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Harnessing the power of Vδ2 cells in cancer immunotherapy

D. W. Fowler and
M. D. Bodman-Smith
Infection and Immunity Research Institute, St. George's University of London, London, UK

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Correspondence: D. W. Fowler, Infection and Immunity Research Institute, St George's University of London, Cranmer Terrace, Tooting, London SW17 0RE, UK.
E-mail: dfowler@sgul.ac.uk

Summary

γδ T cells are a subset of T lymphocytes that have been implicated in immunosurveillance against infections and tumours. In the peripheral blood of humans the γδ T cell pool is made up predominantly of Vδ2 cells, which can detect both foreign and self-metabolites of the isoprenoid biosynthesis pathway. This unique axis of antigen recognition enables Vδ2 cells to respond to a range of pathogenic infections as well as perturbations in endogenous isoprenoid biosynthesis that can occur during cell stress and malignant transformation. There has been growing interest in Vδ2 cells as a potential avenue for cancer immunotherapy, and a number of strategies have been utilized in an attempt to boost the anti-tumour response of Vδ2 cells in patients. In this review we discuss critically the evidence that Vδ2 cells contribute to the cytotoxic response against tumours and evaluate current immunotherapeutic approaches that target these cells in cancer patients, with specific focus on their shortcomings and how they may be improved.

Keywords: cancer immunotherapy, γδ T cells, tumour immunology

Introduction

γδ T cells are a unique subset of lymphocytes that express T cell receptors (TCRs) composed of γ and δ chains. In humans, two subsets of γδ T cell predominate, defined by the variable domain of their δ chain. $V\delta2^+$ γδ T cells are the most abundant subset found in peripheral blood, and are often regarded as sentinels against infection, whereas $V\delta1^+$ γδ T cells are the most abundant subset in mucosal epithelia, forming part of the protective barrier against invading pathogens [1]. In addition, both subsets have been implicated in immunosurveillance against tumours [2–4], and thus manipulation of γδ T cells in order to enhance their anti-tumour properties is a potential approach to cancer immunotherapy. To date, research has focused on peripheral blood Vδ2 cells because this subset has potent reactivity against tumours and is readily accessible. In human peripheral blood, Vδ2 cells typically constitute 1–5% of the total T cell population [5], and all these cells have the potential to target tumour. This is a large pool of tumour-reactive cytotoxic cells compared with their peptide-specific αβ T cell counterparts, and thus a promising cell population to exploit in cancer immunotherapy. In this review we will discuss the current evidence that Vδ2 cells function as anti-tumour immune cells, paying particular attention to the limitations encountered thus far. We will then assess the therapeutic approaches that have been employed to effectively bolster their anti-tumour activity, and discuss how these treatment strategies may be enhanced.

Vδ2 cell tumour recognition

Within the peripheral blood population of Vδ2 cells in humans there are three functionally distinct subsets: naive ($CD45RA^+CD27^+$), central memory ($CD45RA^-CD27^+$) and effector memory ($CD45RA^-CD27^-$) [6]. In response to antigenic stimulation, naive and central memory Vδ2 cells proliferate, with central memory cells displaying a higher magnitude of response compared to naive cells. In contrast, effector memory Vδ2 cells display low levels of proliferation but are potent producers of cytokines such as interferon (IFN)-γ and tumour necrosis factor (TNF)-α. At sites of inflammation, Dieli *et al.* identified a fourth terminally differentiated subset ($CD45RA^+CD27^-$), which displays potent cytotoxicity against target cells. The majority of peripheral

blood Vδ2 cells express the inflammatory homing chemokine receptor CCR5/CD195 and lack expression of the lymph node homing chemokine receptor CCR7/CD197, and thus home to sites of inflammation that are expressing the CCR5/CD195 ligands CCL3, CCL4 and CCL5 [7,8]. These characteristics suggest that Vδ2 cells are patrolling the periphery, awaiting the appropriate migratory signals that will direct them to sites of inflammation where they rapidly proliferate and produce cytokines, and subsequently differentiate into cytotoxic cells that kill infected and/or malignant cells.

