



# Monitoring circulating $\gamma\delta$ T cells in cancer patients to optimize $\gamma\delta$ T cell-based immunotherapy

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The success of  $\gamma\delta$  T cell-based immunotherapy, where the cytotoxic activity of circulating  $\gamma\delta$  T lymphocytes is activated by nitrogen-containing bisphosphonates (n-BP), or possibly by bispecific antibodies or the combination of both, requires a profound knowledge of patients'  $\gamma\delta$  T cells. A possible influence of radio- or chemotherapy on  $\gamma\delta$  T cells as well as their reported exhaustion after repetitive treatment with n-BP or their lack of response to various cancers can be easily determined by the monitoring assays described in this perspective article. Monitoring the absolute cell numbers of circulating  $\gamma\delta$  T cell subpopulations in small volumes of whole blood from cancer patients and determining  $\gamma\delta$  T cell cytotoxicity using the Real-Time Cell Analyzer can give a more comprehensive assessment of a personalized tumor treatment. Possible future directions such as the combined usage of n-BP or phosphorylated antigens together with bispecific antibodies that selectively target  $\gamma\delta$  T cells to tumor-associated antigens, will be discussed. Such strategies induce expansion and enhance  $\gamma\delta$  T cell cytotoxicity and might possibly avoid their exhaustion and overcome the immunosuppressive tumor microenvironment.

**Keywords: monitoring, human,  $\gamma\delta$  T cells, pancreatic ductal adenocarcinoma, bispecific antibodies, phosphorylated antigens, aminobisphosphonate**

