

Synecta™ T1 Cell-Derived Nanoparticles Enhance Lentiviral Transduction and T Cell Manufacturing Efficiency with IRO®:

A Closed, Automated Cell Therapy Manufacturing Platform



Key Findings

- Synecta T1 + IRO® achieved the highest transduction efficiency and total viable transduced cell yield across all conditions tested.
- In Synecta T1-activated conditions, a 48-hour static phase prior to initiating IRO® transduction mixing maximized D7 CAR-T cell output.
- Performance was reproduced across independent runs, supporting platform and workflow robustness.
- Synecta T1 shifted T cells toward a fitter, less glycolysis-skewed metabolic state
 - maintained oxidative phosphorylation with reduced extracellular acidification
 - consistent with a more memory-associated product phenotype.

Background

CAR-T cell therapy manufacturing faces key challenges to deliver therapies at the scale needed to ensure widespread patient access. Overcoming these challenges requires innovation across biology and technology.

Conventional CD3/CD28 bead-based activation adds workflow complexity through bead removal steps, ongoing cytokine supplementation, and delayed transduction — and can impair T cell fitness, metabolic state, and transduction readiness. On the technology side, legacy, non-automated, static culture systems limit expansion capacity, gene delivery efficiency, and scalable throughput.

Synecta™ T1 cell-derived nanoparticles (CDNPs) address the biological bottleneck caused by suboptimal activation. A biodegradable, bead-free activation platform, Synecta T1 delivers localized, multi-signal T cell activation through membrane-bound OKT3 scFv, CD86, 4-1BBL, IL-7, and IL-15/IL-15R α — mimicking the complexity of the immunological synapse. By eliminating bead removal, reducing reliance on exogenous cytokines, and enabling simultaneous Day 0 activation and transduction, Synecta T1 supports a favorable activation state, improved T cell fitness, and a metabolic profile associated with productive expansion and in vivo persistence.

Oribiotech's IRO® platform is the new standard in cell therapy manufacturing technology. IRO automates better biology by combining proprietary hardware, consumables, software and data analytics in a closed, GMP-ready system.

Autologous cell therapies, manufactured from a patient's own cells, require precise, reliable and scalable manufacturing processes. IRO is a closed, automated manufacturing platform with programmable fed-batch culture and dynamic mixing modes — static, rock, and compression — automating critical steps in T cell activation, transduction, and expansion. The platform provides real-time process insights and supports seamless transition from research to GMP manufacturing.

Here, we evaluate the combination of Synecta T1 and IRO® against a polymeric nanomatrix comparator activator (Comparator 2) across multiple bioreactor conditions and characterize the metabolic profile of Synecta T1-activated T cells relative to a bead-based comparator (Comparator 1). The goal: determine whether pairing Synecta T1's biology-driven activation with IRO®'s automated culture environment produces synergistic manufacturing performance.

Materials & Methods

Study Design

Two complementary studies were conducted:

- **Study 1 — Bioreactor Format Comparison:** Head-to-head evaluation of Synecta T1 vs. Comparator 2 across IRO® and static bioreactor formats, with IRO® mixing conditions varied to optimize transduction and yield.
- **Study 2 — Metabolic Profiling:** Seahorse-based mitochondrial stress assay (OCR and ECAR) comparing Synecta T1 vs. Comparator 1 to characterize metabolic fitness of activated T cells.

Conditions (Study 1)

Condition	Vessel	Activator	Protocol
C1	IRO® Platform	Synecta T1	24h static hold post-seed, then mixing
C2	IRO® Platform	Synecta T1	48h static hold post-seed, then mixing
C3	IRO® Platform	Synecta T1	Rock mix from Day 0 (no static period)
C4	IRO® Platform	Comparator 2	24h static hold post-seed, then mixing
C5	Static Bioreactor	Synecta T1	Static culture throughout
C6	Static Bioreactor	Comparator 2	Static culture throughout

Activation & Transduction

Synecta T1 conditions (C1, C2, C3, C5): Day 0 activation and lentiviral transduction. No exogenous cytokines added during culture time. CDNPs are biodegradable — no removal step required.