Underpinning the immune responses of human Vδ2 cells is a unique system of TCR-mediated antigen recognition that is seemingly unique to primates. The Vδ2 cell TCR, which is composed typically of Vδ2 chains paired with Vγ9 chains, recognizes small phosphate-rich metabolites of the isoprenoid biosynthesis pathway, namely (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) of the 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway and isopentenyl pyrophosphate (IPP) of the mevalonate pathway [9]. Purified forms of these phosphoantigens have been shown to activate Vδ2 cells in a TCR-dependent manner, with HMBPP displaying a 30 000-fold higher potency than IPP [10]. Of particular interest to this review is that malignant cells can have a dysfunctional mevalonate pathway, which can cause them to over-express IPP at levels capable of activating Vδ2 cells [11].

It is important to note that not all malignant cells are susceptible to Vδ2 cell killing via IPP recognition, as suggested by the TCR-independent killing observed in certain cell lines by Wrobel *et al.* [12]. There are, however, synthetic drugs, the nitrogen-containing bisphosphonates (NBP), that have been shown to disrupt isoprenoid biosynthesis, and thus increase tumour susceptibility to Vδ2 cell killing [13]. Specifically, NBPs block a particular enzyme of the mevalonate pathway called farnesyl pyrophosphate synthase, which converts dimethylallyl and geranyl pyrophosphate into downstream metabolites [14]. Blocking the activity of this enzyme causes intracellular accumulation of IPP, and *in-vitro* studies have demonstrated that tumour cells pre-exposed to NBPs become more susceptible to Vδ2 cell killing [15–17]. It is important to note that this effect of NBPs is not common to all tumour cell lines, possibly because of reduced cellular uptake and low mevalonate activity in these cells [18]. Moreover, tumour cells are not the only cell type affected by NBPs. It has been shown that peripheral blood mononuclear cells (PBMCs) treated with zoledronic acid (ZA) contain activated Vδ2 cells, an effect that appears to be mediated by the up-regulation of phosphoantigens in peripheral blood monocytes [19].

Although phosphoantigens such as IPP and HMBPP are known to activate Vδ2 cells in a TCR-dependent manner, the underlying mechanism is poorly understood. Early studies demonstrated that recognition of purified phosphoantigen is dependent upon antigen-presenting cells (APCs) of primate origin, but independent of previously identified antigen-presenting molecules such as human leucocyte antigen (HLA) class I, HLA class II and CD1 [20]. Recent advances have been made that implicate a critical role of butyrophilin (BTN) 3/CD277 in the phosphoantigen-mediated activation of human Vδ2 cells [21]. CD277 is a member of the immunoglobulin-supergene family of transmembrane proteins whose extracellular domains share sequence homology to the B7 family [22]. In 2012, Harly *et al.* discovered that an agonist antibody specific for CD277 was able to activate Vδ2 cells in a similar fashion to phosphoantigens and render non-susceptible tumour cells cytolytic targets for Vδ2 cells in a TCR-dependent manner [23]. The authors also found that cytotoxicity against susceptible tumour cells was blocked by antagonist antibodies against CD277, and that the 3A1 isoform of CD277 was a critical mediator of phosphoantigen-induced γδ T cell activation. Wang *et al.* confirmed these observations by showing that APCs treated with the same agonistic antibody were stimulatory for Vδ2 cells [24]. In their studies, the antibody did not alter IPP levels in APCs and its ability to render APCs stimulatory for Vδ2 cells was unaffected by statins, which are 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors that block cholesterol synthesis upstream of IPP. The authors went on to show that silencing BTN3A1 in APCs abrogated the activity of this antibody, as did substituting the intracellular region of BTN3A1 for that of BTN3A3. In a later study by Sandstrom *et al.*, phosphoantigens were shown to bind to an intracellular domain of the BTN3A1 molecule and subsequently confer Vδ2 cell reactivity [25]. Taken together, this research has led to a new wave of hypotheses that phosphoantigens bind the intracellular domains of BTN3A1 and induce conformational changes in the extracellular domains that are then detected by the Vδ2 cell TCR. Harly *et al.* discussed that they were unable to demonstrate direct binding of recombinant Vδ2 TCRs to CD277, and they proposed that BTN3A1 may be an accessory molecule for an as-yet undiscovered antigen for Vδ2 cells [23]. However, in 2013 Vavassori *et al.* demonstrated that soluble Vδ2 cell TCRs interact with phosphoantigen-bound BTN3A1 complexes [26], and subsequently proposed an alternative hypothesis that BTN3A1 is a presenting molecule for phosphoantigens.

