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Next-Generation CAR-NK Immunotherapy for Myeloid Malignancies: From Target Discovery to Clinical Translation

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Abstract

Chimeric antigen receptor-engineered natural killer (CAR-NK) cell therapy has emerged as a promising next-generation immunotherapeutic approach for the treatment of myeloid malignancies, particularly acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and related myeloproliferative neoplasms. Despite significant advances in conventional chemotherapy, targeted therapies, and hematopoietic stem cell transplantation (HSCT), long-term outcomes remain unsatisfactory for many patients, especially those with relapsed or refractory disease. While CAR-T cell therapies have revolutionized the management of B-cell malignancies, their application in myeloid cancers has been limited by target antigen overlap with normal hematopoietic tissues, severe toxicities, and manufacturing complexities. CAR-NK cells offer several potential advantages, including potent innate antitumor activity, lower risk of cytokine release syndrome and graft-versus-host disease, and the feasibility of developing universal "off-the-shelf" cellular products. Recent preclinical studies and early-phase clinical trials have demonstrated encouraging safety profiles and preliminary efficacy of CAR-NK platforms targeting key myeloid antigens such as CD33 and CD123. Furthermore, advances in induced pluripotent stem cell (iPSC)-derived NK cells, gene-editing technologies, and cytokine-enhanced CAR constructs have accelerated the clinical translation of this therapeutic modality. However, several challenges continue to impede widespread clinical implementation. These include antigen heterogeneity, on-target myelotoxicity affecting normal hematopoietic stem and progenitor cells, limited in vivo persistence and trafficking of NK cells, immunosuppressive bone marrow microenvironments, and constraints on scalable manufacturing. Innovative engineering strategies, including multi-antigen targeting, armored CAR constructs, cytokine support systems, checkpoint inhibition, and combination therapies, are being actively explored to overcome these barriers.

This review provides a comprehensive overview of NK-cell biology relevant to CAR engineering, summarizes current preclinical and clinical developments in CAR-NK therapy for myeloid malignancies, and critically examines the major translational challenges facing the field. Finally, it highlights emerging technological innovations and future research priorities required to establish CAR-NK therapy as a safe, effective, and widely accessible treatment option for patients with myeloid cancers.

Keywords: Chimeric antigen receptor-natural killer cells (CAR-NK); Acute myeloid leukemia (AML); Myeloid malignancies; Immunotherapy; CD33; CD123; Hematopoietic stem cell transplantation; Induced pluripotent stem cells (iPSCs); Tumor microenvironment; Off-the-shelf cellular therapy.

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1. Introduction

The therapeutic efficacy of CAR-NK cells is largely determined by the design of the chimeric antigen receptor construct and the engineering approaches used to enhance NK-cell functionality. Although CAR architectures were initially developed for T-cell therapies, increasing evidence suggests that NK cells require tailored receptor designs that account for their unique signaling pathways and biological characteristics[1–3].

A typical CAR construct consists of an extracellular antigen-recognition domain, usually a single-chain variable fragment (scFv), a hinge region, a transmembrane domain, and intracellular signaling domains. First-generation CARs incorporated only the CD3ζ signaling domain and demonstrated limited efficacy due to insufficient activation and persistence. Second- and third-generation CARs subsequently incorporated one or more co-stimulatory domains, such as CD28, 4-1BB (CD137), OX40, or ICOS, resulting in enhanced proliferation, survival, and cytotoxicity[1–3].

Unlike CAR-T cells, NK cells possess distinct intracellular signaling pathways involving molecules such as DAP10, DAP12, FcεRIγ, and 2B4 (CD244). Consequently, NK-specific CAR constructs incorporating DAP12, NKG2D, or 2B4 signaling domains have demonstrated superior activation and antitumor activity compared with conventional T-cell-derived CAR designs[4–6].

These findings have stimulated the development of next-generation CAR-NK platforms specifically optimized for NK-cell biology[4–6].

