

Therapeutic avenues for $\gamma\delta$ T cells in cancer

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ABSTRACT

$\gamma\delta$ T cells are regarded as promising effector lymphocytes for next-generation cancer immunotherapies. In spite of being relatively rare in human peripheral blood, $\gamma\delta$ T cells are more abundant in epithelial tissues where many tumors develop, and have been shown to actively participate in anticancer immunity as cytotoxic cells or as “type 1” immune orchestrators. A major asset of $\gamma\delta$ T cells for tackling advanced cancers is their independence from antigen presentation via the major histocompatibility complex, which clearly sets them apart from conventional $\alpha\beta$ T cells. Here we discuss the main therapeutic strategies based on human $\gamma\delta$ T cells. These include antibody-based bispecific engagers and adoptive cell therapies, either focused on the $V\delta 1^+$ or $V\delta 2^+$ $\gamma\delta$ T-cell subsets, which can be expanded selectively and differentiated or engineered to maximize their antitumor functions. We review the preclinical data that supports each of the therapeutic strategies under development; and summarize the clinical trials being pursued towards establishing $\gamma\delta$ T cell-based treatments for solid and hematological malignancies.

INTRODUCTION

Cancer immunotherapies have recently produced major advances in the treatment of both solid and hematological tumors. In particular, immune checkpoint blockade (ICB) has been successfully implemented in various advanced solid cancers, most notably melanoma and lung carcinoma¹; and T cells expressing chimeric antigen receptors (CAR-T cells) have changed the paradigm in the treatment of B-cell-derived neoplasms.² This notwithstanding, many indications and large proportions of patients still lack efficacious immunotherapies, which are now the major challenge in immuno-oncology.

One of the key concepts towards improving the clinical application of immunotherapy is to tackle immune evasion mechanisms that result from the dynamic interaction between cancer and immune cells. A major immune evasion strategy found in advanced cancers is the downregulation of major histocompatibility complex (MHC; also known as human leukocyte antigen, HLA) molecules, which are required for $\alpha\beta$ T-cell activation on presentation of somatically mutated

“neoantigens” to $\alpha\beta$ T-cell receptors (TCR).³ However, this limitation does not apply to $\gamma\delta$ TCR-expressing T cells ($\gamma\delta$ T cells), which despite being rare in the human peripheral blood, are enriched in epithelial tissues where many cancers develop, and have been shown to actively participate in antitumor immunity.⁴

$\gamma\delta$ T cells make up a small fraction, ranging from 1% to 10%, of the total human $CD3^+$ T-cell population. These cells express a lineage-specific TCR, containing one of seven $V\gamma$ chain isotypes ($V\gamma 2, 3, 4, 5, 8, 9,$ and 11) paired with one of four $V\delta$ chain types ($V\delta 1, 2, 3,$ and 5), which can be highly diverse due to the stochastic nature of the TCR somatic recombination process.⁵ The most common pairing consists in the $V\gamma 9V\delta 2$ TCR that has the particularity of sensing the intracellular accumulation of non-peptidic phosphoantigens (PAg) in target cells. Such an accumulation can either be naturally found in cancer cells, as the result of an overactive mevalonate pathway; or be induced by nitrogen-containing bisphosphonates (n-BP).⁶

Among human $\gamma\delta$ T-cell subsets, $V\delta 2^+$ T cells (particularly those expressing a $V\gamma 9V\delta 2$ TCR) have been more extensively studied as they are the most abundant subpopulation in the peripheral blood, whereas $V\delta 1^+$ T cells represent the major fraction of $\gamma\delta$ T cells in epithelial tissues. However, $V\delta 1$ T cells typically outnumber $V\delta 2$ T cells in cancerous tissue infiltrations; $V\delta 1$ tumor-infiltrating lymphocytes (TIL)-derived cell cultures can exhibit superior in vitro cancer-killing ability compared with $V\delta 2$ TIL cultures^{7,8}; and $V\delta 1$ T cells can persist long-term as tumor-reactive lymphocytes,⁹ which has prompted a more recent focus on $V\delta 1$ T cells.