Tumour immunosurveillance by Vδ2 cells

Due to the lack of appropriate animal models, there is no direct evidence to suggest that human Vδ2 cells eradicate or reduce tumour burden *in vivo*; however, a number of studies imply that Vδ2 cells may contribute to anti-tumour immunity, and are thus a promising target for cancer immunotherapy.

In-vitro experiments, although limited in their extrapolation into a physiological system, have demonstrated

that Vδ2 cells are capable of recognizing tumour cells and killing them through multiple pathways, including granule exocytosis, Fas/Fas-ligand (CD95/CD178)-induced apoptosis, antibody-dependent cell-mediated cytotoxicity and TNF-related apoptosis inducing ligand [15,27–29]. Human Vδ2 cells were found to kill a broad range of tumour cell lines derived from haematological and solid malignancies in both allogeneic and autologous settings [12,30,31]. Mechanistically, the use of γδTCR- and natural killer group 2, member D (NKG2D)-specific antibodies in Vδ2 cell cytotoxicity assays demonstrated that tumour recognition can be TCR- and/or NKG2D-dependent [12]. However, experimental data for whether or not this effect takes place *in situ* are understandably lacking. Immunocompromised mice bearing human tumours have been utilized in the attempt to model a physiological system, and results have shown that human Vδ2 cells exert some degree of protection against tumour growth in these systems [18,32–35]; however, there is a marked degree of uncertainty as to whether the activity of human Vδ2 cells in a xenograft model is the same as in their syngeneic host.

In patients, both positive and negative correlations have been made between clinical responses and tumour-infiltrating Vδ2 cells. For instance, Cordova *et al.* found that γδ T cells, consisting of both $V\delta1^+$ and $V\delta2^+$ cells, were the predominant tumour-infiltrating lymphocytes in melanoma lesions, and that low numbers of tumour-infiltrating γδ T cells correlated with advanced disease [36]. In a cohort of ovarian cancer patients, Raspollini *et al.* reported a positive correlation between the number of tumour-infiltrating γδ T cells and a brief disease-free interval [37]. In contrast, Inman *et al.* found relatively low percentages of tumour-infiltrating γδ T cells in renal cancer patients [38], and Ma *et al.* reported a positive correlation between disease progression and the number of tumour-infiltrating γδ T cells in a cohort of breast cancer patients [39]. Interestingly, despite no correlation between numbers of tumour-infiltrating Vδ2 cells and clinical responses in a cohort of renal cancer patients, Viey *et al.* found that peripheral blood Vδ2 cells were inefficient at migrating towards renal tumour cell lines *in vitro* compared with tumour-infiltrating Vδ2 cells [40]. This observation has important ramifications regarding the utility of peripheral blood Vδ2 cells in the absence of appropriate priming. With such a limited number of studies it remains unclear as to whether peripheral blood Vδ2 cells infiltrate tumours, and whether or not their presence in the tumour microenvironment has any bearing on disease prognosis. Moreover, correlations between the numbers of infiltrating γδ T cells and clinical responses do not address whether the Vδ2 cells detected within the tumour mass are activated and exerting cytotoxic activity against the tumour cells. Indeed, we have already mentioned that not all tumour cells are susceptible to Vδ2 cell killing. More studies that critically assess the phenotype and function of immune cells that infiltrate the tumour microenvironment are required, and further efforts to conduct such studies should be made.