## INTRODUCTION

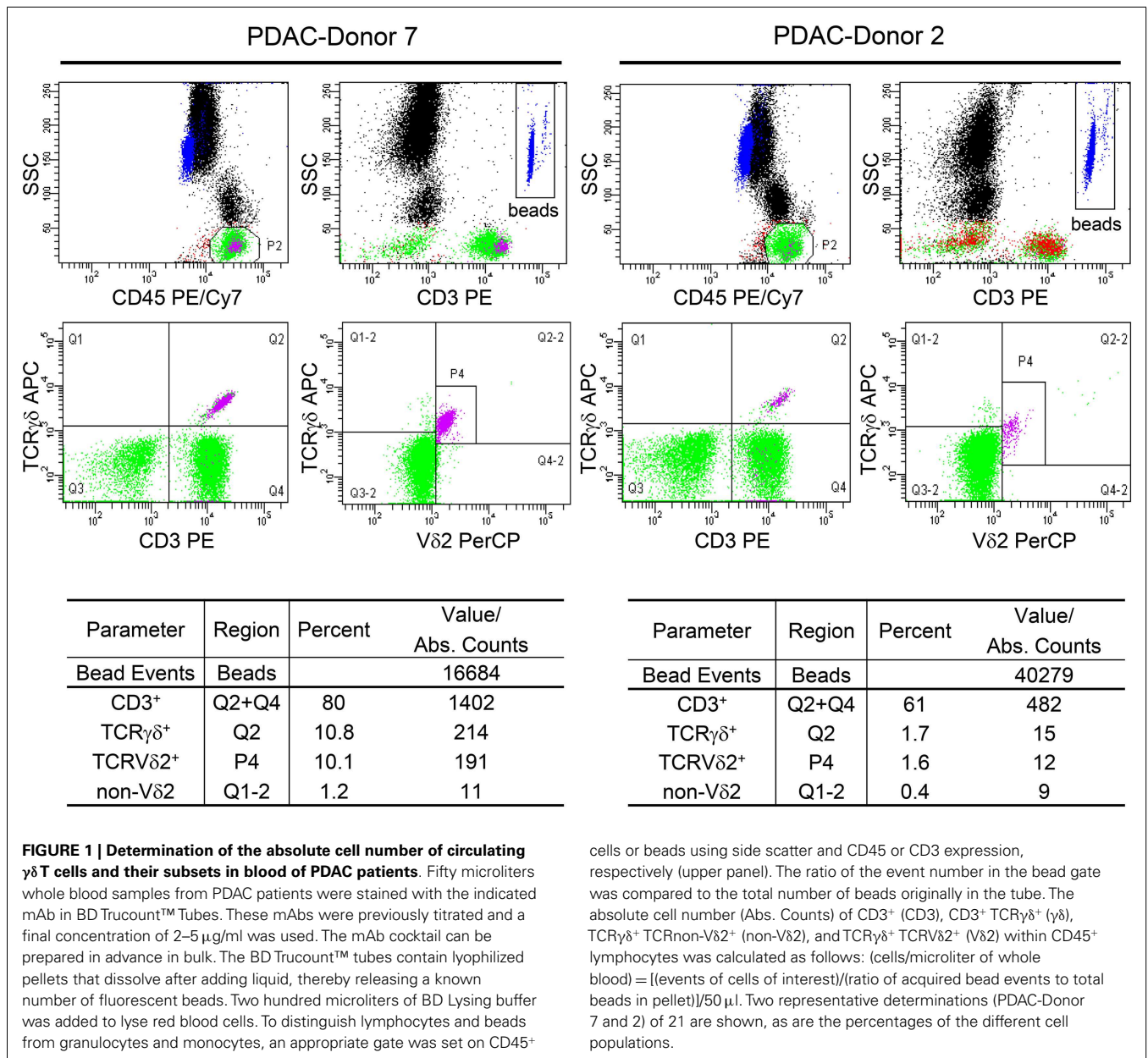
Human  $\gamma\delta$  T cells ( $\gamma\delta$ Tc) represent a small subset (1–10%) of CD3<sup>+</sup> T lymphocytes with several unconventional features. Similar to antigen presenting cells (APC),  $\gamma\delta$ Tc can phagocytose and present soluble antigens to CD3<sup>+</sup>  $\alpha\beta$  T cells (1, 2). Additionally,  $\gamma\delta$ Tc can induce the maturation of dendritic cells (DCs), and kill various tumor cells in a HLA-independent manner (3, 4). Thus, there is a substantial interest in  $\gamma\delta$ Tc in the context of T cell-based immunotherapeutic strategies (5, 6). Several pilot studies have described a partial success of  $\gamma\delta$  T cell-based immunotherapy in different types of cancer after the application of amino-bisphosphonates (n-BP) or phosphorylated antigens (PAG) plus IL-2 *in vivo* or after repetitive transfer of *in vitro* expanded V $\delta$ 2-expressing  $\gamma\delta$ Tc (7–10). Although  $\gamma\delta$  T cell-based immunotherapy has delivered promising results, sustained stimulation of V $\delta$ 2  $\gamma\delta$ Tc by n-BP or PAG often leads to V $\delta$ 2 T cell exhaustion (8, 11, 12). Additionally, a low number of functionally unresponsive  $\gamma\delta$ Tc has been described in patients with chronic lymphocytic leukemia or multiple myeloma (13–15). Novel bispecific antibodies (with concomitant specificity for epitopes on both  $\gamma\delta$ Tc and tumor cells) provide a tool to enhance cytotoxic activity of  $\gamma\delta$ Tc against cancer cells by selectively targeting  $\gamma\delta$ Tc to antigens expressed by tumor

cells (16). Additionally, independent of previous immunotherapeutic strategies and prior to the application of a  $\gamma\delta$  T cell-based immunotherapy, it is mandatory to analyze the number and functional capacity of patients'  $\gamma\delta$ Tc in a simple manner. This article demonstrates that the analysis of absolute cell numbers of circulating  $\gamma\delta$ Tc from patients as well as the determination of the cytotoxic capacity against tumor cells of interest can give a better assessment of subsequent personalized tumor treatment.

