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γδ T Cells in Cancer Immunotherapy: Biology, Mechanisms, and Translational Advances

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Abstract. Traditional cancer treatments and a type of MHC-restricted αβ T cell immunotherapy have notable limitations, underscoring the need for alternative approaches. γδ T cells offer distinct advantages because of their MHC-unrestricted tumor recognition, potent cytotoxicity, and immunoregulatory functions. Therefore, this review will summarize the biological features of γδT cells and their major anti-tumor mechanisms. The key therapeutic strategies, such as in vivo expansion, autologous and allogeneic adoptive transfer, and genetic engineering approaches, including CAR-γδT cells, bispecific T cell engagers, and TCR engineering, will also be discussed in this review. Additionally, advanced technologies, especially single-cell sequencing, have further revealed γδT cell heterogeneity and functional diversity, providing new insights for precision immunotherapy.

Keywords: γδT cell, cancer immunotherapy, potent cytotoxicity

1. Introduction

Cancer, which is defined by uncontrolled cellular growth, can develop in an extensive array of tissues and organs [1]. As the second leading cause of death, cancer accounted for more than 20 million new cases in 2022, presenting formidable pressures to both affected individuals and their respective health structures worldwide. Traditional modalities for cancer, including surgical excision, radiation therapy, and chemotherapy, are each observed to be circumscribed by essential drawbacks. Complete excisional intervention can theoretically cure cancer; however, it often fails to address metastasis and causes considerable organ damage [2]. Techniques grounded in radiation and chemotherapy do effectively kill tumor cells, radiotherapy frequently leaves persisting residual cancer cells, and chemotherapy disturbs physiological immune systems, both engender measurable deterioration in quality of life [2]. Immunotherapy has emerged as an alternative approach for cancer from these therapeutic insufficiencies, which demonstrates potential safety and high specificity across nearly all cancer types and stages [3].

Alertness in the immune surveillance can be alerted by malignant transformation, triggering anti-tumor responses [4]. Cancer immunotherapy tries to harness these immune mechanisms through various pathways to label cancer cells, enhance immune function, and enable effective detection and elimination of these cells [4,5]. The main challenge, however, the tumor can evade immune surveillance by multiple mechanisms within the Tumor Immune Microenvironment (TME), including recruitment of immunosuppressive cells, upregulation of immune checkpoints, and

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accumulation of toxic metabolites [6]. Stemming from cognition of such obstacles, the primary goal of cancer immunotherapy is not only to enhance the immune system's specificity and capacity to recognize and eliminate cancer cells but also to overcome the immunosuppressive TME.

Within the vertebrate adaptive immune architecture, lymphocyte populations can be classified into: B cells, αβ T cells, and γδ T cells [7]. Pertinent literature indicates that αβ T cells recognize antigens presented by major histocompatibility complex (MHC) molecules, limiting their recognition to specific MHC types [8]. Although significant potencies have been achieved through approaches like CAR-T targeting for αβ T cell-based immunotherapies, the restriction imposed by MHC-specificity demonstrably impedes broader application in targeting certain transformed or infected cells [9,10]. This limitation forms the increasing of attention from researchers and clinicians to the γδT cell-based immunotherapy, which can broadly recognize diverse antigens on tumor cell surfaces in an MHC-unrestricted manner [7].

Despite the γδT cells only constituting a minor ratio of the lymphocyte population [11], they exhibit pronounced structural and functional diversity through their distinctive TCR composed of γ and δ chains [12]. In cancer immunotherapy, the γδT cells offer two compelling advantages: Firstly, MHC-unrestricted tumor recognition through receptors such as natural killer cell receptors (NCRs), substantially mitigating escape potential typified by many tumors [13]. Secondly, both direct cytotoxic activity and indirect anti-tumor capabilities possess significant ability to kill cancer [10]. These advantages make the γδT cell-based cancer immunotherapy a promising frontier in biology and medical science [14]. Thus, this review aims to synthesize contemporary research on the γδT cells across fundamental biology, pre-clinical studies, and clinical applications. Furthermore, potential strategies for enhancing the efficacy and reliability of future cancer immunotherapy based on γδT cells are evaluated.

