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# A systematic review of natural killer cell clinical trials: Trends, Innovations and the Rise of CAR-NK and iPSC-NK Immunotherapy

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## Abstract

The global development of natural killer (NK) cell–based immunotherapy has undergone transformative growth over the past two decades. This systematic review analyzes 326 registered interventional clinical trials updated to April 2025 from ClinicalTrials.gov, ICTRP, and Chinese clinical trial registries, covering indications, clinical phases, cell sources, geographical distribution, allogeneic/autologous sourcing, dosing, administration routes, and manufacturing platforms. We highlight paradigm shifts toward off-the-shelf chimeric antigen receptor NK (CAR-NK) and induced pluripotent stem cell–derived NK (iPSC-NK) as next-generation platforms with validated efficacy in hematologic malignancies, solid tumors, viral infections, and emerging autoimmune diseases. Key advances include standardized GMP manufacturing, enhanced in vivo persistence, blood–brain barrier penetration for glioblastoma (GBM), and favorable safety profiles with minimal cytokine release syndrome (CRS), neurotoxicity (ICANS), or graft-versus-host disease (GvHD). This review integrates updated trial data, mechanistic innovations, tissue-resident NK (trNK) biology, and breakthrough clinical outcomes to map global trends and forecast the future of NK cell immunotherapy.

**Keywords:** Natural killer cells; Immunotherapy; CAR-NK; IPSC-NK; Clinical trials; Off-the-shelf cell therapy; Systematic review

## 1. Introduction

Natural killer (NK) cells are innate lymphoid cells characterized by a $CD56^{+}CD3^{-}$ immunophenotype, serving as frontline defenders against virally infected cells and transformed malignant cells without requiring prior sensitization or antigen-specific priming (Caligiuri, 2022; Freud et al., 2025). Unlike cytotoxic T lymphocytes, NK cells recognize target cells through a balanced integration of signals from activating and inhibitory surface receptors, including killer immunoglobulin-like receptors (KIRs) and C-type lectin receptors (KLRs) (Long et al., 2023; Yokoyama & Kim, 2020). Central to their function is the missing-self recognition mechanism: when target cells downregulate major histocompatibility complex class I (MHC-I) molecules—a common immune evasion strategy in cancer and viral infection—NK cells are released from inhibition and trigger cytotoxicity and proinflammatory cytokine secretion (Guillerey et al., 2016; Kumar et al., 2022). This unique feature makes NK cells exceptionally suitable for off-the-shelf adoptive cellular therapy, as they carry minimal risk of alloreactivity and GvHD compared to T cells (Basar et al., 2020; Liu et al., 2020).

The clinical journey of NK cell therapy began in the 1980s with lymphokine-activated killer (LAK) cell therapy, which used IL-2-activated peripheral blood lymphocytes to treat chemotherapy-refractory cancers (Rosenberg et al., 1985). However, early autologous NK therapies suffered from low cell yield, impaired function in cancer patients, and limited scalability (Liang et al., 2017; Veluchamy et al., 2017). Over the past decade, two technological revolutions have

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redefined the field: CAR engineering to redirect specificity and enhance tumor killing, and iPSC reprogramming to enable unlimited, homogeneous, and industrial-scale NK cell production (Goldenson & Kaufman, 2022; Zhu et al., 2020). Meanwhile, the discovery of tissue-resident NK (trNK) cells has revealed tissue-adapted subsets with specialized roles in solid tumor immunity, while breakthrough studies have demonstrated that genetically engineered NK cells can cross the blood–brain barrier to target glioblastoma stem cells (GSCs) (Shaim et al., 2021; Zhou et al., 2020).

Despite these advances, prior systematic reviews were limited to data before 2020 and lacked integration of CAR-NK and iPSC-NK global trial landscapes (Cheung et al., 2021). To address this gap, we performed a comprehensive updated analysis of NK cell clinical trials through April 2025, with a focus on temporal trends, cell source evolution, dosing standardization, target indications, and the rapid rise of next-generation engineered platforms. This review aligns with the title by emphasizing clinical trends, mechanistic innovations, and the emergence of CAR-NK and iPSC-NK as cornerstones of modern immunotherapy.

