The emerging role of γδ T cells in cancer immunotherapy

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A B S T R A C T

The recent successes of chimeric antigen receptor T cells in the treatment of hematological malignancies have clearly led to an explosion in the field of adoptive cell therapy for cancer. Current efforts are focused on the translation of this exciting technology to the treatment of solid tumors and the development of allogeneic 'off-the-shelf' therapies. γδ T cells are currently gaining considerable attention in this field as their unique biology and established role in cancer immunosurveillance place them in a unique position to potentially overcome these challenges in adoptive cell therapy. Here, we review the relevant aspects of the function of γδ T cells in cancer immunity, and summarize clinical observations and clinical trial results that highlight their emerging role as a platform for the development of safe and effective cancer immunotherapies.

Introduction

There is striking evidence that in addition to our adaptive immune system, the innate immune system deals with malignant cells long before visible tumor development. Special interest in this matter is given to the unconventional group of γδ T cells that share all cytotoxic features with αβ T cells but also possess innate-like features, including the expression of various natural killer cell receptors (NCRs) [1]. Evolutionary highly conserved, γδ T cells are unique in that they recognize a variety of antigens [2] in a major histocompatibility complex (MHC)-unrestricted fashion, mature in the thymus and retain a preactivated state, meaning they do not require clonal expansion or differentiation into an effector T cell phenotype upon activation [3]. Showing strong enrichment in epithelial tissues, these cells have adopted efficient ways to monitor other cells for abnormal changes in their physiology in tissues and blood — a function that has been summarized as the 'lymphoid stress-surveillance response' [4,5].

Unique contributions by γδ T cells to broader immunological processes, such as pathogen recognition and clearance [6], attraction and maturation of antigen-presenting cells [7] and direct stimulation of αβ T cells via direct antigen presentation [8], are well established. In addition, γδ T cells contribute to tissue homeostasis and wound healing [9]. Most striking is the phenotype of mice that lack the entirety or specific subtypes of γδ T cells. T cell receptor (TCR) δ chain knockout mice show a significant increase in the occurrence of papillomas which develop into visible tumor development [10]. This increase in malignant events is not shared by mice that lack αβ T cells [11]. Similar protective effects by γδ T cells have been validated in models of colorectal cancer [12], malignant melanoma [13], B cell lymphoma [14] and prostate cancer [15]. These important contributions to tissue homeostasis and cancer immunosurveillance [16] have fuelled scientific interest to further explore the biology of γδ T cells and their potential for clinical translation [17,18].

Self-surveillance, natural killer receptor NKG2D and other NCRs

The activating cell surface receptor NKG2D and its ligands play an important role in cytotoxic immune responses of natural killer (NK) cells, NK-T cells and γδ T cells against tumors [19]. Ligands for NKG2D include MHC class I polypeptide-related sequence A and B (MICA/B) and several UL16-binding proteins (ULBPs) that are poorly expressed in normal tissues but are strongly upregulated in stressed or transformed cells [20]. This stress signal can be induced via the DNA repair response after ultraviolet exposure [21], oncogenes such as Ras [22], osmotic shock and/or oxidative stress via epidermal growth factor receptor signalling [23]. Moreover, MICA can also be upregulated via pharmacological manipulation of the mevalonate pathway [24]. In mice, γδ T cells protect the skin from tumors by responding to increased expression of the MICA homologue Rae1 [25]. Remarkably, this protective contribution not only involves direct cytotoxicity, but also the production of interleukin (IL)-13 [26] and modulation of B cells promoting immunoglobulin E class switching and the accumulation of autoreactive antibodies [27]. Mice...
lacking NKG2D show more susceptibility to spontaneous development of prostate cancer [28], and T cells and NK cells rapidly clear malignant cells injected into mice when they express NKG2D ligands [29]. In human carcinomas of the lung, breast, kidney, ovary, prostate and colon, NKG2D ligands are widely expressed and prompt responses from tumor-infiltrating autologous Vδ1 T cells [30]. In lung cancer, single nucleotide polymorphisms of MICA influence not only disease progression but also susceptibility to platinum chemotherapy [31]. Lung cancer cells that express MICA are recognized and killed by NK cells [32], and in patients with head and neck cancer, the use of cetuximab causes NKG2D− NK cells to recognize MICA, which induces a tumor antigen-specific adaptive response through dendritic cell maturation and consequent activation of cytotoxic T lymphocytes [33]. The activating NCRs Nkp30, Nkp44 and Nkp46 are also expressed on human Vδ1 T cells after activation and costimulation with cytokines enhancing the production of interferon gamma (IFNγ) [34]. Furthermore, the engagement of Nkp30 and Nkp44 on Vδ1+ T cells promotes the recognition and killing of leukemia cells and correlates with increased granzyme expression [35]. Not limited to cytotoxicity alone, activation of Nkp30 on Vδ1+ T cells induces production of the CC chemokine ligands CCL3, CCL4 and CCL5, linking target recognition with the attraction of antigen-presenting cells such as monocytes and conventional αβ T cells [36]. NCRs have also been shown to bind to self-proteins expressed on malignant or stressed cells; for example, binding of B-associated transcript 3 [37] or B7-H6 [38] on target cells by Nkp30 renders these cells prone to killing by NK cells. Similarly, the multiplicity of NCRs expressed [34], especially on intra-epithelial γδ T cells [39], is expected to enable these cells to respond to markers of dysregulation and stress immediately where they reside [4,25].

