Synecta™ Cell-Derived Nanoparticle Platform Offers Versatile Use of Membrane-Bound and Soluble Cytokines for Rapid and Efficient CAR-T Cell Production

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BlueWhale Bio

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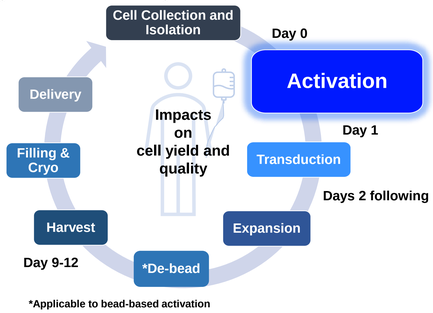
Background

CAR-T cell manufacturing is limited by slow production and inefficient T cell activation. Current methods introduce variability and complicate workflows while not fully addressing a variety of additional technical challenges. Rapid, robust, and consistent T cell activation technology is therefore essential for advancing CAR-T manufacturing.



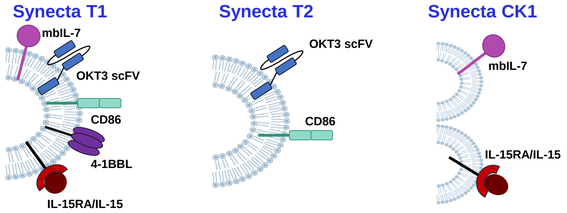
*Applicable to bead-based activation

We developed the Synecta™ Cell-Derived Nanoparticle (CDNP) platform, which entails the surface display of membrane-bound (mb) stimulatory signals and cytokines, thus enabling more consistent, robust, and simplified CAR-T manufacturing. We hypothesized that, compared to soluble cytokines, membrane-bound formats promote more effective expansion of long-lived and highly functional T cells.

Radically simplified process

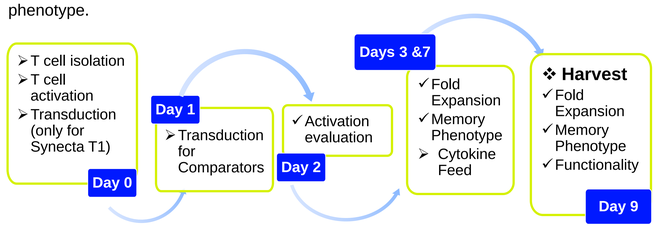
Day 0	Day 3 - 9
Synecta T1 + Transduction	Harvest

Synecta Activation Platform



Methods

Synecta T1 or T2 in combination with Synecta CK 1 or soluble cytokines were evaluated for *in vitro* T cell expansion and activation, as well as their effect on T cell phenotype.



- **Isolation:** T cells were negatively isolated from healthy and patient donors.
- **Activation:** T cells were activated with Synecta or comparators on Day 0.
- **Transduction:** T cells were transduced with CD19 CAR LVV on Day 0 for Synecta T1 and on Day 1 for comparators.
- **Cytokines:** Comparator cultures were supplemented with soluble IL-7 and IL-15 (5 and 10 ng/mL; Figure 1) or IL-2 (100 IU/mL; Figure 4). Synecta T1 was supplemented with IL-7 and IL-15 (5 and 10 ng/mL) and Synecta CK 1 (Figures 2 & 3), while Synecta T2 received IL-2 (100 IU/mL).
- **Expansion:** Cells were counted on Days 3, 7, and 9 using NC-200.
- **Flow Cytometry:** Activation markers (CD69, CD25) were assessed on Day 2; phenotype markers (CD4, CD8, CD45RA, CCR7, CD95) on Days 3, 7, and 9.
- **Functionality:** Chronic Antigen Exposure (CAE) was performed on Day 9 post-harvest.

Results

Synecta T1 drives rapid, functional T-cell expansion

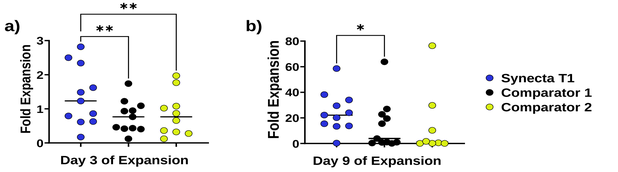


Figure 1. T cell Expansion with Synecta T1 vs Commercial Activators. (a–b) Expansion of patient T cells: Synecta T1 and Comparator 1 (n = 11) and Comparator 2 (n = 10) on Day 3 (a) and Day 9 (b). **Stats:** Mean ± SD; (a) Repeated Measures One-way ANOVA with Sidak's; (b) Wilcoxon paired t-test; *p < 0.05, **p < 0.01.