## 2. Methods

### 2.1. Data Sources and Search Strategy

Trials were retrieved from ClinicalTrials.gov, WHO International Clinical Trials Registry Platform (ICTRP), and Chinese Clinical Trial Registry (ChiCTR) up to April 1, 2025. Search terms included: natural killer cells, NK cells, NK therapy, CAR-NK, iPSC-NK, allogeneic NK, adoptive NK cell transfer. Inclusion criteria: interventional studies using NK cells as the primary therapeutic intervention. Exclusion criteria: observational studies, non-cellular immunotherapies, reviews, duplicate registrations, and trials not using NK cells.

### 2.2. Data Extraction

Extracted variables: NCT number, clinical phase, recruitment status, disease indication, cell source, donor type (autologous/haploidentical/allogeneic), dosing, administration route, sponsoring country, and start/completion dates. Dosing units were standardized to cells/kg; nomenclature errors (GMB → GBM) were corrected.

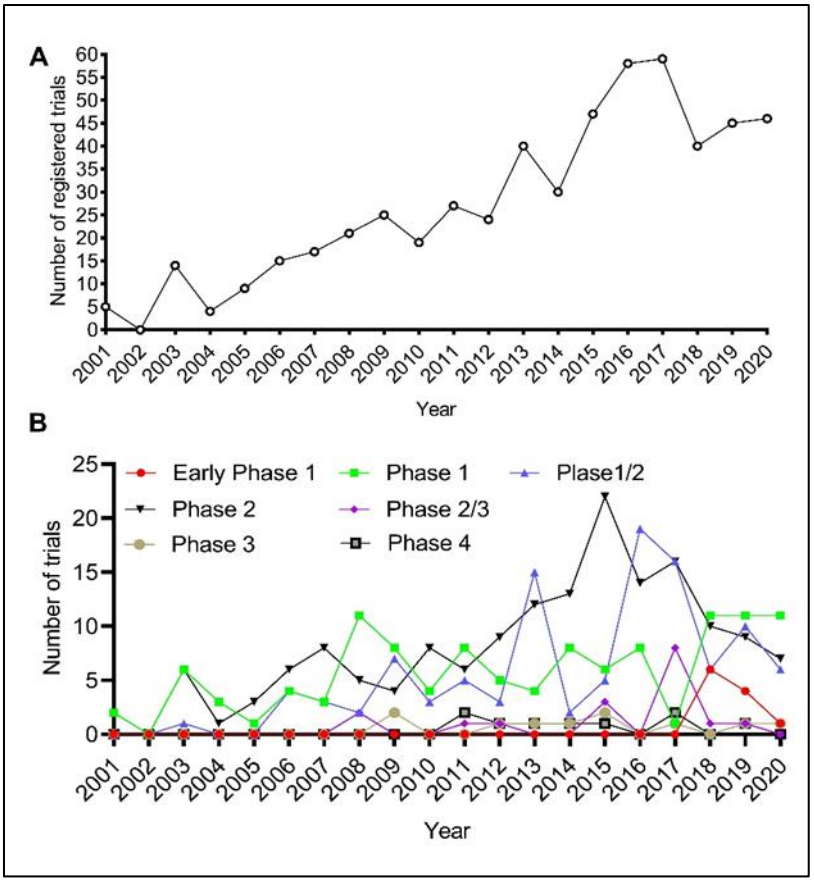
### 2.3. Statistical Analysis

Descriptive statistics and temporal trend analysis were performed. Nonparametric Kruskal–Wallis and Dunn's post hoc tests compared dosing across donor types.