## MONITORING OF ABSOLUTE CELL NUMBERS

The monitoring system that uses the BD Multitest 6-color TBNK (M6T) Reagent with BD Trucount™ Beads (www.bd.com/resource.aspx?IDX=17743, BD Biosciences, San Jose, CA, US) allows determination of absolute cell numbers of  $\alpha\beta$  T and B lymphocytes and NK cells as well as CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets (17, 18). Since  $\gamma\delta$  T lymphocytes and their subpopulations are not detected by the M6T, we adapted  $\gamma\delta$ Tc staining from the BD Trucount™ Tube technical data sheet (version 8/2010) as follows: 50  $\mu$ l whole blood from cancer patients were stained with anti-CD45-PE/Cy7 (clone HI30), CD3-PE (clone SK7) pan-TCR $\gamma\delta$ -APC (clone 11F2, customized) (all from BD Biosciences, Heidelberg, Germany), and V $\delta$ 2-PerCP (clone B6, Biolegend, Fell, Germany) mAbs and occasionally with V $\delta$ 1-FITC mAb (clone TS8.2, Thermo Fisher Scientific, Germany) in BD Trucount™ Tubes as described (16). After staining, red blood cells were lysed with 200  $\mu$ l BD Lysing buffer and analyzed using the FACS Canto flow cytometer and FACS Diva software (both from BD Biosciences). For two

**Abbreviations:** BrHPP, bromohydrin-pyrophosphate;  $\gamma\delta$ Tc,  $\gamma\delta$  T cells; mAb, monoclonal antibody; n-BP, nitrogen-containing bisphosphonate; PAG, phosphorylated antigens; PDAC, pancreatic ductal adenocarcinoma; RTCA, real-time cell analyzer; TCR, T cell-antigen receptor.



cells or beads using side scatter and CD45 or CD3 expression, respectively (upper panel). The ratio of the event number in the bead gate was compared to the total number of beads originally in the tube. The absolute cell number (Abs. Counts) of CD3<sup>+</sup> (CD3), CD3<sup>+</sup> TCR $\gamma\delta$ <sup>+</sup> ( $\gamma\delta$ ), TCR $\gamma\delta$ <sup>+</sup> TCRnon-V $\delta$ 2<sup>+</sup> (non-V $\delta$ 2), and TCR $\gamma\delta$ <sup>+</sup> TCRV $\delta$ 2<sup>+</sup> (V $\delta$ 2) within CD45<sup>+</sup> lymphocytes was calculated as follows: (cells/microliter of whole blood) = [(events of cells of interest)/(ratio of acquired bead events to total beads in pellet)]/50  $\mu$ l. Two representative determinations (PDAC-Donor 7 and 2) of 21 are shown, as are the percentages of the different cell populations.

representative donors, the absolute numbers of total  $\gamma\delta$ Tc as well as V $\delta$ 2 and non-V $\delta$ 2 subsets are shown (Figure 1). Moreover, cells can be stained with anti-V $\delta$ 1 mAb labeled with an additional fluorochrome (data not shown).

Certainly, other bead-based detection systems could be used alternatively to determine absolute cell numbers. Importantly, however, these strategies must allow this determination from a small volume of patient's blood.

In addition, a possible influence of radio- or chemotherapy on circulating immune cell numbers can be easily determined by this monitoring system. For instance, our own data reveal that the absolute number of V $\delta$ 2  $\gamma\delta$ Tc in a cohort of 10 breast cancer patients receiving chemotherapy did not differ from age-matched breast cancer patients without treatment (Adam-Klages et al.,

unpublished data). Moreover, in a cohort of 41 patients with pancreatic ductal adenocarcinoma (PDAC, stage pT3–4, pN0–1, L0–1 and V0–1), we recently observed that the decrease in absolute numbers of V $\delta$ 2  $\gamma\delta$ Tc did not correlate with cancer stage/progression, but rather with patient age (16).

While determination of the absolute  $\gamma\delta$  T cell numbers and that of their subsets provides no information about their cytotoxic capacity, this can be addressed in an additional functional assay.

### DETERMINATION OF CYTOTOXIC CAPACITY

We recently examined the functional capacity of  $\gamma\delta$ Tc from patients with PDAC (16). PDAC is a highly aggressive gastrointestinal malignancy characterized by the presence of