If it is hypothesized that Vδ2 cells indeed play a role in immunosurveillance against malignant transformations why, then, do tumours develop? If this hypothesis were true then one would expect people with low numbers of peripheral blood Vδ2 cells to be more susceptible to cancer and/or the activity of Vδ2 cells in patients to be somehow impaired. Indeed, the activity of γδ T cells from cancer patients has been compared with that of healthy controls, and in melanoma, glioblastoma and nasopharyngeal carcinoma, reduced numbers of peripheral blood γδ T cells and/or impaired functional responses have been noted [41–44]. However, characterization of immune cells in the periphery is not necessarily an indication of what is happening within the tumour microenvironment. One particular study by Yi *et al.* reported that tumour-infiltrating γδ T cells in hepatocellular carcinoma exhibited impaired degranulation and IFN-γ responses compared with γδ T cells isolated from peritumoural tissue, suggesting that the tumour microenvironment may be hampering γδ T cell function [45]. In this study, tumour-infiltrating T regulatory cells and their production of immunosuppressive cytokines such as transforming growth factor (TGF)-β and interleukin (IL)-10 seemed to coincide with γδ T cell suppression. It is thought that tumours may have evolved immune escape mechanisms that enable them to hamper the activity of infiltrating cytotoxic cells [46]. For instance, tumours and tumour-associated macrophages have been reported to express the inhibitory programmed cell death ligand (PDL)-1/CD274 [47], and γδ T cells have been shown to up-regulate the receptor for this ligand (PD-1/CD279) following antigenic stimulation [48]. Accordingly, *in-vitro* studies have demonstrated that Vδ2 cells display reduced cytokine and cytotoxic responses in the presence of $PDL\text{-}1/CD274^+$ tumour cells compared with $PDL\text{-}1/CD274^-$ tumour cells [48]. Interestingly, when $PDL\text{-}1/CD274^+$ tumour cells were treated with ZA, the inhibitory effect on $PD\text{-}1/CD279^+$ Vδ2 cell cytotoxicity was reduced. An additional hypothesis is that suboptimal Vδ2 cell function in cancer patients is linked to genetic mutations; for example, Gaafar *et al.* found that a granzyme B polymorphism was associated with breast cancer, and that this polymorphism coincided with decreased cytotoxic function in peripheral blood Vδ2 cells [49]. Although evidence suggests that Vδ2 cells in cancer patients may be impaired they are not inert, and can respond to antigenic stimulation *in vitro* in the appropriate manner [30,50,51]. Importantly, this suggests that tumour immunosuppression can be overcome, and therapies that can do this will provide useful tools for Vδ2 cell-based cancer immunotherapy. For example, antibodies that block cytotoxic T lymphocyte-associated protein (CTLA)-4/CD152 and PD-1/CD279 are available

as therapeutic drugs for cancer, and may have potential as combinatorial partners for Vδ2 cell-based cancer immunotherapies [52,53].

Vδ2 cells in cancer immunotherapy

Bolstering the activity of peripheral blood Vδ2 cells in cancer patients in order to enhance their capacity to infiltrate and kill tumour has captured growing interest in the field of cancer immunotherapy. To date, two approaches have been employed: the first involves targeting Vδ2 cells *in situ* via intravenous (i.v.) administration of NBPs; the second involves adoptive transfer of *in-vitro*-primed Vδ2 cells. Although both these methodologies have yielded promising results, there are a number of limitations associated with each that, once overcome, may help to further improve their efficacy.

In vitro, NBPs have been shown to rapidly expand peripheral blood Vδ2 cell populations when used in combination with IL-2, as well as increase the sensitivity of tumour cells to Vδ2 cell killing [54,55]. Based on these observations, it was hypothesized that NBPs, when administered i.v. in combination with IL-2 [given either subcutaneously (s.c.) or i.v.], will expand the pool of tumour-reactive Vδ2 cells in the peripheral blood of cancer patients while concomitantly increasing the sensitivity of the tumour to Vδ2 cell killing. Administration of NBPs in combination with IL-2 has been conducted in early-phase clinical trials in patients with acute myeloid leukaemia (AML), breast cancer, malignant melanoma, multiple myeloma (MM), non-Hodgkin's lymphoma (NHL), prostate cancer and renal cell carcinoma (RCC). In a clinical trial by Wilhelm *et al.*, patients with either NHL or MM received i.v. pamidronic acid combined with i.v. IL-2, and significant levels of *in-vivo* Vδ2 cell activation and proliferation were observed in five of nine patients, three of whom showed clinical responses [56]. In a cohort of patients with prostate cancer, Dieli *et al.* reported that i.v. ZA combined with s.c. IL-2 increased the numbers of peripheral blood effector memory Vδ2 cells, an affect which correlated with clinical responses and a decline in prostate serum antigen [57]. Similarly, Meraviglia *et al.* reported that sustained numbers of peripheral blood Vδ2 cells correlated with clinical responses and a decline in serum cancer antigen in breast cancer patients [58]. In contrast, Lang *et al.* reported that i.v. ZA used in combination with s.c. IL-2 in RCC resulted in no clinical responses; however, the authors observed only partial induction of responses in peripheral blood Vδ2 cells, and reported that repeated cycles of ZA and IL-2 reduced overall percentages of peripheral blood Vδ2 cells as well as their capacity to proliferate in response to *in-vitro* restimulation [59]. Kunzmann *et al.* conducted a trial of i.v. ZA and s.c. IL-2 on small cohorts of patients with RCC, malignant melanoma and AML and found that, although expansion and activation of peripheral blood Vδ2 cells was observed in all patients, clinical responses were observed only in AML, the only cancer type of blood/bone origin [60]. Interestingly, in the study by Kunzmann *et al.*, elevated vascular endothelial growth factor levels were found to correlate negatively with clinical response.