Comparator 2 conditions (C4, C6): Day 1 lentiviral transduction (Day 0 activation). Cytokine supplementation: 5 ng/mL IL-7 + 10 ng/mL IL-15 throughout culture.

Harvest & Analytics

Day 7 harvest. Endpoints: total viable cell count, transduction efficiency (GFP+ or CD19+ by flow cytometry), CD4/CD8 ratio, memory phenotype (CD45RA, CCR7, CD95 surface markers — T_n, T_{scm}, T_{cm}, T_{em}, T_{ef} subsets).

Seeding & Scale

150M isolated T cells seeded into IRO®; 15M into static bioreactor. Lentiviral vector (LVV) added at MOI = 1 for GFP+ transduction efficiency assessment (Study 1, Figure 1) and MOI = 0.5 for CD19+ CAR-T analysis (Study 1, Figure 2).

Metabolic Profiling (Study 2)

OCR and ECAR were measured by mitochondrial stress assay following sequential treatment with oligomycin, BAM15, and rotenone plus antimycin A. Synecta T1-activated cells were compared to Comparator 1-activated cells across matched vessel formats.

Results

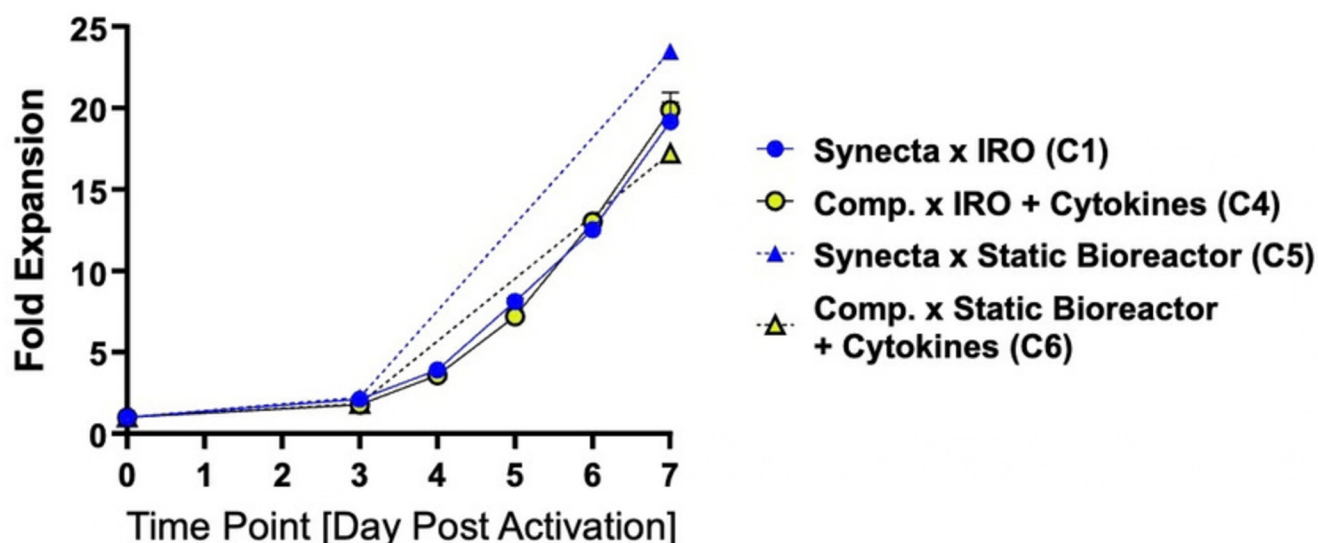
Study 1, Part A: Synecta T1 + IRO® Achieves Highest Transduction and Yield Across Bioreactor Formats

Synecta T1 supported greater expansion than Comparator 2 in the static bioreactor. This advantage was amplified in transduction performance: the combination of Synecta T1 and IRO® produced the highest total viable transduced cell yield across all conditions.