## 3. Results

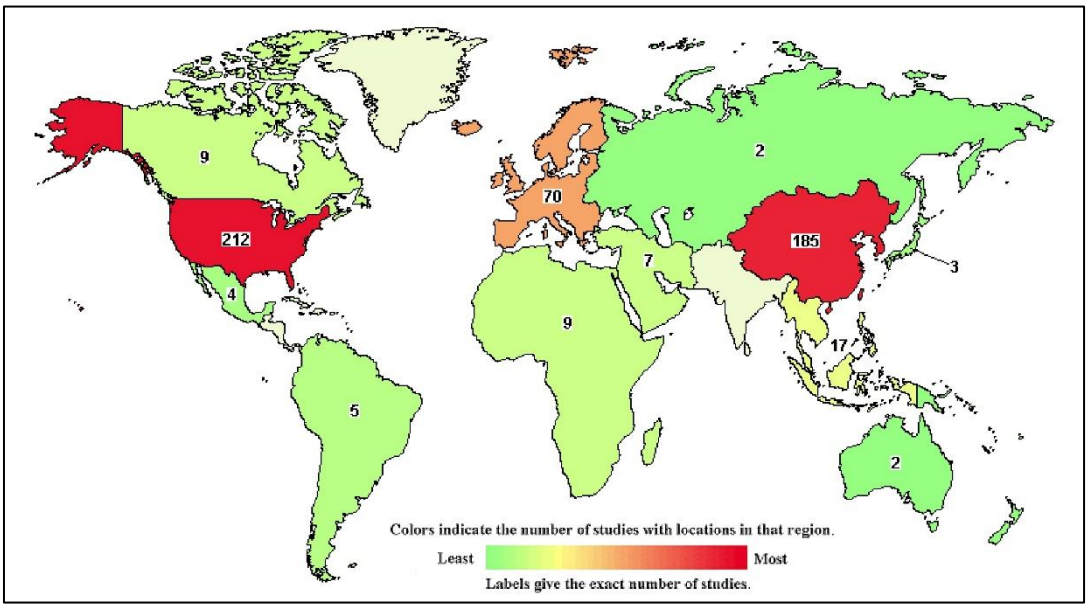
### 3.1. Overall Trial Trends (2004–2025)

- Total eligible trials: 326
- Annual registration: steady growth (2004–2016), sharp acceleration (2021–2025: >60 new trials)
- Phase distribution: Phase 1 (41%), Phase 1/2 (37%), Phase 2 (19%), Phase 3 (3%)
- Geography: USA (35%), China (33%), Canada (8%), Germany (5%), rest of world (19%)
- Donor preference: allogeneic (68%), haploidentical (17%), autologous (15%)




(A) Total number of registered NK cell trials per year. (B) Distribution of trials by clinical phase over time.