An underlying hypothesis for the i.v. administration of NBPs in combination with IL-2 therapy is that peripheral blood Vδ2 cells become activated and subsequently undergo expansion, thus resulting in increased numbers of tumour-reactive Vδ2 cells in the circulation. For these cytotoxic cells to offer optimal protection against tumour they must migrate from the circulation to the tumour mass; however, this has yet to be demonstrated *in vivo*. Peripheral blood Vδ2 cells are predominantly CCR5/CD195$^+$ and CCR7/CD197$^-$, suggesting that they will home to sites of inflammation such as the tumour microenvironment [7]. Indeed, the production of inflammatory chemokines such as CCL3, CCL4 and CCL5 by tumour cells, stromal cells and/or infiltrating immune cells within the tumour microenvironment has been noted, which may potentially draw in CCR5/CD195$^+$ Vδ2 cells from the peripheral blood [61–64]. However, we have shown recently that Vδ2 cells within ZA-treated PBMCs have down-regulated expression of CCR5/CD195 and reduced migration towards CCL5, suggesting that, following i.v. ZA, Vδ2 cells may have reduced homing towards inflammatory sites and possibly tumours [65]. Others have also shown that γδ T cells undergo marked changes in their inflammatory homing programme following antigenic stimulation. In a study by Brandes *et al.*, TCR-activated γδ T cells were reported to have down-regulated CCR5/CD195 and up-regulated CCR7/CD197 expression, which coincided with reduced migration towards CCR5/CD195 ligands and increased migration towards CCR7/CD197 ligands in Transwell assays [7]. This suggests that TCR-mediated activation of peripheral blood γδ T cells results in reduced inflammatory homing and increased lymph node homing, and further supports the notion of inhibited migration towards tumour following ZA treatment. Although the loss of CCR5 was only transient in our studies, *in-vivo* models have shown that an increase in tumour susceptibility to Vδ2 cell killing following i.v. NBPs may also be transient [33]. Taken together, these studies suggest that at the time of optimal tumour susceptibility to Vδ2 cell killing following i.v NBPs, peripheral blood Vδ2 cells have down-regulated expression of the inflammatory chemokine receptors that may be involved in tumour migration. Furthermore, our data suggest that ZA renders peripheral blood monocytes targets for Vδ2 cells, an effect which may result in exhaustion of Vδ2 cells before they reach the tumour [65]. Indeed, Sugie *et al.* and Lang *et al.* have shown that repeated administration of ZA in breast cancer patients can decrease the responsiveness of Vδ2 cells to *in-vitro* restimulation [59,66].

Even if it is assumed that Vδ2 cells are migratory towards a tumour mass following i.v. administration of

NBPs, there remains the question of how susceptible is the tumour to Vδ2 cell killing? NBPs are capable of increasing the susceptibility of a broad range of tumour cell lines to Vδ2 cell cytotoxicity *in vitro*, and Santolaria *et al.* have shown that in immunocompromised mice bearing human tumours, i.v. injections of pamidronic acid enhanced the capacity of cells isolated from the tumour to stimulate human Vδ2 cells *in vitro* [33]. There is little evidence, however, to suggest that a patient's tumour mass becomes more susceptible to Vδ2 cell killing following i.v. infusion of NBPs. This is compounded by the fact that the hydroxyl group of ZA and other NBPs confer high affinity for calcium, and thus bone [67]. Indeed, pharmacokinetic studies have shown that within hours of i.v. infusion, ZA is either deposited on bone surfaces or found in the kidneys, where it is then excreted in the urine [67]. It is therefore questionable as to whether ZA administered by i.v. infusion reaches tumours that are not associated with either the blood or bone. Accordingly, in the clinical trial conducted by Kunzmann *et al.*, in which ZA and IL-2 was administered to patients with either solid or haematological malignancies, objective clinical responses were observed only in the latter [60]. Therefore, rigorous investigations need to be made in order to assess the effect of NBPs on tumour following i.v. infusion in patients.

