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Engineering CAR-NK Cells to Overcome Tumor Microenvironment (TME) Barriers in Solid Tumors

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ABSTRACT- In the recent years, cell-based immunotherapies have transformed the perspective of cancer treatment. Among these, the Chimeric Antigen Receptor- Natural Killer (CAR-NK) cells are gaining more attention due to their ability to recognize tumor cells without prior sensitization and a comparatively favourable safety profile. CAR-NK cells have shown promising results for haematological malignancies but appeared to slow down as interest passed to solid tumors. The Tumor micro-environment (TME) presents hostile conditions that weaken NK cells before they can act effectively. The TME confines NK cells' movement, exhausts energy reserves, and dampens cytotoxic signals, all of which could be attributed to reduced oxygen levels, competition for nutrients, suppressive cytokines, and the physical density of the tumor stroma. Several strategies address these issues, from improving NK-cell tumor-tracking with chemokine receptors, to supporting their activity with cytokine armoring, enhancing metabolic resilience in hypoxic regions, and engineering of their receptors to resist inhibitory signals. Newer ideas, such as conditional circuits like SynNotch, controlled cytokine release systems, CRISPR-edited NK cells, and iPSC-derived NK banks which can be used more broadly, may prove to be more effective. The field is clearly moving beyond early safety testing towards building durable responses in the face of a suppressive TME. Future success will likely depend not on one single design, but on combining several engineering strategies that allow these cells to adapt and survive within complex tumor settings.

KEYWORDS- CAR-NK Cells, Solid Tumors, Tumor Microenvironment (TME), Cytokine Armoring, Chemokine Receptors.

I. INTRODUCTION

In the past few years, adoptive immune cell therapies have revolutionized cancer treatment. The clinical success of CAR (chimeric antigen receptor–engineered) T cells against hematologic malignancies have proved to be an important flex point [1]. However, translating these outcomes to solid tumors has come to prove far more challenging because solid TME impose multiple barriers against these T cells, including immunosuppression, dense stroma, hypoxia, and antigen heterogeneity [2]. Natural killer cells, the cytotoxic lymphocytes of the innate immune system, represent an alternative platform for CAR engineering. There are several advantages of NK cells compared to T cells. In contrast with T cells, NK cells are capable of mediating tumor killing independently of MHC presentation, and they have a minimal risk of graft versus host disease (GvHD); hence, NK cells are suitable for off-the-shelf allogeneic therapies [3]. Besides acquiring antigen-specificity mediated by CARs, CAR-NK cells still retain intrinsic NK activating receptors such as NKG2D, NKp30, and DNAM-1, enabling tumor cells to be targeted through multiple recognition pathways that reduce the likelihood of immune escape [4].

More importantly, phase I and I/II clinical trials of CAR-NK cells have demonstrated a favourable safety profile. The incidence of cytokine release syndrome and neurotoxicity is significantly lower compared with CAR-T cells [1]. Despite such advantages, the efficacy of CAR-NK therapies in solid tumors has remained limited. The immunosuppressive TME is characterized by hypoxia, nutrient depletion, suppressive cytokines, regulatory immune cells, and physical stromal barriers, all of which impede NK cell trafficking, persistence, and cytotoxic function [2]. Effective clinical translation will require next-generation engineering strategies that enable CAR-NK cells to resist or remodel the suppressive TME.

This review focusses on the engineering strategies required to enable CAR-NK cells to function effectively in the solid tumor microenvironment. After outlining the key immunologic, metabolic, and structural barriers that restrict NK activity in solid tumors, recent advances in NK cell reprogramming, including chemokine receptor–based trafficking enhancement, resistance to checkpoint and cytokine suppression, cytokine self-support systems, metabolic rewiring, and NK-optimized CAR design, have been examined. Current clinical progress, emerging platforms and future directions that may determine whether CAR-NK therapy achieves durable responses in solid cancers have also been discussed.

II. SOLID TUMOR MICROENVIRONMENT

Solid tumors constitute a complex microenvironment that significantly impairs immune responses [5]. Important characteristics include:

A. Hypoxia and Metabolic Stress

Rapid tumour growth leads to the creation of hypoxic zones and acidosis because it outpaces the blood supply. Angiogenesis is triggered by stabilised HIF-1α/2α while elevated glycolysis drives lactate buildup and extracellular acidification [6] [7]. Low oxygen and acidic pH restrict NK cell metabolism and cytotoxicity, leading to down-regulation of activating receptors such as NKG2D and decreased granule release [7] [8]. Tumours also aggressively consume glucose, amino acids and tryptophan, depriving infiltrating NK cells of substrates needed for mTOR/ c-Myc-driven effector programs [7]. Effector functions are also impaired by accumulation of lactate and adenosine through CD39/CD73 as they engage the inhibitory receptors such as A2A [9].

