SARTORIUS

Simplifying Progress

Characterization of the NanoSpark® GROW-NK Soluble Activator, a Feeder Cell, and Magnetic Bead-Free Natural Killer Cell Expansion Technology

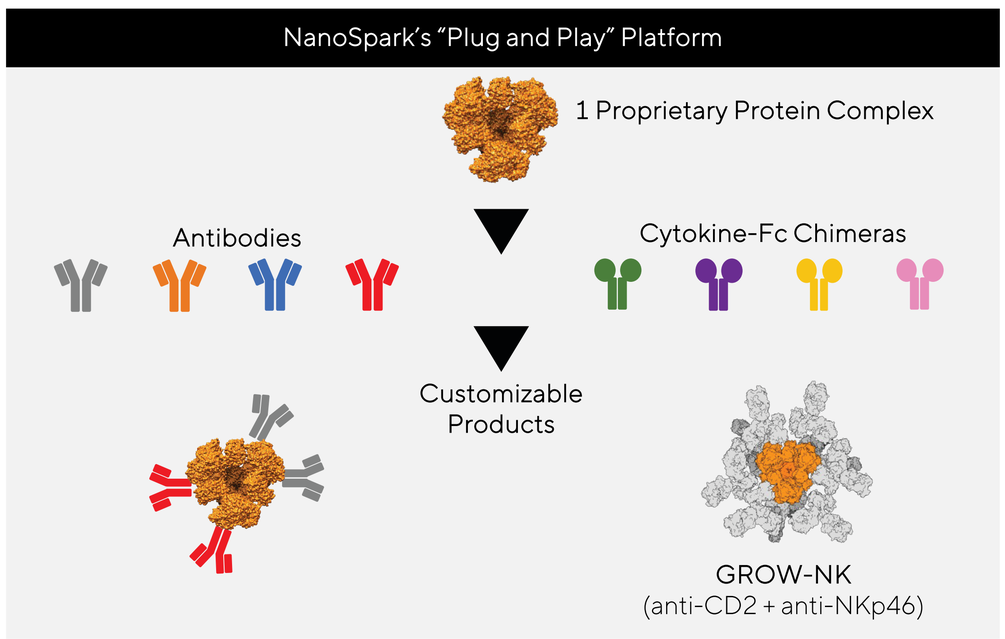
Luke G. Haines, MBE; Zachary I. Imam, PhD; Donna M. Marsh, BA; Curtis D. Hodge, PhD

2950 San Pablo Ave, Berkeley, CA 94702
* Corresponding author: curtis@nanoteintech.com

NANOTEIN TECHNOLOGIES

Introduction

Natural killer (NK) cells have generated strong interest in the cell therapy field for combating various types of cancers. NK cells offer unique advantages to this emerging industry: they require no HLA matching and are excellent candidates for "off-the-shelf" therapies. NK cells can identify and attack cancer cells even without prior stimulation. However, a major limitation of NK cell therapies is the challenge of producing a sufficient quantity of NK cells to achieve a therapeutic effect. The current industry standard for NK cell expansion involves co-culturing NK cells with engineered feeder cell lines. These feeder cells are costly, labor-intensive to maintain, and require purification—a process that is time-consuming and difficult. Even after purification, feeder cells can still contaminate the final cell product. Nanotein's novel platform technology, NanoSpark®, helps overcome these obstacles.



- High performance expander
- Feeder-free and scalable
- Soluble, all protein-based
- No magnetic components
- No purification required
- GMP manufacturing underway

