CONTROL-LAYER SIGNAL ARCHITECTURE FOR DURABLE NK IMMUNOTHERAPY

Reversible Off-Switch Design, Termination Kinetics, and Vaccinal-Effect Validation

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Abstract

Genetically encoded NK-cell therapeutic designs that hard-wire co-stimulatory signal persistence and incorporate cell-intrinsic IL-15 survival support can, in principle, sustain antitumor effector function across ligand-poor and suppressive tumor microenvironments. However, these same architectural choices elevate primary safety risks: autonomous effector persistence, cytokine-driven systemic toxicity, and off-target tissue injury. Accordingly, reversibility must be elevated from an optional feature to a first-order design constraint. This paper formalizes a control-layer framework for engineered NK immunotherapy, emphasizing (i) clinically precedent **cell elimination ("off-switch") mechanisms** and (ii) a rigorous evidentiary plan to support "vaccination-like" claims via innate memory-like durability and/or adaptive "vaccinal effect" through antigen spread and dendritic cell cross-priming. The resulting methodology defines measurable termination kinetics, completeness thresholds, and immunologic durability endpoints suitable for preclinical-to-translational validation.

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1. Rationale: Why an Off-Switch Is a Primary Safety Requirement

Durable NK effector designs that combine persistent co-stimulatory signaling with intrinsic pro-survival cytokine signaling can shift the therapeutic system into a regime where (a) efficacy and (b) toxicity share the same upstream driver: persistent intracellular activation. In this regime, "dose reduction" is an incomplete safety strategy because engineered cells can continue functioning independent of external drug exposure. Therefore, an engineered NK therapy intended for systemic, repeatable, outpatient deployment must include an integrated control layer capable of **rapid termination** of effector function when pre-specified safety criteria are exceeded.

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2. Off-Switch Taxonomy With Translational Precedent

2.1 Class I: Inducible Apoptosis (“Hard Kill Switch”) — iCasp9

Inducible caspase-9 (iCasp9) safety switches were developed to permit rapid elimination of adoptively transferred cells upon administration of a dimerizing agent, providing an explicit mechanism to ablate therapeutic cells during severe adverse events. Clinical and translational reports describe rapid activation of apoptosis in engineered lymphocytes expressing iCasp9 and frame the approach as a practical safeguard for adoptive cell therapy.

Control-layer value proposition: fastest and most deterministic termination modality, particularly important when persistence modules (e.g., IL-15 support) are intentionally built-in.

2.2 Class II: Antibody-Mediated Depletion Markers (“Clinical Depletion Switch”) — EGFRt / EGFRopt

Truncated EGFR (EGFRt) has been widely used as a co-expressed surface marker enabling both selection/tracking and antibody-mediated depletion using clinically available anti-EGFR antibodies (e.g., cetuximab). Preclinical and translational studies demonstrate the feasibility of depleting EGFRt-tagged CAR-engineered cells in vivo, and later optimization work (EGFRopt) reports enhanced and more rapid elimination and termination of effector activity relative to EGFRt.

Control-layer value proposition: leverages approved biologics and established clinical workflows; serves as redundancy and as an operational “depletion handle,” though kinetics may be slower and tissue-distribution dependent compared with inducible apoptosis.

2.3 Class III: Compact Epitope-Based Marker/Suicide Constructs — RQR8

RQR8 is a compact epitope-based marker/suicide construct combining CD34 and CD20 epitopes, enabling positive selection/manufacturing utility and depletion using a clinically available anti-CD20 antibody (rituximab) via complement- and ADCC-mediated mechanisms. Foundational and follow-on publications explicitly define RQR8 as a marker/suicide system for adoptive cellular therapies and validate rituximab-mediated elimination.

Control-layer value proposition: compactness and use of widely deployed clinical reagents; provides both manufacturing advantages and an in vivo elimination path.

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3. Control-Layer Design Requirements (Minimum “Safety Spec”)

A control layer suitable for a durable NK platform should satisfy the following performance specifications:

1. **Termination speed:** demonstrable rapid reduction of engineered-cell effector function after off-switch activation (time-to-functional-termination).

2. **Termination completeness:** high fractional elimination/silencing of engineered cells (residual-risk quantification).

3. **Predictability:** reproducible on-switch and off-switch behavior across donors, manufacturing runs, and relevant in vivo environments.

4. **Redundancy:** at least two independent termination paths (e.g., iCasp9 + depletion marker) to mitigate single-point failure modes.

5. **Non-interference:** the off-switch must not substantially impair baseline cytotoxic function, trafficking, or persistence prior to activation (functional neutrality under “off” state).

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4. “Vaccination Strategy” Claims: Scientifically Defensible Meanings

A “vaccination-like” claim can be made scientifically credible in engineered NK therapy under two distinct paradigms:

4.1 Paradigm A: Innate Memory / Memory-Like NK Durability (“Innate Vaccination”)

There is substantial literature that NK cells can acquire adaptive or memory-like properties and that such features can be harnessed in immunotherapeutic contexts.
Cytokine-induced memory-like NK (CIML NK) studies include first-in-human evidence of proliferation/expansion and robust antileukemia responses following adoptive transfer in AML, supporting the plausibility of durable NK functional enhancement in vivo.