desmoplastic stromal microenvironment where conventional treatment approaches including surgery, chemotherapy, and/or radiation are often not effective (19). The observed decrease in absolute V $\delta$ 2 T cell numbers in untreated patients with advanced PDAC is attributable to age, not disease status, as similar numbers were found in age-matched healthy controls (16). In an attempt to avoid V $\delta$ 2 T cell exhaustion through repetitive n-BP stimulation and overcome the immunosuppressive activity of PDAC stromal cells on cytotoxic  $\gamma\delta$  T cells, novel bispecific antibodies such as [Her2xCD3] and [(Her2) $_2$ xV $\gamma$ 9] were designed. [(Her2) $_2$ xV $\gamma$ 9] is specific for V $\gamma$ 9 on  $\gamma\delta$ Tc (associated with V $\delta$ 2) and for human epidermal growth factor receptor HER2/neu overexpressed on PDAC, breast, and prostate cancer cells. The [(Her2) $_2$ xV $\gamma$ 9] tribody design allows monovalent binding to  $\gamma\delta$ Tc and bivalent HER2-targeting, which enhances avidity to the tumor cell and thereby increases cytolytic activity. Both bispecific antibodies selectively target  $\gamma\delta$ Tc to tumor antigens, thereby enhancing the cytotoxic activity of  $\gamma\delta$ Tc *in vitro* as well as *in vivo* in a PDAC grafted SCID-Beige mouse model (16).

In previous studies, we usually examined the functional capacity of  $\gamma\delta$  T cell lines or freshly isolated  $\gamma\delta$  Tc. Aiming to simplify handling of cells from patients with a low  $\gamma\delta$  T cell number in the following experiments, we investigated the functional capacity of cytotoxic  $\gamma\delta$ Tc within PBMC. We observed that the functional cytotoxic activity of circulating  $\gamma\delta$ Tc from patients can be determined in as few as of  $1-2 \times 10^6$  PBMC, readily obtainable from 2 to 4 ml of patients' blood. We analyzed blood from 21 patients with PDAC after obtaining their informed consent and relevant institutional review board approvals (code number: D401/14). As a read out system for cytotoxic activity of  $\gamma\delta$ Tc within freshly isolated PBMC, the real-time cell analyzer (RTCA) single-plate system (ACEA, San Diego, CA, USA) was used. RTCA measures the impedance of adherent tumor cell monolayers, but not of suspended cells such as PBMCs with electronic sensors. The measurement of impedance in arbitrary cell index units reflects changes in cellular parameters of tumor cells, which allows monitoring of cellular events in real time without the incorporation of labels over time periods of several days. The loss of impedance correlates with the  $\gamma\delta$  T cell-mediated lysis of tumor cells (16). A further advantage of measuring impedance over an extended time is that it enables us to observe whether tumor cells can regenerate when lysis is incomplete.

To ensure adherence of tumor cells, PDAC cells were cultured for 24–27 h in RTCA plates before the addition of  $\gamma\delta$ Tc alone with or without additional substances. Thereafter, PDAC cells were still cultured alone or together with PDAC patient-derived PBMC in (i) medium, (ii) PAG such as bromohydrin-pyrophosphate (BrHPP), or (iii) [(Her2) $_2$ xV $\gamma$ 9]. During the extended time course, we observed that  $\gamma\delta$ Tc within PBMC required almost 24–36 h after initial stimulation to exert their cytotoxic capacity (Figure 2A, red arrow with a star). Moreover, we observed that [(Her2) $_2$ xV $\gamma$ 9] triggered tumor cell lysis more efficiently than PAG in 30% of PDAC patient samples (Figure 2A, responder), while neither substance was effective in 70% of patient samples (Figure 2A, non-responder). The unexpected cytotoxicity against PDAC cells in the absence of a stimulus (medium, orange line) is likely due to the reactivity of NK cells in the presence of IL-2 (Figure 2A),

because additional experiments with untouched, freshly isolated  $\gamma\delta$ Tc demonstrated that cytotoxic activity of  $\gamma\delta$ Tc is not induced by IL-2 alone (16).

Regarding the absolute V $\delta$ 2 T cell numbers presented in Figure 2B (table), we correlated the unresponsiveness of the majority of the tested patient samples [negative [(Her2) $_2$ xV $\gamma$ 9] reactivity] with their low initial V $\delta$ 2 T cell number. PBMC from patients with more than 30 V $\delta$ 2<sup>+</sup>  $\gamma\delta$ Tc/ $\mu$ l blood were responsive (responder in Figure 2A and “positive” in Figure 2B), whereas in samples with <30 V $\delta$ 2<sup>+</sup>  $\gamma\delta$ Tc/ $\mu$ l blood, no induction of cytotoxic activity to PAG or [(Her2) $_2$ xV $\gamma$ 9] stimulation was observed (non-responder in Figure 2A and “negative” in Figure 2B).