The impact of other NK cell-associated inhibitory receptors on γδ T cells (e.g. CD94 heterodimers with NKG2A), which have been shown to be strongly inhibitory for NK cells and conventional cytotoxic αβ T cells in tumors [40], remains unclear as reports investigating the expression on γδ T cells and modulation of function through NKG2A are currently lacking.

γδ T cells in humans: same but different

Human T cells expressing a γδ TCR show functional similarities with mice in that they are highly capable and primed killer cells that almost exclusively produce IFNγ upon activation [41–43]. However, there are fundamental differences between γδ T cells in humans and mice. For example, the signature subset of mouse dendritic epidermal T cells is completely absent in humans, most likely due to a premature stop codon in the Vγ5 selecting protein Skint-1 [44]. Other γδ T cell-specific tissue-selecting proteins do show conservation between mice and humans, namely butyrophilin-like (Btnl) 1/6 in mice and BTN3L3/8 in humans, selecting mouse Vγ7 T cells into the intestinal epithelium or human Vγ4 T cells into the colonic epithelium, respectively [45]. Fascinatingly, these interactions of γδ TCRs and BTNls happen through germline-encoded regions of the TCR, allowing for additional binding of clone-specific antigens through the complementarity-determining regions 1–3 [46].

A striking functional difference in mice is that γδ T cells develop into two functional lineages in the thymus that produce high levels of either INFγ or IL-17 upon activation [47]; the latter is abundant in the dermis, together with its INFγ-producing counterpart. IL-17 producing γδ T cells have been shown to have undesirable effects on tumor growth and promotion in mouse models of breast cancer [48] and ovarian cancer [49]. Although there has been a report of human γδ T cells producing IL-17 in colorectal cancer [50], humans lack the mouse counterpart of the dedicated IL-17γδ T cells at steady state, which is identified by the lack of CD27 expression in mice.

The main difference between γδ T cells in humans and mice is the fact that humans, among other primates, have an additional subset of γδ T cells which express a Vδ9 chain paired to a Vβ2 chain to form the TCR [51]. Rodents completely lack this type of invariant T cell, whereas in humans, this cell type dominates the composition of γδ T cell subtypes in the blood, representing up to 5% of all T cells (Figure 1).

T cells expressing the Vδ2Vγ9 TCR recognize the bacterial metabolite (E)-4-hydroxy-3-methyl-but-2-enyl and show cross-reactivity with the
malignant cells but not normal tissues [55]. Targeting the mevalonate pathway using aminobisphosphonates (N-bis) (e.g. zoledronic acid, which is commonly used in the treatment of osteoporosis) results in accumulation of IPP in cancer cells, thereby further increasing the immunogenicity of cancer cells towards Vδ2 T cells [5]. Moreover, it has been demonstrated that γδ T cells respond to various mevalonate pathway intermediates; this process is influenced by stress-related cytokines [56,57]. Interestingly, the recognition of phosphoantigens by Vδ2Vγ9 T cells involves the modulation of BTN3A1, 2 and 3 [58,59], further supporting the idea of γδ T cell regulation and activation via the family of butyrophilin and butyrophilin-like molecules [60,61].