There is an urgent need for alternative therapeutic approaches based on the limitations of conventional cancer treatments and MHC-restricted αβ T cell immunotherapies. The γδT cells, with MHC-independent recognition capabilities and multifaceted anti-tumor mechanisms, represent a potential strategy in cancer immunotherapy. However, translating the therapeutic potential of γδT cells from bench to clinical application requires a comprehensive understanding of their fundamental biology, careful evaluation of pre-clinical evidence, and critical analysis of clinical outcomes. Thus, this review is structured to address the following three critical dimensions systematically. The basic biology of the γδT cells, for instance, their development, TCR diversity, subset classification, and mechanisms of tumor recognition and cytotoxicity will be first analyzed based on advanced techniques. Subsequent investigation encompasses pre-clinical studies for elucidating the anti-tumor efficacy of the γδT cells across various cancer models and identifying key factors influencing their therapeutic performance. Next, we review clinical trials and case studies that demonstrate the safety, feasibility, and preliminary efficacy of the γδT cell-based immunotherapy in cancer patients. Through these investigations, we can discuss current challenges, emerging strategies for optimization, and future directions to enhance the clinical translation and therapeutic reliability of the indicated cancer immunotherapy. What emerges from this review is a roadmap for advancing the γδT cell immunotherapy from an experimental approach to a robust clinical application.

2. The supportive role of single-cell sequencing technology in the γδT cell research

While traditional cell sequencing techniques like flow cytometry and mass spectrometry offer high throughput, they typically rely on predetermined surface biomarkers and inadequately reveal the functional complexity and heterogeneity of γδT cells [15]. The emergence of single-cell RNA sequencing (scRNA-seq) technology has addressed this limitation by enabling transcriptome

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profiling at single-cell resolution [16]. This leap in resolution facilitates comprehensive analysis of the γδT cell heterogeneity and functional states [16].

2.1. The indicated γδT cells inside the microenvironment

ScRNA-seq technology has been utilized in mapping features of the γδT cell heterogeneity in organisms. For example, Li et al. in 2022 applied this technology to perform subpopulation classification and tissue-specific distribution [17]. They constructed single-cell transcriptome profiles from mouse lymphoid tissues, spleen, and thymus, identifying nine indicated γδT cell clusters with distinct gene expression signatures and providing novel insights for issue-level complexity of the γδT cells [17]. Beyond the classical thymic developmental pathway, scRNA-seq has revealed extrathymic origins of the γδT cells. A study in 2021 employed scRNA-seq on hepatic γδT cells identified several classical subsets (γδ T1, γδ T17, and γδ NKT) alongside two previously uncharacterized subpopulations (C7 and C8) [18]. Furthermore, the discovery of a developing cluster of the γδT cell precursors (C3-L) demonstrated the liver's capacity for autonomous γδT cell generation [18]. Pseudotime trajectory analysis further supported this conclusion that liver-specific hematopoietic progenitor cells give rise to the γδT cell precursors capable of autonomously differentiating into IFN-γ-producing γδ T1 cells independent of thymic involvement [18].

The extensive heterogeneity of the γδT cells can drive tumor cell progression and therapeutic resistance under pathological conditions. This can be demonstrated in hepatosplenic T-cell lymphoma (HSTCL) derived from γδT cells. A scRNA-seq study for patients with examined samples before and after treatment uncovered that malignant HSTCL cells highly express multiple oncogenes while inducing dynamic changes in the TME [19]. Despite originating from a common precursor, scRNA-seq revealed differentiation into two subpopulations with markedly divergent transcriptomes. The chemotherapy-resistant Tumor_2 subgroup undergoes selective enrichment, contributing to therapeutic failure. This study further identified genes associated with chemotherapy resistance in the Tumor_2 subgroup and proposed potential therapeutic targets, including CD38 [19]. Collectively, these findings underscore that a comprehensive understanding of the γδT cell heterogeneity is not merely descriptive but essential for understanding their biological functions.

2.2. Defining the γδT cell functional states through scRNA-seq

Precise characterization of the γδT cell functional states within tumors is essential for developing targeted therapies, and scRNA-seq provides an efficient approach to achieve this objective [20]. Cerapio et al. integrated scRNA-seq data from approximately 150 samples to construct a reference differentiation atlas of the γδT cells, establishing a standardized framework for cross-study comparisons [20]. It can be considered that the functional states of the γδT cells and CD8+ T cells within tumor-infiltrating lymphocytes are closely correlated, contrasting with the previously assumed functional independence of the γδT cells and αβ T cells in tumors. This correlation indicates these two cell types may be driven by similar antigens or exhibit cooperative effects within the TME [20]. More recently, scRNA-seq analysis of rare cutaneous lymphomas derived from γδT cells (TCR-γδ+ mycosis fungoides) demonstrated that these cells intrinsically exhibit cytotoxic properties [21]. Two biomarkers, GNLY and KLRC1, were also identified as unique signatures of γδ T-cell lymphoma and enable discrimination from CD8+ lymphoma [21]. Hence, this result further shows the essential function of scRNA-seq in accurately defining the γδT cell functional states.