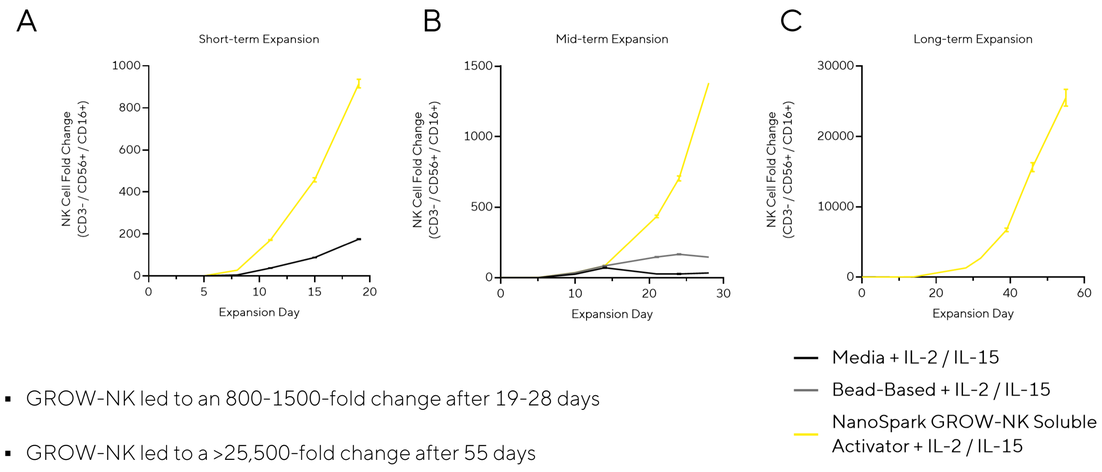
Experimental Approach

- Figure 1 Cryopreserved peripheral blood (PB) NK cells were expanded in CellGenix® GMP SCGM medium with IL-2/IL-15 (35 ng/mL each), HPL, and NanoSpark® GROW-NK (20 µL/mL). Cultures were maintained up to 55 days and analyzed for CD3–CD16+CD56+ by flow cytometry[1].

- Figure 2. Cryopreserved PB NK cells were cultured in CellGenix® GMP SCGM medium with 5% human serum and various cytokine combinations (IL-2/IL-15: 35 ng/mL each or IL-2/IL-15/IL-21: IL-2, IL-15, 35 ng/mL each, and IL-21, 10 ng/mL). NanoSpark® GROW-NK (1–20 µL/mL) was added with or without weekly restimulation. NK phenotype (CD3–CD16+CD56+NKp46+) and cytotoxicity (CD3-CD16+CD107a+, day 28-expanded NK effector cells were co-incubated with K562 target cells at effector-to-target (E:T) ratios 1:1, 5:1,10:1, 4 h) were assessed by flow cytometry[2].

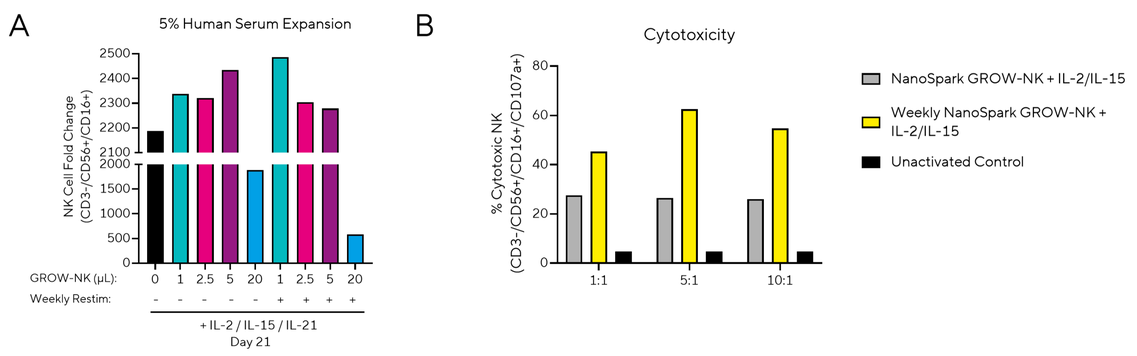
- Figure 3. Cryopreserved PB NK cells were expanded in CellGenix SCGM GMP medium supplemented with 5% human serum, IL-2 (20 ng/mL), with or without IL-15 (20 ng/mL), and IL-21 (10 ng/mL), and 5 µL/mL NanoSpark® GROW-NK (restimulated twice weekly). Co-cultures with feeder cells K562-mbIL21/41BBL served as controls. Cytotoxicity was tested at E:T 5:1 after 24 h.