While relatively few antigens have been implicated in tumor cell recognition by $\gamma\delta$ T cells,^{10,11} it is now well-established that the phosphoantigen sensor (on intracellular binding), BTN3A1 or CD277, plays a key role in the TCR-dependent activities of $V\gamma 9V\delta 2$ T cells.^{12,13} Besides the TCR, $\gamma\delta$ T cells can



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employ various natural killer cell receptors (NKR) in their potent cytotoxic functions. NKG2D, DNAM-1, NKp30 and NKp44 are some of the NKRs that can participate in tumor cell targeting by human $\gamma\delta$ T cells.^{14–16} As with other cytotoxic lymphocytes, the mechanisms of tumor cell elimination are mediated by the secretion of perforin and granzymes, or the expression of ligands that engage death receptors (Fas, TRAIL-R) on target cells.¹⁷ For example, V γ 9V δ 2 T cells are able to induce cell death through the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), or FAS ligand, or antibody-dependent cellular toxicity (ADCC), in which immune cells expressing an Fc receptor recognize antibodies bound to their target cell.^{18–20}

$\gamma\delta$ T cells also play a crucial role in indirectly inducing antitumor immune responses. First, they produce interferon- γ (IFN- γ) and exhibit antigen-presenting cell functions,²¹ which in turn activate $\alpha\beta$ T cells.²² Second, expression of 4-1BB ligand (4-1BBL) on $\gamma\delta$ T cells stimulates natural killer (NK) cells.²³ Additionally, $\gamma\delta$ T cells are involved in inducing antibody class switching in B cells,^{10 24 25} which is crucial for a protective humoral response. Furthermore, $\gamma\delta$ T cells regulate the infiltration of dendritic cells by producing granulocyte-macrophage colony-stimulating factor.^{4 26}

Based on these interesting immune properties, which have been corroborated in syngeneic mouse cancer models,⁴ $\gamma\delta$ T cells are regarded as attractive candidates for next-generation cancer immunotherapies.

Γ T-CELL RESPONSES TO IMMUNE CHECKPOINT BLOCKADE

A major asset of $\gamma\delta$ T cells for tackling advanced cancers is their independence from MHCs, both class I and class II, which clearly sets them apart from conventional $\alpha\beta$ T cells.^{27 28} Along these lines, a recent study demonstrated that $\gamma\delta$ T cells were the major responding immune subset in patients with colorectal cancer with defects in HLA class I presentation that were treated with ICB.²⁹ This study was conducted in DNA mismatch repair deficient (MMR-d) colon cancers with β 2-microglobulin (B2M) genomic inactivation. The investigators concluded that the high mutational status of B2M cells is associated with an enhanced response to programmed cell death protein 1 (PD-1) blockade. Moreover, since B2M is an essential component of HLA class I, these findings suggested the involvement of an immune component independent of the HLA complex. Their results indeed demonstrated the enrichment in two $\gamma\delta$ T-cell subtypes, V δ 1⁺ (in majority) and V δ 3⁺ (in minority), displaying activation and exhaustion markers, infiltrating the tumors of the best responding patients.²⁹ Importantly, all $\gamma\delta$ T-cell subsets, including V δ 2⁺ cells, expressed cytotoxic signatures, and displayed antitumor cytolytic functions *in vitro*. Altogether, these data support an important role for $\gamma\delta$ T cells in mediating cytotoxic responses in HLA class I-negative MMR-d colon cancers.³⁰

In another recent study, Rancan and colleagues investigated whether $\gamma\delta$ T cells could have a clinical impact on human kidney cancer.³¹ The phenotype of $\gamma\delta$ T cells was investigated in cells from healthy individuals and was compared with cells from patients with renal cell carcinoma (RCC). They observed that V δ 2⁺ cells had an identical phenotype in both healthy and patients with RCC. By contrast, tumor-infiltrating V δ 2⁻ cells (which were 50% V δ 1⁺) expressed markers associated with early-activated (CD28 and CD27) or experienced (CD57, PD-1, and 4-1BB) effector cells, clearly distinguishing them from healthy tissue V δ 2⁻ cells. Interestingly, although V δ 2⁻ cells were found to be the population that expressed the highest levels of the immune checkpoints PD-1, T cell immunoreceptor with Ig and ITIM domains (TIGIT), and T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), as well as various transcriptional hallmarks of “exhaustion”, they retained high expression of Ki67 (proliferation marker), cytolytic effector molecules and pro-inflammatory cytokine secretion potential. The “exhausted yet cytotoxic” transcriptional profile of V δ 2⁻ T cells in pretreatment tumor biopsies associated with increased 5-year overall survival on PD-1 blockade in patients with RCC (as well as urothelial) cancers.³¹

Overall, these recent clinical evidences highlight the potential of exploring $\gamma\delta$ T cells to design new strategies that obviate the shortcomings of the available immunotherapies for advanced cancers.⁴ Here, we highlight recent advances in the development of $\gamma\delta$ T cell-specific engagers and in adoptive cellular therapy using expanded or engineered $\gamma\delta$ T cells (figure 1), from the supporting preclinical data to the ongoing clinical trials in patients with cancer.

