



REVIEW

Advancements and Challenges of Gamma Delta ($\gamma\delta$) T Cell- and Invariant Natural Killer T (iNKT) Cell-Based Cancer Immunotherapies

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ABSTRACT: Gamma delta ($\gamma\delta$) T cells and invariant natural killer T (iNKT) cells are unconventional T cells with limited T cell receptor (TCR) diversity. Both can recognize lipid or non-peptide antigens, often through cluster of differentiation 1d (CD1d), rapidly produce cytokines, express natural killer (NK) cell markers, and are mainly found in mucosal and barrier tissues. Acting as a bridge between innate and adaptive immunity, they show great promise for cancer immunotherapy. Developing $\gamma\delta$ T and iNKT cells for treatment involves shared features like thymic origin, MHC-independent recognition, rapid cytotoxicity, low graft-vs.-host disease (GvHD) risk, *ex vivo* expansion, and genetic modification, making them suitable for adoptive cell therapies. While their mechanisms are similar, iNKT cells rely on CD1d-mediated antigen presentation, provided by CD1d-expressing antigen-presenting cells (APCs) or engineered cell lines, to activate their invariant TCR and expand effectively. Chimeric antigen receptors (CAR)-induced functional activations make these cell types viable alternatives to conventional cell-based or CAR-T therapies with additional safety benefits. Early clinical trials have shown encouraging results, and their completion will confirm their potential for future treatments. This review explores the biology and mechanisms of $\gamma\delta$ T and iNKT cells, focusing on how APCs, cytokines, feeder cells, and CARs contribute to boosting their cytotoxic function, cytokine production, and expansion, enhancing their promise as cancer immunotherapies. It also explores the advancements and challenges in developing $\gamma\delta$ T and iNKT cell-based immunotherapies, with preclinical and early clinical outcomes offering promising insights.

KEYWORDS: Gamma delta ($\gamma\delta$) T cells; invariant natural killer T (iNKT) cells; immunotherapy; cancer; feeder cells; K562; cluster of differentiation 1d (CD1d) molecule

1 Introduction: Biology of Gamma Delta ($\gamma\delta$) T Cells and Invariant Natural Killer T (iNKT) Cells

Gamma delta ($\gamma\delta$) T cells are a distinct type of T lymphocyte characterized by specialized γ and δ T-cell receptor (TCR) chains, serving as a bridge $\gamma\delta$ [1]. They comprise 1%–10% of human peripheral blood T cells but are more abundant in epithelial and mucosal tissues, such as the skin, gut, lungs, and reproductive tract [2,3]. Discovered in the 1980s, $\gamma\delta$ T cells were characterized by 1987 as CD3-associated molecules and a heterodimer consisting of a γ chain and a δ chain [4–6]. They develop in the thymus from common progenitors shared with $\alpha\beta$ T cells, emerging early from double-negative thymocytes under the influence of Notch signaling and interleukin-7 (IL-7) [7]. Their lineage commitment is guided by models such as stochastic, instructive, and signal strength-based [7]. Developing in waves, they originate from the fetal liver and thymus, showing distinct tissue dynamics, with V γ 9V δ 2 dominating in the fetal liver and V δ 1 expanding after birth [7]. Their differentiation and function depend on intricate signaling pathways [1,8].



$\gamma\delta$ T cells have semi-invariant TCRs with limited diversity compared to $\alpha\beta$ TCRs, yet they recognize a wide range of ligands, including non-peptide and stress-related antigens [9,10]. Unlike $\alpha\beta$ T cells, they can recognize antigens independently of major histocompatibility complex (MHC) involvement, enabling them to target tumors broadly, respond quickly, and perform diverse roles in cancer defense [7,11]. They quickly produce cytokines like IL-17 and IFN- γ , maintain tissue homeostasis, and exhibit memory-like traits in barrier tissues such as the intestines [12–14]. The main subsets, V δ 1, V δ 2 (primarily V γ 9V δ 2), and V δ 3, vary in location, antigen recognition, and function. V δ 1 T cells reside in tissues like the gut, liver, skin, and spleen, recognizing stress ligands like MICA/B and glycolipids via CD1d [7,15]. They demonstrate strong cytotoxicity, express natural killer (NK) receptors, such as natural killer group 2 member D (NKG2D), and resist activation-induced cell death, thereby sustaining anti-tumor activity [16]. V γ 9V δ 2 T cells, found in the blood, recognize phosphoantigens (pAgs) such as IPP from disrupted tumor mevalonate pathways and are activated by butyrophilin complexes (butyrophilin subfamily 3 member A1 [BTN3A1] and butyrophilin subfamily 2 member A1 [BTN2A1]) [17,18]. These cells exhibit cytotoxicity, secrete cytokines like IFN- γ and tumor necrosis factor- α (TNF- α), and mediate antibody-dependent cellular cytotoxicity (ADCC) through cluster of differentiation-16 (CD16) [19,20]. V δ 3 T cells, less studied, are located in the liver and gut, responding to glycolipids and oxidative stress presented by CD1d [21,22]. In conditions like leukemia and viral infections, V δ 3 cells can also appear in the bloodstream [21].

iNKT cells are a unique type of innate-like T lymphocytes with a semi-invariant T cell receptor (TCR) that recognizes lipid antigens presented by the CD1d molecule [23]. The cytokine environment and availability of ligands regulate iNKT activation, with α -galactosylceramide (α GalCer) serving as a key glycolipid antigen [23,24]. Found in tissues such as the liver, spleen, thymus, and peripheral blood (0.01%–0.1% in humans), iNKT cells bridge innate lipid recognition with adaptive immunity [25–27]. Research highlights their complex development, differentiation, and roles in diseases and therapies. Early microbial exposure plays a role in iNKT development and tissue residency [28–30]. Recent studies highlight skin-homing traits driven by CCR10 expression during thymic development [30,31]. iNKT cells develop in the thymus from CD4⁺CD8⁺ double-positive precursors selected on CD1d-expressing cortical thymocytes, with key transcription factors like promyelocytic leukemia zinc finger (PLZF) playing a key role in their effector functions [32–34]. Subsets are defined by transcription factors: T-bet for iNKT1 (IFN- γ producers), GATA binding protein-3 (GATA-3) for iNKT2 (IL-4 producers), and ROR γ t for iNKT17 (IL-17 producers) [35–37]. Developmental models include the linear maturation model, where iNKT cells progress through stages marked by CD24, CD44, and NK1.1 expression, and the lineage differentiation (branching) model, where iNKT subsets (NKT1, NKT2, NKT17) diverge early from common progenitors based on transcription factor expression rather than surface markers [36,38,39]. iNKT cells rapidly release cytokines like IFN- γ , IL-4, IL-13, and IL-17 upon activation [25]. IL-17 receptor B (IL-17RB)⁺ CD4⁺ iNKT cells produce Th2, Th9, and Th17 cytokines in response to IL-25, while IL-17RB⁺ CD4[–] iNKT cells (ROR γ t⁺) release Th17 cytokines when stimulated by IL-23 [40]. IL-17RB[–] iNKT cells produce IFN- γ , associated with classical effector roles [40,41]. IL-15 is crucial in maintaining certain iNKT subtypes [42]. These subsets originate in the thymus and migrate to peripheral tissues, where they perform functions including direct cytotoxicity. Cytotoxicity is mediated directly via perforin/granzyme and Fas/FasL pathways, as well as indirectly by activating dendritic cells (DCs), NK cells, and T cells [43,44]. Changes in iNKT cell number or function are linked to autoimmune diseases, infections, cancer, and allergic conditions [23,45,46].