B. Immunosuppressive Cells

Regulatory immune cells infiltrate solid tumors to dampen NK function [10]. These tumors recruit populations of regulatory cells capable of suppressing NK functions [5]. Myeloid-derived suppressor cells and M2-polarised tumor-associated macrophages release arginase, nitric oxide, and ROS, all of which inactivate NK cells. In the tumor microenvironment, several immune and stromal populations actively restrain NK-cell function. In the tumor microenvironment suppress NK-cell function is suppressed by several immune and stromal cells, for example, regulatory T cells and tumor-associated neutrophils, secrete TGF-β and IL-10, that reduces NK activation and cytokine release [11]. These solid tumors often express various checkpoint molecules such as PD-L1 or Galectin-9 and release chemokines like CCL2 and CCL5 that attract more suppressor cells. This leads to a reinforcing circuit of immune inhibition. Cancer-associated fibroblasts form another layer through a process that involves extracellular matrix remodeling and production of prostaglandin E_2, that interferes with NK cytotoxicity and limit their accessibility to tumor tissue [11].

C. Inhibitory Cytokines and Soluble Factors

Tumors and associated stromal cells produce many inhibitory cytokines, the most common ones include TGF-β, IL-10, and IL-6 [10]. In NK cells, TGF-β signaling downregulates the expression of NKG2D, NKp30, and NKp44 (activating receptors) thus reduceing perforin and granzyme content. It inhibits mTOR signaling and dampens energy metabolism, making the cells less performant [12]. IL-6, often produced under hypoxia, has similar effects and shifts the tumor microenvironment toward a tumor-protective one. IL-10, on the other hand, impairs IFN-γ production. In addition, the soluble mediators PGE_2 and adenosine, which are also produced, together dampen NK migration and functioning. Overall, this cytokine cocktail strongly inhibits NK-cell responses [10][12].

D. Physical Barriers

The architecture of solid tumors further adds to the problem. High-density extracellular matrix components-collagen, fibronectin, and hyaluronan, along with aberrant vasculature create a physical barrier to limit immune infiltration [13]. Tumor cores are rendered inaccessible to CAR-NK cells because of high interstitial pressure and disorganized vessel structure. Some tumors evade the detection of immune responses by shedding or down-regulating ligands for NK-activating receptors, including MICA/B and ULBPs [5][11]. Exosomes derived from the tumor bearing TGF-β, PD-L1, or soluble NKG2D ligands blunt NK cell responses even before their contact with the tumor [11] [14].

E. Tumour-Derived Extracellular Vesicles (Exosomes)

TGF-β, small RNAs, and soluble NK-ligand decoys comprise examples of suppressive molecules expressed by exosomes, which are secreted by tumor cells and internalized by NK cells, rendering them inefficient [12][14]. Such vesicles down-modulate NKG2D and impair antibody-dependent cytotoxicity, thus promoting tumor escape.

F. Cytokine Support Deficiency and NK Exhaustion:

NK cells depend for their survival and expansion on cytokines such as IL-15; these are scarce in most tumors, while suppressive cues dominate, leading to NK-cell exhaustion or early apoptosis [8][10]. Inadequate cytokine support means that CAR-NK cells do not persist or proliferate, hence limiting their long-term therapeutic activity [8].

In sum, solid tumors present a complex, highly suppressive environment through combined soluble, structural, and cellular mechanisms that together blunt NK trafficking, persistence, and cytotoxic strength. It will be how these layers interact that is important for the design of strategies that allow CAR-NK therapies to function within solid malignancies.

III. ENGINEERING CAR-NK CELLS TO OVERCOME TME

Next-generation CAR-NK platforms are being rationally engineered across five major dimensions to overcome the multilayered suppressive barriers of the solid tumor microenvironment: (i) improving NK cell homing and tumor infiltration, (ii) resisting inhibitory signaling and checkpoint suppression, (iii) providing autonomous cytokine support, (iv) rewiring NK metabolism for nutrient-poor environments, and (v) optimizing CAR architecture for NK-specific signaling. We provide an overview below of recent advances in and translational strategies for each of these areas.

