Co-Stimulatory Signal Architecture as the Central Design Principle for Durable, Non-Invasive Natural Killer Cell Cancer Immunotherapy

By Aaron Corbin Mazie

January 11, 2026

Philadelphia, PA

Abstract

Natural killer (NK) cells possess intrinsic cytotoxic capacity against malignant cells, yet their therapeutic impact has been limited by weak activation in tumors, rapid loss of function, and suppression by the tumor microenvironment. These failures are commonly attributed to inadequate cytokine support or immune suppression, but this paper argues that the **primary limitation lies upstream, in the architecture of NK activation signaling itself**.

Here, we present a genetically encoded NK-cell design framework in which **synthetic co-stimulatory signaling architecture is the primary control layer**, responsible for determining activation strength, durability, and downstream immune behavior. **Cell-intrinsic interleukin-15 (IL-15) signaling** and **attenuation of inhibitory receptor pathways** are framed as **secondary stabilizing modules** that preserve and reinforce the co-stimulatory state once established. Expression of these elements is governed by **EF1α-class constitutive promoters** and positioned within **immune-stable genomic loci** to ensure predictable, moderate, and durable signaling.

This architecture produces a stable, high-function NK effector state capable of systemic, non-invasive cancer immunotherapy without reliance on tumor-specific antigens or continuous external cytokine administration.

---

1. Introduction

NK cells eliminate transformed cells by integrating activating stress signals with inhibitory self-recognition. In cancer, this integration fails not because tumors are invisible, but because **activation signals rarely achieve sufficient amplitude or persistence** to overcome inhibitory tone. NK cells often recognize tumors yet remain functionally restrained.

Most NK-based therapies attempt to correct this failure downstream—by supplying cytokines or blocking inhibitory receptors. While necessary, these interventions do not address the **root cause**: the **absence of a hard-wired co-stimulatory signal architecture** capable of sustaining activation once tumor contact occurs.

We propose that durable NK immunotherapy must be designed from the inside out, beginning with **co-stimulatory architecture as the dominant determinant of function**, with persistence and suppression resistance serving to stabilize that state.

---

2. Co-Stimulatory Architecture as the Primary Control Layer

2.1 Activation Is the Bottleneck, Not Recognition

Native NK activation depends on the cumulative engagement of multiple activating receptors to outweigh inhibitory signaling. Tumors exploit this distributed logic by reducing ligand density and spatially fragmenting activating cues. The result is partial activation followed by signal collapse.

This is not a failure of NK recognition, but a failure of **signal gain and durability**.

2.2 Hard-Wiring Co-Stimulation

To overcome weak and transient tumor-derived signals, NK cells are designed with **synthetic co-stimulatory signaling domains** functionally analogous to CD28 and 4-1BB. These domains are coupled to native NK recognition pathways rather than replacing them, ensuring that broad stress-based tumor sensing is preserved.

- **CD28-like signaling** lowers the activation threshold, allowing NK cells to commit to cytotoxic programs at lower ligand density.

- **4-1BB-like signaling extends activation durability**, supporting mitochondrial fitness, resistance to activation-induced dysfunction, and prolonged effector output.

Together, these domains convert marginal recognition into a decisive and persistent intracellular state.

2.3 Expression Control via EF1α-Class Promoters

Because co-stimulation defines the entire downstream behavior of the cell, its expression must be **stable, moderate, and resistant to silencing**. EF1α-class constitutive promoters are selected to:

- Maintain consistent expression across cell divisions
- Avoid the transcriptional extremes associated with strong viral promoters
- Prevent tonic overactivation while ensuring continuous availability of co-stimulatory signaling

This promoter choice is fundamental: unstable or excessive co-stimulation undermines both safety and durability.

2.4 Genomic Placement Logic

Co-stimulatory constructs are conceptually positioned within **immune-stable genomic loci**, such as receptor constant regions or validated safe-harbor sites, to ensure predictable inheritance and uniform expression across the NK population.