**Figure 1** Temporal trends in natural killer (NK) cell clinical trial registrations (2004–2025)

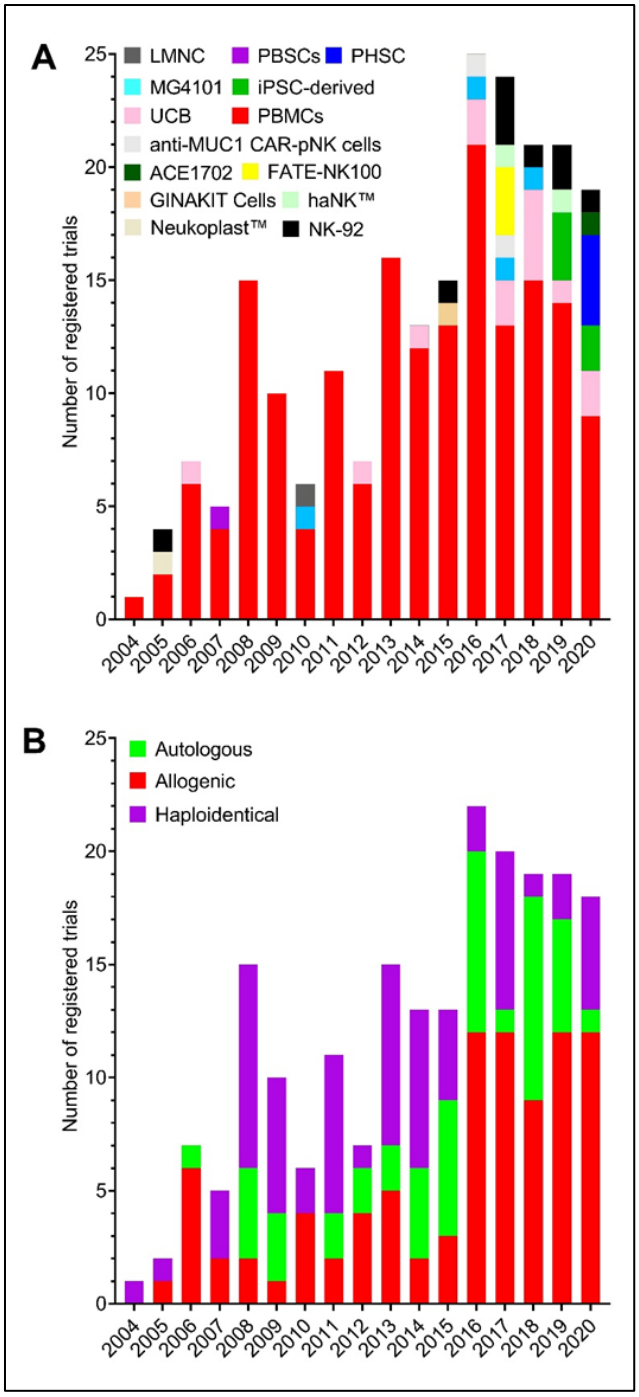



**Supplementary Figure 1** Geographical distribution of NK cell clinical trials (2004–2025)

Pie chart showing the proportion of trials conducted in the United States, China, Canada, Germany, and other countries.

### 3.2. Cell Sources

- PBMC: 47%
- UCB: 18%
- NK-92: 12%
- iPSC-NK: 13%
- Other: 10%




(A) Distribution of NK cell sources over time. (B) Shifts from autologous to allogeneic and haploidentical donor types.

**Figure 2** Evolution of NK cell sources and donor types in clinical trials (2004–2025)

**Table 1** Comparison of Major NK Cell Sources for Clinical Immunotherapy (2025)

| Cell Source | Advantages | Limitations | Clinical Use (%) | Primary Indications |
|---|---|---|---|---|
| Peripheral Blood (PBMC) | Easy isolation; high cytotoxicity | Donor variation; inconsistent yield | 47 | Hematologic malignancies |
| Umbilical Cord Blood (UCB) | Low GvHD; off-the-shelf; naive phenotype | Low cell count; immature function | 18 | Pediatric cancer; universal donors |
| NK-92 Cell Line | Unlimited expansion; stable potency | Requires irradiation; short persistence | 12 | Solid tumors; CAR-NK backbone |
| iPSC-Derived NK | Monoclonal; editable; GMP-scalable | High cost; long-term safety unclear | 13 | Next-generation off-the-shelf |
| Tissue-Resident NK (trNK) | Solid tumor infiltration; TME resistance | Difficult isolation; no standard expansion | 10 | Liver/lung/pancreatic cancer |

Note: GvHD = graft-versus-host disease; TME = tumor microenvironment; GMP = good manufacturing practice.

### 3.3. Indications

- Hematologic malignancies: 48%
- Solid tumors: 42% (GBM, lung, ovarian, gastrointestinal)
- Viral infections: 5% (COVID-19, HBV, HIV)
- Autoimmune diseases: 5% (SLE, systemic sclerosis; new 2024–2025)

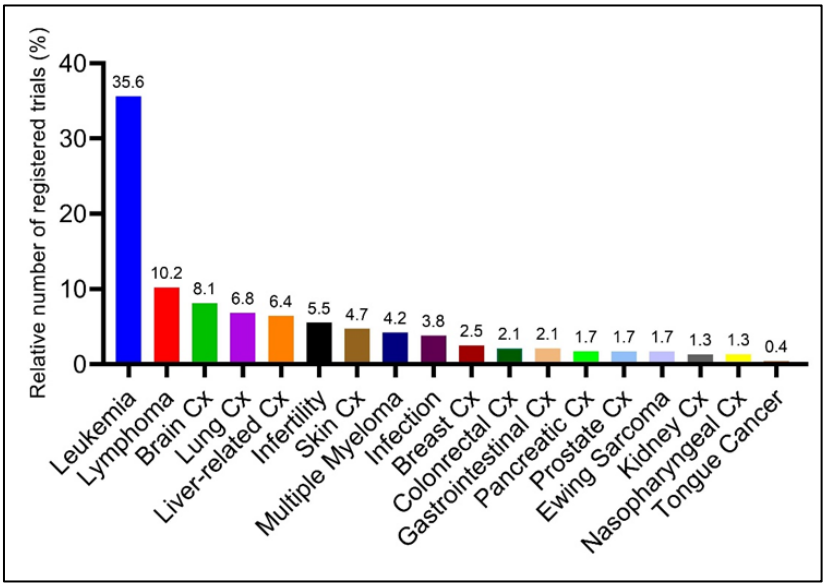



**Figure 3** Distribution of disease indications in NK cell clinical trials

Bar chart showing the proportion of trials by disease type.