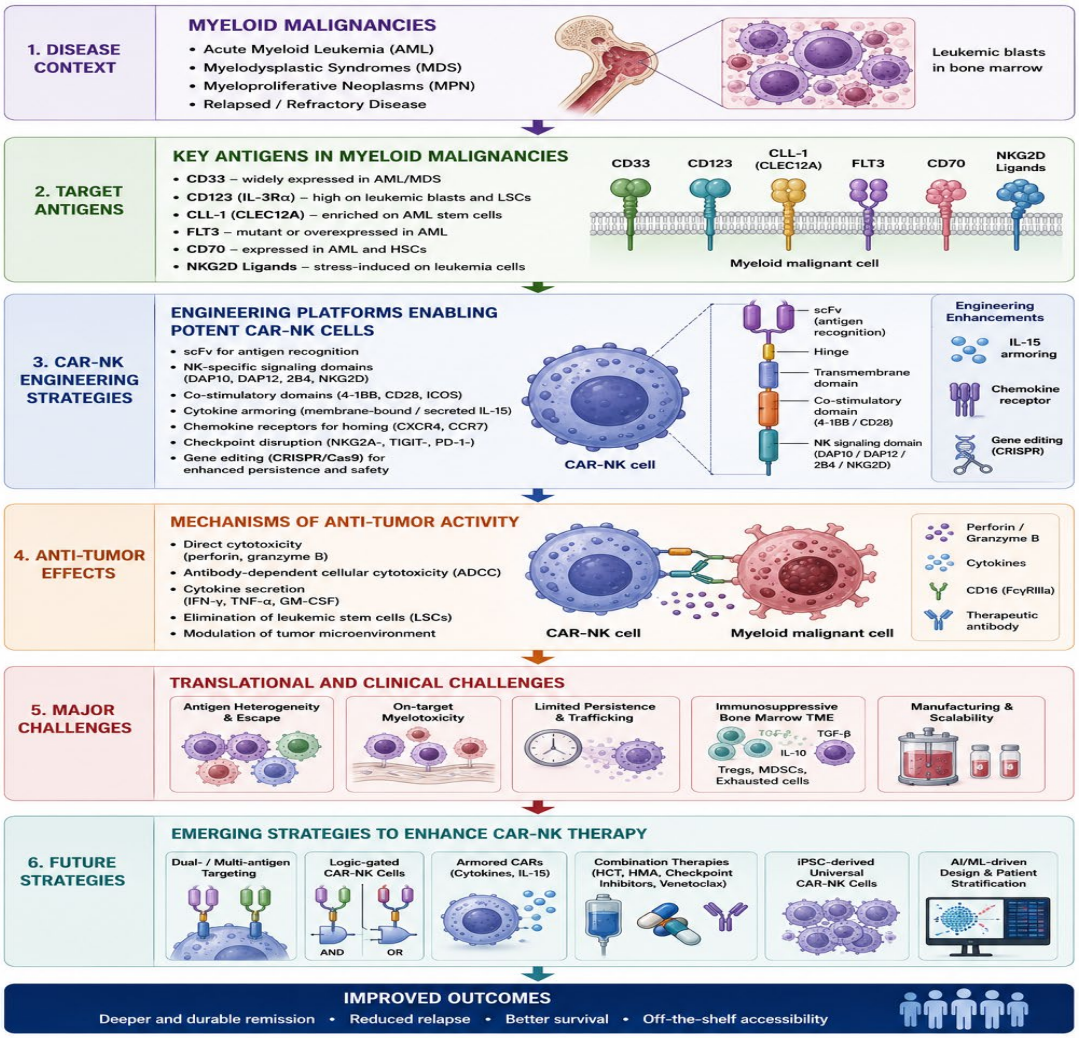
To further improve persistence and therapeutic durability, several engineered CAR-NK products have incorporated cytokine support systems, particularly membrane-bound or secreted interleukin-15 (IL-15). IL-15 promotes NK-cell survival, proliferation, and metabolic fitness while reducing dependence on exogenous cytokine administration[7,8].

Additional engineering strategies include expression of chemokine receptors to improve tumor homing, dominant-negative receptors to counteract immunosuppressive cytokines, and checkpoint-disruption approaches targeting inhibitory pathways such as NKG2A and TIGIT[9,10].

Recent advances in gene-editing technologies, including CRISPR-Cas9 and TALEN-based platforms, have further expanded the potential of CAR-NK therapy by enabling precise genomic modifications. These approaches facilitate targeted insertion of CAR constructs, elimination of inhibitory receptors, enhancement of persistence, and generation of standardized allogeneic products suitable for large-scale manufacturing[11,12].

Collectively, these innovations are driving the evolution of CAR-NK cells toward more potent, durable, and clinically applicable therapeutic platforms[11,12].

Figure 1. Overview of CAR-NK cell therapy for myeloid malignancies**.** The figure summarizes major target antigens, CAR engineering strategies, antitumor mechanisms, translational challenges, and emerging approaches designed to improve the efficacy, persistence, safety, and clinical applicability of CAR-NK cell therapies in acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and related myeloid neoplasms.



2. Target Antigens for CAR-NK Therapy in Myeloid Malignancies

Selection of an appropriate target antigen remains one of the most critical determinants of CAR-NK therapeutic success in myeloid malignancies. Unlike B-cell malignancies, where antigens such as CD19 are largely restricted to dispensable B-cell populations, most myeloid antigens are also expressed on normal hematopoietic stem and progenitor cells (HSPCs). Consequently, antigen selection must balance effective leukemia eradication against the risk of prolonged myelosuppression and hematopoietic toxicity[13,14].

2.1 CD33

CD33 is among the most extensively investigated targets in AML immunotherapy. Expressed in approximately 85–90% of AML cases, CD33 is present on leukemic blasts and leukemic stem cells but is also found on normal myeloid progenitors[15–17].

CAR-NK cells targeting CD33 have demonstrated potent antileukemic activity in multiple preclinical models and remain a leading candidate for clinical translation[15–17].

However, potential myeloablation resulting from CD33 expression on normal hematopoietic cells remains a major challenge[15–17].

2.2 CD123

CD123, the α-chain of the interleukin-3 receptor, is highly expressed on AML blasts and leukemic stem cells while exhibiting relatively lower expression on normal hematopoietic cells[18–20].

Numerous preclinical studies and early-phase clinical trials have demonstrated encouraging efficacy of CD123-directed CAR-NK therapies[18–20].