The above functional aspects of Vδ2 T cell biology and the fact that these cells can easily be grown and expanded ex vivo using N-bis [62] and synthetic phosphoantigens (pAgs), such as bromohydrin pyrophosphate (BrHPP) [63], have motivated investigators to exploit Vδ2 T cells for cancer immunotherapy.

Clinical experiences with Vδ2+ T cell immunotherapy in cancer

Two strategies of Vδ2+ T cell cancer immunotherapy have been developed and applied. The first is to stimulate and expand Vδ2 T cells in vivo by systemic administration of pAgs or N-bis. This approach has been tested in eight pilot/phase 1 clinical trials in hematological malignancies and solid tumors over the last years (Table 1). The use of BrHPP or N-bis (pamidronate or zoledronate), mainly in combination with IL-2, was found to be safe and resulted in Vδ2 T cell expansions in vivo and/or maturation towards an IFN-γ-producing effector phenotype in most patients. Eight out of a total of 121 patients (7%) showed objective responses, but no complete responses were observed.

The second approach that has been clinically applied is the adoptive transfer of autologous Vδ2+ T cells after ex vivo expansion using synthetic pAgs or N-bis. Infusion of ex vivo expanded autologous Vδ2+ T cells alone or in combination with BrHPP or zoledronate and IL-2 was well tolerated across nine different clinical trials (Table 2), and resulted in six objective responses (8%; n=86) and two complete responses (2%; n=86) in total. Intraperitoneal injections of ex vivo expanded autologous Vδ2+ T cells in combination with zoledronate for the treatment of malignant ascites have been reported for seven patients with gastric cancer, resulting in a significant reduction in the number of tumor cells in the ascites and a significant reduction in the volume of ascites in two patients [64].

Allogeneic Vδ2+ T cells have also been used as part of a more heterogeneous cell population in a small pilot study [65]. Four patients with advanced refractory hematological malignancies received CD4+/CD8+–depleted infusions of haploidentical leukapheresis products highly enriched for Vδ2+ T cells after lymphodepleting chemotherapy with cyclophosphamide and fludarabine. A marked in vivo expansion of donor Vδ2+ T cells was observed in all patients without any signs of graft versus host disease (GVHD). Although refractory to all prior therapies, three of four patients achieved complete remissions, which lasted for 8 months in a patient with plasma cell leukemia.

Most recently, Alnaggar et al. [66] published a case report of a patient with stage IV cholangiocarcinoma showing recurrent mediastinal lymph node metastasis after liver transplantation. The patient received eight consecutive infusions of allogeneic Vδ2+ T cells that were expanded from peripheral blood mononuclear cells (PBMCs) of a healthy donor. No adverse effects were observed after cell infusion, and the authors reported a complete response with no detectable peritoneal lymph node metastasis at the end of treatment.

In summary, these clinical results clearly demonstrate that Vδ2+ T cell-based immunotherapy is safe and well tolerated, but the signs of clinical efficacy are highly variable. This might be explained by the very heterogeneous group of diseases treated and the variation in protocols used for ex vivo or in vivo expansion of Vδ2+ T cells, or in the variability of treatment regimens applied in these studies. In vivo activation of Vδ2+ T cells by pAgs or N-bis clearly resulted in activation of circulating Vδ2+ T cells, but no study could provide evidence that this approach also

| Table 1 |
| Pilot/Phase 1 trials evaluating safety and clinical activity of Vδ2 T cells in vivo activation of Vδ2 T cells |
| Year | Disease | Treatment | n | OR | CR | Reference |
| 2003 | MM | Pamidronate + IL-2 | 19 | 3/0 | 19 | 94 |
| 2003 | Prostate cancer | Zoledronate | 9 | 0/0 | 9 | 95 |
| 2007 | Prostate cancer | Zoledronate + IL-2 | 18 | 3/0 | 18 | 96 |
| 2010 | Breast cancer | Zoledronate + IL-2 | 10 | 0/0 | 10 | 97 |
| 2010 | RCC | BrHPP + IL-2 | 28 | 0/0 | 28 | 98 |
| 2011 | RCC | Zoledronate + IL-2 | 12 | 0/0 | 12 | 99 |
| 2012 | RCC | Zoledronate + IL-2 | 21 | 2/0 | 21 | 100 |
| 2016 | Neuroblastoma | Zoledronate + IL-2 | 4 | 0/4 | 4 | 101 |