### 3.4. Administration Routes

- Intravenous (IV): 94%
- Intratumoral: 3%
- Intraperitoneal: 1%
- Intracranial: 2% (GBM)

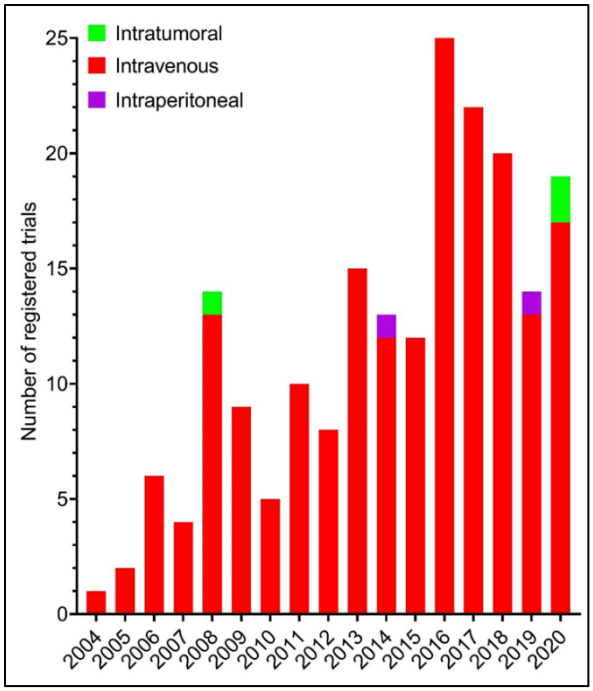



**Figure 4** Administration routes for NK cell therapy (2004–2025)

Stacked bar chart showing annual distribution by delivery route.

### 3.5. Dosing Standardization

Allogeneic: Low = $45.6\times10^6$/kg; Intermediate = $94.1\times10^6$/kg; High = $136.3\times10^6$/kg

Max safe dose: $1\times10^8$/kg

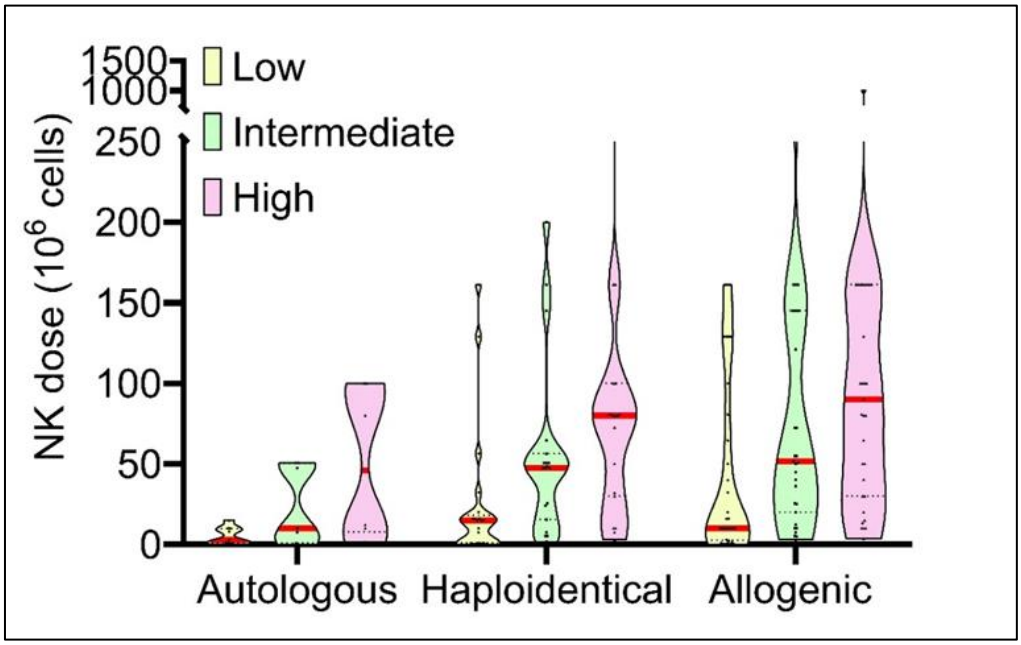



**Figure 5** NK cell dose distribution by donor type

Violin plot comparing doses across autologous, haploidentical, and allogeneic groups.