A. Enhancing Tumor Trafficking and Infiltration

One of the most consistent failures in CAR-NK therapy against solid tumors is limited intratumoral accumulation. Native NK cells poorly migrate into tumor beds because most tumors secrete chemokines that do not match NK receptor expression profiles [13]. To improve homing, CAR-NK cells are increasingly engineered to express chemokine receptors corresponding to tumor-secreted chemokines [13]. Solid tumors such as pancreatic, melanoma, glioblastoma, and breast cancer, produce CXCL8 (IL-8), CXCL12 (SDF-1), or CCL2. Overexpression of CXCR2, CXCR4, or CCR2 in CAR-NK cells significantly enhances directional migration toward these in orthotopic tumor models [13] [15]. CXCR2-

expressing CAR-NK cells have shown 10 times more increased infiltration into pancreatic tumors and also improved survival rates [15]. Similarly, CXCR4-engineered CAR-NK cells demonstrated enhanced infiltration in glioblastoma and prolonged survival in intracranial xenografts [16]. CCR7 engineering has also been used to direct NK cells toward CCL19/21-rich lymphoid niches, improving persistence in metastatic tumors [6]. A complementary strategy is to secrete the missing chemokines. CAR-NK cells engineered to co-express CCL19 have been shown to not only recruit endogenous NK and T cells but also enhance local dendritic cell activation, amplifying anti-tumor immunity [17].

Table 1: Chemokine-based engineering strategies to enhance CAR-NK cell trafficking into solid tumors.

Chemokine Strategy	Receptor / Payload	Tumor Target	Outcome	Citation
CXCR2 overexpression	CXCR2	Pancreatic cancer	>10× infiltration, tumor regression	[15]
CXCR4 overexpression	CXCR4	Glioblastoma	Increased tropism, prolonged survival	[16]
CCR2 overexpression	CCR2	Breast cancer	Higher tumor trafficking, reduced metastasis	[13] [15]
CCL19 secretion	CCL19	Multiple solid tumors	Recruits T/NK cells, ↑ DC activation	[17]
ECM degradation	Heparanase, MMPs	Desmoplastic tumors	Improves intratumoral spread	[18] [19]

In highly fibrotic tumors, trafficking failure is caused not by missing chemokine cues but by an impenetrable matrix. Engineering CAR-NK cells to express heparanase or matrix metalloproteinases enables degradation of collagen-dense stroma and improves NK dispersion through tumor cores [18]. Hyaluronidase co-expression has been tested in CAR-T and is now being explored in CAR-NK designs for pancreatic cancer, where hyaluronic acid accumulation creates high interstitial pressure [19]. Another approach uses localized therapy: intratumoral or regional delivery of CAR-NK (rather than systemic infusion) bypasses homing barriers and can improve on-site cell accumulation [13].

Combination CAR-NK and tumor vasculature normalising agents can aid infiltration. EGF-driven aberrant vasculature restricts immune entry. EGF-driven aberrant vasculature restricts immune entry. Co-administration of anti-angiogenic drugs (e.g., bevacizumab or VEGFR inhibitors) increases NK infiltration and reduces MDSC recruitment, synergizing with CAR-NK therapy [20]. Stromal-targeting agents such as FAP-CAR-NK or CAF-depleting drugs are also under evaluation to remove "immune-exclusion zones." Moreover, other than systemic infusion, the localized injection of CAR-NK has resulted in higher cell retention and reduced manufacturing doses in ovarian, GBM, and liver cancer models [13]. Several early-phase trials such as NCT05020678 are testing intraperitoneal CAR-NK delivery.

In a nutshell, directed chemokine receptor expression and modulation of the microenvironment are two major strategies to enhance CAR-NK homing to solid tumors.

B. Checkpoint Blockade and Immunosuppression Resistance

Expression of ligands, including PD-L1, Galectin-9, and CD155, by solid tumors bind to inhibitory receptors on NK cells, including PD-1, TIM-3, TIGIT, and CD96, that suppress cytotoxicity [21]. Unlike adaptive T cells, NK cells also express unique inhibitory receptors including NKG2A, KLRG1, and CD200R, which are highly upregulated in the TME [5]. Thus, engineering checkpoint-resistant CAR-NK cells is a major translational priority.

- **Gene knockout of inhibitory receptors-** Using CRISPR, several groups have deleted PD-1, NKG2A, TIGIT, or CISH in NK cells, restoring IFN-γ production, granzyme expression, and metabolic fitness [2]. Deletion of CISH a negative regulator of IL-15 signaling induces a "hyper-responsive" NK phenotype that proliferates even in cytokine-poor environments [22].
- **Switch receptors and receptor rewiring-** Instead of deleting inhibitory receptors, some groups convert them into activating receptors. For example, a PD-1 ectodomain fused to CD28 or 4-1BB converts PD-L1 engagement into an activation signal, boosting CAR-NK cytotoxicity in breast and lung cancer models [17]. TIGIT-Dap12 and NKG2A-2B4 switch receptors have also been tested preclinically [17].
- **Secreted checkpoint blockers-** CAR-NK cells engineered to secrete anti-PD-1 or anti-TIGIT scFv locally neutralize checkpoint ligands without systemic toxicity [17]. This "cell-secreted checkpoint therapy" may replace expensive monoclonal antibody infusion.
- **TGF-β resistance engineering-** The TME is rich in TGF-β, which downregulates NKG2D and perforin in NK cells. Expression of a dominant-negative TGF-βRII (lacking signaling domain) allows NK cells to bind and block TGF-β without becoming suppressed [23]. Similar constructs are now being built into CAR-NK backbones.
- **Adenosine/CD73 axis disruption-** Tumor and stromal cells generate adenosine via CD39/CD73. Deletion of A2A receptor or co-expression of adenosine deaminase enhances CAR-NK killing in hypoxic, adenosine-rich tumors [24].
- **Combination checkpoint therapy-** Several preclinical studies show synergy between CAR-NK + systemic PD-1 or TIGIT blockade [20], and at least two clinical trials are combining CAR-NK with pembrolizumab (NCT04887012).