Notably, current scRNA-seq technology suffers from a limitation in the γδT cell identification known as "dropout" [22]. Due to limited mRNA content in individual cells and detection instability,

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scRNA-seq data may contain extensive zero counts, then insufficient for identifying adequate numbers of target cells [22]. To address this deficiency and improve the identification accuracy for γδT cells, one study proposed a method for processing scRNA-seq data based on TCR gene module scoring [23]. This approach successfully labeled γδT cells as populations with high γδTCR scores and low αβTCR scores [23].

3. The application of γδ T cells

3.1. The mechanistic aspect of the immune function of γδ T cells

The γδT cells which are known as innate and adaptive immune characteristics [24]. A typical example of this innate capability is the Vγ9Vδ2 T cell subset, which recognizes tumors without being restricted by MHC, usually by binding to the surface of tumor cells through a complex of butyrophilin (BTN) [25]. As shown in Figure 1, by interacting with two immunoglobulin superfamily members, BTN2A1 and BTN3A1, Vγ9Vδ2 T cells can respond to phosphorylated antigens (PAgs), thereby activating and proliferating [25]. In 2024, a research found that two factors, BTN3A2 and BTN3A3, also state a key role in destroying the tumor cells by Vγ9Vδ2T cells [26]. After activation, the γδT cells can exert strong anti-tumor effects in various ways [27]. Specifically, the γδT cells can induce tumor cell apoptosis directly by releasing perforin or granzyme B, or through the Fas/FasL and TRAIL pathways, and exhibit a mechanism similar to natural killer (NK) cells, widely recognizing and killing tumor cells using activated receptors such as NKG2D and DNAM-1 [28]. Moreover, the γδT cells are able to remold the immunosuppressive microenvironment by secreting Th1 cytokines, for example, IFN-γ and TNF-α, and mediate antibody-dependent cytotoxicity (ADCC) via their surface FcγRIII [28].

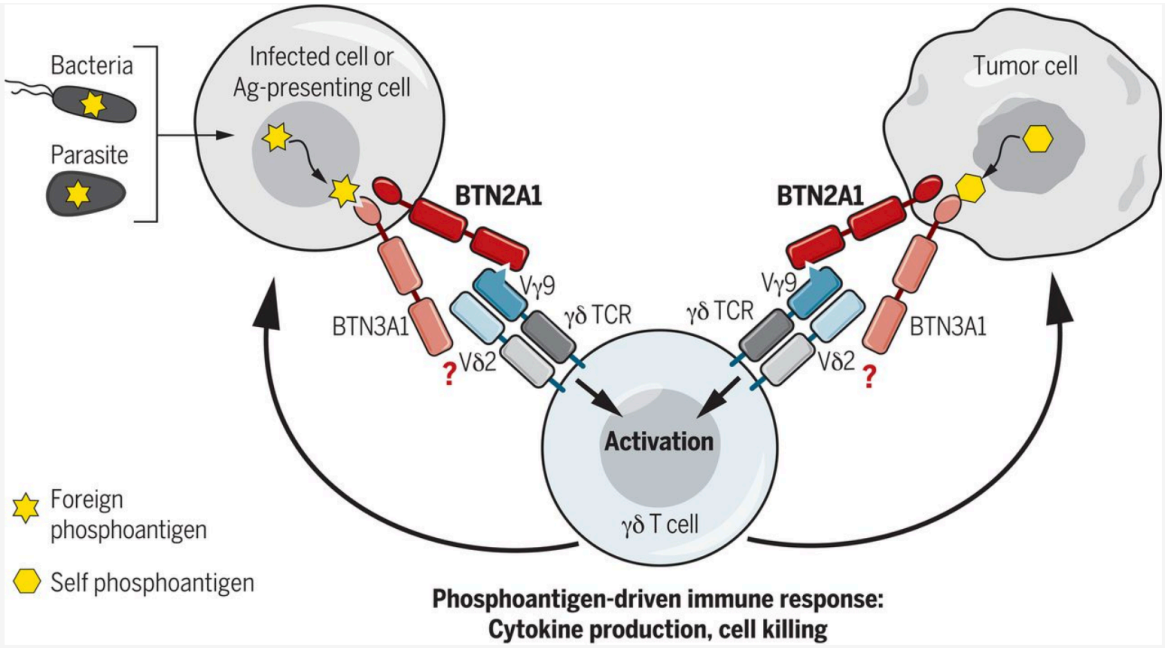

Figure 1. Butyrophilin 2A1 (BTN2A1) is essential for human γδ T cell activation by phosphoantigens. Our study reveals that BTN2A1 binds directly to the γδ TCR and cooperates with BTN3A1 to mediate the sensing of microbial and tumor-derived phosphoantigens [25]