| MM, multiple myeloma; NHL, non-Hodgkin lymphoma; RCC, renal cell cancer; AML, acute myeloid leukemia. |

| Table 2 |
| Pilot/phase 1 trials evaluating safety and clinical activity of adoptively transferred autologous ex vivo-expanded Vδ2 T cells |
| Year | Disease | Treatment | n | OR | CR | Reference |
| 2007 | RCC | Vδ2 T cells + zoledronate + IL-2 | 7 | 3/0 | 7 | 102 |
| 2008 | RCC | Vδ2 T cells + BrHPP + IL-2 | 10 | 0/0 | 10 | 103 |
| 2009 | MM | Vδ2 T cells + zoledronate + IL-2 | 6 | 0/0 | 6 | 104 |
| 2010 | NSCLC | Vδ2 T cells + zoledronate + IL-2 | 10 | 0/0 | 10 | 105 |
| 2011 | RCC | Vδ2 T cells + zoledronate + IL-2 | 11 | 1/1 | 11 | 106 |
| 2011 | Melanoma | Vδ2 T cells + zoledronate | 18 | 3/1 | 12 | 107 |
| 2011 | Colon cancer | Vδ2 T cells + zoledronate | 12 | 12 | |
| 2011 | NSCLC | Vδ2 T cells + zoledronate + IL-2 | 15 | 0/0 | 12 | 108 |
| 2013 | Colon cancer | Vδ2 T cells | 6 | 0/0 | 6 | 109 |
| 2014 | NSCLC | Vδ2 T cells | 15 | 0/0 | 12 | 110 |
| 2014 | Gastric cancer | Vδ2 T cells + zoledronate | 7 | |

| BrHPP, bromohydrin pyrophosphate; CR, complete response; IL, interleukin; MM, multiple myeloma; NSCLC, non-small cell lung cancer; OR, objective response; RCC, renal cell cancer. |
resulted in activation of the small number of tissue-resident \( \gamma \delta \) T cells or resulted in recruitment of \( \gamma \delta \) T cells from the circulation to the tumor site. In addition, \( \gamma \delta \) T cells are dysfunctional in some cancer patients, are susceptible to activation-induced anergy, and repeated stimulation of \( \gamma \delta \) T cells may induce terminal differentiation and exhaustion [67–69]. This might explain why the adoptive transfer of ex vivo expanded \( \gamma \delta \) T cells seems to be the more effective approach resulting in complete responses in some patients. Strikingly, four of five patients treated with allogeneic \( \gamma \delta \) T cells showed complete responses, compared with only two complete responses observed in 98 patients treated with autologous \( \gamma \delta \) T cells. Although the number of patients treated with allogeneic cells is too small to draw definitive conclusions, these results might indicate that allogeneic \( \gamma \delta \) T cells expanded from healthy donors have a functionally superior phenotype compared with autologous patient-derived \( \gamma \delta \) T cells. The fact that allogeneic \( \gamma \delta \) T cells do not induce GvHD, together with the possibility to generate large numbers and batches of cells from a single healthy donor, will certainly advance the use of healthy donor-derived \( \gamma \delta \) T cells in future clinical studies.

**Role of \( \gamma \delta \) T cells in cancer**

The fact that mice are protected from malignant events by tissue-resident \( \gamma \delta \) TCR chain-expressing T cells and lack the \( \gamma \delta \) T cell subtype entirely has sparked great interest in studying the tumor-protective role of \( \gamma \delta \) T cells in human cancer. Human tissues contain large numbers of \( \gamma \delta \) T cells, especially the intestine, colon and dermis [3,46], but preclinical research on \( \gamma \delta \) T cells was held back in the past by a lack of imaging reagents to discriminate \( \gamma \delta \) T cells from \( \alpha \beta \) T cells and, more importantly, to differentiate \( \gamma \delta \) T cells from \( \gamma \delta \) T cells. Although commercial antibodies to stain \( \gamma \delta \) T cells in tissues are available [45,70], we still rely on tissue digestion or PBMC isolation and flow cytometry to identify \( \gamma \delta \) T cell clonotypes.