In light of these discussions, it would be interesting to investigate ways of improving the exposure of solid tumours not associated with blood or bone to NBPs. Indeed, nanoparticle-based drug delivery systems have been utilized in the attempt to reduce NBP binding to bone and thus increase its extraskeletal bioavailability. This technology also has the potential to target NBPs specifically to tumours and immunosuppressive cell types, such as tumour-associated macrophages [68], and increased efficacy of ZA has been reported in preclinical models [69,70]. Alternative routes of administration such as intratumoural injection may also prove successful, and the efficacy of such an approach could be tested in easily accessible lesions such as those associated with melanoma. This approach could be combined with other therapeutic strategies capable of priming anti-tumour responses in peripheral blood Vδ2 cells. For instance, the attenuated preparation of *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) can induce clinical responses when administered intravesicularly in bladder cancer, and may have potential when given intradermally in other cancers such as melanoma [71]. We have shown recently that BCG can boost anti-tumour responses in peripheral blood Vδ2 cells [72,73]. Specifically, BCG activated IFN-γ production by Vδ2 cells and enhanced their cytotoxicity against susceptible tumour target cells in a manner that is dependent upon myeloid dendritic cells and memory $CD4^+$ αβ T cells. In support of our *in-vitro* observations, Cairo *et al.* have reported that intradermal injections of BCG in *Macaca fascicularis* induced Vδ2 cell proliferation *in vivo*, and enhanced PBMC IFN-γ production and Vδ2 cell proliferation in response to *in-vitro* restimulation with IPP [74]. Data suggest that BCG may also down-regulate chemokine receptors on Vδ2 cells; for example, Glatzel *et al.* have demonstrated that human PBMCs treated with heat-killed *M. tuberculosis* extract contain Vδ2 cells with down-regulated CCR5/CD195 expression, an effect that is dependent upon CCR5/CD195 ligands [8]. However, combination therapy would enable clinicians to optimize the timing of intradermal BCG and intratumoural injections of ZA so that tumour susceptibility and peripheral blood Vδ2 cell activity are co-ordinated effectively.

An alternative approach to Vδ2-based cancer immunotherapy has involved the adoptive transfer of *in-vitro*-expanded populations of Vδ2 cells. This approach has been tested in early-phase clinical trials in breast cancer, lung cancer, MM and RCC patients. In these trials, PBMCs were isolated from patients and treated *in vitro* with either ZA or synthetic phosphoantigens combined with IL-2 for 2 weeks. The resulting cell population is enriched with $V\delta2^+CD3^+$ cells, exhibits marked cytotoxicity against tumour target cells and expresses high levels of CCR5/CD195 and CXCR3/CD183 [31,75]. In addition to testing the safety and feasibility of this technique, some of these trials demonstrated clinical responses. In 2007, Kobayashi *et al.* reported reduced tumour growth in three of five RCC patients receiving adoptively transferred γδ T cells [76]. The authors then repeated this trial in 2011 using adoptively transferred γδ T cells in combination with ZA and IL-2 therapy, and found reduced tumour growth in 11 of 11 RCC patients [77]. Similarly, in 2011 Nicol *et al.* tested adoptive transfer of γδ T cells in combination with ZA and IL-2 therapy in patients with solid malignancies, and reported clinical responses in those patients who were concomitantly receiving other therapies [78]. Furthermore, Abe *et al.* observed stable levels of serum M-protein in four of six MM patients receiving injections of *in-vitro*-stimulated γδ T cells, and reported that soluble MHC class I chain-related protein A was detected only in the sera from the two non-responding patients [79], which may represent a potential tumour escape mechanism [80,81]. However, in trials conducted by Bennouna *et al.*, Nakajima *et al.* and Sakamoto *et al.*, no clinical responses were reported in cohorts of patients with RCC or non-small cell lung cancer receiving adoptive transfer of *in-vitro*-stimulated γδ T cells [82–84].