Recent research highlights the varied roles of iNKT subsets, shaped by their tissue residency or circulation [25]. Tissue-resident iNKTs maintain local balance and secrete specific cytokines, while circulating iNKTs act as cytotoxic sentinels for antitumor and antiviral defense [25]. These subsets differ in transcriptional profiles, surface markers, cytotoxic molecules, and immune functions, influencing host

defense, regulation, and therapies [25]. Tissue-resident iNKTs, found in organs like the liver, thymus, spleen, lungs, intestines, and adipose tissue, are usually CD244–CXCR6+ with T cell-like traits. They secrete cytokines such as IFN- γ , IL-4, and IL-17, activated by tissue-derived cytokines like IL-7 and IL-15. They support local immunity, homeostasis, and tissue repair, with tissue imprinting adding retention molecules like P2RX7 and CXCR6 [25,47]. Their restricted TCR repertoires reflect local antigen exposure and clonal expansion shaped by the microenvironment [47]. Tissue-resident iNKTs are locally specialized, moderately cytotoxic, and exhibit T cell-like signaling, while circulating iNKTs are mobile, highly cytotoxic, and rapidly respond to infections and tumors [25]. Circulating iNKTs, typically CD244+CXCR6+ with NK cell-like traits, primarily secrete IFN- γ and are activated by IL-15 from medullary thymic epithelium [34]. They are crucial for systemic antitumor and antiviral defense, with functional differences defined by transcription factors, cytokines, and epigenetics [25].

Both $\gamma\delta$ T cells and iNKT cells are categorized as unconventional or innate-like T lymphocytes, bridging innate and adaptive immunity with their rapid responses, bypassing classical MHC-restricted peptide antigen presentation [48]. Their TCR diversity is limited compared to conventional $\alpha\beta$ T cells; iNKT cells express an invariant $\alpha\beta$ TCR, while $\gamma\delta$ T cells have heterodimeric γ and δ chains that often detect non-peptide antigens [49]. Both recognize non-peptidic antigens—iNKT cells respond to lipid antigens presented by the CD1d molecule (an MHC-I-like molecule), while some $\gamma\delta$ T cell subsets also recognize CD1d-presented lipid antigens or respond to phosphoantigens and stress-induced molecules independently of classical MHC presentation. Unlike conventional $\alpha\beta$ T cells, they do not rely on MHC class I or II peptides for activation. Upon activation, both can quickly produce a wide range of cytokines such as IFN- γ , TNF- α , IL-4, and IL-17, supporting diverse immune responses (Th1-, Th2-, Th17-like). They influence other immune cells like NK cells, DCs, and conventional T and B lymphocytes, modulating immune responses. These cells exhibit functional plasticity, playing both pro-inflammatory and immunoregulatory roles. Both subsets share NK cell-related surface markers like CD16 and NKG2D, reflecting their intermediate phenotype between T cells and NK cells. They develop primarily in the thymus but are enriched in epithelial and mucosal tissues (e.g., iNKT cells in the liver, $\gamma\delta$ T cells in the skin and gut mucosa). Acting as first-line defenders at barrier sites, they recognize stressed, infected, or transformed cells, contributing to tumor immunosurveillance.

This review discusses the role of antigen-presenting cells (APCs), cytokines, feeder cells, and chimeric antigen receptors (CARs) in advancing $\gamma\delta$ T cell- and iNKT cell-based immunotherapies. Explored the latest $\gamma\delta$ T cell- and iNKT cells-based immunotherapies, the challenges they faced, and highlighted the critical areas to be addressed to push immunotherapies forward.

2 Role of APC in Advancing $\gamma\delta$ T and iNKT Cell-Based Immunotherapies

APCs play a vital role in initiating adaptive immune responses by presenting processed antigens to $\gamma\delta$ T and iNKT cells along with necessary co-stimulatory signals [50]. APCs can be natural or artificial. Natural APCs (nAPCs) are derived from human cells, while artificial APCs (aAPCs) are engineered to replicate their function [50,51]. Both can stimulate $\gamma\delta$ T and iNKT cells, but they differ in concept, mechanism, and practicality [51]. Examples of nAPCs include dendritic cells (DCs), macrophages, and B lymphocytes, which specialize in capturing, processing, and presenting antigens through MHC class I, MHC class II, or non-classical MHC molecules like CD1 for lipid antigens. Co-stimulatory signals are delivered via naturally expressed receptors such as CD80/CD86, ICAM-1, LFA-3, CD40, and cytokines like IL-12, IL-15, IL-18, and type I interferons. Found in tissues like the skin, mucosa, and lymphoid organs, nAPCs create dynamic environments for immune interactions. aAPCs use engineered platforms that can be cell-based, such as human leukemia lines, or acellular, like microbeads, nanoparticles, liposomes, or supported lipid bilayers combined with MHC or non-classical ligands and co-stimulatory molecules [51,52]. They can present

antigens using phosphoantigens or tumor-associated antigens and achieve MHC-independent presentation with engineered ligands [51]. Controlled co-stimulation allows precise expression of CD80/CD86, ICAM-1, LFA-3, 4-1BBL, and co-stimulatory monoclonal antibodies.

Studies have demonstrated that aAPCs like K562 induce long-term proliferation, enhanced activations, and anti-tumor effects compared to nAPCs, suggesting their use to advance clinical applications and to engineer $\gamma\delta$ T cell- and iNKT cells [53,54]. Cytokines like IL-2, IL-15, or IL-21 can also be tethered or released to enhance immune cell proliferation *ex vivo* [51,52]. The interaction between APCs and innate T cell groups like $\gamma\delta$ T cells and iNKT cells is key to advancing immunotherapies. By understanding these relationships, we can develop better cancer treatments that utilize the unique traits of these T cells, improving their effectiveness and patient outcomes [55]. With ongoing research, the potential for $\gamma\delta$ T and iNKT cells in clinical use looks promising, encouraging deeper exploration of their roles and how they can be modulated within the immune system [55,56]. Key advantages and limitations of nAPCs and aAPCs are listed in Table 1.