Several studies have shown that \( \gamma \delta \) T cells are an important component of tumor-infiltrating lymphocytes (TILs) in patients with different types of cancer, and a recent analysis of \( \sim 18,000 \) transcriptomes from 39 human tumors identified tumor-infiltrating \( \gamma \delta \) T cells as the most significant favorable cancer-wide prognostic factor. The same study also showed NKG2D to be positively associated with better outcome [71]. Although this study could not discriminate between \( \gamma \delta \) and \( \gamma \delta \) T cells, other studies showed that \( \gamma \delta \) T cells represent the predominant tumor-infiltrating \( \gamma \delta \) T cell subtype [72,73].

More direct clinical evidence to support the tumor-protective features of \( \gamma \delta \) T cells comes from a larger study in patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) who received \( \gamma \delta \) T cell-depleted bone marrow grafts from partially human leukocyte antigen (HLA)-mismatched donors [74]. Disease-free survival at 30 months post transplant was significantly better in those patients in whom the percentage of \( \gamma \delta \) T cells exceeded 10% of the total lymphocyte count in the blood. No significant difference in the incidence of acute or chronic GvHD was observed, suggesting an enhanced graft versus leukemia effect in the absence of GvHD. In an extended 42-month follow-up study, these data were confirmed [75], and a further 8-year follow-up study with additional patients (\( n = 153 \)) showed significantly better 5-year leukemia-free survival and overall survival for patients who recovered with an increased proportion of \( \gamma \delta \) T cells [76]. The expanded \( \gamma \delta \) T cell subtype in \( > 90\% \) of long-term survivors in this study was predominantly \( \gamma \delta \), suggesting that these cells were involved in long-term clearance of leukemia.

Increases in \( \gamma \delta \) T cells have also been correlated with cytomegalovirus (CMV) reactivation in patients with leukemia following allogeneic hematopoietic stem cell transplantation (HSCT) [77,78]. When isolated, these \( \gamma \delta \) T cells not only kill CMV-infected cells but also leukemic cells and other tumor cells in vitro via HLA- and NKG2D-independent mechanisms [78,79]. Moreover, \( \gamma \delta \) T cells that are specifically expanded in patients with CMV reactivation are more cytotoxic against primary ALL and AML cells compared with \( \gamma \delta \) T cells from patients without CMV reactivation [80]. This may explain, at least in part, the favourable effect of CMV reactivation after HSCT on the risk of relapse [81], further supported by a 2–6-year follow-up study in patients after kidney transplantation, where expanding numbers of \( \gamma \delta \) T cells associated with CMV reactivation strongly correlated with a significantly reduced occurrence rate of malignancies [82].

Taken together, these data warrant clinical testing of \( \gamma \delta \) T cells as a novel effector cell type for cancer immunotherapy, and the period following HSCT in patients with leukemia seems to be a promising therapeutic window for adoptive transfer of \( \gamma \delta \) T cells to prevent relapse. However, the lack of clinical-grade protocols to selectively expand \( \gamma \delta \) T cells in vivo or ex vivo has prevented the conduct of clinical trials to harness the therapeutic potential of \( \gamma \delta \) T cells to date.

**V\( \gamma \delta \) T cells and their development for cancer immunotherapy**

The use of \( \gamma \delta \) T cells for preclinical research and clinical development is currently limited to isolation of very small cell numbers from human PBMCs or isolation from human tissues using enzymatic digestion or alternative methods. \( \gamma \delta \) T cells expanded from blood using a combination of IL-7 and phytohemaglutinin controlled tumor growth in an NSG mouse model for colon cancer much better than \( \gamma \delta \) T cells [83], but this protocol is not applicable for clinical-grade expansion of \( \gamma \delta \) T cells. A system using genetically modified antigen-presenting cells linked to anti-\( \gamma \delta \) TCR antibodies generated a mixed population of expanded \( \gamma \delta \) T cells comprising \( \gamma \delta \) T cells and non-\( \gamma \delta \) T cells. Whilst all \( \gamma \delta \) T cells in this system exerted cytotoxicity against GD2-expressing neuroblastoma