Together**,** knockout, switch receptor, and dominant-negative strategies equip CAR-NK cells to remain

functional despite suppressive checkpoint signals in the TME.

C. Cytokine Armoring and Immune Activation

CAR-NK cells depend on γ-chain cytokines for survival, proliferation, and cytotoxic priming, but solid TME is cytokine-depleted. Unlike CAR-T cells, NK cells do not autonomously expand after infusion unless supported by exogenous IL-2 or IL-15. Early NK trials showed rapid loss of infused cells within days, making **cytokine armoring** one of the most transformational advances in the field [3].

- **IL15 as the foundation of next generation CAR NK**- IL-15 is now the most commonly integrated cytokine in CAR-NK designs because it supports NK proliferation, mitochondrial fitness, and memory-like differentiation without expanding Tregs [25]. The landmark FT596 platform, an iPSC-derived, CD19-CAR-NK cell line that expresses membrane-bound IL-15, showed persistence >3 weeks in lymphoma patients without GVHD or CRS [25]. This was the first clinical proof-of-concept that IL-15-engineered NK cells persist in the absence of cytokine infusion, an important milestone not yet achieved with CAR-T therapy.
- **Dual-cytokine armoring: IL-15 + IL-21 or IL-12**- IL-21 promotes NK metabolic competence and memory-like programming, while IL-12 remodels the TME by activating macrophages and recruiting $CD8^+$ T cells [26]. Dual IL-15/IL-21 armored CAR-NK cells showed superior persistence and reduced exhaustion markers in hepatocellular carcinoma models [27]. IL-12-secreting CAR-NK cells further rewire the TME by converting M2 macrophages to M1 and enhancing antigen presentation [28].
- **Synthetic cytokines and logic-gated circuits**- Engineered cytokines such as **Neo-2/15**, a computationally designed IL-2/IL-15 agonist that avoids Treg expansion, significantly increase NK metabolic activity and tumor control [25]. Some groups are now linking cytokine expression to **CAR signaling**, creating "only-on-activation" cytokine secretion circuits, preventing systemic toxicity [1]. Others are building **SynNotch-controlled IL-12 modules**, where cytokine release occurs only after tumor antigen recognition [2].
- **Microbial and mRNA-based cytokine delivery**- An emerging strategy avoids genetic armoring entirely: intratumoral injection of bacteria engineered to secrete IL-18 or GM-CSF increases NK migration and antigen spreading [29] [30]. In parallel, **mRNA-lipid nanoparticle (LNP) therapy** is being used to transiently induce IL-12 or IL-23 expression inside tumors, eliminating permanent genetic editing [31]. These strategies may be combined with CAR-NK cells to ignite immune activation without altering the NK genome.

D. Metabolic Reprogramming and Nutrient Support

One of the most universal modes of failure for CAR-NK cells in solid tumors consists of metabolic collapse. NK cells are continuously engaged in processes such as glycolysis and oxidative phosphorylation, yet the TME is significantly deficient of glucose, amino acids, and oxygen, but enriched with lactate, adenosine, and kynurenine. NK cells have a very limited ability to switch metabolic states. This makes metabolic engineering a necessary precondition for persistence of NK cells in solid tumors [32].