Transduction efficiency was highest in the Synecta T1 + IRO® conditions, consistent with the benefit of Day 0 transduction in a receptive, activated T cell population combined with the dynamic mixing environment of the IRO® system.

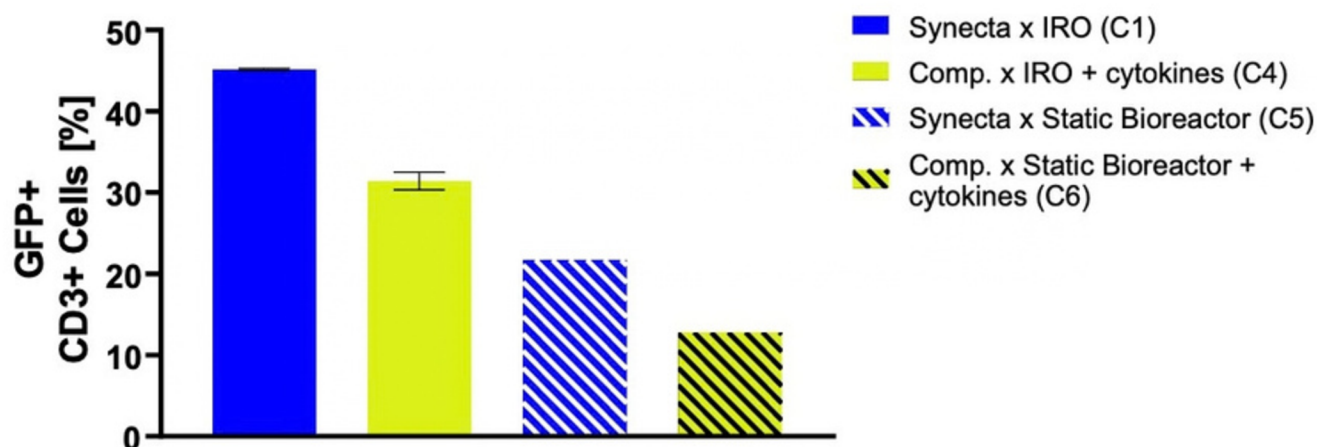
CD4/CD8 composition remained balanced across all conditions. Memory phenotype distribution at Day 7 was broadly comparable, with central memory T cells (T_{cm}) as the predominant subset across conditions — a favorable phenotype associated with in-vivo persistence.

FIGURE 1A — Fold Expansion Over Time



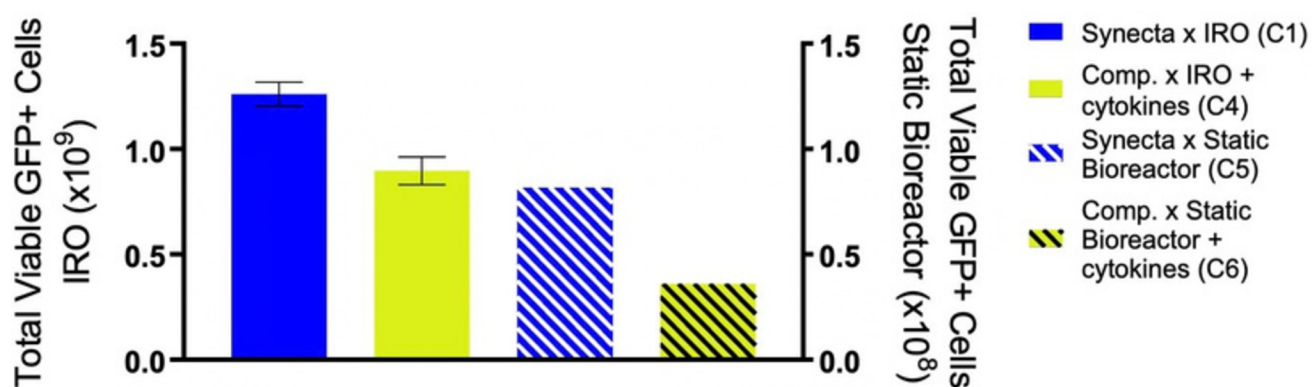
T cell fold expansion in IRO and static bioreactor culture for Synecta T1 and Comparator 2 across dynamic IRO and static conditions. N=2 for IRO conditions; N=1 for static. MOI=1.