Γ T CELL-BASED THERAPEUTIC APPROACHES

Engagers

Bispecific antibodies which combine a tumor-binding domain with a T-cell engager domain are designed to mobilize killer T cells against cancer cells. After intense development in the conventional T-cell field, bispecific modalities have also been generated specifically for $\gamma\delta$ T cells.

In one of the earliest studies, a bispecific antibody binding Her2 and the V γ 9 TCR chain was developed and tested for its capacity to increase V γ 9V δ 2 T cell-mediated cytotoxicity against ductal adenocarcinoma pancreatic cells.³² The background for this investigation was a cohort of 21 patients with histologically verified pancreatic ductal adenocarcinoma (PDAC), which overall had decreased V γ 9V δ 2 T-cell numbers compared with controls. Patient-derived V γ 9V δ 2 T cells showed limited cytotoxicity against various tumor cell lines (PanTu-I, Colo357 and Panc89), but this could be rescued by the addition of PAG or n-BP, although this sustained stimulation frequently led to exhaustion of the V γ 9V δ 2 T cells. Based on these foundations, a bispecific antibody was developed in the tribody format which enables bivalent Her2-targeting and

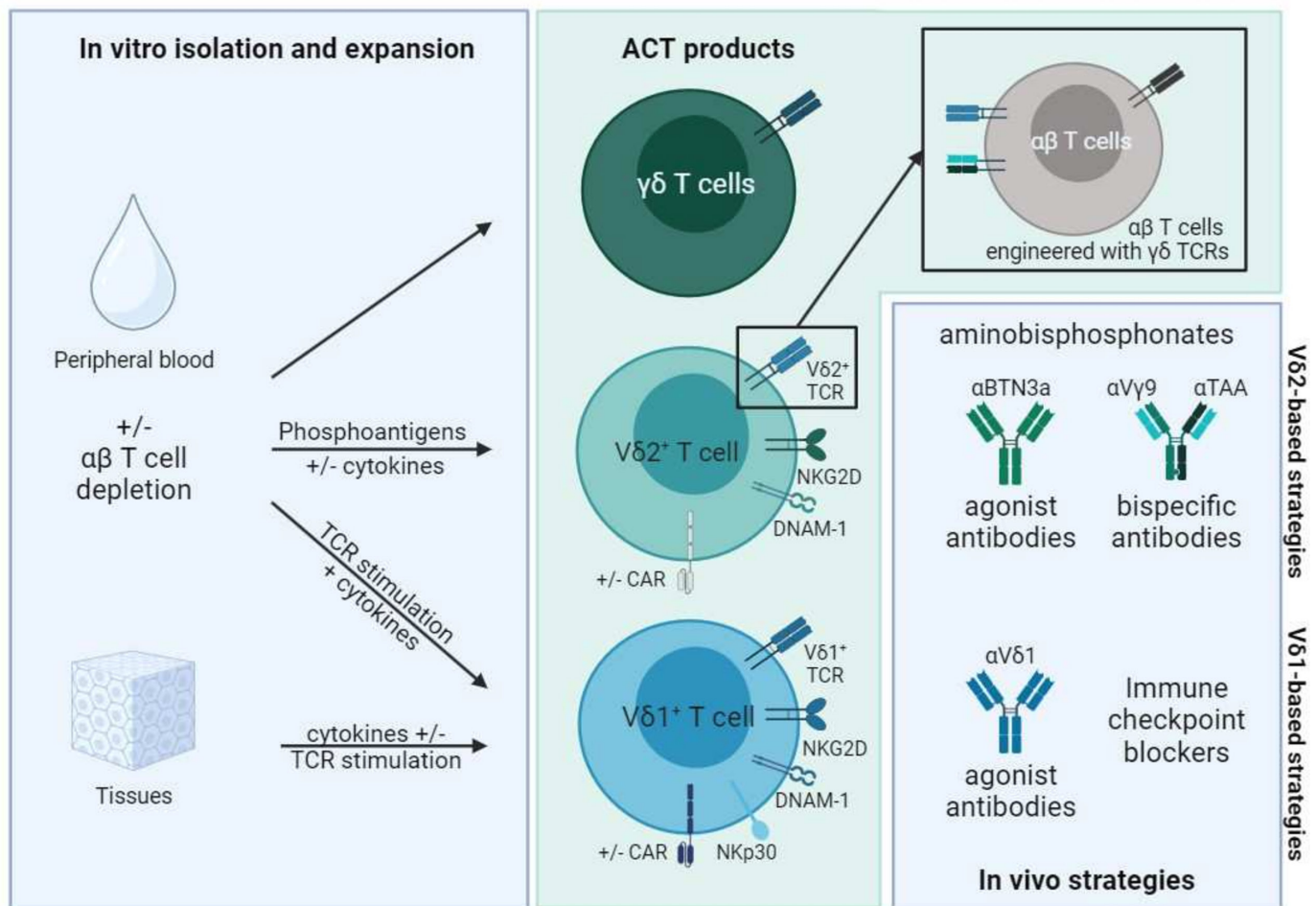


Figure 1 Strategies for therapeutic application of $\gamma\delta$ T cells in cancer immunotherapy. Adoptive cell therapy products based on $\gamma\delta$ T-cell subsets; and agonists (of stimulatory pathways) or antagonists (immune checkpoint blockers) administered to patients. Each strategy is detailed in the text. ACT, adoptive cell therapy; CAR, chimeric antigen receptors; TAA, tumor-associated antigen; TCR, T-cell receptors.

monovalent binding to $V\gamma 9^+$ $\gamma\delta$ T cells ($(\text{Her}2)_2 \times V\gamma 9$). Besides demonstrating its potency in various in vitro assays against PDAC cell lines, the authors showed that the $((\text{Her}2)_2 \times V\gamma 9)$ tribody, when given together with short-term activated $\gamma\delta$ T cells plus interleukin (IL)-2, produced a significant reduction in tumor burden in a subcutaneous PancTu-1 xenograft mouse model. Clique ou toque aqui para introduzir texto.