Table 1: Key benefits and challenges of natural antigen-presenting cells (nAPCs) and artificial antigen-presenting cells (aAPCs) in advancing immunotherapies for gamma delta ($\gamma\delta$) T and invariant natural killer T (iNKT) cells

Advantages or limitations	nAPCs	aAPCs
Advantages	- Physiological signal integration depends on multiple co-stimulatory and inhibitory pathways, enabling context-specific fine-tuning of immune cell activation [57,58]. - Ability to stimulate memory-like cell responses [59].	- Standardized production allows for customization, making it ideal for patient-independent therapies [51]. - Enhanced traits improved the activation and expansion of immune cells [60,61]. - High reproducibility and scalability make adoptive cell-based therapies highly promising [62–64]. - Can selectively activate immune cells without the need for patient-derived nAPCs [60].
Limitations	- Sourcing relies on the patient [65,66]. - Variations in APC maturity and phenotype affect reproducibility [65]. - <i>Ex vivo</i> expansion therapies face challenges with limited scalability [62,66].	- Lack tissue-specific cues and physiological regulatory signals [67]. - Lack of fully replicated complex antigen processing or interactions can potentially lead to less refined effector differentiation or exhaustion during extended culture periods [67].

Note: nAPC: natural antigen-presenting cell.

3 Role of Cytokines in Advancing $\gamma\delta$ T and iNKT Cell-Based Immunotherapies

Tumor recognition without human leukocyte antigen (HLA) dependency makes $\gamma\delta$ T and iNKT cells excellent candidates for allogeneic adoptive cell transfer (ACT) [68]. However, their low frequencies in peripheral blood require *ex vivo* expansion and functional improvements. Cytokines are crucial for

enhancing their proliferation, activation, and cytotoxicity, especially in cancer immunotherapy. Advances in cytokine biology and protein engineering have significantly improved these cells' therapeutic potential. Cytokines like IL-2, IL-15, IL-7, and IL-21 promote their proliferation, survival, and cytotoxic abilities [69]. IL-2 fosters proliferation, boosts cytotoxicity, and encourages an effector memory phenotype [70,71]. IL-15 combined with IL-2 supports long-term memory, while IL-21 improves cytotoxic functions and facilitates expansion with or without feeders [72,73]. IFN- γ aids in anti-tumor activity. In the tumor microenvironment, cytokines help $\gamma\delta$ T and iNKT cells avoid exhaustion from continuous antigen exposure [74,75]. However, natural cytokines face challenges like short half-life, pleiotropy, and systemic toxicity, complicating safe clinical use. To overcome these obstacles, various engineering strategies have been developed [76–78]. Engineered cytokines help restore cytotoxicity of these cells in immunosuppressive conditions [79,80].

Mutant or designer cytokines, called “superkines” (e.g., IL-2 superkine, Neo-2/15), specifically target IL-2R $\beta\gamma$ on cytotoxic lymphocytes while avoiding IL-2R α on Tregs, improving anti-tumor effects and minimizing off-target risks [79,81,82]. These superkines activate effector $\gamma\delta$ T and iNKT cells without triggering Tregs, enhancing anti-tumor activity with reduced toxicity. Orthogonal cytokine-receptor pairs enable engineered $\gamma\delta$ T and iNKT cells to respond only to modified cytokines, lowering systemic immunotoxicity [81]. Cytokine-polymer conjugates, like PEGylated IL-2 or IL-10 (e.g., Bempegaldesleukin), extend cytokine half-life, enhance tumor targeting, and decrease systemic exposure [83]. Fusion proteins and immunocytokines, created by linking cytokines with Fc domains, albumin, or antibodies, improve serum half-life and allow tumor-specific delivery [77]. Immunocytokines and prodrug cytokines, activated in the tumor microenvironment, provide localized delivery, reduce systemic toxicity, and stimulate $\gamma\delta$ T and iNKT cell activation and expansion at tumor sites. *Ex vivo* protocols use cytokines like IL-2, IL-18, and IL-15 with phosphoantigens or feeder cells to optimize efficiency [77]. Prodrug or masked cytokines selectively activate in the tumor microenvironment, reducing systemic toxicity. Cellular delivery vehicles, such as engineered $\gamma\delta$ T and iNKT cells, can locally secrete cytokines, boosting autocrine and paracrine signaling to enhance anti-tumor effects without systemic side effects [84]. Incorporating engineered cytokines into $\gamma\delta$ T and iNKT cell therapy aims to increase therapeutic levels, broaden cytotoxicity against various tumors, and lower toxicities linked to high-dose cytokine use. These findings highlight the potential of cytokines in engineered $\gamma\delta$ T and iNKT cell platforms as a clinical strategy to improve anti-tumor immunity [77].

iNKT cell activation depends greatly on cytokine signaling, particularly through the JAK/STAT pathway, which regulates their growth, cytotoxicity, and survival [85,86]. Engineered cytokine receptors in iNKT cells activate JAK/STAT signaling independently of external cytokines, ensuring activation only in response to antigens and preventing off-target effects or unwanted differentiation [87]. Controlled cytokine signaling helps maintain memory-like phenotypes and avoids early exhaustion. Enhanced STAT5/STAT1 activation boosts granzyme and perforin levels, increasing iNKT cell cytotoxicity [87,88]. Strategies like generating iNKT cells from induced pluripotent stem cells (iPSCs) with CAR constructs (e.g., HER2 CAR) can be enhanced by cytokines to improve activation [89]. CAR-iNKT cells release cytokines such as IL-2, IL-15, and IFN- γ upon recognizing antigens, promoting dendritic cell maturation, M1 macrophage polarization, and endogenous CD8⁺ T cell activation [90,91]. Cytokine release reshapes the tumor or tissue environment to enhance anti-tumor responses while targeted cytokine delivery (e.g., fusion proteins, receptor engineering) minimizes systemic toxicity, avoiding pleiotropic effects and vascular leak syndrome seen with traditional IL-2 therapies [92,93].

By utilizing cytokines and incorporating techniques like protein engineering, antibody-cytokine fusions, PEGylation, and cellular programming, cytokines can effectively boost $\gamma\delta$ T and iNKT cell activity while minimizing systemic toxicity [79]. These methods introduce a novel perspective, transforming cytokines from mere soluble mediators into precision tools designed to enhance the effectiveness and

durability of $\gamma\delta$ T and iNKT cells. CAR- $\gamma\delta$ T and CAR-iNKT combined with cytokines make them highly scalable, setting the stage for a feeder-free approach to generate next-generation $\gamma\delta$ T and iNKT cell immunotherapies for clinical applicability.