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Besides, the γδT cells can exert immune effects by secreting cytokines or directly participating in immune cell interactions [29]. Activated γδT cells (especially Vγ9Vδ2 T cells) can function as specialized antigen-presenting cells, efficiently presenting ingested tumor antigens by upregulating MHC molecules, co-stimulatory molecules, and lymph node homing receptors, then activating αβ T cells, and inducing CD4+ and CD8+ T cell responses [30]. For NK cells, activated γδT cells can upregulate signals such as CD137/CD137L, enhancing the cytotoxicity of NK cell units towards tumor cells [30]. In humoral immunity, the γδT cells can promote B cell growth and immunoglobulin production by secreting factors such as IL-4 [28]. In addition, the γδT cells can recruit and activate neutrophils and dendritic cells through secreting chemokines [28,30]. These synergistic effects enable the γδT cells to transcend the simple effector function and work together with some others to establish the anti-tumor immune microenvironment, which also reflects their characteristic of connecting innate immunity and long-lasting adaptive immunity.

3.2. Main strategies of γδ T cells in immunotherapy

3.2.1. Expansion of γδ T cells

Because the γδT cells constitute only one to ten percent of circulating T lymphocytes, efficient methods for their isolation and large-scale expansion are essential for clinical application [31]. Advances in the characterization of the γδT cell antitumor activity have led to two major therapeutic strategies.

The first strategy involves in vivo activation and expansion, in which pharmacological agents—most notably aminobisphosphonates—are used to directly stimulate and proliferate the γδT cells within the patient, offering a relatively simple clinical workflow [32]. The second strategy is adoptive cell transfer, wherein the γδT cells are expanded ex vivo to clinically relevant numbers and subsequently reinfused [32]. Although technically more demanding, ex vivo expansion provides substantially greater control over cell quality, phenotype, and function [32].

Despite its feasibility, in vivo activation using bisphosphonates and IL-2 often produces transient immune responses, and repeated stimulation may drive the γδT cell exhaustion [33]. Recent studies show that zoledronic acid combined with IL-2 can also stimulate potent in vitro expansion of Vγ9Vδ2 T cells, with extended culture duration further enhancing cytotoxic potency [34]. Beyond classical phosphoantigen-dependent activation, antigen-independent pathways have also been identified; for example, combined IL-12 and IL-18 stimulation can directly activate Vγ9Vδ2 T cells to produce robust IFN-γ independently of phosphoantigen recognition [35].

To overcome the limited availability of starting the γδT cells and achieve true clinical-scale expansion, more advanced approaches have employed genetically engineered artificial antigen-presenting cells (aAPCs). Notably, K-562-derived aAPCs engineered to co-express CD3scFv, CD137L, CD28scFv, and IL-15RA provide potent activation and costimulatory cues, supporting robust proliferation of healthy-donor γδT cells and generating cell numbers compatible with therapeutic application (Figure 2)[36].

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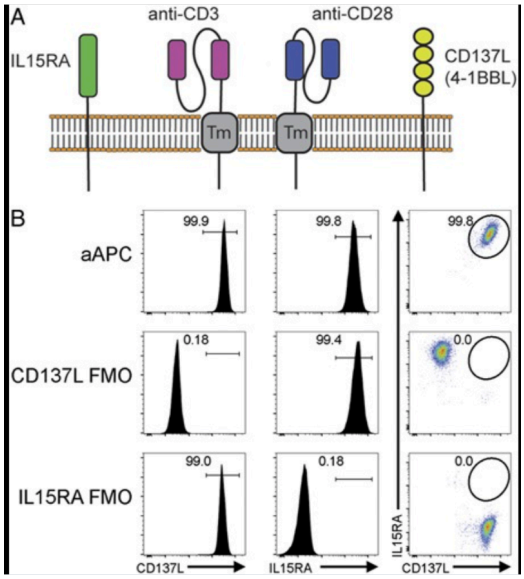

Figure 2. Characterization of K-562 aAPCs (CD3scFv/CD137L/CD28scFv/IL15RA). (A) Schematic design of the modified aAPCs. (B) Post-sorting flow cytometry analysis, including histograms and FMO (fluorescence minus one) controls [36]

3.2.2. Engineering the γδT cell–based immunotherapy

As understanding of the above mentioned γδT cell biology deepens, genetic engineering strategies designed to enhance their antigen specificity and functional potency have become a major research focus. Engineering-based γδT cell therapies generally fall into three categories: CAR-T approaches, bispecific antibody platforms, and TCR engineering [31]. Table 1 presents the general view of these strategies.