Figure 1: GROW-NK Enables Rapid and Long-Term Expansion of Peripheral Blood NKs Under Serum-Free, Feeder-Free Conditions



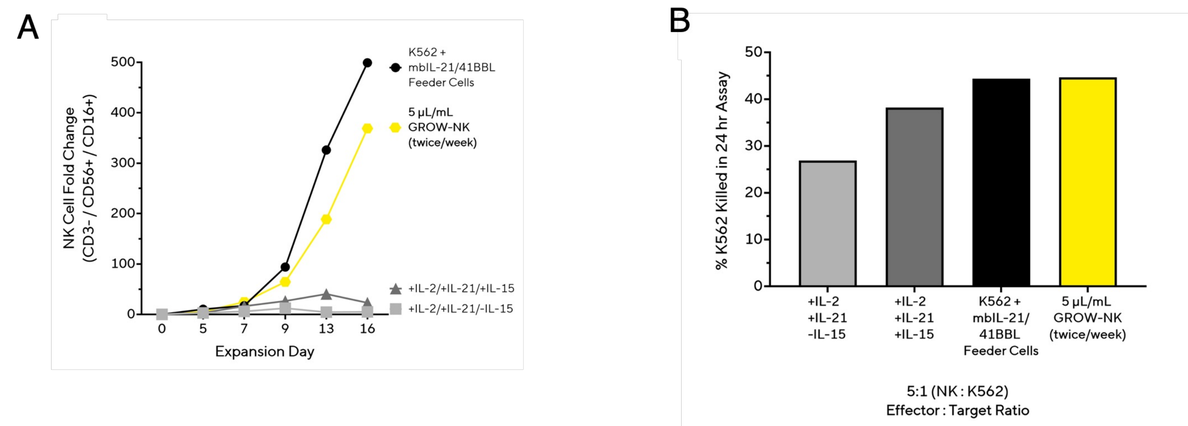
- GROW-NK led to an 800-1500-fold change after 19-28 days
- GROW-NK led to a >25,500-fold change after 55 days
- In the mid-term expansion, GROW-NK outperformed bead-based activator while showing no signs of exhaustion
- Cell viability for all GROW-NK soluble activator groups was>95% at the final timepoint

Figure 2: Optimizing GROW-NK Dosing for Maximal NK Expansion and Cytotoxicity Under Feeder-Free Conditions with 5% Human Serum



- A single dose of 5 uL/mL GROW-NK resulted in the highest fold expansion (2,433-fold), with the optimal range observed between 1-5 uL/mL
- Weekly dosing at 1 uL/mL achieved the highest fold expansion overall (2,486-fold), also within the 1-5 uL/mL range
- Weekly GROW-NK treatment led to enhanced cytotoxicity compared to a single-dose regimen

Figure 3: Feeder-Free NK Expansion Using GROW-NK Achieves Equivalent Cytotoxicity and Proliferation



- Cells cultured with GROW-NK exhibited comparable fold expansion compared to those co-cultured with engineered feeder cells
- Equivalent killing functionality was observed in GROW-NK groups compared to those co-cultured with engineered feeder cells
- GROW-NK and feeder groups both had >95% viability at the final time point of the expansion

Conclusion

- Our findings demonstrate a new way to expand NK cells without the need for feeder cells, bead-based activators, or magnetic components
- GROW-NK soluble activator has a comparable expansion and killing profile compared to engineered feeder cells – without any post-expansion purification needed
- This novel, entirely protein-based, modality represents a significant advancement in the activation and expansion of NK cells at scale, paving the way for improved scalable manufacturing for cell therapies against various types of cancers

References

1. Del Zotto, G., Antonini, F., Pesce, S., Moretta, F., Moretta, L., & Marcenaro, E. (2020). Comprehensive phenotyping of human PB NK cells by flow cytometry. *Cytometry* Part A, 97(9), 891-899.

2. Aktas, E., Kucuksezer, U. C., Bilgic, S., Erten, G., & Deniz, G. (2009). Relationship between CD107a expression and cytotoxic activity. *Cellular immunology*, 254(2), 149-154.

Explore more about NanoSpark® immune cell activators