Operational definition (for claims): persistence + preserved cytotoxic competence + recall-like enhancement upon re-encounter with malignant targets, relative to baseline NK comparators.

4.2 Paradigm B: Adaptive "Vaccinal Effect" via Antigen Spread (In Situ Vaccination Cascade)

A second—and often more clinically meaningful—vaccination-like mechanism is "vaccinal effect," where tumor cell killing leads to antigen release and dendritic cell (DC) cross-presentation, resulting in durable, systemic T-cell immunity. Reviews of in situ vaccination emphasize use of the tumor as an antigen source to generate systemic and lasting antitumor immunity.
Direct evidence indicates NK cells can be necessary for distant antitumor responses mediated by CD8+ T cells following in situ tumor vaccination, supporting a plausible NK→DC/T cell propagation pathway.

Constraint (must be acknowledged): NK cells can also regulate, and in some contexts limit, vaccine-induced T-cell responses (e.g., PD-L1–dependent suppression noted in therapeutic vaccination models), meaning "vaccination-like" effects are not guaranteed and must be empirically demonstrated for the specific NK architecture.

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5. Methodical Scientific Instructions (Evidentiary Program; Non-Protocol)

The following is a structured validation program expressed as hypotheses, endpoints, controls, and decision gates—suitable for a grant, pre-IND narrative, or translational package—without providing operational wet-lab instructions.

Aim 1 — Demonstrate Controllability (Off-Switch Efficacy and Termination Kinetics)

Hypothesis: activation of the integrated off-switch rapidly terminates engineered NK effector function and reduces systemic inflammatory outputs.

Primary endpoints (minimum set):

- **Time-to-functional-termination:** objective assay demonstrating rapid reduction in cytotoxic output post-activation.

- **Completeness of elimination/silencing:** fraction of engineered cells remaining functional after off-switch engagement.

- **Inflammatory signature reversal:** reduction in key inflammatory mediators after off-switch engagement.

Controls:

- Engineered NK without control layer

- Engineered NK with nonfunctional control element

- Unmodified NK baseline

Decision gate: failure to meet predefined termination speed/completeness thresholds disqualifies the construct for claims of systemic durable deployment, regardless of efficacy.

Supporting precedent: iCasp9 as rapid ablation safety switch in adoptive cell therapy.

Aim 2 — Demonstrate Durable NK Functional Enhancement (Innate Memory-Like Profile)

Hypothesis: engineered NK cells maintain enhanced functional competence over time and exhibit preserved responsiveness after a rest period and re-encounter.

Primary endpoints:

- Persistence of engineered NK phenotype and function over time

- Recall-like enhancement vs baseline NK

- Exhaustion/metabolic fitness trendlines (durability vs burnout)

Benchmark comparator: CIML NK as a reference durability class with in vivo expansion/persistence evidence.

Decision gate: if durability requires excessive activation tone that approaches toxicity thresholds, the design must be re-architected (e.g., gating and/or restrained persistence modules).

Aim 3 — Demonstrate Adaptive Vaccinal Effect (Antigen Spread and DC→T-cell Propagation)

Hypothesis: tumor killing by engineered NK induces DC activation and cross-presentation sufficient to generate systemic, tumor-reactive CD8+ T-cell immunity and protection upon re-challenge.

Primary endpoints:

- DC activation/cross-presentation signatures consistent with in situ vaccination principles
- Expansion and functionality of tumor-reactive CD8+ T cells
- Protection against re-challenge / systemic control of distant tumor sites (abscopal-like phenotypes where model-relevant)

Key mechanistic test: demonstrate NK dependence of the downstream CD8+ response (literature supports NK necessity in a specific in situ vaccination setting).

Decision gate: absence of durable, antigen-specific T-cell memory (or evidence of NK-mediated suppression of adaptive responses) precludes “vaccination-like” claims under Paradigm B.

Aim 4 — Define the Safety Envelope (Failure Modes and Off-Switch Mitigation)

Hypothesis: toxicity emerges through predictable systemic inflammation and/or off-target tissue injury; off-switch engagement mitigates these events within acceptable time.

Primary endpoints:

- CRS-like systemic inflammatory measures
- Tissue injury proxies in high-risk organs (liver, lung, endothelium)
- Evidence of autoimmunity signatures
- Demonstrated mitigation after off-switch activation

Decision gate: toxicity at exposures near minimum-efficacy range requires design changes (signal gating, restrained persistence, or alternative attenuation strategy).

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6. Discussion: Claim Language Suitable for External Dissemination

To preserve scientific credibility and reduce overclaim risk in outreach materials, the recommended phrasing is:

- "**Vaccination-like durable immune surveillance**" (conditional on data)
- "**Innate memory-like persistence and recall**" (Paradigm A)
- "**Vaccinal effect via antigen spread and DC cross-priming**" (Paradigm B)

The manuscript should explicitly acknowledge that NK cells can also negatively regulate vaccine-induced T-cell responses in some settings, making empirical validation essential.

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