Other researchers generated a bispecific antibody against CD40, which is overexpressed in many B-cell malignancies such as chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). CD40 has an important anti-apoptotic function by providing survival signals to malignant cells. The CD40-specific $V\gamma 9V\delta 2$ T cell-engaging construct not only prompted $V\gamma 9V\delta 2$ T cell-mediated tumor cell lysis, but also hampered CD40 stimulation in CLL cells. This study was performed using patient-derived samples and an in vivo xenograft model (in immunodeficient NOD SCID mice).³³ Mice that were administered with both the bispecific antibody and $V\gamma 9V\delta 2$ T cells showed a significant increase in lifespan,

with a median overall survival rate of 80 days compared with 47 days in the control group.

In another investigation, a CD1d-specific $V\gamma 9V\delta 2$ -T cell engager was designed based on CD1d upregulation in several hematological malignancies, especially in advanced stages of CLL, MM, acute myeloid leukemia (AML) or Hodgkin's lymphoma. The engager activated $V\gamma 9V\delta 2$ T cells to release pro-inflammatory cytokines, capable of maturing dendritic cells (DCs) and other effector cells.³⁴ Moreover, the CD1d-specific $V\gamma 9V\delta 2$ -T cell engager enhanced the antitumor activity against various cell lines (the cell line MM.1s, the mantle cell lymphoma cell line Jeko-1, and the T acute lymphoblastic leukemia cell line CCRF-CEM) via secretion of perforin and granzymes.³⁴ Additionally, some strategies were used to further boost this process, namely all-trans retinoic acid to increase CD1d expression on target cells; and n-BP to enhance the activation of $V\gamma 9V\delta 2$ T cells isolated from peripheral blood and lymph node biopsies from untreated patients with CLL (or healthy donor peripheral blood mononuclear cells (PBMCs)).³⁴

An anti-CD123/anti-V γ 9 bispecific antibody was produced for AML, since CD123 is highly expressed on AML blasts. The investigators concluded that the bispecific antibody was capable of inducing the recruitment, activation and proliferation of V γ 9⁺ $\gamma\delta$ T cells from patients with AML in order to effectively eliminate AML blasts and AML cell lines. This evidence was obtained in vitro (against AML cell lines Kasumi-3, MOLM-13 and KG-1) but also in a xenograft model, where KG-1 cells were injected into NOD-SCID mice.³⁵