Table 1. Main strategies of engineered γδ T cell immunotherapy

Strategy	Principle	Advantage	Deficiency	Representative Case
CAR-γδ T	By redirecting γδ T cells through synthetic receptors (CARs), tumor surface antigens can be recognized.	MHC unrestricted; Inhibit antigen escape; High security	Insufficient persistence of cells in the body	[37,38]
Bispecific T cell engagers (bsTCE)	Design an antibody that binds to tumor antigens and γδ TCR (such as Vδ2) at the same time, bridging and activating γδ T cells.	High efficacy and tolerability; Low toxicity; High production efficiency	Restricted by the extent of target expression in the antigen	[39]
TCR engineering	Enhancing or endowing γδ T cells with new antigen recognition capabilities by modifying TCR	High flexibility; Integrating innate and adaptive immunity	Complex technology	[40,41]

3.2.2.1. Car-γδT cells

CAR-T therapy is currently the most widely explored engineered γδT cell platform. CARs are synthetic receptors that can redirect T cells to recognize and eliminate tumor cells expressing predefined antigens [42]. The γδT cells offer inherent advantages as CAR carriers, including reduced risk of antigen escape, lower relapse rates, and a more favorable safety profile [38]. In murine models, CAR-modified Vγ9Vδ2 T cells targeting CD19 effectively eliminated $CD19^{+}$ tumors while

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retaining intrinsic CAR-independent cytotoxicity against $CD19^{-}$ tumors. However, their in vivo persistence was inferior to conventional αβ CAR-T cells [38].

A study conducted in 2022 established a simplified, GMP-compliant workflow for expanding and engineering Vδ1 T cells using reagents adapted from αβ CAR-T manufacturing [37]. Early-phase depletion of αβT and $CD56^{+}$ cells combined with OKT-3 and IL-15 stimulation enabled efficient Vδ1 expansion and yielded high-purity products. These Vδ1 cells exhibited strong innate antitumor activity and can be efficiently transduced with B7-H3-CAR constructs, conferring potent antigen-specific killing and improved persistence [37].

3.2.2.2. Bispecific T cell engagers (bsTCEs)

Bispecific T cell engagers (bsTCEs) are engineered antibodies designed to simultaneously integrate tumor-associated antigens and T cell receptor (TCR). Then it can redirect T cell cytotoxicity toward tumor cells. These molecules combine high therapeutic potency with low systemic toxicity and favorable manufacturability [43]. For instance, de Weerdt et al. engineered a bispecific antibody with one arm targeting CD1 (a molecule frequently overexpressed in hematologic malignancies) and the other arm integrating the Vδ2 chain of Vγ9Vδ2 T cells [39]. This precise molecular bridge robustly activated Vγ9Vδ2 T cells for promoting IFN-γ and TNF-α secretion, degranulation, and efficient cytotoxicity against $CD1d^{+}$ tumor cells. Furthermore, tumor sensitivity was enhanced when combined with all-trans retinoic acid (ATRA) and aminobisphosphonates, which upregulated CD1d expression and potentiated the γδT cell activation [39]. The combination of high tumor selectivity and low off-target effects indicates the translational potential of bsTCE-mediated γδT cell redirection.

3.2.2.3. T cell receptor engineering

TCR molecules on the surface of T cells play a critical role in recognizing abnormal cells, mediating cell activation, and regulating immune surveillance [44]. In this context, TCR engineering aims to enhance antigen specificity and sensitivity by modifying these receptors [44]. One innovative approach is bidirectional TCR replacement between αβT and γδT cells. Transfer of specific αβ TCRs into the γδT cells enables the acquisition of MHC-restricted antigen recognition while retaining intrinsic MHC-unrestricted cytotoxicity without introducing risks of TCR mismatch [41]. Conversely, inducing γδ TCR expression in αβT cells brings broad MHC-independent anti-tumor capabilities, which combine the innate persistence of αβT cells in vivo with the innate-like cytotoxicity of the γδT cells [41].

A recent study provided a case of the potential of synthetic TCR design for addressing tumor heterogeneity and antigen escape. Using structural redundancy in the CDR3 region of the δ2 chain, Davies et al. constructed a dual-specificity Vγ9Vδ2 TCR by inserting an A20 peptide targeting αvβ6 integrin into a key position of the G115 receptor [40]. The modified TCR retained phosphate antigen reactivity while gaining specific recognition of αvβ6 integrin, which effectively integrates innate and adaptive immune functions in a single receptor [40]. This strategy verifies that engineering TCRs can simultaneously target multiple tumor antigens and provide a potential solution to tumor heterogeneity and antigenic escape.