FIGURE 1B — Transduction Efficiency at Day 7



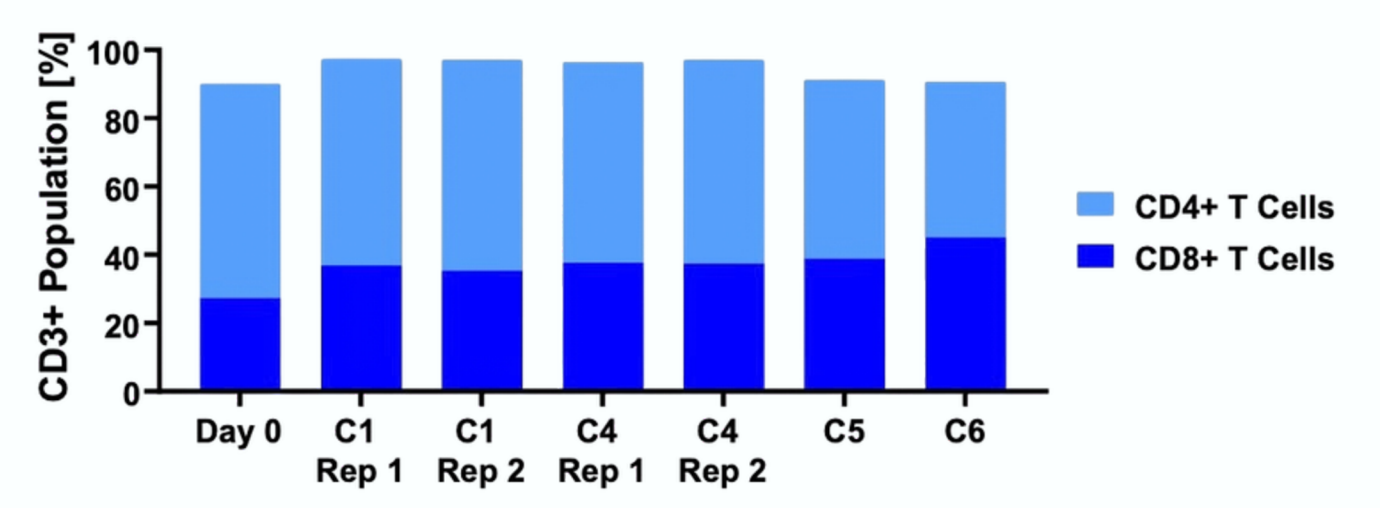
Day 7 transduction efficiency (GFP+) for Synecta T1 and Comparator 2 across IRO and static bioreactor formats. MOI=1; N=2 for IRO; N=1 for static.