4 Role of Feeder Cells in Advancing $\gamma\delta$ T Cell and iNKT Cells-Based Immunotherapies

Feeder cells play a crucial role in providing signals and presenting antigens for the development, activation, proliferation, and expansion of $\gamma\delta$ T and iNKT cells, especially when starting with limited populations, such as those from peripheral blood [94,95] (Fig. 1). Well-characterized feeder cells for $\gamma\delta$ T and iNKT cell expansion are K562 cells (human chronic myelogenous leukemia cell line), EBV-transformed lymphoid cell lines (EBV-LCLs), irradiated or mitocycin-C-treated PBMCs, and untreated or Zoledronate-treated osteoclasts [96–98]. Most commonly used feeder cells, such as K562 cells, lack MHC class I and II expression, reducing allogeneic immune responses and minimizing GvHD risk [99]. Genetically engineered to express co-stimulatory molecules and membrane-bound cytokines, K562 cells function as aAPCs for $\gamma\delta$ T cells. Typical transgenes include CD19, CD64 (Fc γ RI), CD86, CD137L (4-1BBL), and membrane-bound IL-15 or IL-15/IL-15R α complexes, providing activation signals and cytokine support for sustained $\gamma\delta$ T cell proliferation [96,97]. The expansion process of $\gamma\delta$ T cells using K562 feeder cells often starts with activating peripheral blood mononuclear cells (PBMCs) using agents like zoledronate (a bisphosphonate) and IL-2 to enrich the $\gamma\delta$ T subpopulation, particularly V γ 9V δ 2 cells. The activated PBMCs are then co-cultured with irradiated, modified K562 feeder cells, driving rapid expansion and achieving several thousand-fold increases in $\gamma\delta$ T cells within 10–17 days. Large-scale growth is supported in cell culture vessels like G-Rex flasks, designed for enhanced gas exchange and compliance with Good Manufacturing Practice (GMP) standards [96]. K562 feeder systems offer significant advantages, including higher expansion rates (~1000–500,000 fold depending on subset and protocol) compared to cytokine-only or bisphosphonate methods [96]. They help maintain the functional properties of $\gamma\delta$ T cells, including their cytotoxic potential and memory phenotypes, and enable the expansion of polyclonal $\gamma\delta$ T populations (V δ 1, V δ 2, and V δ 3 subsets), broadening therapeutic applications [96]. Studies have shown that osteoclasts treated with zoledronate [100,101] or combined with probiotic bacteria [102] as potent feeder cells to induce cell expansion and functional activation of $\gamma\delta$ T cells. Osteoclasts may provide CD1d-mediated antigen presentation, supporting iNKT cell expansion and activation; however, this remains to be investigated.

In addition to above above-listed feeder cells, key feeder cells specifically for iNKT cells include CD1d-expressing APCs, such as double-positive (CD4⁺CD8⁺) thymocytes during thymic development, and dendritic cells (DCs), macrophages, and B cells in peripheral tissues [23]. *In vitro*, stromal feeder cells like OP9-DLL1 are used to promote iNKT differentiation from stem cells. CD1d, a non-polymorphic MHC-like molecule, presents glycolipid antigens to the invariant TCR of iNKT cells [103,104]. Other CD1d-expressing lymphoid and myeloid cells or Epstein-Barr virus-transformed lymphoblastoid cell lines (EBV-LCLs) also aid iNKT and $\gamma\delta$ T cell activation and expansion by presenting antigens [105,106].

Addressing challenges and considerations, removing residual feeder cells after expansion is essential, typically done through irradiation or cell sorting, as feeders are irradiated before culture [107]. Maintaining balanced co-culture ratios, cytokine supplementation (like IL-2, IL-15, IL-21), and appropriate duration is key for achieving optimal expansion without causing $\gamma\delta$ T cell exhaustion [94]. The purity of $\gamma\delta$ T cell populations is often enhanced using prior isolation methods such as magnetic bead separation and flow cytometric sorting [94]. K562-derived feeder cells enable off-the-shelf expansion of large quantities of $\gamma\delta$ T cells suitable for adoptive cell therapy (ACT), avoiding the need for extensive leukapheresis [96]. They support the expansion of $\gamma\delta$ T and iNKT cells while preserving their cytotoxicity and memory phenotypes

against tumor cells in an MHC-independent way [96]. These feeder-based protocols have been adapted to GMP-compliant workflows critical for clinical manufacturing [96].

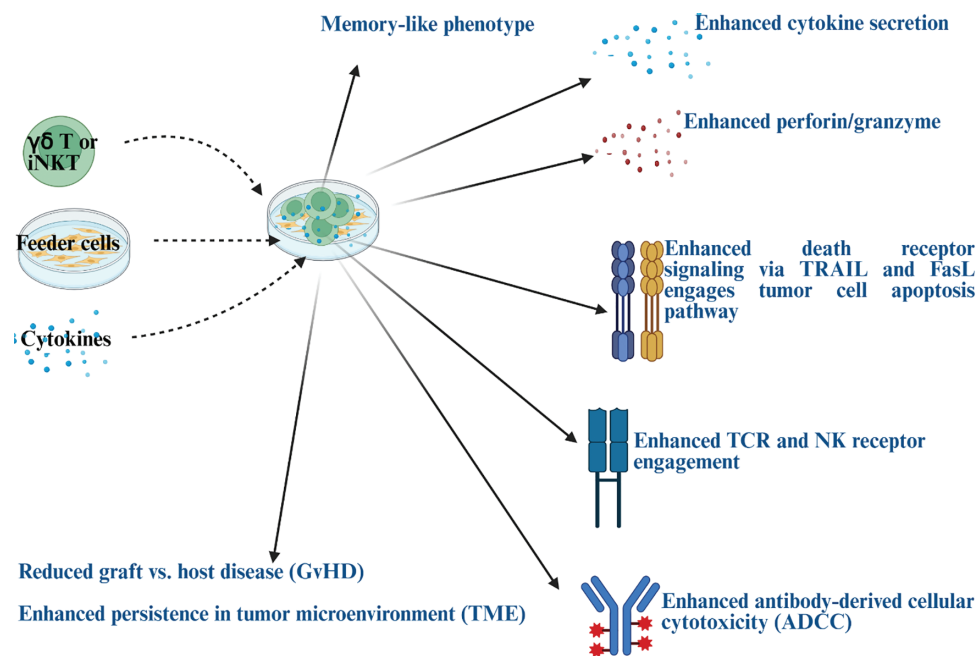


Figure 1: Illustration to demonstrate anti-cancer activity in gamma delta ($\gamma\delta$) T and invariant natural killer T (iNKT) cells after they are co-cultured with feeder cells in the presence of cytokines. Blue color text represents the characteristics of engineered $\gamma\delta$ T cells and iNKT generated using feeder cells and cytokines. Note: TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; FasL: fas ligand; TCR: T-cell receptor; NK: natural killer. Created in BioRender. Kaur, K. (2025) <https://BioRender.com/4mu9q7s> (accessed on 29 October 2025)

5 Role of CAR in Advancing $\gamma\delta$ T and iNKT Cell-Based Immunotherapies

CARs have revolutionized cellular immunotherapy, allowing T cells to recognize and destroy specific tumor antigens without needing MHC presentation. While traditional CAR-T therapies primarily target conventional $\alpha\beta$ T cells, extending CAR technology to $\gamma\delta$ T cells and iNKT cells addresses some of the challenges in current adoptive cell therapies and provides unique therapeutic advantages. Therefore, CAR- $\gamma\delta$ T and CAR-iNKT cells offer an exciting alternative to traditional CAR-T therapy, addressing many of its limitations with unique advantages [11,108,109]. These cells combine CAR-specific targeting with the innate cytotoxic abilities of $\gamma\delta$ T or iNKT cells, allowing them to recognize tumor-associated antigens and stress-induced ligands, making them highly effective for allogeneic treatments [11].