The selective overexpression of CD123 on leukemic populations has positioned it as one of the most promising targets for AML immunotherapy[18–20].

2.3 CLL-1 (CLEC12A)

C-type lectin-like molecule-1 (CLL-1), also known as CLEC12A, has emerged as a particularly attractive AML target because of its high expression on AML blasts and leukemic stem cells with limited expression on normal hematopoietic stem cells[21,22].

This differential expression profile may reduce the risk of prolonged hematopoietic toxicity while maintaining effective antileukemic activity[21,22].

Consequently, CLL-1-directed CAR-NK and CAR-T platforms have attracted significant research interest[21,22].

2.4 FLT3

FMS-like tyrosine kinase 3 (FLT3) is frequently overexpressed or mutated in AML and is associated with aggressive disease biology and poor prognosis[23,24].

FLT3-directed CAR-NK cells have demonstrated promising preclinical efficacy and may provide a therapeutic option for patients with FLT3-mutated AML who develop resistance to tyrosine kinase inhibitors[23,24].

2.5 Emerging Targets

Several additional targets are currently under investigation, including NKG2D ligands, CD70, TIM-3, WT1, LILRB4, and mesothelin-associated antigens[25–27].

Furthermore, dual-target and multi-target CAR strategies are being developed to address antigen heterogeneity and prevent immune escape through antigen loss[25–27].

Such approaches may prove particularly valuable in AML, where clonal evolution and target variability frequently contribute to disease relapse[25–27].

Despite substantial progress, no ideal AML antigen has yet been identified[13–27].

Future success will likely depend on combinatorial targeting approaches, advanced gene-engineering technologies, and strategies capable of selectively eliminating leukemic cells while preserving normal hematopoiesis[13–27].

3. Tumor Microenvironment in Myeloid Malignancies

3.1 Bone Marrow Niche and Immunosuppression

The bone marrow microenvironment (BMME) plays a pivotal role in the pathogenesis, progression, and therapeutic resistance of myeloid malignancies, particularly acute myeloid leukemia (AML). Rather than serving solely as a physical reservoir for leukemic cells, the bone marrow niche undergoes extensive remodeling by malignant blasts, creating a highly immunosuppressive ecosystem that promotes leukemia survival while impairing antitumor immune responses[13,14].

AML cells interact dynamically with mesenchymal stromal cells, endothelial cells, osteoblasts, adipocytes, and immune cells within the marrow microenvironment. These interactions

facilitate the secretion of immunosuppressive cytokines and growth factors, including transforming growth factor-β (TGF-β), interleukin-10 (IL-10), vascular endothelial growth factor (VEGF), and granulocyte-macrophage colony-stimulating factor (GM-CSF), which collectively suppress immune effector functions and support leukemic proliferation[2,4,13].

In addition, AML blasts upregulate immune checkpoint molecules such as programmed death-ligand 1 (PD-L1), Galectin-9, and CD200, which inhibit both innate and adaptive immune responses. Increased accumulation of regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and dysfunctional dendritic cells further contributes to immune evasion. NK cells residing within the leukemic microenvironment frequently exhibit reduced expression of activating receptors, including NKG2D, NKp30, and DNAM-1, accompanied by impaired cytokine production and diminished cytotoxic capacity[13,15].

Metabolic alterations within the leukemic niche represent another significant barrier to effective immunotherapy. AML cells consume substantial quantities of glucose, amino acids, and oxygen, creating nutrient-deprived, hypoxic, and acidic conditions that impair immune-cell metabolism. Furthermore, increased expression of indoleamine 2,3-dioxygenase (IDO), arginase, and adenosine-producing enzymes promotes metabolic exhaustion of infiltrating lymphocytes and NK cells. These factors collectively diminish the persistence and functional activity of CAR-engineered immune cells following infusion[13,16].

The immunosuppressive nature of the AML bone marrow microenvironment therefore represents a major obstacle to successful CAR-NK therapy. Overcoming these inhibitory signals will likely require a combination of cellular engineering approaches and adjunctive therapies capable of reprogramming the leukemic niche toward a more immune-permissive state[2,4,13].

3.2 Impact on CAR-NK Cell Trafficking and Persistence

Effective CAR-NK therapy depends not only on potent cytotoxic activity but also on efficient trafficking to disease sites and sustained persistence within the bone marrow microenvironment. Following systemic administration, CAR-NK cells must successfully home to leukemic niches, infiltrate tumor-rich regions, survive in an immunosuppressive environment, and maintain functional activity long enough to eradicate both leukemic blasts and leukemia stem cells[3,13].

NK-cell trafficking is regulated primarily through chemokine receptor-ligand interactions. Chemokine receptors such as CXCR4, CXCR3, CCR5, and CX3CR1 guide NK-cell migration toward corresponding chemokine gradients within tissues. In AML, however, disruption of normal chemokine signaling pathways and extensive remodeling of the bone marrow niche can impair NK-cell recruitment and retention. Altered CXCL12/CXCR4 signaling, in particular,

has been implicated in leukemic cell retention within protective marrow niches while simultaneously limiting immune-cell infiltration[13,17].