On a different design, a bispecific molecule capable of linking the extracellular domains of tumor reactive V γ 9V δ 2 TCRs to a CD3-binding structure, called gamma delta TCR anti-CD3 bispecific molecules (GABs), was generated. In addition to being able to target multiple hematological and solid tumor cell lines and primary samples, GABs were shown to promoted T-cell infiltration in tumor microenvironment, contributing to decreased tumor growth in vivo, following subcutaneous injection of RPMI 8226MM cells into NSG mice.³⁶

Finally, since V γ 9V δ 2 T cells, like NK cells, also exhibit CD16-dependent cytotoxicity, they can be mobilized by CD16-binding engagers. For example, a “tribody” binding CD16 (monovalent) and CD19 (bivalent) was shown to effectively engage both NK and $\gamma\delta$ T cells against malignant B-cell lines (RAJI, NALM-6, SEM, NAMALWA and ARH-77), with an ADCC activity that surpassed that of the therapeutic antibody rituximab.³⁷

If proven successful in clinical trials, bispecific engagers will be a powerful and important avenue for $\gamma\delta$ T cell-based immunotherapy, especially given their relative low cost when compared with adoptive cellular therapy.

Adoptive cell therapy

Expanded $\gamma\delta$ T-cell subsets

$\gamma\delta$ T cells can be isolated either from PBMCs or from tissues, such as skin (figure 1). The use of PBMCs as source of $\gamma\delta$ T cells has the clear advantages of being easier to access and providing larger numbers of cells. Selective expansion of V γ 9V δ 2 T cells from PBMCs is obtained by phosphoantigen or bisphosphonate stimulation, which given the specificity for V γ 9V δ 2 T cells, does not require $\alpha\beta$ T-cell depletion. By contrast, to expand V δ 1 T cells from peripheral blood or skin explants, an initial step of $\alpha\beta$ T-cell depletion (or $\gamma\delta$ T-cell isolation) is usually required, especially when using a pan-TCR stimulus like agonist CD3 antibody (eg, OKT3), to drive preferential V δ 1 T-cell proliferation. Cytokines are also crucial to support cell proliferation (especially IL-2 or IL-4) and their differentiation into potent cytotoxic cells (especially IL-15).⁹

Several clinical studies have been conducted over the last two decades to evaluate the safety and effectiveness of activated/expanded V γ 9V δ 2 T cells.³⁸ More recently, Xu and his team have developed a novel expansion method that involves culturing V γ 9V δ 2 T cells with zoledronate, IL-2, IL-15, and vitamin C. Compared with incubation with just zoledronate and IL-2, this protocol resulted in

more proliferation and cytotoxicity against cancer cell lines. When these cells were administered to humanized mice bearing lung tumors, their lifespan was extended. Additionally, the allogeneic V γ 9V δ 2 T cells that were expanded in vitro were tested in a phase I clinical trial involving 132 patients. Of these patients, 18 with advanced lung and liver cancer exhibited prolonged survival.³⁹ Although these findings are promising, they emphasize the importance of identifying biomarkers that can predict a response to immunotherapy based on V γ 9V δ 2 T cells.

Apart from V γ 9V δ 2 T cells, V δ 1 T cells have also been considered for therapeutic purposes. By devising a clinical-grade protocol for their expansion,⁹ our laboratory has overcome the major obstacle for using this subset in clinical application—their small numbers in the blood—while also differentiating V δ 1 T cells into more potent effectors expressing high levels of several NKRrs (including NKp30, NKG2D and DNAM-1), which we termed “Delta One T” (DOT) cells. In fact, an innovation of our methodology consisted in a two-step protocol, whereby the expansion and differentiation of the immune cells were done sequentially, given that the key ILs to promote each of these processes in V δ 1 T cells, IL-4 and IL-15, respectively (instead of suboptimal IL-2), have antagonistic actions that prevent using them simultaneously. In order to evaluate the efficacy of DOT-cell adoptive cell therapy (ACT) in vivo, multiple xenograft models have been employed. In the first study, the CLL line, MEC-1, was implanted subcutaneously in NSG mice, which in control animals led to dissemination to multiple organs, namely the bone marrow, spleen and liver. Strikingly, flow cytometry analysis and histological samples collected at the end of the experiment revealed the general absence of tumor cells in all such organs of animals treated with DOT cells. Furthermore, this study did not reveal signs of treatment-associated toxicity in any of the multiple organs analyzed.⁹