Preclinical studies show strong anti-tumor activity, durability, and safety of CAR- $\gamma\delta$ T and CAR-iNKT cells. CAR- $\gamma\delta$ T cytotoxicity works through granule exocytosis (perforin/granzyme) and death receptor pathways (FasL/TRAIL), while cytokine secretion (IFN- γ , TNF- α) supports their function without requiring extensive external cytokine support [11]. CAR- $\gamma\delta$ T and CAR-iNKT cells reprogram the tumor microenvironment by targeting immunosuppressive cells like tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs), while activating dendritic cells, endogenous CD8⁺ T cells, and promoting M1 macrophage polarization to enhance anti-tumor immunity. With naturally expressed chemokine receptors (e.g., CXCR6, CCR1, CCR6), CAR- $\gamma\delta$ T and CAR-iNKT cells can migrate and stay in peripheral tissues, improving infiltration into solid tumors where CAR-T cells often struggle due to physical barriers like dense stroma and fibrotic extracellular matrix [108]. Their multiple cytotoxic pathways

reduce the risk of tumor antigen escape, a common problem in CAR-T therapy, especially in heterogeneous or relapsed leukemias. Being non-alloreactive, they are safe for allogeneic use across HLA barriers without causing GvHD. Early studies show lower risks of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity (ICANS), making them safer than CAR-T cells. Additionally, iPSC-derived CAR- $\gamma\delta$ T, CAR- $\gamma\delta$ T, and CAR-iNKT cells have shown effectiveness without continuous cytokine exposure, offering a scalable, consistent platform that reduces batch variability and supports off-the-shelf applications [108,110].

CAR-iNKT cells with dual-targeting abilities, such as CD19/CD133, have shown impressive results in eradicating high-risk acute lymphoblastic leukemia (ALL) in mouse models, achieving durable responses in bone marrow and meninges, unlike conventional CAR-T cells, which only provided temporary clearance [91]. Early human trials for B-cell lymphomas revealed that CAR-iNKT cells had better tissue infiltration and persistence without causing GvHD. In solid tumors, preclinical studies with GD2 (neuroblastoma), CLDN18.2 (pancreatic cancer), and HER2 CAR-iNKT cells demonstrated enhanced anti-tumor activity, tissue penetration, persistence, and TME modulation compared to conventional CAR-Ts, suggesting their potential superiority in this challenging setting [108]. Anti-CD38-CAR- $\gamma\delta$ T cells have shown impressive cytotoxicity against multiple myeloma in both *in vitro* and *in vivo* studies, demonstrating promise for treating blood cancers [111]. CAR- $\gamma\delta$ T cells also show potential against solid tumors, with dual-targeting CAR strategies addressing tumor heterogeneity with early-stage trials of CAR- $\gamma\delta$ T have also shown promising results [108,109]. However, clinical data on CAR- $\gamma\delta$ T and CAR-iNKT cells remain limited, and direct comparisons to CAR-T cells in human trials have yet to occur. Obstacles like manufacturing challenges, regulatory hurdles, and reliable large-scale expansion persist, along with the need for more research into solid tumor infiltration and persistence across diverse human cancers [108].

CAR- $\gamma\delta$ T and CAR-iNKT cells bring several benefits compared to traditional CAR-T cells, including dual innate and adaptive cytotoxicity, the ability to modulate the tumor microenvironment, lower immunotoxicity, and convenient, ready-to-use availability (Table 2). These features position them as strong candidates to surpass CAR-T therapy, particularly in high-risk blood cancers and solid tumors. Although early results and clinical data are encouraging, comprehensive clinical trials, especially phase II/III studies, are essential to establish clear comparisons, validate their effectiveness, and assess their persistence and long-term safety across a wider range of solid tumor types.

Table 2: Key CAR constructs, disease indicators, and therapeutic benefits in advancing immunotherapies for $\gamma\delta$ T and iNKT cells

Key points	$\gamma\delta$ T cells	iNKT cells
CAR construct	CD19 [112,113]	GD2 [108,114–116]
	CD33 [112]	BCMA [117,118]
	CD123 [112]	CD19 [91,119,120]
		CD133 [91]
		CD117 [121]
		CSPG4 [122]
	Acute myeloid leukemia (AML) [112,113]	Acute myeloid leukemia (AML) [121]
	Acute lymphocytic leukemia (ALL) [112,113]	Acute lymphocytic leukemia (ALL) [91,120]

(Continued)

Table 2 (continued)

Key points	$\gamma\delta$ T cells	iNKT cells
Disease indicators	Chronic myeloid leukemia (CML) [113] Solid cancer-Ongoing trial	Chronic myeloid leukemia (CML) [123] Chronic lymphocytic leukemia (CLL) [120] Non-Hodgkin lymphoma (NHL) [120] Multiple myeloma (MM) [117,118] Neuroblastoma [114–116] Pancreatic cancer [124] Sarcomas [125] Melanoma [122]
Therapeutic benefits	- Improved safety profile through selective targeting, reducing harm to healthy tissues [112] - Better therapeutic index compared to standard CAR-T cells, especially for antigens found on essential healthy cells [112] - Broader potential applications in cancers where conventional CAR-T therapy causes severe off-tumor toxicity [112] - IL-15 co-expression enhances the durability and effectiveness of $\gamma\delta$ CAR-T cells [112] - Leverages the innate tumor-recognition ability of $\gamma\delta$ T cells, which identify various stress-induced antigens without relying on MHC [113]	- Improved safety profile through selective targeting, reducing harm to healthy tissues [114,120,121,125]. - Enhanced tumor detection through the integration of CAR, native semi-invariant TCR, and NK receptor pathways [114,121,125] - Effective TME modulation to overcome immunosuppressive barriers [125] - Reduced risk of severe cytokine-release syndrome and neurotoxicity [116,117,124] - Feasible allogeneic “off-the-shelf” application due to lower GvHD risk [120,121,124,125] - Comprehensive immune coordination, linking innate and adaptive immunity [125,126] - Quick functional response and cytokine release without <i>ex vivo</i> priming, boosting early anti-tumor activity [125,126]

Note: CAR: chimeric antigen receptor; CD: cluster of differentiation; GD2: disialoganglioside; BCMA: B-cell maturation antigen; CSPG4: chondroitin sulfate proteoglycan 4; TCR: T-cell receptor; NK: natural killer; TME: tumor microenvironment; GvHD: graft vs. host disease; MHC: major histocompatibility complex; IL: interleukin.