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**Table 3**

| Company          | Modality | T-cell type | Source | Autologous/allogeneic | Engineering | Comments                        |
|------------------|----------|-------------|--------|-----------------------|------------|---------------------------------|
| Adipect Bio      | Cell therapy | V\( \delta \) | Blood  | Allogeneic          | CAR        | -                               |
| Beijing Dojing Biomedical | Cell therapy | V\( \delta \) | Blood  | Autologous           | Unmodified/CAR | -                               |
| Cytomed Therapeutics | Cell therapy | V\( \delta \) | Blood  | Allogeneic          | CAR        | -                               |
| Gadeta           | Cell therapy | V\( \delta \) | Blood  | Autologous          | V\( \delta \) TCR | -                               |
| GammaCell Biotechnologies | Cell therapy | V\( \delta \) | Blood  | Autologous/allogeneic | Unmodified          | -                               |
| GammaDelta Therapeutics | Cell therapy | V\( \delta \) | Skin/blood | Allogeneic          | Unmodified/CAR | -                               |
| Hebei Senlang Biotechnology | Cell therapy | V\( \delta \) | Blood  | Autologous          | CAR/\( \alpha \beta \) TCR | -                               |
| Immatics         | Cell therapy | V\( \delta \) | Blood  | Allogeneic          | \( \alpha \beta \) TCR | -                               |
| Incysys          | Cell therapy | V\( \delta \) | Blood  | Autologous          | Engineered | Engineered for chemotherapy resistance |
| Therapeutics     | Cell therapy | V\( \delta \) | Blood  | Autologous          | -          | -                               |
| Phosphogam       | Cell therapy | V\( \delta \) | Blood  | Allogeneic          | Unmodified          | -                               |
| TC BioPharm      | Cell therapy | V\( \delta \)/V\( \delta \) | Blood  | Autologous/allogeneic | Unmodified/CAR | -                               |
| Imcheck Therapeutics | Antibodies | V\( \delta \) | -      | -         | -          | Activation of V\( \delta \) T cells (BTNA3A) |
| Lava Therapeutics | T-cell engager | V\( \delta \) | -      | -         | -          | Redirection of V\( \delta \) T cells against tumors |
| Nyho             | Antibodies | Pan \( \gamma \delta \) | -      | -         | -          | Depletion of inhibitory \( \gamma \delta \) T cells |

**Notes:**

- CAR, chimeric antigen receptor; TCR, T cell receptor.
cells, Vδ2+ T cells relied on antibody-dependent cellular cytotoxicity via the expression of CD16, whilst non-Vδ2+ T cells, including Vδ1+ T cells, did not [84]. A more easily translatable system for the expansion of Vδ1+ T cells specifically, using a combination of common γ chain cytokines and the CD3 engaging antibody OKT3, was developed recently [35]. These Vδ1+ T cells show favourable expression of NCRs, exhibit cytotoxicity against hemagglutination tumor lines in vitro and show the capacity to infiltrate the tumor core, bone marrow, liver and spleen thus controlling tumor growth over several weeks in an NSG mouse model of subcutaneously induced chronic lymphoid leukemia [85]. These blood-derived and expanded Vδ1+ T cells show cytotoxicity against primary, patient-derived AML cells that are resistant to chemotherapy. Whilst the mechanism of recognition and killing most likely did not depend on the TCR, it was dependent on the expression of NKp30 and B7-H6 on target cells. Adoptive transfer of Vδ1+ T cells into human AML xenograft mice improved survival significantly, decreasing tumor load in the blood and target organs [86].