Following that report on lymphoid leukemia, we then explored the potential of DOT cells to target AML, the deadliest of hematological malignancies. During this investigation, we came across some interesting properties of DOT cells: we found them to be highly polyclonal and lacking dominant clones (at the end of the 3-week process), instead expanding naïve-like CD27⁺ V δ 1⁺ T cells with a strikingly diverse TCR repertoire, at the expense of terminally differentiated CD27⁻ V δ 1 T cells. The resulting DOT-cell products exhibited potent cytotoxicity against a panel of AML cell lines and primary samples, while sparing normal leukocyte populations (both myeloid and lymphoid). Moreover, it was also demonstrated the capacity of DOT cells to target chemoresistant AML cells, and to control AML growth in various xenograft models.^{40 41} After providing preclinical proof-of-concept for DOT cells (branded TK012 by Takeda) in blood cancers, and initiating a clinical trial in relapsed/refractory AML (NCT05886491; table 1), we are now investigating their potential to target solid tumors.

Table 1 $\gamma\delta$ T cell-based immunotherapies under evaluation in cancer clinical trials

Type of therapy	Details	Indications	Product/clinical trial
Engager	BTN3A agonist	Hematological and solid tumors	ICT01/NCT04243499, NCT05307874
	V γ 9 TCR-CD1d	Chronic lymphocytic leukemia acute myeloid leukemia (AML) and multiple myeloma (MM)	LAVA-051/NCT04887259
	PSMA-targeting bispecific $\gamma\delta$ -T cell engager	Metastatic castration-resistant prostate cancer	LAVA-1207/NCT05369000
ACT - unmodified	Expanded allogeneic V γ 9V δ 2 T cells	Lung cancer and liver cancer	NCT03183232, NCT03183219
	Expanded allogeneic V δ 1 T (DOT) cells	r/r AML	TAK012/NCT05886491
	Chemotherapy-resistant $\gamma\delta$ T cells	Glioblastoma	DRI/NCT04165941, DeltEX/NCT05664243
	Allogeneic $\gamma\delta$ T cell-enriched cells	Leukemias and myelodysplastic syndromes	EAGD/NCT03533816
	Allogeneic $\gamma\delta$ T cell-enriched cells	AML	AAPC/NCT05015426
	NK/ $\gamma\delta$ T cell-enriched cell therapy product	AML, acute lymphocytic leukemia (ALL), myelodysplastic myeloproliferative and lymphoproliferative disorders	NCT03939585
	Expanded $\gamma\delta$ T cells	non-Hodgkin's lymphoma, peripheral T-cell lymphoma	NCT04696705
	Expanded allogeneic $\gamma\delta$ T cells	r/r AML	TCB008, NCT05358808
	Expanded matched/haploidentical $\gamma\delta$ T cells	r/r AML	OmnImmune, NCT03790072
	Expanded allogeneic $\gamma\delta$ T cells	AML, ALL, myelodysplastic syndromes and lymphoma	NCT04764513
	Expanded allogeneic $\gamma\delta$ T cells	Solid tumors	NCT04765462
	Expanded allogeneic $\gamma\delta$ T cells	r/r or progressive neuroblastoma	NCT05400603
ACT - CAR transduced	NKG2D ligand-specific CAR-transduced V γ 9V δ 2 T cells	Advanced solid and hematological tumors	CTM-N2D/NCT05302037
	CD20-specific CAR-transduced V δ 1 T cells	B-cell lymphoma	ADI-001/NCT04735471, NCT04911478
ACT - $\gamma\delta$ TCR-engineered T cells	$\alpha\beta$ T-cell product retrovirally transduced with V γ 9V δ 2 TCRs	r/r MM	TEG-002/NCT04688853
	CD19-specific antibody $\gamma\delta$ TCR - transduced T cells	B-cell lymphoma	ET019003/NCT04014894
	GPC3-specific antibody $\gamma\delta$ TCR - transduced T cells	B-cell lymphoma	ECT204 T cells/NCT04864054
	Alpha-fetoprotein-specific antibody $\gamma\delta$ TCR -transduced T cells with gypican-3-specific co-stimulatory molecule	Hepatocellular carcinoma	ET140203/NCT04502082; NCT04634357

ACT, adoptive cell therapy; CAR, chimeric antigen receptor; DOT, Delta One T; NK, natural killer; PSMA, prostate-specific membrane antigen; r/r, relapsed, refractory; TCR, T-cell receptor.