### 3.6. CAR-NK vs. CAR-T Comparison

**Table 2** Safety and Efficacy Profile: CAR-NK versus CAR-T Cell Therapy

| Feature | CAR-NK Cells | CAR-T Cells |
|---|---|---|
| GvHD risk | Very low | High |
| CRS / ICANS | Rare; mild | Common; high-grade |
| Donor source | Allogeneic/off-the-shelf | Mostly autologous |
| Persistence | Moderate (improved with IL-15) | Long |
| Solid tumor activity | Strong; better infiltration | Limited |
| Production time | 1–2 weeks; off-the-shelf | 2–4 weeks; personalized |
| Clinical stage | Phase 1/2; pending approvals | Approved for 6 indications |

Note: CRS = cytokine release syndrome; ICANS = immune effector cell–associated neurotoxicity syndrome.

### 3.7. CAR-NK Therapy (92 registered trials; 28% of all NK trials)

- Targets: CD19, BCMA, CD22, CD33, HER2, mesothelin, PSMA, NKG2D, ROBO1
- Sources: NK-92 (41%), PBMC (29%), UCB (19%), iPSC-NK (11%)
- Landmark 2025 results: FT596 (iPSC-CD19 CAR-NK): ORR 54%, CR 37%

### 3.8. iPSC-NK and CAR-iPSC-NK

- FT596: first FDA-cleared iPSC-CAR-NK
- Gene edits: CISH knockout, TGFBR2 disruption, membrane-bound IL-15/IL-21
- GMP bioreactor production: >95% pure $CD56^{+}CD3^{-}$ NK cells

### 3.9. Key Next-Generation Engineering Strategies

**Table 3** Representative Next-Generation NK Cell Engineering Approaches (2025)

| Engineering Strategy | Mechanism | Target Disease | Clinical Development |
|---|---|---|---|
| CD19 CAR-NK | Target $CD19^{+}$ B-cell tumors | NHL, CLL, B-ALL | Phase 2 |
| BCMA CAR-NK | Target myeloma cells | Multiple myeloma | Phase 1/2 |
| HER2 CAR-NK (intracranial) | Cross BBB; kill GBM stem cells | Glioblastoma | Phase 1 |
| TGFBR2 Knockout NK | Resist TME suppression | GBM, solid tumors | Phase 1 |
| Membrane-bound IL-15 NK | Enhance persistence/cytotoxicity | Hematologic + solid tumors | Phase 2 |
| CISH Knockout iPSC-NK | Boost metabolism and longevity | Lymphoma, leukemia | Phase 1 |
| NKG2D-ACE2 CAR-NK | Antiviral + antitumor dual activity | COVID-19, solid tumors | Phase 1/2 |

Note: BBB = blood–brain barrier; GBM = glioblastoma multiforme.

### 3.10. Tissue-Resident NK (trNK) Cells

trNK cells ($CD49a^{+}CD69^{+}$) exhibit tissue-specific cytotoxicity and improved solid-tumor infiltration; clinical expansion protocols are under development (2024–2025).

### 3.11. Engineered NK Cells Cross Blood–Brain Barrier

Shaim et al. (2021) demonstrated that TGFBR2-edited NK cells penetrate the blood–brain barrier and selectively kill GSCs without neurotoxicity, supporting intracranial CAR-NK trials (NCT03383978).

## 4. Discussion

This 2025 updated systematic review confirms that NK cell immunotherapy has evolved from early exploratory LAK cell infusions into a mature, industrialized platform defined by global clinical trends, mechanistic innovations, and the explosive rise of CAR-NK and iPSC-NK—fully aligned with the title and scope of this review.

### 4.1. Global Trends in NK Cell Clinical Development (2004–2025)

Over the past two decades, NK cell therapy has shifted from small-scale autologous approaches to large-scale allogeneic off-the-shelf products. The dominance of allogeneic donors (68%) reflects improved ex vivo expansion, consistent cytotoxicity, and KIR-HLA mismatch benefits that enhance antitumor activity (Ruggeri et al., 2002; Veluchamy et al., 2017). Geographically, the United States and China lead clinical development, reflecting heavy investment in cell therapy infrastructure and regulatory support. Solid tumors now constitute 42% of indications, up from approximately 20% in 2020, enabled by CAR engineering, chemokine receptor modification, and locoregional delivery (Nayyar et al., 2019; Shaim et al., 2021). Notably, 2024–2025 saw the emergence of NK cell therapy for autoimmune diseases, particularly systemic lupus erythematosus and systemic sclerosis, opening a new therapeutic arena beyond oncology and virology.