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3.2.3. Combination therapy of the γδT cells immunotherapies or with other treatments

Although the γδT cells immunotherapy has demonstrated considerable therapeutic potential, its efficacy as monotherapy is often constrained by the TME. Consequently, combination strategies with different therapies can control or reshape the TME and have emerged as a promising approach to overcome these therapeutic limitations [45]. For instance, low-dose radiotherapy has been shown to remodel the TME by polarizing tumor-associated macrophages toward a pro-inflammatory M1 phenotype, inhibiting TGF-β signaling, and creating conditions conducive to T cell homing and activation [45]. These findings provide both a theoretical rationale and a clinical basis for synergistic strategies integrating the γδT cell immunotherapy with other strategies.

In the context of solid tumors such as diffuse midline gliomas (DMG), combination therapy with oncolytic viruses has shown encouraging preclinical results [46]. Vazaios et al. demonstrated that when administered together with low concentrations of oncolytic viruses, the engineered γδT cells present a synergistic cytotoxic effect on DMG cells without the requirement of additional activation through phosphonate drugs [46]. This drug-independent combination approach may overcome the limitations of low intratumoral delivery efficiency of phosphate antigens, making it particularly suitable for treating solid tumors characterized by heterogeneous antigen expression or resistance to traditional immunotherapies [46].

Furthermore, advanced strategies in the γδT cell engineering can enhance therapeutic efficacy through combination or integration approaches. A recent study developed an AbTCR chimeric receptor that merges the Fab region of an antibody with the constant region of the γδ TCR, therefore combining the advantages of CAR and TCR technologies [47]. This approach not only avoids the high activation threshold limitations of traditional CAR-T therapy but also preserves stem cell–like memory subpopulations and improves in vivo persistence, offering a promising strategy for enhancing antitumor activity [47].

3.3. Application and challenges in recent clinical research

3.3.1. Clinical validation of the main strategies of the γδT cell immunotherapy

Currently, the primary clinical application for the γδT cell-related immunotherapy includes autologous adoptive transfer and allogeneic adoptive transfer therapy [48]. Table 2 presents clinical application cases of various γδ T cell immunotherapies.

Table 2. Clinical application cases related to different strategies of γδ T cell immunotherapy

Time Period	Strategy	Clinical Progress	Disease	References
2012-2018	Autologous transfer therapy	Phase 2	Refractory non-small-cell lung cancer	[49]
2011-2014	Autologous transfer therapy	Phase 1	refractory neuroblastoma	[50]
2017-2019	Allogeneic transfer therapy	Phase 1 and Phase 2	Pancreatic, lung, liver, and breast cancer	[51]
2018-2020	Allogeneic transfer therapy	Phase 1	Relapsed or refractory acute myeloid leukemia	[52]
2021-2025	Allogeneic CAR^+ Vδ1 γδ T treatment	Phase 1	B-cell malignancies	[53]
2024-(unfinished)	Dual-targeting mRNA-engineered Nb-CAR.BiTE-Vδ2 γδT therapy	Phase 1 and Phase 2	Refractory non-small-cell lung cancer	[54]

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Despite the potential of the γδT cell immunotherapy, non-engineered autologous therapy remains largely ineffective in advanced solid tumors. In a phase II clinical trial of patients with refractory non-small cell lung cancer, zoledronic acid was used to expand Vγ9Vδ2 T cells in vitro for subsequent autologous reinfusion [49]. While the therapy was safe and capable of achieving durable in vivo persistence, its clinical efficacy was limited with a median progression-free survival of only 95 days and an objective response rate of 4%. These results indicate that unmodified autologous Vγ9Vδ2 T cell monotherapy is insufficient for advanced tumors characterized by high heterogeneity [49]. There were similar observations reported in neuroblastoma studies, which highlight additional barriers in mechanisms [50]. Even with in vivo amplification using zoledronic acid and IL-2, the γδT cell numbers generally increased only to physiological levels [50]. This result may be inadequate for significant antitumor effects. Efficacy is further constrained by low baseline γδT cell counts, particularly in heavily pretreated patients [50]. The absence of predictive biomarkers such as BTN3A1 expression, RhoB GTPase activity, or phosphate antigen abundance also leads to empiric treatment strategies [49]. Additionally, the TME can impede the reinfusion of γδT cells from penetrating tumors and achieving full activation, while exogenous IL-2 can simultaneously expand regulatory T cells, further limiting therapeutic efficacy.