Even when CAR-NK cells successfully reach the bone marrow, their persistence remains a significant challenge. Unlike T cells, NK cells generally exhibit shorter in vivo lifespans and depend heavily on cytokines such as IL-2, IL-15, and IL-21 for survival and proliferation. Exposure to inhibitory cytokines, chronic antigen stimulation, oxidative stress, and metabolic deprivation within the AML microenvironment can accelerate NK-cell dysfunction and apoptosis, thereby limiting therapeutic durability[7,13].

To address these limitations, several engineering strategies are under active investigation. These include overexpression of chemokine receptors such as CXCR4 to enhance bone marrow homing, incorporation of IL-15 transgenes to improve persistence, and genetic modifications that confer resistance to suppressive cytokines such as TGF-β. Additional approaches include checkpoint-resistant CAR-NK constructs, dominant-negative receptors, cytokine-armored CARs, and synthetic switch receptors capable of converting inhibitory signals into activating stimuli[3,4,18].

Emerging evidence suggests that optimizing trafficking and persistence may be as critical as improving cytotoxic potency for achieving durable clinical responses. Consequently, future generations of CAR-NK therapies will likely integrate enhanced homing capabilities, metabolic resilience, and resistance to immune suppression to maximize efficacy in patients with AML and other myeloid malignancies[2,3,13,18].

4. Current Landscape of CAR-NK Therapy in Myeloid Malignancies

4.1 Preclinical Evidence

Over the past decade, substantial preclinical research has established the therapeutic potential of CAR-NK cells against myeloid malignancies, particularly acute myeloid leukemia (AML). Studies utilizing peripheral blood-derived NK cells, cord blood-derived NK cells, NK-92 cell lines, and induced pluripotent stem cell (iPSC)-derived NK cells have consistently demonstrated potent antileukemic activity against AML cell lines and primary patient samples[8,15–18].

Among the investigated targets, CD33 and CD123 remain the most extensively studied. CD33-directed CAR-NK cells have exhibited robust cytotoxicity against AML blasts in vitro and significant leukemia burden reduction in xenograft models. Similarly, CD123-targeted CAR-NK constructs effectively eliminate AML cells through both CAR-mediated recognition and intrinsic NK-cell cytotoxic mechanisms[2,4,15].

Importantly, several studies have demonstrated that CAR-NK cells retain the ability to recognize and kill antigen-low tumor cells through their endogenous activating receptors, potentially reducing the risk of complete immune escape[2,4,15].CLL-1 (CLEC12A)-directed CAR-NK cells have emerged as particularly promising candidates because of the restricted expression of CLL-1 on leukemic stem cells and its minimal presence on normal hematopoietic stem cells[16,17].

Preclinical investigations indicate that CLL-1-targeted approaches can achieve potent leukemia eradication while potentially minimizing long-term hematopoietic toxicity[16,17].To enhance therapeutic durability, several next-generation CAR-NK platforms have incorporated cytokine support systems such as IL-15 transgenes, resulting in improved proliferation, persistence, and antitumor efficacy[3,11,18].NK-specific signaling domains including DAP10, DAP12, and 2B4 have further enhanced cytotoxic activity compared with conventional T-cell-derived CAR constructs[3,11,18].More recently, dual-target and multi-antigen CAR-NK strategies targeting combinations such as CD33/CLL-1 and CD123/CLL-1 have demonstrated superior control of antigen-heterogeneous leukemia populations in preclinical models[4,15,18].

These approaches significantly reduce the likelihood of antigen escape while maintaining potent antileukemic activity[4,15,18].Collectively, preclinical studies strongly support the continued clinical development of CAR-NK therapies for AML and related myeloid disorders[8,15–18].

4.2 Clinical Trials in AML and Related Disorders

Although CAR-NK therapy remains at an earlier stage of development than CAR-T therapy, clinical investigation has accelerated considerably in recent years[7,8,19].Multiple phase I and phase I/II trials are currently evaluating CAR-NK products targeting CD33, CD123, CLL-1, and other myeloid antigens in patients with relapsed or refractory AML and high-risk myelodysplastic syndromes (MDS)[7,8,19].Among the most advanced programs are CD33-directed allogeneic CAR-NK therapies[8,19].

Early clinical studies have demonstrated encouraging safety profiles and preliminary evidence of antileukemic activity in heavily pretreated AML patients[8,19].Several participants achieved morphologic leukemia-free states, partial remissions, or complete remissions without experiencing severe cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), or graft-versus-host disease (GVHD)[8,19].

Universal donor “off-the-shelf” CAR-NK products derived from cord blood or iPSCs are also progressing through clinical evaluation[10,19,20].These platforms offer important logistical advantages by eliminating the need for patient-specific manufacturing and enabling rapid treatment administration[10,19,20].Early reports suggest that such products can be administered

safely following lymphodepleting chemotherapy, with evidence of in vivo expansion and measurable antileukemic responses[10,19,20].

In parallel, several clinical studies are investigating CD123-targeted CAR-NK therapies, frequently incorporating IL-15 support systems or NK-specific engineering strategies designed to improve persistence and functionality[7,20].