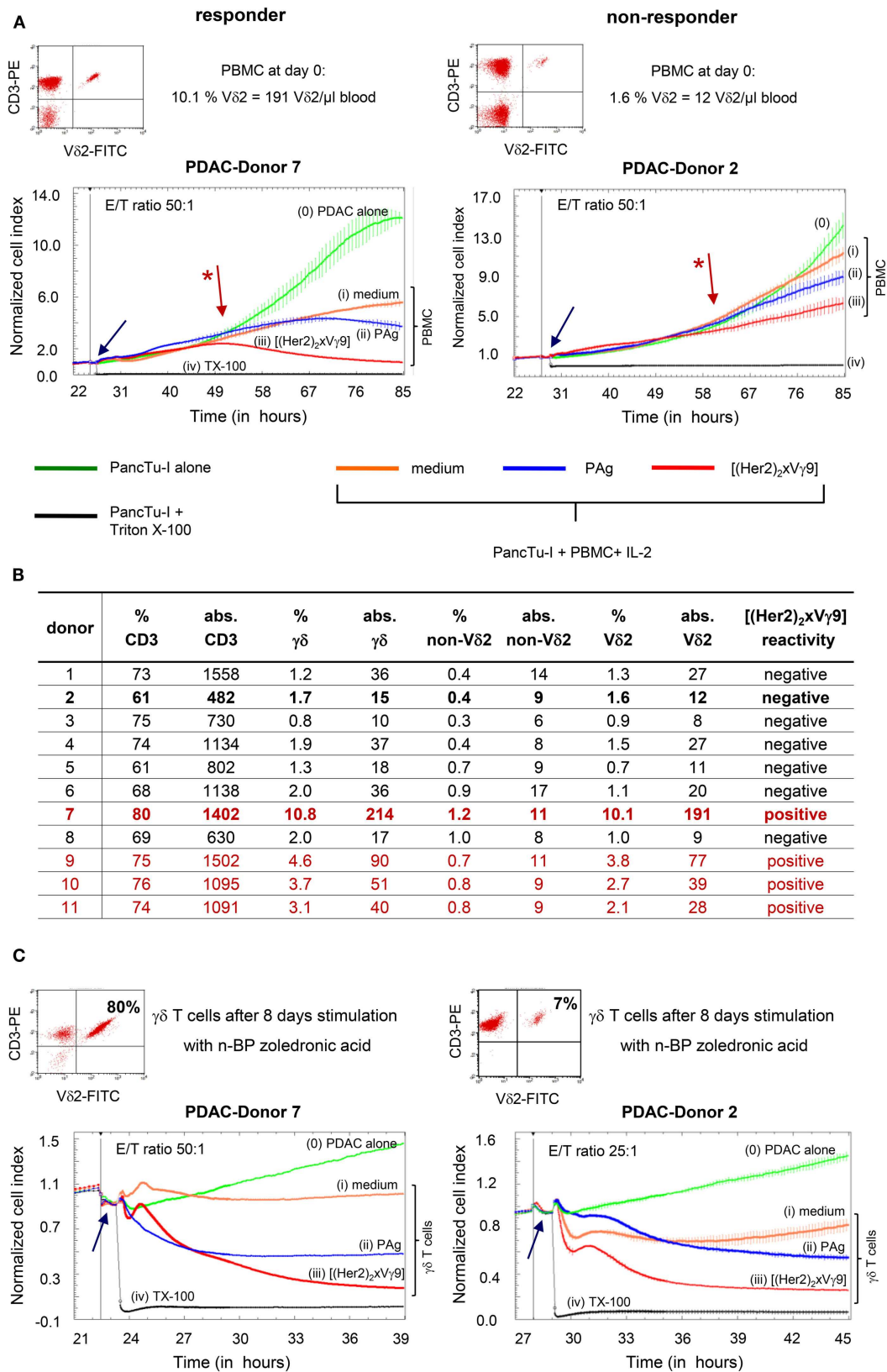
The weak capacity of bispecific antibodies to induce  $\gamma\delta$  T cell proliferation could explain the observed unresponsiveness to [(Her2) $_2$ xV $\gamma$ 9]. Therefore, PBMC from the same patients were stimulated with the PAG BrHPP or, as presented in Figure 2C, with n-BP zoledronic acid for 7–14 days. Although the responder cells expanded to 80%  $\gamma\delta$ Tc in culture, while non-responders comprised only 7% after n-BP stimulation, this small population of non-responders exhibited nearly the same degree of cytotoxicity as responders after re-stimulation with [(Her2) $_2$ xV $\gamma$ 9], despite the lower effector/target ratio (Figure 2C).