Adoptive cell transfer is an appealing approach for cancer immunotherapy. It has the potential to generate consistently large pools of tumour-reactive cells because the cells are stimulated outside the immunosuppressive environment of their tumour-bearing host. Furthermore, the cells can be harvested and used at the point at which they display optimal effector functions and migratory function. However, the success of adoptive transfer of Vδ2 cells is dependent upon how susceptible the tumour is to Vδ2 cell

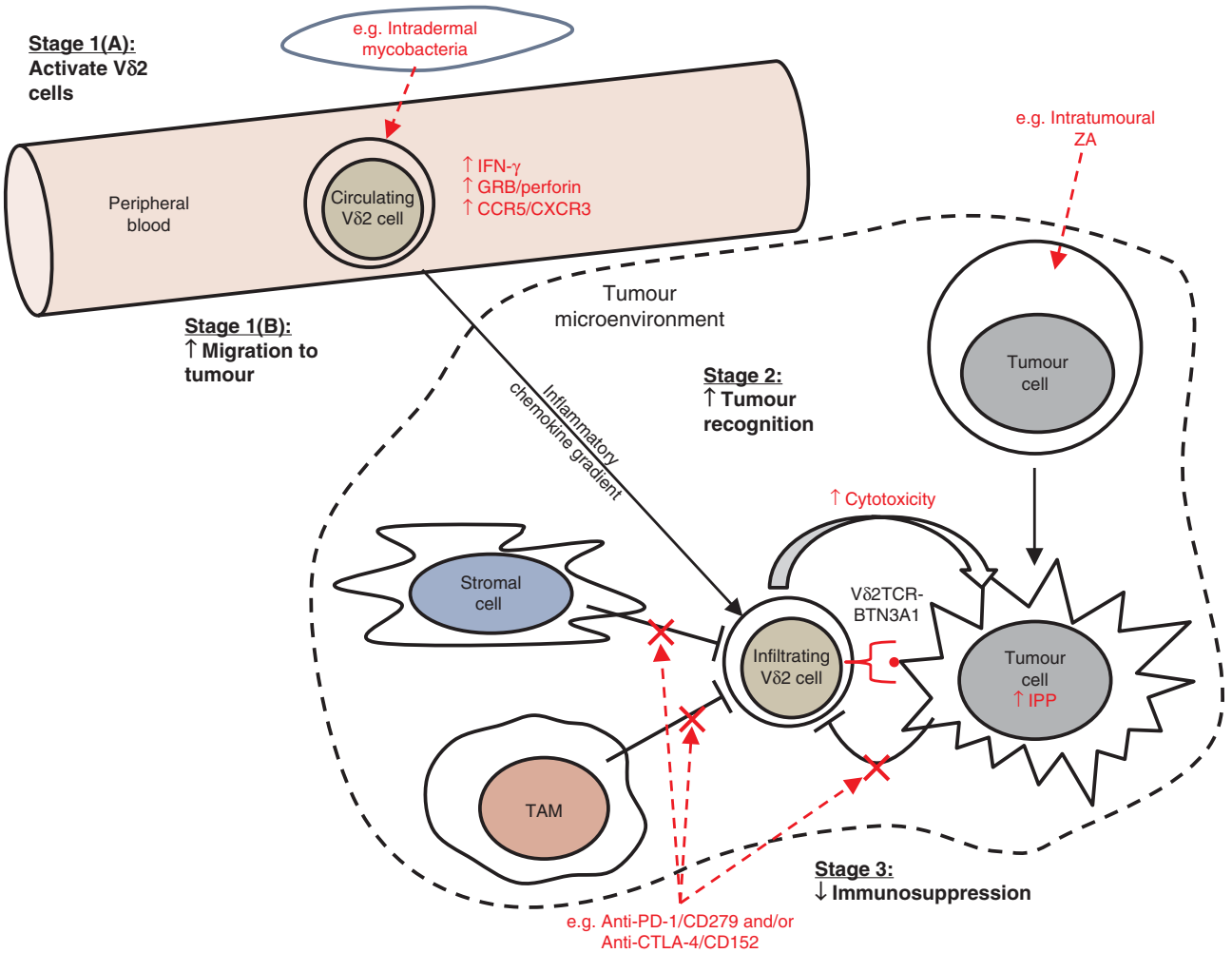

Fig. 1. Schematic overview of potential strategies to harness the power of Vδ2 cells in cancer immunotherapy. In this schematic, we have depicted and compartmentalized into key stages the hypothetical processes involved in achieving optimal Vδ2 cell targeting of tumour and the potential ways in which clinical intervention may be used to manipulate the activity of Vδ2 cells in patients. 'Stage 1(A): activate Vδ2 cells': peripheral blood Vδ2 cells must be primed in order to enhance their anti-tumour response. Intradermal administration of mycobacterial preparations such as bacillus Calmette–Guérin (BCG) may achieve this by increasing Vδ2 cell expression of cytolytic effector molecules [e.g. granzyme B (GRB) and perforin] and production of proinflammatory cytokines [e.g. interferon (IFN)-γ]. 'Stage 1(B): increase migration to tumour': primed Vδ2 cells must then migrate from the peripheral blood to the site of tumour. This may involve inflammatory chemokines, and thus require optimal expression of inflammatory chemokine receptors on Vδ2 cells following the priming stage. 'Stage 2: increase tumour recognition': Once Vδ2 cells have successfully infiltrated the tumour mass, tumour cells must be susceptible to Vδ2 cell cytotoxicity. Vδ2 cell recognition of tumour can be manipulated in certain cancers by delivering nitrogen-containing bisphosphonates (NBPs) such as zoledronic acid (ZA) to the site of the tumour. 'Stage 3: decrease immunosuppression': immunosuppressive elements within the tumour microenvironment must be counteracted in order to ensure an uninhibited Vδ2 cell response against tumour. Examples of potential immunosuppressive pathways include programmed death (PD)-1/CD279 and cytotoxic T lymphocyte antigen-4 (CTLA-4)/CD152, both of which can be blocked using monoclonal antibodies.

killing. Interestingly, of the two clinical trials conducted by Kobayashi *et al.*, one using adoptively transferred Vδ2 cells alone and the other in combination with i.v. ZA, the combination treatment regimen generated a higher proportion of clinical responses [76,77]. Although these two treatment strategies were not tested in parallel, one could speculate that the combination therapy was more effective because i.v. ZA renders the tumour mass more susceptible to the adoptively transferred Vδ2 cells. Moreover, it remains unclear as to whether adoptively transferred Vδ2 cells migrate to sites of tumour. In one study by Nicol *et al.