Late-Breaking Abstract: Improved T-Cell Expansion, Viability, and Metabolic Fitness with Synecta™ Cell-Derived Nanoparticles Across Clinically Relevant Media Conditions

Authors
Chiquita Hanindya[1], Asma Ayari, PhD[2]; Bakir Valentic[3], Peter Keller[1], David Sheehan[2], Roddy O'Connor[3]
[1]BlueWhale Bio, Philadelphia, PA, USA
[2]Nucleus Biologics, San Diego, CA, USA
[3]University of Pennsylvania, Philadelphia, PA, USA

Background
Robust and consistent T-cell activation during ex vivo manufacturing is a critical determinant of engineered T-cell therapy success. Conventional bead-based activation systems are often associated with variable expansion and serum dependence, reflecting strong, non-physiologic stimulation that increases glycolytic demand and metabolic stress commonly linked to exhausted T-cell phenotypes during prolonged culture. These limitations contribute to batch variability, manufacturing delays, and reduced patient access. Synecta™ is a cell-derived nanoparticle (CDNP) platform engineered to deliver membrane-bound activation and co-stimulatory signals in a biologically relevant configuration. This late-breaking analysis evaluates the impact of Synecta™ on T-cell expansion, viability, activation consistency, and metabolic fitness using various clinically relevant media including NB-ROC™ as expansion media and Physiologix XF as supplement. NB-ROC is a xeno-free, serum-free T cell culture media that can significantly boost transduction efficiency. Physiologix XF, human growth factor concentrate, is a GMP-grade, xeno-free media supplement for stem cells and T cells that replaces human serum. Synecta CDNP activation in ROC medium promotes a metabolic profile characterized by greater reliance on oxidative metabolism compared with conventional activation approaches, enabling higher expansion with reduced cellular stress. Such metabolic features have been associated with improved persistence and cytotoxic functional activity in engineered T cells. Developing CAR-T cells using a strategy that combines compatible products helps reduce manufacturing failures, shorten vein-to-vein timelines, and increase the likelihood that patients ultimately receive an optimized and successful therapy.

Methods
Isolated T cells from both healthy donors and patient samples were activated using either Synecta™ CDNPs (30 µL or 60 µL per million cells) or CD3/CD28 magnetic beads. Cultures were maintained from Day 0 to Day 7 in a G-Rex® 24 across the following conditions: Media A, Media A + 2% Physiologix and NB-ROC™ + 2% Physiologix.

Endpoints

- **Primary readouts** included T-cell expansion kinetics, population doubling, activation profiling, memory phenotype, and transduction efficiency by flow cytometry. Measurements were taken at Days 2, 3, and 7 and analyzed descriptively across conditions to assess consistency and relative performance.
- **Exploratory readouts** included cytotoxicity (killing) assessed by luminescence-based assays and metabolic endpoints evaluating cellular metabolic reliance, defined by relative glycolytic versus oxidative metabolism. Metabolic analyses were informed by independent extracellular flux (Seahorse) profiling of Synecta™-activated T cells demonstrating reduced glycolytic dependence compared with bead-activated controls.

Type of Analysis and Statistical Methods

This will be a comparative in vitro performance analysis. Results are presented as mean values with observed variability across conditions. No formal hypothesis testing was prespecified. The planned final analysis date was **January 2026**, following completion of all Day-7 readouts.

Disclosures

Chiquita Hanindya
BSc
BlueWhale Bio
Philadelphia, PA, USA
Chanindya@bluewhale.bio
Employment with BlueWhale Bio

Peter Keller
MSc
BlueWhale Bio
Philadelphia, PA, USA
Pkeller@bluewhale.bio
Employment with BlueWhale Bio

Asma Ayari
PhD
Nucleus Biologics
San Diego, CA, USA

aayari@nucleusbiologics.com
Employment with Nucleus Biologics

David Sheehan
MBA
Nucleus Biologics
San Diego, CA, USA
dsheehan@nucleusbiologics.com
Employment with Nucleus Biologics

Bakir Valentic
BA
Perelman School of Medicine
Center for Cellular Immunotherapies
Philadelphia, PA, USA
bakirv@sas.upenn.edu
Employment with University of Pennsylvania

Roddy S.O'Connor,
PhD
Perelman School of Medicine
Center for Cellular Immunotherapies
Philadelphia, PA, USA
oconnorr@pennmedicine.upenn.edu
Dr O'Connor has equity in Nucleus Biologics and Stoic Bio