Additional trials are exploring combination approaches involving CAR-NK cells and hypomethylating agents, venetoclax, checkpoint inhibitors, or cytokine-based therapies to enhance therapeutic efficacy[7,20].Although patient numbers remain relatively small and follow-up durations are limited, current clinical findings support the feasibility, safety, and preliminary efficacy of CAR-NK therapy in myeloid malignancies[7,8,19,20].Larger studies will be required to establish optimal dosing strategies, durability of response, and long-term clinical benefit[7,8,19,20].

4.3 Safety Profile Relative to CAR-T Cells

One of the most compelling advantages of CAR-NK therapy is its favorable safety profile compared with CAR-T-cell therapy[21,22].While CAR-T therapies have demonstrated remarkable efficacy in B-cell malignancies, their use is frequently complicated by severe cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), prolonged cytopenias, and manufacturing-related delays[21,22].In contrast, CAR-NK cells generally produce lower levels of inflammatory cytokines such as interleukin-6 (IL-6), a key mediator of CRS[3,7,21].

Early clinical studies across hematologic malignancies have reported substantially lower rates and severity of CRS and neurotoxicity following CAR-NK infusion[3,7,21].

Furthermore, because NK cells do not mediate classical alloreactive responses in the same manner as T cells, the risk of graft-versus-host disease remains extremely low even when donor-derived cells are administered across HLA barriers[3,7,21].The reduced toxicity profile of CAR-NK therapy has facilitated the development of allogeneic and universal donor products, potentially enabling broader patient access and more rapid treatment delivery[3,7,21].Nevertheless, safety challenges remain[2,4,15].

Myelosuppression and cytopenias continue to be major concerns when targeting antigens such as CD33 and CD123 because these molecules are expressed on normal hematopoietic progenitors[2,4,15].

Distinguishing treatment-related hematopoietic toxicity from disease-associated marrow failure can be difficult, particularly in heavily pretreated AML patients[2,4,15].Another potential

limitation is the relatively short persistence of NK cells in vivo[3,11,18].While transient persistence contributes to reduced toxicity, it may also limit long-term leukemia control compared with highly persistent CAR-T-cell products[3,11,18].Consequently, ongoing engineering efforts aim to enhance CAR-NK durability through cytokine support, optimized signaling domains, and repeated dosing strategies without compromising their inherent safety advantages[3,11,18].

Overall, current evidence suggests that CAR-NK therapy offers a favorable balance between efficacy and safety, positioning it as a highly promising next-generation cellular immunotherapy platform for AML and other myeloid malignancies[21,22].

5. Major Challenges in Advancing CAR-NK Therapy for Myeloid Malignancies

5.1 Antigen Heterogeneity and Antigen Escape

Despite encouraging advances in CAR-NK therapy, antigen heterogeneity remains one of the most significant barriers to achieving durable responses in myeloid malignancies[21–24].AML is characterized by extensive genetic, phenotypic, and clonal diversity, resulting in variable expression of target antigens among patients and even within individual leukemic populations[21–24].

Leukemic stem cells (LSCs), which are responsible for disease initiation and relapse, often display antigen-expression patterns distinct from bulk leukemic blasts, further complicating effective targeting[25,26].Most currently investigated CAR-NK targets, including CD33, CD123, CLL-1, and FLT3, exhibit heterogeneous expression levels across AML subtypes and disease stages[27–29].Under selective pressure from antigen-specific immunotherapy, antigen-low or antigen-negative subclones may expand and eventually dominate the leukemic population, leading to treatment resistance and disease recurrence[27–29].

This phenomenon, commonly referred to as antigen escape, has already been observed in CD19-directed CAR-T therapies and is increasingly recognized as a potential limitation of CAR-NK approaches[30,31].To overcome this challenge, researchers are developing dual-target, tandem, and logic-gated CAR-NK constructs capable of simultaneously recognizing multiple leukemia-associated antigens[29,32].These strategies may reduce the likelihood of immune escape while improving overall treatment efficacy[29,32].

5.2 On-Target Off-Tumor Myelotoxicity

The lack of truly leukemia-specific antigens represents a major obstacle for CAR-based therapies in AML[33,34].Unlike CD19 in B-cell malignancies, most AML-associated antigens are also expressed on normal hematopoietic stem and progenitor cells (HSPCs), creating the risk

of on-target off-tumor toxicity[33,34].CD33 and CD123 are prime examples of therapeutically relevant targets that are expressed on both leukemic and normal myeloid progenitors[35,36].

Consequently, CAR-NK-mediated elimination of malignant cells may also result in profoundmyelosuppression, neutropenia, thrombocytopenia, and impaired hematopoietic recovery[35,36].While transient myeloablation may be manageable in conjunction with hematopoietic stem cell transplantation, prolonged marrow aplasia could result in life-threatening complications[37].

Several approaches are being investigated to minimize hematologic toxicity, including transient CAR expression using mRNA electroporation, incorporation of suicide switches, inhibitory CAR systems, and selective targeting of antigens preferentially expressed on leukemic stem cells rather than healthy progenitors[38–40].These innovations may improve the therapeutic window of CAR-NK therapies while preserving normal hematopoiesis[38–40].