Allogeneic γδT cell therapy addresses key limitations of autologous approaches, including limited cell availability and suboptimal functional status. Due to their MHC-unrestricted cytotoxicity, the γδT cells carry a very low risk of GVHD, which enables large-scale, standardized manufacturing from healthy donors [51]. Indeed, a novel culture way superior in vitro cytotoxicity was developed to expand Vγ9Vδ2 T cells from the healthy donors to yield cells with enhanced metabolic activity and improve effector molecule expression (e.g., IFN-γ and TNF-α). In 132 patients with solid tumors, 414 infusions were well tolerated [51]. No severe adverse events (SAEs), including cytokine release syndrome (CRS) or GVHD, were observed [51]. Notably, patients with advanced lung and liver-related tumors showed prolonged survival exceeding 10 months, and five patients maintained normal quality of life for 30–35 months [51]. In 2023, allogeneic γδT cell therapy was further validated in relapsed/refractory acute myeloid leukemia using partially HLA-matched family donors, demonstrating safety without dose-limiting toxicity, infusion reactions, neurotoxicity, or GVHD, though depth and persistence of response remain areas for improvement [52].

Engineered γδT cells have shown enhanced cytotoxic potential in preclinical studies, though clinical evidence remains limited. For example, CD20 CAR-Vδ1 γδT cells have been successfully expanded under cGMP conditions, maintaining a memory phenotype and resting, non-exhausted state comparable to CARαβ T cell products [53]. These cells express chemokine and natural killer receptors facilitating tumor homing and cytotoxicity. Preclinical xenograft models confirmed tumor suppression without GVHD, making a phase I clinical trial for the B-cell non-Hodgkin lymphoma (NCT04735471) [53]. In solid tumors, mRNA-engineered dual-targeting strategies enable Vδ2 γδT cells to co-express an HLA-G–targeting CAR and locally secrete PD-L1–targeting BiTEs, effectively clearing heterogeneous tumor populations without observation of toxicity [54]. This approach has entered phase I/II trials (NCT06150885), indicating the translational potential of engineered γδT cells in oncology.

3.3.2. Γδ T cell as a clinical prognostic factor

γδT cells are more and more recognized as complex prognostic biomarkers in oncology [14]. Their association with patient outcomes is highly dependent on functional subtypes [14]. This emphasizes

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the necessity for high-resolution analytical approaches, such as single-cell RNA sequencing and spatial transcriptomics, to accurately evaluate their prognostic potential [14].

A recent multi-omics study integrating scRNA-Seq, bulk transcriptomics, and immunohistochemistry has elucidated the prognostic significance of the γδT cell infiltration in cervical cancer [55]. High levels of γδT cell infiltration were significantly associated with improved survival, and the study suggested the feasibility of non-invasive assessment of γδT cell density through MRI-based radiomic analysis [55]. Moreover, patients exhibiting elevated γδT cell infiltration demonstrated enhanced responsiveness to traditional treatments, including chemotherapy, radiotherapy, and immune checkpoint inhibitors [55].

In a broader pan-cancer analysis of γδ TCR repertoires across 33 cancer types using the TRUST4 algorithm, Yu et al. highlighted both the complexity and clinical relevance of the γδT cells [56]. Specifically, TRG/TRD gene expression levels serve as convenient markers of γδT cells' activity, and the prognostic impact of the cells exhibits pronounced cancer-type specificity. For instance, in HPV-positive head and neck squamous cell carcinoma and MSI-high tumors, high γδT cell diversity and clonality correlate with favorable survival, while HPV-negative and MSI-low tumors show the opposite trend. Furthermore, responders to immunotherapy generally exhibit higher γδT cell enrichment and γ chain TCR diversity [56], supporting the potential utility of the γδT cells as predictive biomarkers for prognosis and immunotherapy efficacy.

Moreover, the prognostic effect of the γδT cells is not universally beneficial. In gallbladder cancer, the IL-17-producing γδT17 subset correlates with tumor angiogenesis and poor survival [57]. Similarly, in colorectal cancer (CRC), γδT17 cells are the predominant IL-17 source, with tumor-associated dendritic cells producing IL-23 to activate these cells. Activated γδT17 cells recruit and expand polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), establishing an immunosuppressive microenvironment conducive to tumor progression [58]. These findings locate γδT17 cells as negative prognostic factors in specific tumor contexts.