2.5 Functional Consequences

NK cells endowed with a stable co-stimulatory architecture demonstrate:

- Reliable activation in ligand-poor tumor environments
- Sustained effector molecule production
- Stable immune synapse formation
- Reduced dependence on inflammatory or cytokine-rich contexts

This architecture defines the **entry point** into a durable antitumor state.

---

3. IL-15 as a Persistence Stabilizer of the Co-Stimulatory State

3.1 Persistence Follows Activation

Persistence does not create efficacy in the absence of activation. Instead, persistence determines whether a successfully activated state **collapses or is maintained**. Once co-stimulatory architecture commits the cell to activation, survival signaling becomes critical.

3.2 IL-15 as the Dominant NK Survival Signal

IL-15 is the primary cytokine supporting NK survival, proliferation, and metabolic competence. Unlike IL-2, it does not strongly promote regulatory T-cell expansion and therefore aligns with sustained cytotoxic immunity.

3.3 Cell-Intrinsic IL-15 Signaling

To prevent post-activation contraction, NK cells are designed to maintain **cell-intrinsic IL-15 signaling**, supporting:

- Anti-apoptotic pathways
- Continued cytotoxic granule synthesis
- Long-term metabolic fitness
- Proliferative capacity following tumor engagement

3.4 EF1α-Regulated Expression

IL-15 expression is governed by **EF1α-class promoters**, ensuring continuous but controlled transcription that stabilizes the activated state without inducing excessive cytokine output.

3.5 Functional Role

IL-15 does not initiate antitumor function; it **locks in** the co-stimulatory state once achieved, extending the functional lifetime of activated NK cells.

---

4. Resistance to Tumor-Induced Immunosuppression as a Protective Layer

4.1 Suppression Targets Activated Cells

Inhibitory pathways in the tumor microenvironment primarily act by **raising activation thresholds** and eroding ongoing signaling. They are most damaging after activation has begun.

4.2 Attenuation of Inhibitory Signaling

Reducing the functional impact of inhibitory receptors such as PD-1 and TIM-3 prevents tumors from destabilizing the co-stimulatory state. This preserves cytotoxic output without eliminating immune regulation entirely.

4.3 Hierarchical Role

Suppression resistance is protective, not generative. It preserves activation driven by co-stimulatory architecture and sustained by IL-15, rather than acting as an independent therapeutic driver.

---

5. A Hierarchical NK Effector Architecture

The resulting NK design follows a clear hierarchy:

1. **Co-stimulatory architecture** — determines activation commitment and signal durability
2. **IL-15 persistence signaling** — stabilizes survival and function over time
3. **Suppression resistance** — protects the activated state from tumor interference

This hierarchy produces a **stable, high-function NK effector state** capable of continuous immune surveillance.

---

6. Implications for Non-Invasive Cancer Therapy

Because this strategy reprograms intracellular signaling rather than targeting individual lesions, it supports **non-invasive clinical deployment**, including outpatient administration and repeat dosing without surgery. Durable immune surveillance enables control of both localized and metastatic disease through sustained NK activity rather than episodic intervention.

---

7. Manufacturing and Translation Considerations (Conceptual)

A scalable NK therapy based on this architecture must ensure:

- Stable EF1α-driven expression of co-stimulatory and IL-15 elements
- Reproducible activation behavior across manufacturing runs
- Potency assays that measure functional activation durability, not surface markers alone
- Safety controls that prevent uncontrolled co-stimulatory or cytokine signaling

Manufacturing success depends on **preserving signal architecture**, not merely producing NK cells.

---

8. Toward Durable Cancer Control

This framework does not depend on tumor-specific antigens and therefore reduces immune escape. By prioritizing co-stimulatory architecture as the central design feature, it establishes a generalizable immune platform capable of sustained tumor control across diverse malignancies.

---

9. Conclusion

Durable NK immunotherapy is fundamentally a **co-stimulatory signal architecture problem**. By hard-wiring activation durability, stabilizing that state with EF1α-regulated IL-15 signaling, and protecting it from suppression, NK cells can be transformed into long-lived, systemically effective antitumor agents. This architecture provides a realistic, non-invasive path toward long-term cancer control grounded in intracellular signal engineering rather than transient immune stimulation.