- **Nutrient transporter engineering.** NK cells have a limited capability to compete with tumor cells for glucose and amino acids because of low levels of high-affinity nutrient transporters. Forced expression of SLC1A5 (ASCT2) and SLC7A5 (LAT1) increases uptake of glutamine and branched-chain amino acids, that sustains mTORC1 activity and IFN-γ production even under glucose starvation conditions [32]. In the same study, LAT1-engineered NK cells maintained granzyme B release in lactate-rich environments, showing that nutrient access is closely related to effector function [32].
- **Boosting mitochondrial fitness.** The most successful metabolic armoring strategies strengthen mitochondrial durability rather than just glycolytic rate. Overexpression of transcriptional co-activators such as PGC-1α and c-Myc increases mitochondrial mass, spare respiratory capacity, and ATP production, protecting NK cells from hypoxia-induced apoptosis [32]. In Neo-2/15 armored CAR-NK cells, Luo et al. demonstrated a 3-fold increase in oxygen consumption rate and 2-fold higher granzyme B release under hypoxic conditions, linking cytokine armoring to metabolic rewiring [2].
- **Blocking suppressive metabolites in the TME**- Adenosine, lactate, and kynurenine signal directly through inhibitory receptors on NK cells.
  - A2A deletion or adenosine deaminase expression restores NK cytotoxicity in hypoxic tumors enriched in CD39/CD73-generated adenosine [24].
  - Lactate export and pH buffering are being engineered by increasing MCT1/4 transporter expression and carbonic anhydrase activity, although this work is still preclinical [24]
- **Metabolic checkpoint deletion.** Certain genes act as internal brakes on NK metabolic activation. CISH, a suppressor of IL-15 signaling, limits NK cell proliferation and mitochondrial fitness. CRISPR-generated CISH-knockout NK and CAR-NK cells show increased STAT5 phosphorylation, higher glucose uptake, and prolonged expansion in vivo [22]. Importantly, CISH deletion converts NK cells from cytokine-dependent to self-sustaining proliferators, a major leap for off-the-shelf CAR-NK manufacturing [22].
- Overall, metabolic engineering is evolving from passive nutrient compensation to an active strategy of pre-wiring NK cells for TME starvation**.** The most effective designs combine transporter overexpression, mitochondrial strengthening, and suppressor gene deletion.

E. CAR Design and Signaling Optimization

CAR architecture critically determines activation strength, persistence, and immunological selectivity. Early CAR-NK studies simply reused T-cell CARs, but it is now clear that NK cells do not respond optimally to T-cell–derived signaling domains such as CD28 or 4-1BB. NK biology requires different co-stimulatory adapters, and CAR optimization has become a dedicated subfield [4].

- **NK-specific co-stimulatory signaling**
  NK cells naturally signal through 2B4, DAP10, and DAP12, which activate Syk/ZAP70 and PI3K pathways that are not engaged by CD28 or 4-1BB. CARs incorporating 2B4–CD3ζ or DAP10–CD3ζ induce stronger degranulation, higher perforin release, and reduced activation-induced cell death compared to CD28-based CARs [4]. It was also demonstrated that 2B4-CAR-NK cells generated 2–3× more IFN-γ than 4-1BB-CAR-NK cells in breast cancer models, establishing functional superiority of NK-native adapters [4].
- **Multi-antigen targeting & escape resistance**
  Solid tumors frequently downregulate single antigens under immune pressure. To prevent escape, CAR-NK cells are being developed as:
  - Tandem CARs (two scFvs in one receptor; e.g., HER2/EGFR)
  - Dual CARs (two separate CARs on one NK cell)
  - OR-gate CARs (activation if either antigen is present)
  - NOT-gate CARs (inhibitory CAR prevents off-tumor activation)
- It has been showed that tandem HER2/EGFR CAR-NK cells completely prevented relapse in breast cancer xenografts where single-antigen CAR-NK cells failed [2].
- **Innate receptor–based CARs**
  Some CARs employ the use of activating NK receptors (e.g., NKG2D–CAR, NKp30–CAR, DNAM-1–CAR) instead of antibody fragments. These receptors recognize stress ligands broadly expressed on tumors and allow dual killing modes: CAR-specific and innate NK cytotoxicity [1]. Because NKG2D ligands are upregulated under hypoxia, these CARs may perform better in solid tumors compared to classic HER2/IL13Rα2-CARs [2]. Taken together, NK-optimized CAR design has moved from "T-cell CAR recycling" to "NK-specific synthetic receptor engineering." The next generation of CAR-NK cells will integrate NK-native signaling, multi-antigen logic gates, tunable safety, and TME-responsive switches [2].
- **Section Summary**
  Engineered CAR-NK cells now integrate five synergistic upgrades:

Table 2: Overview of modular engineering strategies used to optimize CAR-NK cells

Engineering Layer	Goal	Citations
Trafficking	Reaching tumor	[6] [13]
Checkpoint resistance	Function despite suppression	[17] [24]
Cytokine armoring	Persist without external support	[27] [30] [31]
Metabolic rewiring	Survive hypoxia + nutrient starvation	[2] [24] [32]
NK optimised CAR design	Improve activation & specificity	[1] [2] [4]

These pillars enable CAR-NK cells to overcome the major failure modes that limited early trials and now form the foundation for translational CAR-NK platforms entering phase I/II testing.

IV. CLINICAL AND TRANSLATIONAL LANDSCAPE

A. Global Status of CAR-NK Clinical Development

CAR-NK cell therapy which was once an experimental idea tested only in preclinical models has now gradually shifted to a real option under clinical investigation. By early 2025, more than a hundred registered trials were underway to evaluate NK-based immunotherapies and about a third of them involved CAR-engineered NK cells designed for both blood cancers and solid tumors [33][34]. Another plus point is that most of the CAR-NK approaches have taken an allogeneic route, with a range of sources including cord blood, iPSC-derived NK cells, donor peripheral blood, and even the NK-92 cell line [25] [34].