FIGURE 1C — Total Viable GFP+ Cell Yield at Day 7



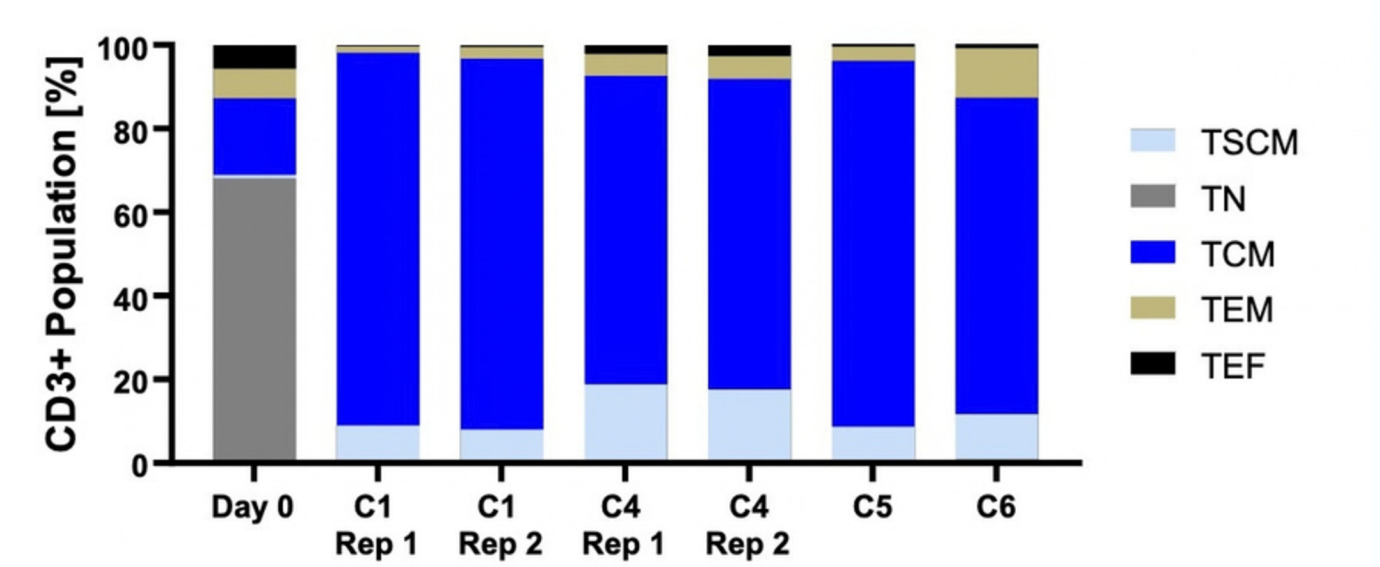
Total viable GFP+ T cell count on Day 7 across all conditions. Synecta T1 + IRO produced the highest yield. MOI=1; N=2 for IRO; N=1 for static.

FIGURE 1D — CD4/CD8 Population at Day 7



CD4+ and CD8+ T cell distributions at Day 0 and Day 7 across conditions. N=2 for IRO; N=1 for static.