5.3 Limited Persistence and Expansion of CAR-NK Cells

Although limited persistence contributes to the favorable safety profile of CAR-NK therapy, it simultaneously represents a significant challenge for achieving sustained antitumor responses[41,42].Unlike memory T cells, NK cells possess relatively short lifespans and demonstrate limited proliferative capacity following infusion[41,42].Clinical studies evaluating both conventional and CAR-engineered NK-cell therapies have shown that transferred NK cells often persist for only a few days to several weeks in vivo[43,44].

This limited persistence may be insufficient to eradicate residual leukemic stem cells, which can subsequently drive disease relapse[43,44].Furthermore, expansion of NK cells depends heavily on cytokine support, particularly IL-2, IL-15, and IL-21, making their survival vulnerable to cytokine deprivation within the leukemic microenvironment[45].To address this limitation, next-generation CAR-NK platforms frequently incorporate cytokine transgenes such as IL-15 or membrane-bound cytokine constructs that promote autonomous survival and proliferation[46–48].Additional strategies include optimization of NK-specific signaling domains, repeated dosing schedules, feeder-cell expansion technologies, and genome-editing approaches designed to enhance metabolic fitness and resistance to apoptosis[46–48].Balancing prolonged persistence with maintenance of safety remains a critical objective, as excessive activation or expansion may increase toxicity and compromise the intrinsic advantages of NK-cell therapy.

5.4 Immunosuppressive Microenvironment and Functional Exhaustion

The leukemic bone marrow microenvironment exerts profound immunosuppressive effects that can substantially limit CAR-NK therapeutic efficacy[49,50].AML blasts actively remodel the bone marrow niche through secretion of suppressive cytokines, expression of immune checkpoint

ligands, recruitment of regulatory immune cells, and induction of metabolic stress[49,50].Exposure to transforming growth factor-β (TGF-β), interleukin-10, indoleamine 2,3-dioxygenase (IDO), adenosine, and other inhibitory mediators suppresses NK-cell activation, proliferation, and cytotoxicity[51–53].In parallel, checkpoint molecules such as PD-1, TIM-3, TIGIT, and NKG2A contribute to progressive immune dysfunction and exhaustion[51–53].

Functional exhaustion is characterized by reduced cytokine secretion, impaired cytotoxic granule release, diminished activating receptor expression, and decreased responsiveness to tumor-associated signals[54,55].Chronic antigen stimulation within the leukemic microenvironment may further accelerate exhaustion of infused CAR-NK cells, thereby reducing therapeutic durability[54,55].Current strategies to overcome microenvironmental resistance include checkpoint blockade, cytokine armoring, dominant-negative TGF-β receptors, metabolic reprogramming, and synthetic switch receptors capable of converting inhibitory signals into activating stimuli[56–58].Such approaches are expected to play a crucial role in the next generation of CAR-NK therapeutics[56–58].

5.5 Manufacturing, Scalability, and Regulatory Hurdles

Large-scale manufacturing remains one of the most important translational barriers facing CAR-NK therapy[59].

Compared with CAR-T products, NK cells are more challenging to expand consistently while maintaining functional potency and phenotypic stability[59].Peripheral blood-derived NK cells often exhibit donor-to-donor variability and limited expansion potential, whereas cord blood-derived NK cells require extensive ex vivo activation[60,61].Although NK-92 cell lines and induced pluripotent stem cell (iPSC)-derived NK cells offer greater scalability and product uniformity, they introduce additional regulatory and safety considerations[60,61].

Manufacturing processes must comply with Good Manufacturing Practice (GMP) standards and incorporate rigorous quality-control measures for identity, purity, potency, sterility, genomic stability, and transgene expression[62,63].

Furthermore, large-scale implementation requires automated and cost-effective production platforms capable of generating standardized off-the-shelf cellular products[62,63].Regulatory agencies also face unique challenges in evaluating gene-edited and allogeneic CAR-NK products, particularly with respect to insertional mutagenesis, long-term persistence, genomic integrity, and traceability of master cell banks[64].As the field advances, harmonization of manufacturing standards and regulatory frameworks will be essential for ensuring widespread clinical adoption and commercial accessibility of CAR-NK therapies for myeloid malignancies[64].

6. Emerging Strategies and Future Perspectives

Despite significant advances in CAR-NK therapy, several biological and technological barriers continue to limit its full clinical potential in myeloid malignancies[65,66].Recent innovations in synthetic biology, genome engineering, cell manufacturing, and combination immunotherapy are driving the development of next-generation CAR-NK platforms designed to improve efficacy, persistence, safety, and accessibility[65,66].These emerging approaches may ultimately transform CAR-NK therapy from an experimental treatment into a standard therapeutic modality for patients with AML and related myeloid disorders[65,66].

6.1 Multi-Antigen and Logic-Gated CAR-NK Cells

Antigen escape remains a major cause of treatment failure in cellular immunotherapy[67,68].

To address this challenge, researchers are developing dual-target, tandem, and logic-gated CAR systems capable of recognizing multiple leukemia-associated antigens simultaneously[67,68].

