CD16, NKG2D, NKp46, CD3, granzyme B. STAT3 phosphorylation assessed at 30–40 minutes post co-culture.

Results



C)

Feature	Soluble Cytokine	RBC Interface	RBC+ sbPCI-NK
Physical Contact Surface	N	Y	Y
NK Phenotype Support	±	Y	Y
Proliferative Drive	Y	±	Y
Defined Ligand Density & Spatial Control	N	N	Y
Co-Stimulatory Signaling (4-1BBL)	N	N	Y
IL-21, IL-15, IL-2 Presentation (Synapse-Localized)	N	N	Y
Self-Terminating	N	N	Y
Feeder/Genetic Modification	N	N	N
Donor-to Donor Reproducibility	±	±	+/Y

Preliminary; n = 3

Figure 1. Programmable cellular interfaces enable quantitative control of immune synapse signaling. (A) sbPCI are generated from modular membrane organizers, spatial organizers, and immunomodulatory biomolecules. **(B)** G-Rex NK workflow: sbPCI-NK Expander added at Day 0 with optional restimulation; no NK isolation or soluble cytokine addition required. **(C)** sbPCI-NK combines RBC biological benefits with programmable ligand presentation, costimulatory signaling, and self-termination — features absent from unmodified RBCs or soluble cytokines.

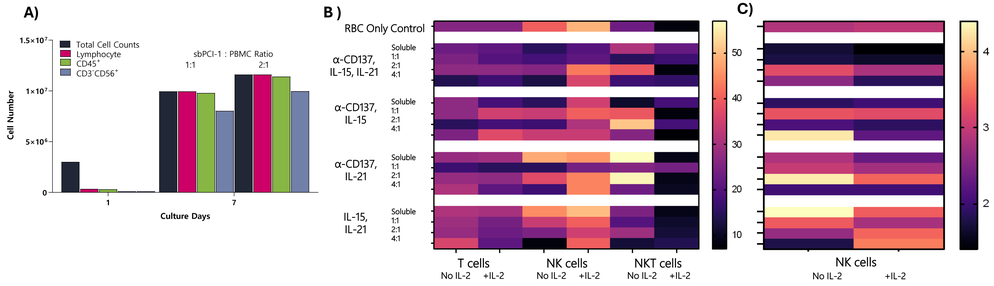
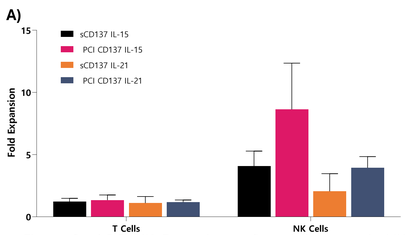
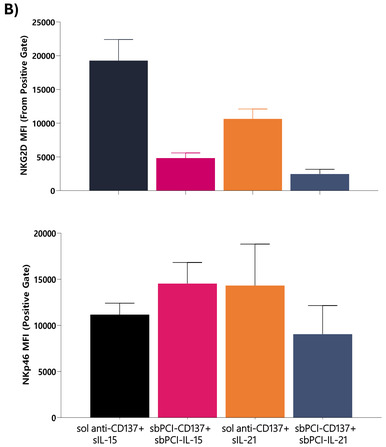
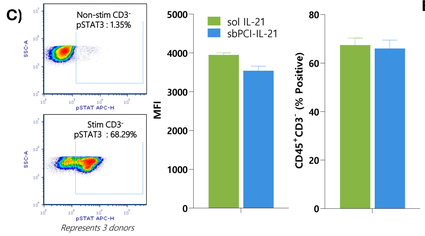
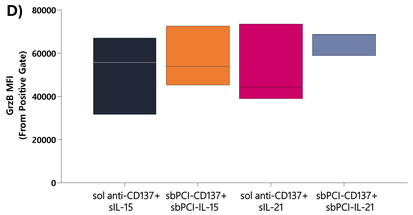


Figure 2. High-throughput screening identifies lead sbPCI-NK formulations for bioreactor scale-up. (A) T75 feasibility confirms NK expansion on RBC-based sbPCI at 7 days; 2:1 outperforms 1:1 (93× vs 75× fold expansion; n = 2). **(B)** Day 5 composition and expansion (96-well): varying ligand density, spatial organization, and PBMC:sbPCI ratio without NK cell depletion produces distinct NK:T outcomes from identical molecular inputs — presentation architecture, not just identity, shapes cellular response. CD137/IL-21 and CD137/IL-15 were advanced to G-Rex (Figure 3). **(C)** NK fold expansion (Day 3 → Day 5). Soluble IL-2 diminished the NK-selective effect and was excluded from G-Rex translation.









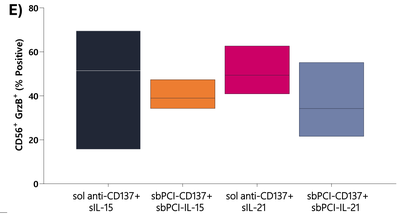


Figure 3. sbPCI-NK Expander preferentially expands NK cells and differentially modulates activating receptor expression in G-Rex. PBMCs from 3 donors were cultured with sbPCI-NK or soluble cytokine controls. (A) sbPCI CD137/IL-15 drives ~8.5× NK expansion with minimal T cell outgrowth (~1×), confirming selective NK expansion without isolation or depletion. **(B) TOP:** NKG2D MFI is reduced in sbPCI groups, consistent with STAT3-dependent receptor dynamics. **BOTTOM:** NKp46 is retained across conditions, reflecting STAT3-independent regulation. This differential response demonstrates that sbPCI composition selectively influences distinct NK signaling axes.

Figure 4. Surface-presented IL-21 engages STAT3 equivalently to soluble delivery but produces distinct cytotoxic arming profiles. (C) sbPCI-IL-21 induces STAT3 phosphorylation in PBMCs at 40 minutes comparable to soluble IL-21, confirming equivalent JAK/STAT3 engagement. **(D)** % GrzB+ NK cells is comparable across conditions; however, GrzB MFI **(E)** trends higher in sbPCI groups — particularly PCI CD137/IL-21 — suggesting synapse-localized delivery promotes deeper per-cell cytotoxic arming. These data, combined with differential NKG2D/NKp46 modulation (Figure 3B), indicate that presentation mode, not signal strength, shapes NK phenotype and function.

Conclusions

- sbPCI-NK Expander drives feeder-free NK expansion from unfractionated PBMCs without NK-specific media, T cell depletion, or exogenous cytokines.
- Equivalent STAT3 activation from surface-presented and soluble IL-21 produces distinct receptor (NKG2D modulated; NKp46 stable) and cytotoxic (higher per-cell GrzB) profiles - presentation mode, not signal strength, shapes NK phenotype.
- Lead configurations translate from 96-well screening to G-Rex bioreactor, establishing a path toward scalable, tunable NK manufacturing.