FIGURE 1E — Memory Phenotype at Day 7

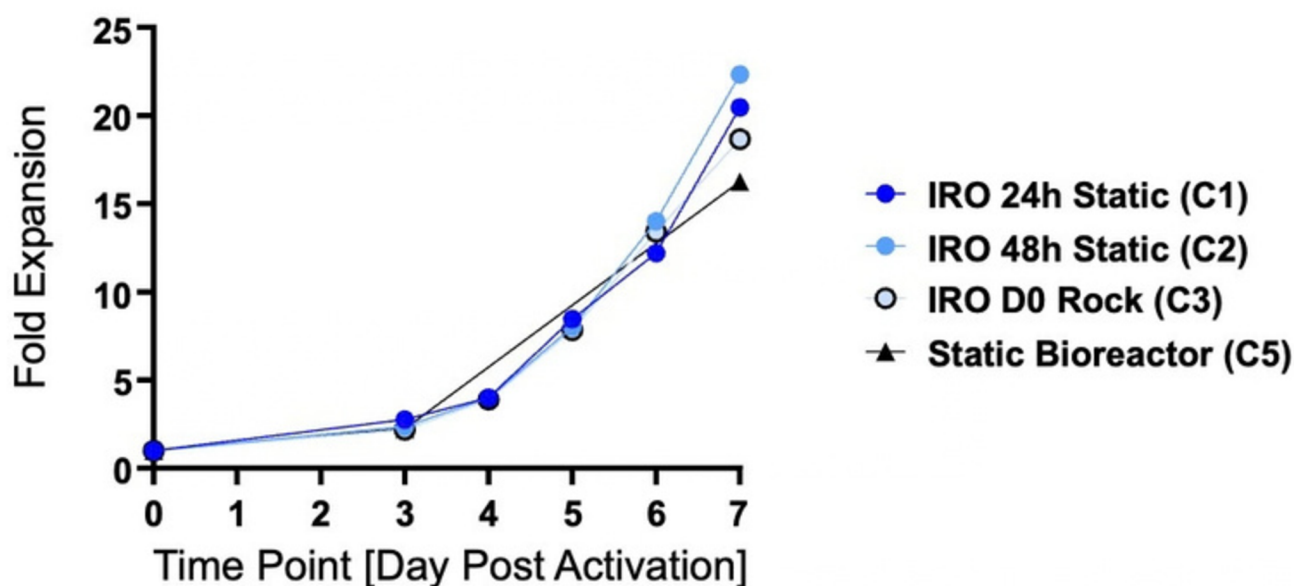


Memory T cell subset distribution (Tn, Tscm, Tcm, Tem, Tef) by CD45RA, CCR7, CD95 at Day 0 and Day 7. N=2 for IRO; N=1 for static.

Study 1, Part B: 48-Hour Static Hold Maximizes CAR-T Yield in IRO®

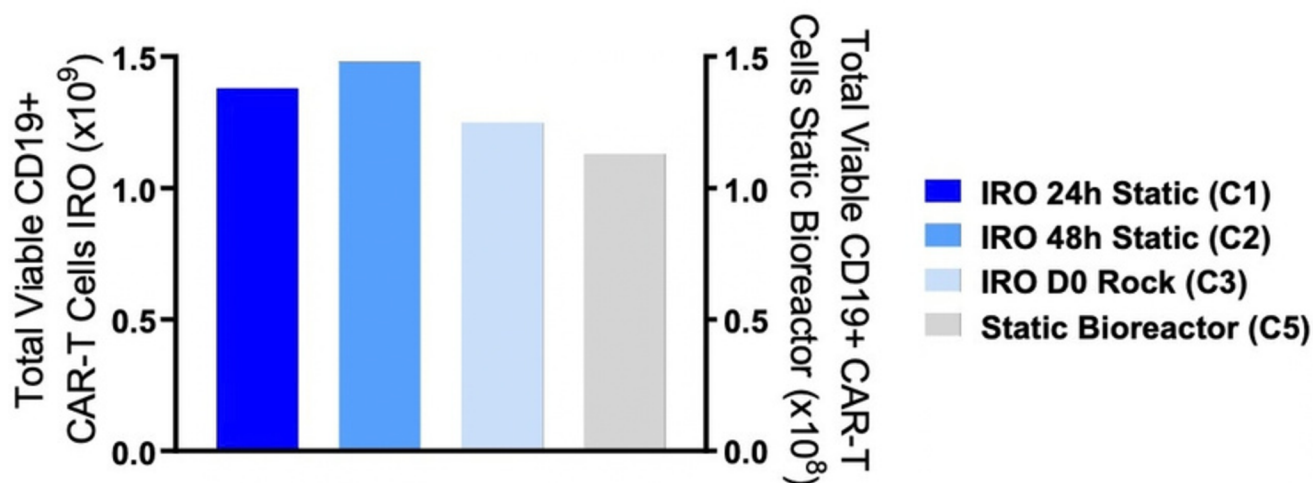
Among Synecta-activated conditions, mixing initiation timing within the IRO® workflow influenced final CAR-T yield. Conditions incorporating an initial static phase followed by IRO® mixing outperformed the fully static condition, with the 48-hour static hold condition (C2) yielding the highest total viable cell expansion and total viable CD19+ CAR-T cells at Day 7. The lower CAR+ yield observed in the fully static bioreactor condition (C5) suggests that the benefit was associated with the combination of an initial static phase and subsequent mixing, rather than static culture alone. IRO® conditions overall yielded higher CD19+ CAR-T cell output than the static bioreactor (C5), highlighting the advantage of the closed, automated system. These results were reproduced across independent runs, demonstrating process robustness.