Although human clinical studies testing the safety and efficacy of enriched and purified preparations of autologous or allogeneic Vδ1+ T cells have yet to be conducted, patients have been treated with high numbers of Vδ1+ T cells as part of a more heterogenous cell population. Adoptive transfer of autologous TILs has shown impressive clinical results in patients with metastatic melanoma. The efficacy of this personalized immunotherapy based on preconditioning chemotherapy followed by infusion of TILs and IL-2 has been confirmed in several independent studies. Objective response rates of 40–50%, including complete tumor regressions in 10–20% of treated patients, have been reported consistently [87,88]. In metastatic melanoma, Vδ1+ T cells can represent the major TIL subset, accounting for ~50% of the total CD8+ population [72]. Indeed, detectable amounts of Vδ1+ T cells in clinical-grade TIL preparations were found in 20 of 27 patients analysed in a recent study of adoptive TIL transfer, and infusion products from 10 patients contained, on average >1 x 10^6 of these cells [89]. Notably, one patient achieving a complete response was infused with 7.8% Vδ1+ T cells, approximately 6.5 x 10^6 cells in total. Since all cell products also contained CD8+ T cells, no conclusions can be drawn on the contribution of Vδ1+ T cells to the clinical antitumor activity observed, but when tested, these cells showed high cytotoxicity against melanoma cells in vitro. In summary, infusion of Vδ1+ T cells together with high numbers of αβ T cells in a clinical trial was safe and well tolerated, and the authors concluded that Vδ1+ T cells should be further scrutinized as a potentially useful tool for the treatment of patients with metastatic melanoma.

Conclusion and outlook

Harnessing the unique biology of γδ T cells for cellular or targeted immunotherapy holds considerable promise for the treatment of different types of cancer. First, γδ T cells do not recognize and kill tumor cells dependent on the expression of a single antigen. In contrast, they recognize most cancer types through a broad pattern of different NCRs expressed on their cell surface in a non-clonally expanded fashion, minimizing tumor immune escape mediated by single antigen loss. Second, γδ T cells distribute and reside in abundance within tissues. The natural tissue tropism of γδ T cells, especially Vδ1+ T cells, could give these cells an advantage over conventional αβ T cells to migrate into tissues and infiltrate solid tumors more efficiently and execute their functions in more hypoxic environments. Third, the ability to recognize target cells in an MHC-independent manner and the low risk for all-reactivity will allow the development of allogeneic cell products without the need for further genetic engineering. Finally, γδ T cells have been shown to interact with antigen-presenting cells and other members of the adaptive immune system, enabling the orchestration of secondary immune responses post activation.

Whilst these combined features of γδ T cells make them an attractive source for unmodified cell-based adoptive immunotherapy approaches, γδ T cells may also be harnessed for genetic manipulation. Either as a vehicle for chimeric antigen receptors (CARs) or αβ T cell-derived TCRs [90], γδ T cells could combine tissue resident biology and innate target recognition with antigen-specific activation and selection. Furthermore, a better understanding of γδ T cell interaction with BNT/BNTL molecules, as well as their regulation and activation in normal tissues and tumors, may allow for therapeutic manipulation in situ using targeted or checkpoint therapies.

Not surprisingly, several companies are now developing next-generation γδ T cell immunotherapies (Table 3). Most of these approaches focus on Vδ1+ T cells as a platform for autologous or allogeneic cell therapies engineered to express CARs. Alternative methods of expanding and/or activating Vδ2+ T cells in vivo that do not depend on the administration of N-bis or pAgs are also in development. A better understanding of Vδ2+ TCR biology and the role of BTN3A in Vδ2+ T cell activation has led to the development of activating antibodies that potentially eliminate the need for TCR overstimulation [91]. Other approaches include the redirection of Vδ2+ T cells towards specific tumor antigens using bispecific Vδ2+ T cell-engaging molecules [92], or genetically engineered chemotherapy resistance of Vδ2+ T cells that can be administered during the therapeutic window when chemotherapy increases the immunogenicity of tumors by upregulating NKGD2 ligands [93].

Moreover, advances in the isolation and expansion of Vδ1+ T cells from blood and the very first protocol to isolate and grow tissue-resident Vδ1+ T cells in large numbers for clinical application (authors’ unpublished data) have paved the way to add Vδ1+ T cells to the growing armamentarium of cancer immunotherapy.

It will be exciting to see these different approaches being tested in clinical trials over the coming years to prove that γδ T cells provide a safe and effective platform for allogeneic ‘off-the-shelf’ cell therapies for cancer.

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