Taken together, our results demonstrate that prior analysis of absolute circulating cell numbers of immune cell subsets as well as determination of their cytotoxic capacity against tumor cells of interest may provide a better assessment of whether a particular personalized tumor treatment will be effective.

#### WHAT CAN WE LEARN FROM THIS MONITORING SYSTEM?

$\gamma\delta$  T cell monitoring can provide an estimate for a potential treatment of cancer patients. Although knowledge of the functional capacity of  $\gamma\delta$ Tc within PBMC does not provide information about their migration and infiltration into the tumor, characterization of these circulating  $\gamma\delta$ Tc is useful since they are activated by intravenous n-BP or PAG administration (8, 10). In clinical trials where  $\gamma\delta$ Tc were repetitively activated with n-BP or PAG together with low-dose IL-2, effects on tumor growth were observed; however, this was associated with exhaustion, anergy, or depletion of  $\gamma\delta$ Tc due to repetitive stimulation (8, 11, 12). In light of these observations, it is necessary to optimize cytotoxic activity, which can be achieved with bispecific antibodies such as the tribody [(Her2) $_2$ xV $\gamma$ 9]. Adoptive transfer of  $\gamma\delta$ Tc with [(Her2) $_2$ xV $\gamma$ 9] and IL-2 significantly reduced growth of pancreatic tumors grafted into SCID-Beige mice in comparison to adoptively transferred  $\gamma\delta$ Tc together with n-BP and IL-2 (16).

V $\delta$ 2  $\gamma\delta$ Tc used for adoptive transfer are cells within PBMC that are initially activated with n-BP or PAG plus IL-2 (7, 20). Such initial activation with n-BP or PAG plus IL-2 causes selective V $\delta$ 2 T cell-expansion, while [(Her2) $_2$ xV $\gamma$ 9] does not induce strong proliferation of  $\gamma\delta$ Tc (unpublished data). Independently of the proliferative response of  $\gamma\delta$  Tc, the cytotoxic activity of PAG or n-BP expanded V $\delta$ 2 T cell lines can be significantly enhanced after re-stimulation with [(Her2) $_2$ xV $\gamma$ 9]. Moreover, the addition of [(Her2) $_2$ xV $\gamma$ 9] did not induce cell death of V $\delta$ 2 T cells, in contrast to restimulation of V $\delta$ 2 T cell lines with PAG (unpublished data). Thus, [(Her2) $_2$ xV $\gamma$ 9] provides a tool to further enhance cytotoxic activity of adoptively transferred  $\gamma\delta$ Tc, whereas PAG or



**FIGURE 2 | Correlation between absolute cell number and functional capacity of Vδ2 T cells.**

(Continued)

**FIGURE 2 | Continued**

**(A)** Flow cytometric analysis of CD3<sup>+</sup> V $\delta$ 2<sup>+</sup>  $\gamma\delta$ Tc within PBMC, and RTCA of PBMC from two representative donors (Donors 7 and 2) of 21; **(B)** list of the relative and absolute numbers (abs.) of CD3,  $\gamma\delta$ , V $\delta$ 2, and non-V $\delta$ 2 T cells in whole blood from 11 representative PDAC patients out of 21 as well as reactivity to the tribody; **(C)** flow cytometric analysis of selective expansion of CD3<sup>+</sup> V $\delta$ 2<sup>+</sup>  $\gamma\delta$ Tc after PAg-activation within PBMC for 8 days, and RTCA with these short-term expanded  $\gamma\delta$ Tc from Donors 7 and 2. Two representative donors of 21 are shown. **(A,C)** For RTCA,  $5 \times 10^3$  PDAC cells (PancTu-1) were cultured in 10% FCS RPMI medium for 24–27 h on an E-plate covered at the bottom with electronic sensors that measure the impedance of the cells expressed as an arbitrary unit called cell index (CI). The CI was analyzed every 5 min to determine adherence and thus cell growth. Since the initial adherence in different wells can differ slightly, the CI