*, *in vitro*-stimulated γδ T cells were labelled with indium (III)-oxine and injected i.v. into three patients (two with melanoma and one with colorectal cancer), and the trafficking of these cells monitored *in situ*. These cells were shown to express high levels of tissue homing CCR5/CD195 and CXCR3/CD183, and traffic to the lungs, liver and spleen, as well as metastatic lesions [78]. However, although it seems possible to gener-

ate large numbers of tumour-homing Vδ2 cells for adoptive transfer they may still come under the influence of the immunosuppressive microenvironment of the tumour, and therefore continued research into the effect of the tumour microenvironment on the function of infiltrating Vδ2 cells is necessary. Lastly, with bespoke adoptive cell transfer therapies there are considerable limitations in terms of the associated costs, the time taken to generate sufficient clinically usable material and whether such treatments, if proved to be successful, will be widely available.

Conclusions

There is a growing body of *in-vitro* data that supports the notion that Vδ2 cells can exert potent responses against tumour. Moreover, initial clinical evaluations of therapies aimed specifically at boosting Vδ2 cell anti-tumour immunity have supported this approach to cancer immunotherapy. In this review we have highlighted three areas within the field of Vδ2 cell-based cancer immunotherapy that need further investigation: (1) Vδ2 cell migration, (2) tumour susceptibility to Vδ2 cell killing and (3) tumour immunosuppression of Vδ2 cell responses. Of foremost importance in Vδ2 cell-based cancer immunotherapy is ensuring that Vδ2 cells can migrate to the site of a tumour, and therefore the features that enable peripheral blood Vδ2 cells to infiltrate tumours need to be defined more clearly, and therapeutic regimens that manipulate Vδ2 cell migratory behaviour appropriately need to be identified. Once Vδ2 cells have infiltrated a tumour mass, the susceptibility of the tumour to killing, and how this can be increased, is a critical parameter to investigate in order to fully gauge the effectiveness of this approach. Furthermore, a better understanding of the immunosuppressive nature of the tumour microenvironment on the function of infiltrating Vδ2 cells will help us to identify potential combinatorial partners that can be used to counteract this, thus allowing Vδ2 cells to optimally carry out their effector response. Further research into these areas will enhance the progression of Vδ2 cell immunotherapy and enable us to effectively prime the migratory and cytotoxic capacity of peripheral blood Vδ2 cells, while simultaneously increasing tumour susceptibility to Vδ2 killing. A schematic overview of the topics discussed in this review is given in Fig. 1.

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Disclosure

The authors declare no competing interests.

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