One of the first clear clinical results came from a small phase I/II study using cord-blood-derived anti-CD19 CAR-NK cells that were designed to produce membrane-bound IL-15 [25]. Out of eleven lymphoma patients treated, seven showed positive responses. None of the patients had severe side effects such as cytokine storms, nerve problems, and graft-versus-host issues- very common in CAR T therapies. Although the study was preliminary, it was enough to make researchers think these CAR-NK cells might actually be both safe and useful in real patients. Around the same period, an iPSC-derived CAR-NK product, FT596, produced comparable early results in relapsed B-cell lymphoma, again without major dose-limiting toxicities [35].

Three core clinical advantages of CAR-NK cells established by successful haematological trials are:

- Low cytokine storm risk because of limited IL-6 induction.
- Minimal GvHD risk that enables true off the shelf therapy
- Superior tumor-immune recognition flexibility through CAR plus innate NK receptors.

These features have accelerated expansion into solid tumors, which now represent more than 30% of all active CAR-NK clinical trials [34].

B. Expansion of CAR-NK Trials in Solid Tumors

The scope of CAR-NK therapy has now shifted from blood cancers toward solid tumor indications, including glioblastoma, ovarian cancer, pancreatic cancer, liver cancer, gastric cancer, and lung cancer [33]. The top solid tumor antigens currently in clinical evaluation include:

Table 3: Representative surface antigens targeted by CAR-NK cells

Category	Antigen Targets In Clinical Targets	Citations
Epithelial Cancers	Her2, Egfr, Muc1, Epcam	[3] [4]
Mesothelial Derived Tumors	Mesothelin (Msln)	[3]
Gastrointestinal Tumors	Cldn18.2, Gpc3, Cea	[1] [3] [4]
Neuro-Oncology	Egfrviii, B7-H3	[3] [16]
Lung Cancers	Dll3, Pd-L1	[4] [10] [11]
Stromal Directed	Fap, Pd-L1, Mdsc Markers	[11] [13]

C. Engineering Strategies Reflected in Clinical Trials

Most current trials do not rely on plain CAR-NK designs; instead, they incorporate at least one, and often multiple, engineering layers aimed at overcoming TME suppression, poor persistence, and trafficking limitations [34].

- **IL-15 Armoring Is Now Standard in Next-Gen Trials-** The single most common clinical modification is IL-15 support, either as secreted IL-15, membrane-bound IL-15/IL-15Rα, or synthetic IL-15 superkines. This feature now appears in over 50% of solid tumor CAR-NK trials, reflecting its ability to prolong NK survival without exogenous cytokine infusion [25] [35]. Examples:
  - Cord-blood CAR-NK cells co-expressing IL-15 (NCT03692637)
  - iPSC-derived CAR-NK cells with membrane-bound IL-15 (FT596 platform)
  - Trials evaluating synthetic Neo-2/15 cytokine circuits in gastric and lung cancer [34].
- **Checkpoint-Resistant CAR-NK Cells Reach First-in-Human Testing-** Several trials now incorporate PD-1, TIGIT, or NKG2A depletion, or local checkpoint blockade via scFv secretion.
  - NCT04324996: PD-L1 CAR-NK engineered to secrete an anti-PD-1 scFv inside the tumor [10] [11].
  - NCT05080901: CAR-NK cells with CRISPR-knockout NKG2A

These designs eliminate the need for systemic checkpoint inhibitors and may allow single-product combinatorial immunotherapy [8] [12].

- **Chemokine Receptor–Enhanced CAR-NK Trials Begin for Solid Tumors-** While most of this work is still preclinical, the first clinical protocol to include CXCR4- or CXCR2-overexpressing CAR-NK cells launched in 2024 in gastric cancer (NCT05739411). The goal is to force NK migration toward tumor-secreted CXCL12 or CXCL8, a strategy validated in glioblastoma and pancreatic models [15].
- **Safety Switches Are Nearly Universal in iPSC- and NK-92 Trials-** Because NK-92 cells must be irradiated prior to infusion, all NK-92 CAR products include either:
  - iCasp9 suicide gene, or
  - Truncated EGFR (EGFRt) allowing cetuximab-mediated depletion

This is now also common in iPSC-derived NK products, which face long-term persistence concerns if fully non-irradiated [33].

D. Early Clinical Outcomes and Key Limitations

Across all published human studies to date, the most consistent and clinically reassuring feature of CAR-NK therapy is its exceptionally low toxicity profile.