was normalized to 1 shortly before the time of addition of suspended cells  $\pm$  substances (vertical black line). After 24–27 h, PDAC cells were treated again with medium [green line (0)] or with PBMC **(A)** or short-term expanded  $\gamma\delta$ Tc **(C)** together with medium [orange line (i)], 300 nM PAg BrHPP [dark blue line (ii)], or 1  $\mu$ g/ml [(Her2)<sub>2</sub>xV $\gamma$ 9] [red line (iii)] at the indicated E:T ratio over the indicated time. As a positive control for maximal lysis, PDAC cells were treated with Triton X-100 [TX-100, black line (iv)]. The addition of substances, PBMC or expanded  $\gamma\delta$ Tc is indicated by the blue arrow. CI was then measured every minute for analysis of precise cytotoxicity time point for >15 to 55 h as indicated. The loss of tumor cell impedance and thus a decrease of the Normalized CI correlates with  $\gamma\delta$  T cell-mediated lysis of PDAC cells. The red arrow with the \* points out the initiation of cytotoxicity. The average of triplicates and standard deviation were calculated; one representative experiment is shown.

n-BP failed because they induce cell death in almost half of the activated cells (unpublished data).

The observation that the majority of elderly people has a low frequency of  $\gamma\delta$ Tc hampers the expansion of autologous  $\gamma\delta$ Tc required for adoptive transfer. Considering these challenges, one might suggest adoptively transferring allogeneic or haploidentical  $\gamma\delta$ Tc from (younger) healthy donors or activating  $\gamma\delta$ Tc within PBMC *in vivo* with bispecific antibodies (21–23). To investigate the effect of bispecific antibodies on unstimulated  $\gamma\delta$ Tc, we monitored whether [(Her2)<sub>2</sub>xV $\gamma$ 9] can induce cytotoxic activity in  $\gamma\delta$ Tc within PBMC. As described above, no or weak responses to [(Her2)<sub>2</sub>xV $\gamma$ 9] were obtained with PBMC from PDAC donors with a lower frequency of V $\delta$ 2  $\gamma\delta$ Tc (non-responder), whereas PBMC with a higher V $\delta$ 2  $\gamma\delta$  T cell frequency responded to [(Her2)<sub>2</sub>xV $\gamma$ 9] resulting in enhanced cytotoxicity (responder) (Figure 2A). Interestingly, n-BP- or PAg-mediated enrichment of non-responder  $\gamma\delta$ Tc within PBMC for 7–14 days led to enhanced cytotoxic activity after restimulating the cells with [(Her2)<sub>2</sub>xV $\gamma$ 9] (Figure 2C).

The validity of this monitoring system to determine  $\gamma\delta$  T cell-reactivity within PBMC needs to be confirmed in patients undergoing  $\gamma\delta$  T cell-targeting therapy. Based on our experience, one might suggest initially administration of n-BP together with IL-2 in cancer patients to induce proliferation of V $\delta$ 2  $\gamma\delta$ Tc followed by treatment with bispecific antibodies engaging  $\gamma\delta$ Tc plus IL-2 in order to avoid the V $\delta$ 2 T cell exhaustion observed in patients mediated by repetitive application of n-BP plus IL-2.

### WHAT ARE THE BENEFITS OF COMBINING $\gamma\delta$ T CELL-BASED IMMUNOTHERAPY WITH BISPECIFIC ANTIBODIES?

Therapeutic antibodies such as rituximab (anti-CD20 mAb) and trastuzumab or pertuzumab (both anti-HER2 mAb) as well as different combined therapies have clearly improved the treatment outcome of patients with B-cell lymphoma or breast cancer, respectively (24, 25). Furthermore, combining these therapeutic antibodies with  $\gamma\delta$  T cell-based immunotherapy seems very promising. Rituximab enhanced cytotoxic activity of *ex vivo* expanded CD16<sup>+</sup> (Fc $\gamma$ RIII)  $\gamma\delta$ Tc against CD20<sup>+</sup> chronic lymphocytic leukemia, while Trastuzumab increased  $\gamma\delta$  T cell cytotoxicity against HER2<sup>+</sup> breast cancer cells (26).