Such constructs may improve tumor specificity while reducing the likelihood of antigen-negative relapse[67,68].Dual-target CAR-NK cells directed against combinations such as CD33/CLL-1, CD123/CLL-1, and CD33/CD123 have demonstrated superior leukemia control in preclinical models compared withsingle-target approaches[69,70].More sophisticated Boolean logic-gated CARs utilizing “AND,” “OR,” and “NOT” signaling principles are also being explored to enhance discrimination between malignant and normal hematopoietic cells[69,70].

6.2 iPSC-Derived Off-the-Shelf CAR-NK Platforms

Induced pluripotent stem cell (iPSC)-derived NK cells represent one of the most promising advances in the field[71].Unlike donor-derived NK cells, iPSC-based platforms enable the generation of unlimited quantities of genetically uniform CAR-NK cells from a single engineered master cell line[71].This approach facilitates large-scale manufacturing, standardized product quality, and incorporation of multiple genetic modifications prior to differentiation[72,73].

Several iPSC-derived CAR-NK products are currently undergoing clinical evaluation and have demonstrated encouraging safety and preliminary efficacy in hematologic malignancies[72,73].The development of universal off-the-shelf CAR-NK therapies may

significantly reduce manufacturing time, improve patient access, and lower treatment costs compared with individualized CAR-T-cell production processes[72,73].

6.3 Combination Therapies with Targeted Agents and Immune Modulators

Combination strategies are increasingly recognized as a promising avenue for enhancing CAR-NK efficacy in AML[74,75].Hypomethylating agents, BCL-2 inhibitors, FLT3 inhibitors, and monoclonal antibodies may sensitize leukemic cells to NK-cell-mediated killing while simultaneously modulating the tumor microenvironment[74,75].Checkpoint inhibitors targeting PD-1, PD-L1, TIGIT, TIM-3, and NKG2A are also being investigated in combination with CAR-NK therapies to reverse immune suppression and restore antitumor activity[76,77].Additionally, cytokine support systems involving IL-15 superagonists and NK-cell engagers may further augment CAR-NK expansion and persistence in vivo[76,77].These combinatorial approaches may prove particularly valuable in relapsed or refractory AML, where multiple resistance mechanisms frequently coexist[76,77].

6.4 Genome Editing and Synthetic Biology Approaches

Advances in genome-editing technologies such as CRISPR-Cas9, TALENs, and zinc-finger nucleases have expanded opportunities for precise engineering of CAR-NK cells[78].These tools enable targeted insertion of CAR constructs, deletion of inhibitory receptors, enhancement of cytokine responsiveness, and optimization of cellular metabolism[78].Gene-editing strategies are being used to disrupt checkpoint molecules such as CISH, NKG2A, and TIGIT, thereby enhancing NK-cell activation and persistence[79,80].Synthetic biology approaches further allow the development of programmable cellular circuits, inducible CAR systems, synthetic cytokine networks, and controllable safety switches[79,80].Such technologies may substantially improve the therapeutic index of CAR-NK therapies while minimizing toxicity and enhancing clinical durability[79,80].

6.5 Precision and Personalized CAR-NK Therapy

Future CAR-NK therapies may increasingly incorporate precision medicine principles through integration of genomic, transcriptomic, proteomic, and immunophenotypic profiling[81].

Identification of patient-specific antigen expression patterns could facilitate individualized target selection and optimized CAR design[81].Artificial intelligence and machine-learning algorithms may further support antigen discovery, prediction of treatment response, manufacturing optimization, and patient stratification[82,83].

Personalized multi-antigen targeting strategies tailored to individual leukemic profiles may help overcome disease heterogeneity and improve long-term outcomes[82,83].As understanding

of AML biology continues to evolve, precision-engineered CAR-NK therapies are expected to become increasingly sophisticated and clinically effective[82,83].

6.6 Future Outlook

The next decade is likely to witness a transition from first-generation CAR-NK products toward highly engineered cellular platforms incorporating enhanced persistence, multi-antigen recognition, microenvironmental resistance, and scalable manufacturing technologies[84,85].Continued progress in cell engineering, synthetic biology, and translational research will be critical for overcoming current limitations and unlocking the full therapeutic potential of CAR-NK therapy in myeloid malignancies[84,85].

7. Discussion

The emergence of CAR-NK cell therapy represents a significant advancement in the field of adoptive cellular immunotherapy for myeloid malignancies[85–88].While CAR-T-cell therapies have transformed the treatment landscape of B-cell malignancies, their application in AML has been hindered by the lack of leukemia-specific antigens, severe treatment-related toxicities, and manufacturing limitations[85–88].CAR-NK cells offer several theoretical and practical advantages, including reduced risk of cytokine release syndrome (CRS), minimal graft-versus-host disease (GVHD), and the possibility of developing universal off-the-shelf products[85–88].