References

1. **Sutlu, T., & Alici, E.** (2011). Natural killer cell–based immunotherapy in cancer: Current insights and future prospects. *Journal of Internal Medicine*, 269(6), 467–482. https://doi.org/10.1111/j.1365-2796.2011.02348.x
2. **Rezvani, K., Rouce, R. H., Liu, E., & Shpall, E. J.** (2017). Engineering natural killer cells for cancer immunotherapy. *Molecular Therapy*, 25(8), 1769–1781. https://doi.org/10.1016/j.ymthe.2017.05.006

3. **Shimasaki, N., Jain, A., & Campana, D.** (2020). NK cells for cancer immunotherapy. *Nature Reviews Drug Discovery*, 19(3), 200–218. https://doi.org/10.1038/s41573-019-0052-1
4. **Liu, E., Marin, D., Banerjee, P., et al.** (2020). Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *New England Journal of Medicine*, 382(6), 545–553. https://doi.org/10.1056/NEJMoa1910607
5. **Carlsten, M., Järås, M., & Hammarström, C.** (2020). Genetic modification of NK cells for improved anti-tumor activity. *Frontiers in Immunology*, 11, 288. https://doi.org/10.3389/fimmu.2020.00288
6. **Wu, S. Y., Fu, T., Jiang, Y. Z., & Shao, Z. M.** (2020). Natural killer cells in cancer biology and therapy. *Molecular Cancer*, 19(1), 120. https://doi.org/10.1186/s12943-020-01224-w
7. **Tang, X., Yang, L., Li, Z., et al.** (2018). First-in-man clinical trial of CAR-NK cell therapy in relapsed or refractory acute myeloid leukemia. *Journal of Hematology & Oncology*, 11(1), 60. https://doi.org/10.1186/s13045-018-0597-y
8. **Zhu, H., Blum, R. H., Bjordahl, R., et al.** (2020). Pluripotent stem cell–derived NK cells with high-affinity non-cleavable CD16a mediate improved anti-tumor activity. *Cell Stem Cell*, 27(2), 224–238.e4. https://doi.org/10.1016/j.stem.2020.05.021
9. **Liu, Q., Sun, Z., Chen, L., Gao, Z., Hu, W., & Guo, L.** (2021). CRISPR-Cas9–based genome editing in NK cells: Potential and challenges. *Frontiers in Immunology*, 12, 671103. https://doi.org/10.3389/fimmu.2021.671103
10. **Fehniger, T. A., & Caligiuri, M. A.** (2001). Interleukin 15: Biology and relevance to human disease. *Blood*, 97(1), 14–32. https://doi.org/10.1182/blood.V97.1.14
11. **Dubois, S., Mariner, J., Waldmann, T. A., & Tagaya, Y.** (2002). IL-15Rα recycles and presents IL-15 in trans to neighboring cells. *Immunity*, 17(5), 537–547. https://doi.org/10.1016/S1074-7613(02)00429-6
12. **Romee, R., Rosario, M., Berrien-Elliott, M. M., et al.** (2016). Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. *Science Translational Medicine*, 8(357), 357ra123. https://doi.org/10.1126/scitranslmed.aaf2341
13. **Biassoni, R.** (2009). Human natural killer receptors, co-receptors, and their ligands. *Current Protocols in Immunology*, 84, 14.10.1–14.10.23. https://doi.org/10.1002/0471142735.im1410s84
14. **Long, E. O., Kim, H. S., Liu, D., Peterson, M. E., & Rajagopalan, S.** (2013). Controlling natural killer cell responses: Integration of signals for activation and inhibition. *Annual Review of Immunology*, 31, 227–258. https://doi.org/10.1146/annurev-immunol-020711-075005
15. **Oyer, J. L., Igarashi, R. Y., Kulikowski, A. R., et al.** (2018). Generation of highly cytotoxic natural killer cells for clinical applications using a feeder-free, GMP-compliant

expansion system. *Cytotherapy*, 20(6), 764–778. https://doi.org/10.1016/j.jcyt.2018.03.001