CAR-transduced $\gamma\delta$ T cells

The intrinsic cytotoxic features of $\gamma\delta$ T cells and their potential to be used in the allogeneic setting (due to lack of MHC/HLA restriction), generated great interest in genetically engineering them for cancer immunotherapy.

In one of the first studies to generate CAR- $\gamma\delta$ T cells, human peripheral blood $\gamma\delta$ T cells were expanded *in vitro* in the presence of zoledronate, thus enriching for V γ 9V δ 2 T cells; and then they were transduced with recombinant retrovirus encoding GD2 or CD19-specific

chimeric receptors. Upregulation of CD69 and high amounts of secreted IFN- γ were detected after exposure to antigen-expressing tumor target cells; and Burkitt's lymphoma cells were efficiently eliminated *in vitro*.⁴²

In a subsequent study, gene transfer via Sleeping Beauty-mediated transposition was shown to lead to the sequential propagation of a highly polyclonal population of a CD19 CAR-expressing $\gamma\delta$ T cells containing both V δ 1 and V δ 2 clones. These cells secreted pro-inflammatory cytokines in response to CD19, lysed CD19⁺ tumor targets,

and exhibited anti-leukemia activity in vivo (xenografted mice).⁴³

Another research team mobilized both V δ 1 and V δ 2 T cells with a GD2-targeting CAR. Three T-cell stimulation methods, namely anti-CD3 antibody, zoledronate, and concanavalin A, were used to expand and transduce these subsets; and resulted in an increase in $\gamma\delta$ T-cell cytotoxicity against GD2-expressing cancer cell lines.⁴⁴

Focused on V γ 9V δ 2 T cells, a recent study on an MUC1-Tn-specific CAR showed similar or superior cytotoxicity, both in vitro and in vivo (against gastric cancer cell lines), when transduced into V γ 9V δ 2 T cells compared with CAR- $\alpha\beta$ T cells. However, on consecutive exposure to tumor cells, V γ 9V δ 2 T-cell cytotoxicity was lost and required IL-2 provision to be restored.⁴⁵

Rather than using an antibody recognition domain, others have employed an NKG2D CAR to target NKG2D-ligand expressing tumors, on CAR messenger RNA electroporation into V γ 9V δ 2 T cells. Although multiple injections of CAR-modified effector cells were needed to reach a considerable cytolytic activity, it allowed for better control of cytokine storms. The antitumor response was evaluated in two in vivo mouse models based on SKOV3-Luc ovarian cancer and HCT116-Luc colorectal cancer cell lines. A significant reduction in ovarian cancer was observed, but only a slight reduction was observed in colorectal cancer.⁴⁶ A clinical trial (NCT05302037) is ongoing to test this strategy in relapsed or refractory solid tumors.

V δ 1 T cells have been gaining momentum as potential host cells for CAR engineering, based on new methodologies to expand and transduce them. We have recently shown that DOT cells transduced with a CD123-CAR are more efficient than mock-transduced DOT cells in controlling patient with AML-derived xenografts, particularly on tumor rechallenge.^{41 47} Similarly, anti-CD20 CAR-transduced V δ 1 cells also showed high cytotoxicity in vitro studies, not only in B-cell lymphoma cell lines but also in rituximab resistant cell lines. In addition, this study demonstrated tumor control in xenograft models (Raji and JVM-2),⁴⁸ and led Adicet Bio to evaluate the clinical activity of CD20 CAR-V δ 1 T cells against B-cell malignancies (NCT04735471, NCT04911478).

Another V δ 1-based CAR T-cell product developed by Adicet Bio and Regeneron consists of a glypican-3-specific CAR-V δ 1 T cell, engineered to produce soluble IL-15.⁴⁹ It is relevant because IL-15 bestowed a proliferative edge, enhanced toxicity over a protracted period, and superior antitumor activity in a hepatocellular carcinoma (HepG2) xenograft model.⁴⁹ Despite the constitutive expression of soluble IL-15, the authors observed no clinical signs (up to at 45 days post-treatment) of graft-versus-host disease in tissues (liver, skin, lung, and spleen) of mice receiving CAR-V δ 1 T cells.⁴⁹

$\gamma\delta$ TCR-engineered T cells

An alternative approach consists, not in the use of $\gamma\delta$ T cells themselves, but their TCR. Marcu-Maligna and

colleagues have transduced $\alpha\beta$ T cells with a V γ 9V δ 2 TCR, which then required pamidronate for cytokine secretion and leukemia growth control in vivo.⁵⁰ Based on this research, Gadeta developed ($\alpha\beta$) T cells transduced with gamma-delta TCR (“TEG”) that were shown to target a broad range of solid and hematological tumors, while also inducing functional maturation of DCs^{13 51 52} and initiated a phase I clinical trial in patients with MM (NCT04688853).