A key observation from current preclinical and clinical studies is that CAR-NK cells retain both antigen-specific CAR-mediated killing and intrinsic NK-cell cytotoxicity[89–92].This dual mechanism may provide a unique advantage in AML, where tumor heterogeneity and antigen loss frequently contribute to therapeutic resistance[89–92].Unlike CAR-T cells, which rely predominantly on single-antigen recognition, CAR-NK cells may continue to eliminate leukemic cells through natural activating receptors even when target antigen expression is reduced[89–92].Nevertheless, whether this advantage is sufficient to prevent long-term disease relapse remains uncertain and requires validation in larger clinical studies[89–92].Target antigen selection remains one of the most critical determinants of therapeutic success[93–96].Although CD33 and CD123 have emerged as leading candidates, both antigens are expressed on normal hematopoietic progenitors, creating concerns regarding prolonged myelosuppression and marrow toxicity[93–96].Similarly, while CLL-1 appears more selective for leukemic cells, its clinical utility remains under active investigation[93–96].Consequently, future CAR-NK strategies will likely require multi-antigen targeting approaches capable of improving specificity while minimizing off-tumor toxicity[93–96].

Another important consideration is the limited in vivo persistence of NK cells[97–100].Compared with CAR-T cells, NK cells generally exhibit shorter survival and reduced proliferative capacity following infusion[97–100].Although this characteristic contributes to their favorable

safety profile, it may also compromise sustained leukemia control[97–100].Several innovative approaches, including IL-15 armoring, NK-specific costimulatory domains, cytokine support systems, and gene-editing technologies, have shown promise in enhancing persistence and functionality[97–100].However, achieving an optimal balance between prolonged activity and acceptable safety remains a major challenge for future development[97–100].The immunosuppressive bone marrow microenvironment further complicates therapeutic efficacy[101–104].

AML cells actively remodel the marrow niche through secretion of inhibitory cytokines, induction of metabolic stress, and expression of checkpoint ligands that suppress NK-cell function[101–104].Emerging evidence suggests that overcoming microenvironmental resistance may be equally important as improving CAR design itself[101–104].Strategies involving checkpoint blockade, metabolic reprogramming, dominant-negative TGF-β receptors, and synthetic switch receptors are therefore attracting increasing attention as potential methods to enhance CAR-NK durability and antitumor activity[101–104].From a translational perspective, manufacturing scalability and product standardization will strongly influence future clinical adoption[105–108].Peripheral blood-derived NK cells, cord blood-derived NK cells, NK-92 cell lines, and iPSC-derived NK cells each offer distinct advantages and limitations regarding expansion potential, persistence, genetic engineering, and regulatory compliance[105–108].Among these, iPSC-derived CAR-NK platforms appear particularly attractive because they enable generation of genetically uniform, off-the-shelf products at industrial scale[105–108].Continued optimization of manufacturing processes and regulatory frameworks will be essential for ensuring widespread accessibility and cost-effective implementation[105–108].The future direction of CAR-NK therapy will likely involve integration of synthetic biology, genome engineering, artificial intelligence-assisted target discovery, and personalized immunotherapy approaches[109–113].Multi-antigen targeting systems, programmable cellular circuits, and precision-engineered universal donor products have the potential to significantly improve efficacy while maintaining the inherent safety advantages of NK-cell therapy[109–113].Furthermore, combination strategies incorporating hypomethylating agents, venetoclax, FLT3 inhibitors, and immune checkpoint inhibitors may provide synergistic antileukemic effects and help overcome mechanisms of resistance[109–113].Overall, the current evidence indicates that CAR-NK therapy has progressed from a promising experimental concept to an emerging clinical reality[114–118].

Although substantial biological and technological challenges remain, continued advances in cell engineering, manufacturing technologies, and clinical trial development are expected to accelerate translation into routine clinical practice[114–118].Future large-scale clinical studies with extended follow-up will ultimately determine whether CAR-NK therapy can achieve durable remissions and become a standard therapeutic option for patients with AML and other myeloid malignancies[114–118].

7. Conclusions

Chimeric antigen receptor-engineered natural killer (CAR-NK) cell therapy represents a promising advancement in adoptive cellular immunotherapy for myeloid malignancies. By combining the innate cytotoxic potential of NK cells with the specificity of CAR-mediated targeting, this approach offers a unique opportunity to overcome several limitations associated with conventional therapies and CAR-T-cell platforms. The favorable safety profile of NK cells, together with the feasibility of developing allogeneic off-the-shelf products, positions CAR-NK therapy as an attractive therapeutic option for patients with acute myeloid leukemia and related disorders.

Substantial progress has been made in understanding NK-cell biology, optimizing CAR design, identifying clinically relevant target antigens, and developing scalable manufacturing platforms. Preclinical studies and early clinical trials have demonstrated encouraging antileukemic activity and manageable toxicity profiles. However, important challenges remain, including antigen heterogeneity, antigen escape, on-target hematopoietic toxicity, limited cellular persistence, and the immunosuppressive bone marrow microenvironment.

Emerging technologies such as multi-antigen targeting strategies, cytokine-armored CAR constructs, genome editing, synthetic biology approaches, and iPSC-derived universal donor platforms have the potential to significantly enhance the efficacy and accessibility of CAR-NK therapies. In parallel, combination approaches with targeted therapies, immune checkpoint inhibitors, and microenvironment-modulating agents may further improve clinical outcomes.

Although considerable work remains before CAR-NK therapy becomes a standard treatment modality, ongoing scientific and clinical advances continue to accelerate its development. With further optimization and validation, CAR-NK cells have the potential to become an effective, safe, and widely accessible immunotherapeutic platform for patients with myeloid malignancies, ultimately improving long-term disease control and survival outcomes.

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