6 Advancements in $\gamma\delta$ T Cell- and iNKT Cells-Based Immunotherapies

$\gamma\delta$ T cells show great potential in cancer immunotherapy, especially for tumors with low MHC expression or high heterogeneity that pose challenges to traditional treatments [127,128]. Their anti-tumor effects include directly killing tumor cells, activating the immune system through cytokines, and supporting

adaptive responses [129,130]. These cells eliminate tumors via granule exocytosis, releasing perforin and granzyme B, activating death receptor pathways like tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and fas ligand (FasL), and performing ADCC by binding CD16 to antibody-coated tumor cells [129,131–133]. They also release pro-inflammatory cytokines such as IFN- γ and TNF- α , shaping the tumor microenvironment and stimulating other immune cells [132,134]. Both non-engineered and engineered $\gamma\delta$ T cell treatments are under clinical investigation, highlighting their adaptability and promise in cell-based and engager therapies [135]. Non-engineered therapies use *ex vivo* expanded autologous or allogeneic $\gamma\delta$ T cells (primarily V γ 9V δ 2), stimulated by aminobisphosphonates (like zoledronate or pamidronate) and cytokines (like IL-2) to enhance proliferation and activation before infusion [129,135,136].

Clinical trials for hematologic and solid tumors have demonstrated safety, scalable production, and some objective responses, though complete response rates remain low [135,137]. Phase 1 trials using *ex vivo* expanded donor-derived allogeneic $\gamma\delta$ T cells after hematopoietic cell transplantation in high-risk acute myeloid leukemia patients showed promising safety profiles, with no reports of cytokine release syndrome (CRS), immune neurotoxicity (ICANS), or GVHD [138,139]. Early efficacy signs include sustained complete remission (CR) and minimal residual disease (MRD) negativity [140]. *In vivo* activation of $\gamma\delta$ T cells can be achieved using aminobisphosphonates and synthetic phosphoantigens, along with cytokines like IL-2, to stimulate endogenous V γ 9V δ 2 T cells [141,142]. While limited by cell exhaustion and moderate efficacy, this approach remains a valuable adjunct. Bispecific $\gamma\delta$ T cell engagers are synthetic molecules that bind $\gamma\delta$ TCRs and tumor antigens, specifically redirecting and activating $\gamma\delta$ T cells at tumor sites to boost cytotoxicity [143,144]. Examples include bispecifics targeting Her2, CD40, or CD123 to leverage $\gamma\delta$ T cell cytotoxicity [145]. The development of banked $\gamma\delta$ T cell products for universal use offers an exciting strategy to lower costs and improve accessibility [146].

Advances in genetic engineering, expansion protocols, and clinical trials are driving the development of next-generation treatments, especially for solid tumors and malignancies that resist conventional T cell therapies [147]. Genetic modifications aim to address challenges like limited expansion, persistence, and tumor targeting [147]. CAR- $\gamma\delta$ T cells, engineered with chimeric antigen receptors targeting tumor antigens, boost specificity and potency [148,149]. These cells merge the innate tumor recognition of $\gamma\delta$ TCRs with CAR targeting, offering improved infiltration and reduced GvHD risks [68]. CAR- $\gamma\delta$ T cells may outperform $\alpha\beta$ CAR-T therapies by reducing toxicity, enhancing tissue penetration, and targeting tumors through multiple receptors [109,150,151]. Preclinical and early clinical studies have demonstrated promising safety and cytotoxicity, including engineered V δ 1 CAR-T cells and $\gamma\delta$ T cells derived from induced pluripotent stem cells (iPSCs) [152,153]. TCR gene transfer introduces high-affinity $\gamma\delta$ TCRs into T cells to strengthen tumor recognition and anti-tumor responses [154]. CRISPR/Cas9 techniques involve deleting inhibitory receptors to prevent exhaustion or inserting cytokine genes (e.g., IL-15) to enhance proliferation and persistence [155,156].

iNKT cells' unique innate-adaptive function and rapid cytokine response make them valuable in cancer immunotherapy [157]. They can directly kill CD1d-expressing tumor cells through cytotoxic granules (perforin, granzymes) and Fas/FasL-mediated apoptosis [158,159]. While some tumors evade detection by downregulating CD1d, iNKT cells can still target tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs), reshaping the TME [160]. When activated (e.g., by α -galactosylceramide (α -GalCer) loaded dendritic cells), iNKT cells release IFN- γ and other cytokines that help mature dendritic cells via CD40–CD40L interaction, boost priming of tumor-specific CD8⁺ and CD4⁺ T cells, and activate NK cells to eliminate MHC-negative tumor cells [161,162]. These mechanisms amplify anti-tumor immunity and create lasting immunological memory. Adoptive cell transfer can be achieved by infusing *ex vivo* expanded autologous or allogeneic iNKT cells [163]. Addressing low iNKT counts in patients

through iPSC-derived iNKT cell generation offers scalable cellular solutions [164]. Early-phase trials using α -GalCer-pulsed DCs and expanded iNKT cells have shown safety and some clinical benefits in cancers like non-small cell lung cancer (NSCLC) and head and neck cancer [165–167]. Allogeneic iNKT therapies, such as agenT-797, exhibit durability, tumor infiltration, immune activation, and manageable effects in solid tumors [168]. Challenges include low iNKT cell frequency in humans, tumor metabolic factors (e.g., glycolytic environment, lactic acid) impairing iNKT function, and the need for better manufacturing, expansion, and persistence of therapeutic iNKT cells, as well as overcoming tumor immune evasion through CD1d downregulation [44,158,169]. CAR-engineered iNKT cells maintain their natural immunomodulatory functions while achieving antigen-specific tumor targeting [108]. They offer safety benefits over CAR-T cells, such as a lower risk of graft-vs.-host disease. Clinical trials targeting GD2 (neuroblastoma), CD19 (B-cell malignancies), and other tumor antigens are showing promising early outcomes [108]. Combining iNKT therapies with checkpoint inhibitors, chemotherapy, or vaccines could boost responses, overcome immunosuppressive challenges, and improve therapy persistence [170].

Both $\gamma\delta$ T and iNKT cell types exhibit a low risk of GvHD, making them ideal for allogeneic (off-the-shelf) immunotherapies targeting cancer and infectious diseases [171]. They can be expanded and activated *ex vivo*, with $\gamma\delta$ T cells responding to phosphoantigens or aminobisphosphonates (e.g., zoledronate) plus IL-2, and iNKT cells responding to α -galactosylceramide (α -GalCer) lipid antigens presented by CD1d. Both can be genetically modified with CARs to enhance tumor antigen specificity while preserving natural recognition pathways. Advances in deriving $\gamma\delta$ T and iNKT cells from iPSCs now allow scalable and consistent production of cytotoxic lymphocytes with defined phenotypes for clinical applications. With their MHC-unrestricted antigen recognition, strong cytotoxic activity, and immunoregulatory roles combined with low alloreactivity, $\gamma\delta$ T and iNKT cells represent complementary, promising platforms for next-generation adoptive cell therapies.