Synecta T1 promotes robust CAR-T cell expansion without inducing exhaustion

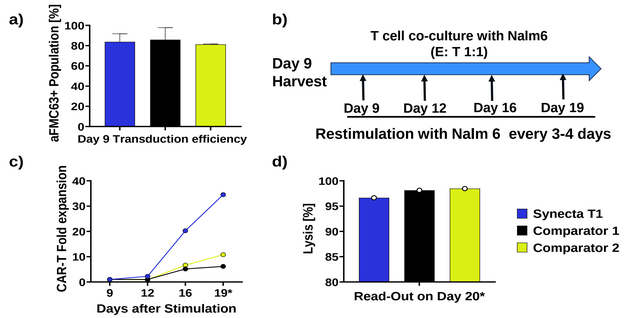


Figure 2. T Cell Functionality: Synecta T1 vs. Commercial Activators. (a) Transduction efficiency of T cells activated with Synecta T1 (Day 0) versus commercial activators (Day 1), n = 2, Mean ± SD. (b) Schematic of the CAE assay. (c)CAR-T expansion comparison. (d)Synecta T1-derived CAR-T cytotoxicity at E: T 1:1 (Day 20).

Synecta CK1 boosts Synecta T1-mediated T-cell expansion while maintaining a memory phenotype

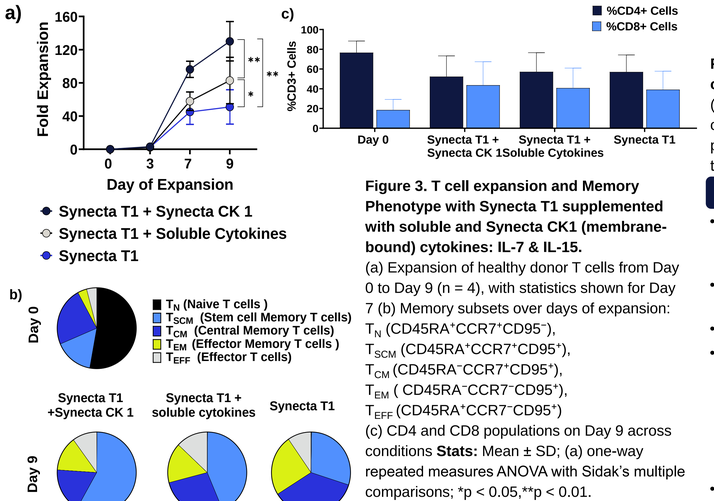


Figure 3. T cell expansion and Memory Phenotype with Synecta T1 supplemented with soluble and Synecta CK1 (membrane-bound) cytokines: IL-7 & IL-15.
(a) Expansion of healthy donor T cells from Day 0 to Day 9 (n = 4), with statistics shown for Day 7 (b) Memory subsets over days of expansion: T_N ($CD45RA^+CCR7^+CD95^-$), T_{SCM} ($CD45RA^+CCR7^+CD95^+$), T_{CM} ($CD45RA^-CCR7^+CD95^+$), T_{EM} ($CD45RA^-CCR7^-CD95^+$), T_{EFF} ($CD45RA^+CCR7^-CD95^+$)
(c) CD4 and CD8 populations on Day 9 across conditions **Stats:** Mean ± SD; (a) one-way repeated measures ANOVA with Sidak's multiple comparisons; *p < 0.05,**p < 0.01.

Synecta T2 supports versatile cytokine supplementation

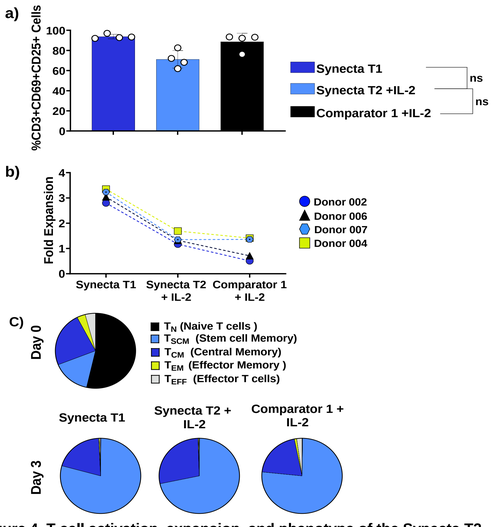


Figure 4. T-cell activation, expansion, and phenotype of the Synecta T2 compared with Comparator 1 and Synecta T1
(a) Activation indicated by $CD69^+CD25^+$ double-positive cells on Day 2 across conditions. (b) Expansion in healthy donors on Day 3 (n = 4). (C) Memory phenotype (as defined in Fig. 3) on Day 3. **Stats:** Mean ± SD; (a) Friedman test with Dunn's post hoc; $p < 0.05$ significant, ns = not significant

Conclusions

- Synecta CDNPs mimic APCs through membrane-bound presentation of stimulatory ligands and cytokines, eliciting synapse-like activation kinetics that promote robust T cell expansion.
- Modular design enables combining a broad range of membrane signals
- Established large-scale manufacturing at 50 L
- Three off-the-shelf products for distinct use cases:
  - **Synecta T1:** Optimized activation via IL-7 and IL-15/IL-15Rα complex
  - **Synecta T2:** Flexible pairing with soluble cytokines (e.g., IL-2)
  - **Synecta CK1:** Proliferation booster (e.g., for allogeneic manufacturing)
- Applicable to multiple immune cell types; expanding towards stem cell applications

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