Nonetheless, other studies indicate a protective role of γδT cells at the population level. A nomogram-based analysis in CRC demonstrated that high total γδT cell infiltration correlates with improved survival and chemotherapy response [59]. Additionally, most Vδ1 T cell subtypes in tumor and adjacent tissues exhibit limited IL-17 production capacity [60]. These divergences likely reflect differences in analytical focus: Wu et al. emphasized pathogenic roles of specific γδT17 subsets [58], whereas Ma et al. assessed the overall prognostic impact of whole abilities of the γδT cells [59].

3.3.3. Current challenges and bottlenecks

Despite promising clinical potential, the γδT cell–based immunotherapy faces multiple challenges related to functional variability, TME-mediated immunosuppression, and industrial-scale production.

The functional outcomes of the γδT cell infiltration are context-dependent, influenced by both microenvironmental cues [61] and signaling pathways [62]. For example, under Th1-polarizing conditions enriched in IL-12, the γδT cells differentiate into IFN-γ–producing subsets with anti-tumor activity. In contrast, Th17-polarizing environments rich in IL-23 and IL-1β promote differentiation into IL-17-producing γδT17 cells, which may facilitate tumor progression [63]. Consequently, identical γδT cell populations can exert opposing effects depending on the TME or therapeutic context, highlighting the need to precisely guide differentiation toward anti-tumor phenotypes while preventing protumor differentiation.

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The TME also imposes significant constraints on the γδT cell efficacy. Heterogeneous colorectal cancer subtypes vary between highly infiltrated dMMR/MSI-H and fibrotic, immune-desert tumors. The γδT cells may become functionally exhausted under these conditions to upregulate multiple inhibitory receptors and lose cytotoxicity despite their MHC-independent tumor recognition [64]. Regulatory cells and immunosuppressive cytokines (for instance, TGF-β, IL-10) further restrain the γδT cell activity, while abnormal chemokine expression limits intratumoral infiltration [65]. Clinically, this contributes to the limited efficacy of monotherapies, including immune checkpoint inhibitors, CAR-T therapy, or γδT cell adoptive transfer, particularly in solid tumors such as CRC [64].

In addition to biological challenges, the industrialization and commercialization of the γδT cell therapies remain constrained. Although early-phase clinical trials have demonstrated the safety of Vγ9Vδ2 activation and allogeneic Vδ1 adoptive transfer, large-scale clinical deployment is obstructed by the complexity, cost, and technical requirements of live cell manufacturing, particularly for engineered products incorporating CARs or bispecific T cell engagers (bsTCEs) [66]. Establishing a reliable, large-scale production and supply chain within regulatory frameworks constitutes a critical barrier to broad clinical implementation [31].

4. Conclusion

The γδT cells, characterized by MHC-unrestricted tumor recognition, potent cytotoxicity, and a bridging role between adaptive and innate immunity, have been a uniquely promising approach in cancer immunotherapy. Therapeutic strategies ranging from conventional in vitro expansion to advanced genetic engineering approaches—including CAR-γδT cells and bispecific T cell engagers (bsTCEs)—have been explored, and early-phase clinical trials have demonstrated their feasibility and safety.

Despite these advances, the translation of the γδT cell immunotherapy into widely effective clinical treatments remains constrained by three principal challenges. First, the intrinsic functional variability of the γδT cells renders their phenotypic characteristics unstable in the TME. Based on local signals, they may differentiate into anti-tumor effector subpopulations or, conversely, adopt tumor-promoting γδT17 phenotypes. Second, the immunosuppressive TME imposes substantial limitations on therapeutic efficacy by inducing cellular exhaustion, recruiting inhibitory immune populations, and impairing the γδT cell infiltration. Third, the industrial-scale production of the γδT cell-based therapies faces significant logistical and regulatory barriers, including high costs, complex manufacturing processes, and challenges in aligning supply chains with regulatory requirements.

Future clinical success can integrate strategies at multiple levels. Biologically, high-resolution tools such as single-cell sequencing are essential for analyzing the γδT cell heterogeneity and identifying subpopulations with optimal therapeutic potential. Therapeutically, reshaping the TME through combinatorial approaches, including conventional modalities, immune checkpoint blockade, and targeted cytokine therapy, may enhance efficacy. Technologically, rational genetic engineering designs aimed at improving persistence, resistance to exhaustion, and functional stability will be critical. Finally, successful industrialization will depend on close collaboration between academic research and industry to overcome challenges in large-scale cell manufacturing and clinical application.

With these combined efforts, γδT cell immunotherapy holds the potential to evolve from an experimental research tool into a robust and clinically reliable strategy for the treatment of cancer, particularly solid tumors.

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