7 Challenges in Advancing Clinical Trials and Persistence of $\gamma\delta$ and iNKT Therapies

While significant progress has been made, developing $\gamma\delta$ T cell- and iNKT cell-based therapies still encounters challenges, such as limited *in vivo* expansion, persistence post-transfer, and the immunosuppressive tumor microenvironment (TME) that reduces their efficacy [127,128,172,173]. The complexity and adaptability of these cell subsets make achieving consistent outcomes difficult, and understanding of their ligands and activation hierarchies, especially for their subsets, remains limited [7]. Current efforts aim to enhance $\gamma\delta$ T and iNKT cell persistence and functional activation within TME [172]. Interim clinical data show good tolerance, manageable side effects, and durable immune recovery, supporting further dose-escalation and efficacy studies [138,139]. Engineering strategies can boost therapeutic precision and robustness, while combining these therapies with checkpoint inhibitors, monoclonal antibodies (to leverage ADCC), chemotherapy, radiotherapy, or other immunomodulators may improve their effectiveness [174,175]. Emerging delivery platforms, such as nanoparticle-encapsulated agonists, offer promise for optimizing pharmacodynamics and reducing systemic toxicity [175–177]. Clinical trials are advancing rapidly in both hematologic cancers and solid tumors to refine dosing, persistence, and overall efficacy [138,139].

Clinical trials using $\gamma\delta$ and iNKT therapies often fall short compared to preclinical models for several reasons. Common challenges include limited *in vivo* persistence and expansion, as these cells struggle to maintain sufficient numbers post-infusion, reducing their ability to target tumor cells effectively [172]. The TME adds to the problem with high levels of immunosuppressive cytokines like TGF- β and IL-10, along with inhibitory checkpoint signals that weaken $\gamma\delta$ /iNKT cytotoxic functions [172,178]. Suboptimal agonist stimulation is another issue, as clinical protocols using aminobisphosphonates (n-BPs) or phosphoantigens (pAgs) face poor pharmacokinetics, low bioavailability, and limited tumor penetration [172]. Additionally,

ligand expression in tumors leads to variability in antigen recognition by $\gamma\delta$ or iNKT TCRs, further limiting clinical outcomes. To address these hurdles, current strategies focus on combining these therapies with checkpoint inhibitors, providing cytokine support like IL-2 and IL-15, and using lipid nanocarriers to deliver optimized agonists.

Production or scalability of $\gamma\delta$ T and iNKT therapies for clinical use is complex and resource-intensive. Autologous products are personalized for each patient, making them time-consuming and difficult to scale, while allogeneic sources face the challenge of immune rejection. The largely manual expansion and activation processes lead to batch variability, complicating consistent potency at scale. Since $\gamma\delta$ T and iNKT cells are rare in the bloodstream, achieving clinical doses requires billions of functional cells per patient, necessitating large-scale expansion while maintaining stable phenotype and function [172]. Moreover, strict GMP standards for viability, purity, and contamination must be adhered to for each batch. Potential solutions include automated bioreactors, closed-system culture platforms, modular cleanrooms, and artificial intelligence (AI)-driven process controls to reduce variability and enhance production efficiency.

Repeated activation during *ex vivo* expansion or within the tumor microenvironment causes $\gamma\delta$ T and iNKT cells to become functionally exhausted, characterized by higher levels of exhaustion markers like PD-1, LAG-3, and TIM-3, along with reduced cytokine production [172]. This exhaustion leads to weaker effector function, lower proliferation, and limited *in vivo* persistence, ultimately reducing therapeutic effectiveness [172]. Strategies to address this include enhancing culture media and feeder cells to prevent terminal differentiation, using transient cytokine support (IL-15, IL-21) to improve survival, and employing genetic modifications such as checkpoint gene knockouts or co-expression of memory-related transcription factors.

These issues are interconnected: poor scalability worsens cell exhaustion, leading to therapeutic failures. The field is progressing toward next-generation off-the-shelf allogeneic products that integrate automated, scalable production with engineered exhaustion resistance and better agonist designs for *in vivo* stimulation.

8 Future Perspective of $\gamma\delta$ T Cell and iNKT Cells-Based Immunotherapies

Most current preclinical and early clinical research emphasizes hematological malignancies, while solid tumors remain a significant challenge for immunotherapy. Future efforts aim to enhance $\gamma\delta$ T cells and iNKT cells' infiltration and persistence in solid tumors, addressing tumor microenvironment barriers. Combining $\gamma\delta$ T or iNKT cell therapies with treatments like checkpoint inhibitors, chemotherapy, radiotherapy, and monoclonal antibodies shows promise but requires further study. Adding probiotics to these therapies might also improve outcomes for resistant patients [179]. Chemotherapy or adjuvant therapeutic preconditioning may enhance $\gamma\delta$ T or iNKT cell homing and persistence, while experimental approaches like nanoparticle and ligand-targeted delivery and catheter-based methods deserve further investigation. Identifying biomarkers to predict which patients will benefit most from these therapies is vital, as is exploring the tumor's immunological landscape to discover such markers. Overcoming efficacy and safety challenges in clinical trials is crucial, and ongoing research focuses on manipulating the tumor microenvironment to better support $\gamma\delta$ T and iNKT cell activity for more effective treatment options. Therefore, the future of $\gamma\delta$ T cell and iNKT cell-based immunotherapies holds great promise, offering innovative approaches to treating various diseases, including cancer.

9 Conclusion

$\gamma\delta$ T and iNKT cells play vital roles at the crossroads of innate and adaptive immunity; key characteristics, therapeutic use, and limitations are listed in Table 3. They respond quickly to stressed, infected, or transformed cells through unconventional antigen recognition that bypasses classical MHC pathways.

Advances in molecular markers and single-cell technologies have enhanced our knowledge of their development, diversity, and functions. These discoveries open doors for targeted cancer therapies by focusing on specific $\gamma\delta$ T and iNKT subsets or developmental pathways. APCs/feeder cells, cytokines, or CARs can provide continuous activation, proliferation, and anti-cancer support for $\gamma\delta$ T and iNKT cells, enabling efficient and scalable expansion for immunotherapy (Fig. 2). Non-engineered methods include *in vivo* or *ex vivo* expansion with specific activation signals, while engineered therapies involve CARs, TCR gene transfer, bispecific engagers, and genetic editing to address challenges like persistence and immunosuppression. These strategies aim to develop effective, ready-to-use immunotherapies with wide applications against blood and solid cancers. Current clinical trials utilize their unique MHC-independent ability to recognize tumor-associated and stress ligands, enabling tumor killing, cytokine release, and immune modulation. Although preclinical and early clinical results are encouraging, issues such as TME resistance, cell expansion, and plasticity remain key research areas, and completion of ongoing clinical trials will provide solutions and answers to these.