CD4/CD8 composition and memory phenotype were preserved across all IRO® mixing conditions, with no notable differences attributable to mixing timing. Central memory T cells remained the predominant subset at Day 7 across all IRO® conditions.

FIGURE 2A — Fold Expansion by IRO® Mixing Condition

Total viable cell count over time for C1 (24h static), C2 (48h static), C3 (Day 0 rock), and C5 (static bioreactor). N=1.

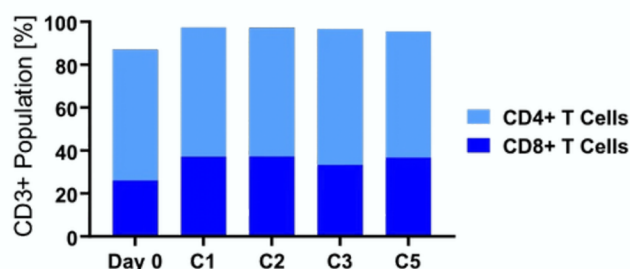
FIGURE 2B — Total Viable CD19+ CAR-T Cells at Day 7



Total viable CD19+ CAR-T cell count at Day 7 across IRO mixing conditions and static bioreactor. MOI=0.5; N=1.

FIGURE 2C

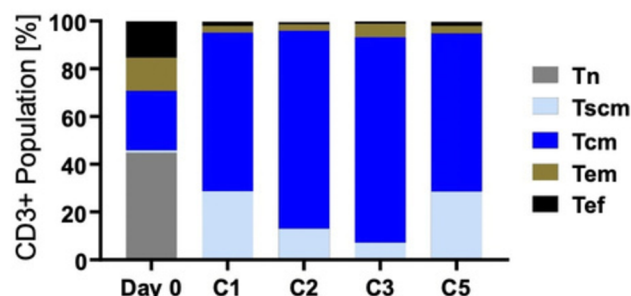
CD4/CD8 Population by IRO® Condition



CD4+ and CD8+ T cell distributions at Day 0 and Day 7 for C1, C2, C3 and C5.

FIGURE 2D

Memory Phenotype by IRO® Condition



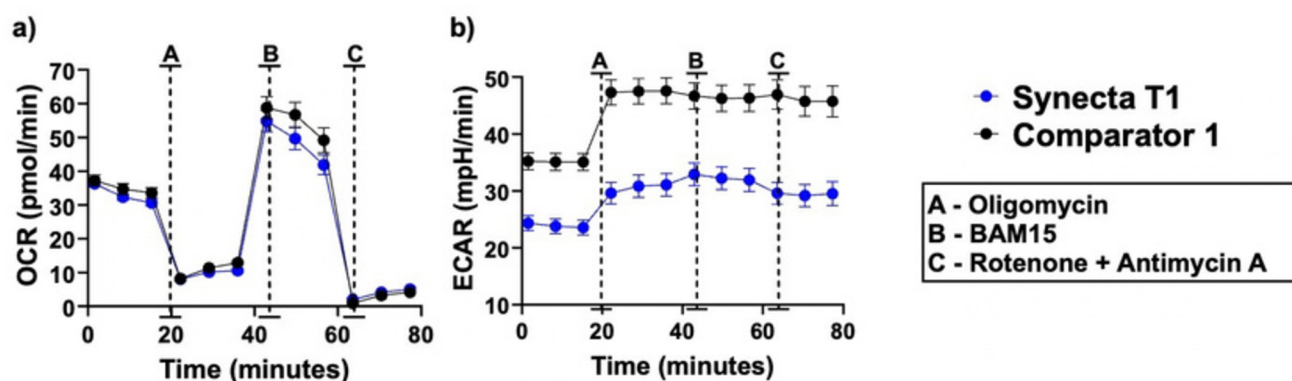
Memory T cell subset distribution at Day 7 across C1, C2, C3 and C5.