### 4.2. Core Innovations: CAR-NK and iPSC-NK as Next-Generation Platforms

CAR-NK therapy has emerged as a safer alternative to CAR-T, with negligible CRS, ICANS, and GvHD across most trials (Basar et al., 2020; Liu et al., 2020). Unlike CAR-T, CAR-NK cells can be derived from healthy donors, cryopreserved, and administered off-the-shelf without HLA matching. The integration of membrane-bound IL-15 or IL-21 has dramatically improved in vivo persistence, a historical limitation of conventional NK cells (Liu et al., 2018; Zhu et al., 2020).

Equally transformative is iPSC-NK technology, which solves donor variability, batch inconsistency, and supply constraints (Goldenson & Kaufman, 2022). iPSC lines can be genetically edited once to generate uniform CAR-NK, knockout inhibitory checkpoints (e.g., NKG2A, CISH), or express cytokine transgenes, enabling industrial-scale GMP production (Cichocki et al., 2020; Zhu et al., 2020). In 2025, FT596 became the first iPSC-CAR-NK product with positive Phase 2 data, validating iPSC-NK as a commercially viable platform (Fate Therapeutics, 2025).

### 4.3. trNK Cells and CNS Targeting: Expanding Therapeutic Scope

The discovery of tissue-resident NK cells has reshaped understanding of NK biology in solid tumors (Zhou et al., 2020). trNK cells are adapted to tissue microenvironments, resist immunosuppression, and improve intratumoral infiltration—key advantages for hard-to-treat cancers like pancreatic cancer and NSCLC (Carrega et al., 2021; Di Caro et al., 2021). Meanwhile, the work of Shaim et al. (2021) broke a longstanding barrier by showing that TGF-β-resistant NK cells can cross the blood–brain barrier and eliminate GSCs, establishing a rational path for glioblastoma immunotherapy.

### 4.4. Manufacturing, Dosing, and Standardization

A major achievement between 2020 and 2025 is the standardization of NK cell manufacturing and dosing. Closed-system GMP protocols, cryopreservation, and closed-circuit expansion have improved cell purity, yield, and consistency. Allogeneic dosing is now consistently higher than autologous dosing, reflecting robust expansion from healthy donors and superior functional competence. Defining minimal effective dose (MED) and maximum tolerated dose (MTD) remains a priority, but shared clinical data and negative result reporting are accelerating this process (Mehta, 2019).

### 4.5. Current Challenges and Mitigation Strategies

Despite rapid progress, challenges remain:

- Tumor microenvironment suppression: mitigated by TGFBR2 editing, checkpoint fusion, and IL-15 armoring (Shaim et al., 2021; Zhu et al., 2020).
- Limited persistence: improved via membrane-bound cytokines and CISH knockout (Cichocki et al., 2020; Liu et al., 2018).
- Solid-tumor penetration: enhanced by chemokine receptor engineering and intratumoral delivery.
- Off-target genome editing: reduced by high-fidelity CRISPR and whole-genome sequencing.

### 4.6. Future Outlook (2025–2030)

In the next five years, we anticipate:

- Approval of the first off-the-shelf CAR-NK products in the United States, Europe, and China.
- Widespread use of iPSC-NK as first-line therapy for lymphoma, myeloma, and solid tumors.
- Combination strategies with checkpoint inhibitors, chemotherapy, and radiation.
- Expansion into autoimmune, inflammatory, and neurodegenerative diseases.
- Standardized global dosing and manufacturing guidelines.

## 5. Conclusion

This review demonstrates that NK cell therapy has entered a new era defined by standardized clinical development, mechanistic innovation, and the rise of CAR-NK and iPSC-NK. From early LAK cells to today's off-the-shelf engineered products, NK immunotherapy has become a safe, scalable, and highly effective modality across oncology, virology, and autoimmunity. Continued investment in gene editing, manufacturing, and clinical translation will solidify NK cells as a cornerstone of 21st-century medicine.

## Compliance with ethical standards

*Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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