Eureka Therapeutics has created an artificial receptor, known as ARTEMIS, by combining an antigen-binding region of an antibody (a Fab fragment) with the signaling part of a $\gamma\delta$ TCR, resulting in an antibody-T-cell receptor (AbTCR). To achieve this, a segment of the heavy chain domain of the Fab fragment is fused to a part of the δ chain, while the light chain domain is fused to a portion of the γ chain. By means of lentiviral transduction, conventional T cells can receive this synthetic receptor, which then interacts with the CD3 complex in order to activate T cells. The use of a $\gamma\delta$ TCR instead of an $\alpha\beta$ TCR has several advantages, including MHC-independence and the higher affinity for the CD3 complex, which leads to more enhanced signaling, as well as the absence of pairing with the host cells’ endogenous $\alpha\beta$ TCR, thus avoiding the need to disrupt the endogenous TCR. In vitro and in vivo studies with Raji tumor cells showed that anti-CD19 AbTCR-T cells had the same antitumor capacity as conventional CAR-T cells bearing the same anti-CD19 binder, but AbTCR-T cells were less likely to secrete inflammatory cytokines, thus reducing the risk of cytokine release syndrome.⁵³ While transferring this technology to the clinic, Liu and colleagues further added a single-chain variable fragment (scFv)/CD28 co-stimulatory molecule to boost activation, and the so-called “ET019003” cells produced durable complete responses in six out of eight patients with relapsed or refractory diffused large B-cell lymphoma (NCT04014894). In addition, the authors created a new version of AbTCR design that uses an antibody fragment recognizing a biomarker of hepatocellular carcinoma, alpha-fetoprotein peptide, which is presented by HLA-A*02. To enhance the efficacy of the construct against tumor cells, they combined it with an scFv/CD28 co-stimulatory molecule, which is specific for glypican-3, a molecule that is usually overexpressed in liver cancer. In an HepG2-xenografted mouse model, these cells led to a significant reduction in tumor load.⁵⁴ Furthermore, among the first six patients treated with these “ET140203 cells”, two experienced partial responses and one (that had lung metastases) experienced a complete response. There are two ongoing clinical trials further testing this product in more patients with hepatocellular carcinoma (NCT04502082, NCT04634357; table 1).

CONCLUSION

Human $\gamma\delta$ T cells are associated with favorable prognostic in most cancer types, especially when considering V δ 1⁺ cells.^{55 56} These naturally home to mucosal tissues and

are often enriched within carcinomas, whereas $V\delta 2^+$ cells predominate in the blood and lymphoid organs. This interesting dichotomy offers two avenues to explore at the therapeutic level in diverse cancer types. As a result, the adoptive transfer of either $V\delta 1^+$ or $V\delta 2^+$ cells, activated and expanded in vitro, is being evaluated in the clinic, often combined with their engineering with specific TCRs or CARs. Alternatively, cell engagers directed at one of these $\gamma\delta$ T-cell subsets are to be tested for their ability to mobilize enough effectors into the tumor mass(es) and drive clinical responses (figure 1).

Compared with conventional $\alpha\beta$ T cells, $\gamma\delta$ T cells offer as advantages their independence on MHC class I-mediated antigen presentation, which makes them applicable in MHC I-deficient tumors^{29,30} and better suited to allogeneic cell therapy. Furthermore, their antitumor functions are independent of somatic mutational load, rendering them efficacious against many cancers that are much less mutated than melanoma or lung carcinoma.³⁰

On the other hand, $\gamma\delta$ T cells also offer advantages over NK cells, such as their increased ability (especially of $V\delta 1^+$ cells) to infiltrate tumors; and the low expression of critical inhibitor molecules, namely killer inhibitory receptors, that interfere with the activation of NK cells.⁵⁷ In fact, $\gamma\delta$ T cells can be regarded as combining “the best of both worlds”, that is, antitumor properties of $\alpha\beta$ T cells and NK cells, namely the capacity to deploy both TCR and NK cell receptors to increase the potential and breath of cancer targeting. As such, we anticipate that ongoing clinical trials will soon produce positive results to enable $\gamma\delta$ T cell-based immunotherapies for the benefit of many patients with cancer.

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