Study 2: Synecta T1 Shifts T Cell Metabolism Toward Oxidative Phosphorylation

Metabolic profiling by mitochondrial stress assay revealed a distinct metabolic signature in Synecta T1-activated T cells relative to Comparator 1-activated cells. Synecta T1-activated cells maintained oxygen consumption rate (OCR) while exhibiting lower extracellular acidification rate (ECAR), indicating reduced glycolytic reliance and greater dependence on oxidative phosphorylation.

This metabolic profile is consistent with a less-exhausted, more memory-associated T cell state. As described by Fraietta et al. (2016), T cells with a more oxidative metabolic profile at harvest are associated with superior in vivo persistence and long-term antitumor activity. The Synecta T1 metabolic advantage was observed independently of vessel format, confirming that the metabolic benefit is driven by the activation biology rather than the culture environment.

FIGURE 3 — Metabolic Profiling by Mitochondrial Stress Assay



OCR (A) and ECAR (B) over time for Synecta T1 vs. Comparator 1 following sequential treatment with oligomycin, BAM15, and rotenone/antimycin A. Lower ECAR with maintained OCR in Synecta T1-activated cells indicates reduced glycolic skewing and greater oxidative metabolic fitness.

Conclusions

These data demonstrate that Synecta™ T1 and IRO® together address key bottlenecks in CAR-T cell manufacturing — activation biology and process automation — and that combining them produces additive manufacturing performance gains.

Synecta T1 is fully compatible with the closed, automated IRO® platform, enabling Day 0 activation and transduction in a bead-free workflow without exogenous cytokines. The Synecta T1 + IRO® combination achieved the highest transduction efficiency and total transduced cell yield across all conditions tested. A 48-hour static phase before mixing further amplified output and showed reproducible performance across independent runs — a critical quality attribute for process transfer and scale-up confidence.

Synecta T1 generates TCM-dominant T cells with balanced CD4/CD8 ratios at Day 7 across vessel formats, supporting consistent product quality regardless of bioreactor system. The metabolic profiling data adds a mechanistic dimension to this phenotypic consistency: Synecta T1- activated cells maintain oxidative metabolism with reduced glycolytic skewing, consistent with the metabolic programming associated with T cell memory development and in vivo durability (Fraietta et al., 2016).

Together, Synecta T1 and IRO® offer an integrated manufacturing solution that pairs a transduction-supportive, physiological activation strategy with an automation-enabled culture system — reducing process complexity, improving transduced cell yield, and producing a final product with a favorable metabolic and phenotypic profile for clinical translation.

Reference

Fraietta JA, et al. Determinants of response and resistance to CD19 cimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nat Med. 2018;24(5):563–571. (Metabolic programming and T cell persistence.)

About OriBiotech

Ori**biotech**

Ori Biotech is a London- and Philadelphia-based manufacturing technology company on a mission to enable widespread patient access to life-saving cell and gene therapies. IRO®, Ori's next-generation manufacturing platform, automates better biology, accelerates product development and enables therapy developers to scale their products' clinical and commercial impact by seamlessly transitioning from R&D to GMP on one platform. The IRO platform has received FDA Advanced Manufacturing Technology (AMT) designation, recognizing it as a leading next-generation solution for cell and gene therapy manufacturing. For news and updates, visit oriotech.com/news-insights.

About BlueWhale Bio

BlueWhale
Bio

BlueWhale Bio is building the manufacturing platform that will define the next generation of cell-based therapies. Synecta™ is a cell-derived nanoparticle (CDNP) platform designed to replicate physiological immune system mechanisms, providing biological control over manufacturing to drive the speed, yield, and cell quality the industry needs. Synecta is in clinical use at the University of Pennsylvania (NCT04684563; first patient dosed July 5, 2025) and has received FDA Advanced Manufacturing Technology (AMT) designation (November 5, 2025). GMP supply is available through Avantor. For more information, visit bluewhale.bio.