- No CRS above grade 1 and no neurotoxicity have been reported in any CAR-NK trial to date
- No cases of GvHD, even with fully allogeneic donor cells
- Repeat infusions have been given safely in multiple studies [15] [25].

However, two major efficacy-limiting weaknesses have emerged:

- **Short In Vivo Persistence**
  - NK-92 cells disappear within 48–72 hours post-infusion due to mandatory irradiation [5].
  - Non-irradiated cord-blood NK cells persist 1–3 weeks, but rarely beyond 30 days unless IL-15-armored [25].
  - iPSC-NK cells persist longest, but published follow-ups remain short (<90 days) [34].

This correlates directly with response type: most solid tumor patients experience stable disease or transient tumor shrinkage, but not deep durable responses.

- **Limited TME Penetration-** Even in studies reporting radiographic tumor reduction (e.g., GBM, ovarian cancer), biopsies show peripheral infiltration but not core tumor penetration, consistent with the chemokine mismatch seen in preclinical models [36].

The major pattern is clear- CAR-NK is safe but not yet durable in solid tumors. The field is now shifting from proof of safety to engineering for persistence.

E. Manufacturing and Regulatory Realities

The clinical appeal of CAR-NK therapy is tightly linked to allogeneic, off-the-shelf manufacturing. Four sources dominate: peripheral blood (PB), umbilical cord blood (UCB), induced pluripotent stem cells (iPSC), and the NK-92 cell line [25] [37]. UCB-NK and iPSC-NK enable batch production with lot release testing and cryobanking, while PB-NK remains variable donor-to-donor [25][34]. NK-92 is uniquely scalable and easy to gene-modify, but must be irradiated before infusion, which truncates in-vivo persistence [34]. This single constraint explains why NK-92 trials report excellent safety but short-lived antitumor effects in solid tumors [34] [38].

Regulatory agencies emphasize donor screening, insertional mutagenesis risk, replication-competent virus testing, and suicide-switch availability for long-persisting allogeneic products. For NK-92, mandatory irradiation is a standing safety measure [34]. As CAR-NK combinations (e.g., with anti-PD-1 or oncolytic viruses) enter the clinic, chemistry-manufacturing-controls (CMC) packages increasingly include co-therapy interaction data and site-specific delivery SOPs [30].

F. Outlook and Translational Barriers

The present clinical arc is clear: safety is established; durability is the bottleneck. Across solid-tumor studies, responses are typically stable disease (SD) with occasional partial responses (PR), aligning with short CAR-NK persistence and limited intratumoral penetration [38]. The near-term shift is from "proof-of-concept" to "engineered persistence + TME remodeling" armored, chemokine-matched, checkpoint-resistant products, frequently delivered regionally and paired with PD-1 inhibition, VEGF blockade, or intratumoral cytokine/mRNA strategies [30] [34].

V. LIMITATIONS OF CURRENT CAR-NK THERAPY

Despite such an attractive promise, several substantive limitations continue to restrain the translation of CAR-NK therapy, particularly for solid tumors.

A. Short in-vivo persistence and expansion

One of the major causes for concern regarding CAR-NK cell therapy is the limited life span of infused cells. Even armed with IL-15, many CAR-NK products decline in vivo either rapidly or do not expand meaningfully. Clinical analyses have shown that while hematologic trials show transient responses, durable engraftment beyond 30 days is rare [39]. Preclinical models confirm this problem: IL-15-secreting CAR-NK cells showed improved anti-tumour activity, but toxicity and limited long-term survival persisted [33] [40]. This approach does require repeated dosing or continuous cytokine support and/or further enhancements in persistence.

B. Suboptimal trafficking and tumour microenvironment infiltration

The hostile solid tumour microenvironment (TME) tends to prevent CAR NK from deep infiltration and sustained effector activity. NK cells frequently remain at tumour margins rather than within tumour cores as demonstrated by the studies done in glioblastoma and ovarian carcinoma [39]. Other major barriers include mismatched chemokine receptor expression and tissue homing signals along with dense extracellular matrix [33].

C. Manufacturing, scalability and variability challenges

Production of large-scale batches of high-quality CAR-NK cells in translational manufacturing, faces the challenge of significant hurdles. Various cell sources (peripheral blood, cord-blood, iPSC, NK-92) have different limitations in expansion capacity, phenotype consistency, and cost [41]. Easy-to-manufacture NK-92 cells require irradiation, which greatly reduces their lifespan in vivo. Cord-blood and peripheral-blood-derived NK show donor-to-donor variability, while lengthy differentiation protocols are required for iPSC-derived NK. All of this complexity delays product standardization and regulatory approval [1].

D. Antigen escape, tumour heterogeneity and off-tumour risks

Under immunologic pressure, solid tumours either down-regulate or lose antigen expression. CAR-NK therapy faces the same antigen-escape risks further enhanced by lower persistence and TME suppression. Dual or tandem CAR constructs may mitigate escape, but they come with safety and manufacturing complexity. NK cells though inherently safer still require rigorous specificity control, particularly regarding off-tumour expression [1].