Table 3: Key characteristics, therapeutic benefits, and challenges of $\gamma\delta$ T and iNKT cells

Classification	$\gamma\delta$ T cells	iNKT cells
Subsets	V δ 1 (tissue-resident) V δ 2 (circulating, V γ 9V δ 2) V δ 3 (found in the liver and intestine)	Invariant TCR (V α 24-J α 18)
Location	Peripheral blood	Tissues (Liver, spleen, etc.)
Antigen recognition	MHC-independent Stress-induced antigens Phosphoantigens Lipid antigens through BTN3A1/2A1 or CD1d for V δ 1/3	-lycolipid antigens through CD1d
Cytotoxic mechanism	Perforin/granzymes FasL/TRAIL ADCC (CD16)	Perforin/granzyme FasL/TRAIL
Cytokines release	IFN- γ , TNF- α , and GM-CSF	IFN- γ , IL-4, and IL-17
Engineered therapies	CAR- $\gamma\delta$ T cells TCR- $\gamma\delta$ Bispecific $\gamma\delta$ T cell engagers	CAR-iNKT cell Bispecific iNKT cell engagers
Feeder cells	K562 Engineered HEK293T Irradiated PBMCs EBV-transformed lymphoblastoid cell lines Dendritic cells Untreated or Zolendronte-treated osteoclasts	Dendritic cells K562 Mitomycin-treated fibroblasts EBV-transformed lymphoblastoid cell lines
Co-Stimulation	4-1BBL, CD80, CD83, CD40L	CD1d, CD40, CD80, CD86. 4-1BBL

(Continued)

Table 3 (continued)

Classification	$\gamma\delta$ T cells	iNKT cells
Cytokines-induced activation	IL-2, IL-15, IL-18, IL-21	-IL-2, IL-7, IL-12, IL-15
Safety profile	Well-tolerated Low risk of GvHD	Promising safety profiles Low risk GvHD Manageable cytokine-related toxicities.
Clinical approach	Phase I/II trials involve <i>ex vivo</i> expanded autologous and allogeneic transfers V γ 9V δ 2 T cells are expanded using bisphosphonates (zoledronate/pamidronate) with or without IL-2 V δ 1 T cells (DOT cells) trials are ongoing for AML and solid tumors CAR- $\gamma\delta$ T cell trials are being explored for targets like CD19, GD2, GPC-3, and CD20	Early-phase clinical trials are underway for solid tumors and hematologic malignancies, exploring CAR-iNKT and glycolipid-expanded iNKT therapies Combinations with checkpoint blockade or cytokine support are also being investigated.
Challenges/Limitations	Low frequency in peripheral blood Difficult post-infusion expansion and persistence TME results in exhaustion Immune checkpoints can inhibit via PD-1, TIGIT, and TIM3. Subset heterogeneity (V δ 1 vs. V δ 2) influences the cytotoxic spectrum and tissue infiltration. Certain subsets, like IL-17-producing $\gamma\delta$ Tregs, may have pro-tumoral effects. Need enhanced ligand identification and <i>ex vivo</i> stimulation	Low frequency in circulation Challenging to expand large clinical-grade populations TME suppresses potential functional activity Need optimization for targeting specificity and persistence in solid tumors. Cytokine balance (Th1 vs. Th2 skewing) must be regulated to prevent immune deviation. Manufacturing scalability remains a hurdle for off-the-shelf allogeneic CAR-iNKT cells.

Note: V δ : gamma delta; TCR: T-cell receptor; MHC: major histocompatibility complex; CD: cluster of differentiation; BTN3A1/2A1: butyrophilin subfamily 3 member A1/2A1; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; FasL: fas ligand; ADCC: antibody-derived cellular cytotoxicity; IFN- γ : interferon gamma; TNF- α : tumor necrosis factor alpha; IL: interleukin; GM-CSF: granulocyte-macrophage colony-stimulating factor; K562: chronic myelogenous leukemia cell line; PBMCs: peripheral blood mononuclear cells; EBV: Epstein Barr virus; GvHD: graft vs. host disease; AML: acute myeloid leukemia; DOT: delta one T; GPC3: glypican3; PD-1: programmed cell death protein 1; TIGIT: T cell immunoreceptor with immunoglobulin; TIM3: T cell immunoglobulin and mucin domain-containing protein 3; Treg: regulatory T; Th: T helper.

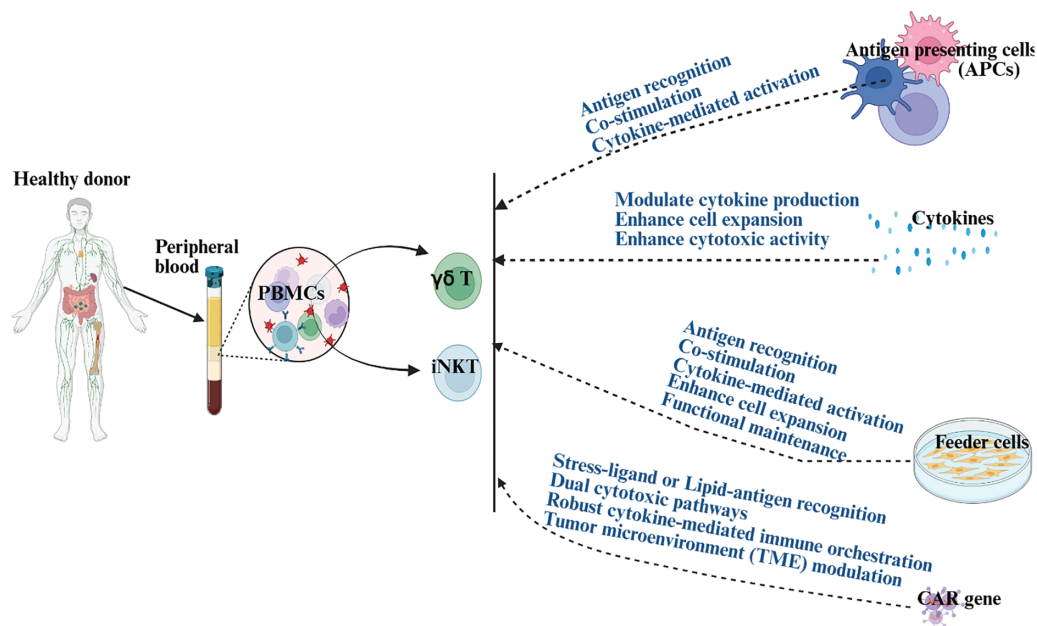


Figure 2: Illustration demonstrating the role of antigen-presenting cells (APCs), cytokines, feeder cells, and chimeric antigen receptors (CARs) in activating and expanding $\gamma\delta$ T and iNKT for immunotherapies. Blue text demonstrates the mechanisms through which APC, cytokines, feeder cells, or CAR induce activation or proliferation in $\gamma\delta$ T and iNKT cells. Created in BioRender. Kaur, K. (2025) <https://BioRender.com/hc7ixak> (accessed on 29 October 2025). Note: PBMCs: peripheral blood mononuclear cells; CAR: chimeric antigen receptor

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