The success of such therapeutic antibodies has inspired antibody engineers to improve the antibody efficacy. One promising approach to enhance cytotoxicity and selectively target T cells to

tumor-associated antigens is based on the usage of single-chain bispecific antibody constructs. One such construct is Blinatumomab with specificity for CD19 on lymphoma or leukemia and CD3 on T cells, which has proved efficient for the treatment of patients with hematological malignancies (27). The short half-life of only a few hours in serum requires continuous intravenous infusion of Blinatumomab, which induces an almost complete molecular response and prolonged leukemia-free survival in patients with minimal residual B-lineage acute lymphoblastic leukemia (28). The favorable characteristics of bispecific antibodies such as high specificity, high cytotoxic potential, and low immunogenicity, led us to design a bispecific antibody targeted to V $\gamma$ 9 instead of CD3 and to HER2 expressed on several PDAC as well as on breast and prostate cancer, which could be easily replaced by another tumor target antigen of interest.

Of course, the question arises as to what differentiates bispecific antibodies with specificity for  $\gamma\delta$ Tc and those with specificity for CD3 T cells. For instance, a target group could be patients with advanced hematological malignancies (e.g., AML) who require allogeneic stem cell transplantation. A major advantage of  $\gamma\delta$  T cell-based immunotherapy is the HLA-independent killing of tumor cells, thereby reducing the risk of graft-versus-host disease often caused by alloreactive CD3<sup>+</sup>  $\alpha\beta$  T cells (21, 22, 29, 30). A successful anti-tumor activity was described for patients with refractory hematological malignancies after adoptive transfer of haploidentical  $\gamma\delta$ Tc (23). Labeling *ex vivo* expanded haploidentical  $\gamma\delta$ Tc with bispecific antibodies could perhaps further enhance the cytotoxic capacity of these cells. A further advantage could be envisioned with respect to the innate lymphocyte capacity of  $\gamma\delta$ Tc to phagocytose and present antigens to  $\alpha\beta$  T cells, an activity that may be enhanced in the presence of a bispecific antibody. In the treatment of solid tumors, the initial administration of n-BP/IL-2 followed by infusion of bispecific antibody together with IL-2 could probably enhance cytotoxic activity of  $\gamma\delta$ Tc, which infiltrate several different tumor types at low frequency.

### CONCLUDING REMARKS

Bispecific antibodies have been designed in different formats. Clinical trials with bispecific antibodies such as Catumaxomab (TriomAb [EpCAMxCD3]), Ertumaxomab (Triomab [HER2xCD3]), and Blinatumomab (Bispecific T Cell Engager (BiTE) [CD19xCD3]) have delivered impressive therapeutic results. Additional clinical studies are certainly required to deeper

evaluate and improve their therapeutic potential. Bispecific antibodies with specificity for CD3 enhance the cytotoxic potential of  $\alpha\beta$  as well  $\gamma\delta$  T cells. However, under certain circumstances, it would be desirable to activate only  $\gamma\delta$ Tc rather than a polyclonal population of T cells. For instance, CD8<sup>+</sup>  $\gamma\delta$ Tc were presented at low frequency but at higher number than CD8<sup>+</sup>  $\alpha\beta$  T cells in ductal epithelium and nearby stroma in PDAC tissues. This  $\gamma\delta$ Tc accumulation suggests an important role of  $\gamma\delta$ Tc in the immune response against PDAC, which is apparently suppressed by the pronounced immunosuppressive PDAC-microenvironment.

Together with the monitoring system described in this article, the tribody [(Her2)<sub>2</sub>xV $\gamma$ 9], which selectively targets  $\gamma\delta$ Tc and enhances their cytotoxic activity, provides a tool to determine the functional capacity of  $\gamma\delta$ Tc within the blood or within tumor-infiltrating T lymphocytes isolated from fresh tumor tissue of tumor patients. Whether bispecific antibodies targeting  $\gamma\delta$ Tc have the capacity to overcome the immunosuppressive stroma in PDAC patients, has yet to be investigated in further *in vivo* studies.

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