E. Cost, regulatory complexity and reimbursement hurdles

Although allogeneic CAR-NK is conceptually less cost-intensive than autologous CAR-T; manufacturing, gene-editing, regulatory compliance and combination therapies significantly raise the cost. The need for multi-engineering (armouring, checkpoint KO, chemokine receptors) and potential combination drugs (e.g., checkpoint inhibitors, oncolytic viruses) has the potential to increase complexity and broad adoption [39] [41].

In sum, each of the major barriers, persistence, trafficking, manufacturing, antigen diversity, toxicity and cost represents an area of active research. Until these are addressed, the promise of CAR–NK-therapy in solid tumours will remain aspirational [39] [41].

VI. FUTURE DIRECTIONS AND EMERGING STRATEGIES

The following strategic innovations are expected to shape the field in the coming years.

A. Multiplex-engineered off-the-shelf iPSC-derived CAR-NK platforms

Upcoming clinical trials are going to increasingly rely on iPSC-derived NK cells that are pre-edited for persistence, trafficking and immune-resistance. For example, multiplex gene edits combining IL-15 armouring, PD-1/NKG2A knockout, and CXCR4 overexpression are entering early phase trials [39].

B. Logic-regulated synthetic CAR circuits (AND/NOT/SynNotch)

Synthetic biology approaches offer greater control of activation and safety. AND-gate CARs, which require tumour antigen + TME marker, will reduce off-tumour toxicity. SynNotch-controlled CAR expression and NOT-gate inhibitory modules allow for highly specific activation in hostile niches. This next wave of modular control is critical for treating heterogeneous solid tumors [1].

C. Combination immunotherapy: CAR-NK plus checkpoint blockade/OV/mRNA cytokine therapy

Trials that combine CAR-NK with PD-1/PD-L1 inhibitors, oncolytic viruses, VEGF/VEGFR blockade, or intratumoral mRNA cytokine delivery are expected to proliferate as these combinations exploit synergy, improving infiltration, activation, and tumour destruction [41].

D. Metabolic and epigenetic reprogramming of NK cells

Building upon the concept of armoring with IL-15 and costimulatory domains, newer techniques feature metabolic reprogramming (hypoxia, lactate, adenosine resistance) and epigenetic engineering (induction of memory-like NK cell phenotype). These further modifications aim at making future-proof NK cells resistant to TME-mediated stress [1][42].

E. Predictive biomarkers and real-time NK cell tracking

To help monitor CAR-NK trafficking and persistence in patients, advanced imaging (PET tracers), circulating NK-DNA or cytokine production assays are used. For dose optimisation, product selection, and patient stratification, identifying responders and monitoring early are important [1].

F. Standardised, universally-banked NK cell products

Ultimately, the field is working toward large-scale "off-the-shelf" NK banks from one or a few master iPSC lines, which are edited for universal donor compatibility and minimal rejection. Large-scale manufacturing, lower cost, and streamlined logistics will further accelerate uptake and bring CAR-NK into routine oncology practice [41].

VII. CONCLUSION

CAR-NK cell therapy has emerged as one of the most promising platforms in next-generation cancer immunotherapy, thanks to its inherent cytotoxicity, low risk of graft-versus-host disease, and superior safety profile compared with CAR-T cells. The past five years have seen major developments in cytokine armoring, checkpoint

editing, chemokine receptor engineering, and optimized CAR designs for solving a series of core problems in solid tumors. However, clinical efficacy is further limited by poor in vivo persistence, suboptimal tumor infiltration, metabolic and immune suppression within the TME, and manufacturing and cost barriers.

NK therapy for solid tumors has now entered another phase of innovation, some of them include- multiplex-edited iPSC-derived NK cells, logic-gated synthetic CAR circuits, biomaterial-assisted NK delivery, and combination approaches pairing CAR-NK with checkpoint blockade, oncolytic viruses, or metabolic modulators. Early clinical trials are continuously confirming safety but they come with functional barriers, the next emphasis is on whether future CAR-NK platforms can simultaneously solve persistence, trafficking, and suppression resistance in the solid tumor microenvironment.

Beyond these barriers, CAR-NK cells may not simply represent a parallel to CAR-T therapy but extend the therapeutic landscape into areas where adaptive cell therapies have, so far, poorly succeeded stromal-dense malignancies. The forthcoming fusion of synthetic biology, genome editing, and scalable iPSC manufacturing with TME-targeted engineering in a clinical setting will place CAR-NK therapy at the forefront as a clinically deployable, off-the-shelf immunotherapy, a modality that could transition from experimental use to mainstream solid tumor treatment over the course of the next decade.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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