Monitoring circulating γδT cells in cancer patients to optimize γδT cell-based immunotherapy

Hans-Heinrich Oberg1, Christian Kellner2, Matthias Peipp3, Susanne Sebens3, Sabine Adam-Klages1, Martin Gramatzki2, Dieter Kabelitz1 and Daniela Wesch1,*

1 Institute of Immunology, Christian-Albrechts-University of Kiel, Kiel, Germany
2 2nd Medical Department, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts-University of Kiel, Kiel, Germany
3 Institute for Experimental Medicine, Christian-Albrechts-University of Kiel, Kiel, Germany

INTRODUCTION
Human γδ T cells (γδTc) represent a small subset (1–10%) of CD3⁺ T lymphocytes with several unconventional features. Similar to antigen presenting cells (APC), γδTc can phagocytose and present soluble antigens to CD3⁺ αβ T cells (1, 2). Additionally, γδTc 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 γδTc in the context of T cell-based immunotherapeutic strategies (5, 6). Several pilot studies have described a partial success of γδTc-based immunotherapeutic strategies (5, 6) and targeted γδTc for the specific killing of multiple myeloma (13). Novel bispecific antibodies (with concomitant specificity for epitopes on both γδTc and tumor cells) provide a tool to enhance cytotoxic activity of γδTc against cancer cells by selectively targeting γδTc to antigens expressed by tumor cells (16). Additionally, independent of previous immunotherapeutic strategies and prior to the application of a γδ T cell-based immunotherapy, where the cytotoxic activity of circulating γδ 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’ γδ T cells. A possible influence of radio- or chemotherapy on γδ 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 γδ T cell subpopulations in small volumes of whole blood from cancer patients and determining γδ 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 γδ T cells to tumor-associated antigens, will be discussed. Such strategies induce expansion and enhance γδ T cell cytotoxicity and might possibly avoid their exhaustion and overcome the immunosuppressive tumor microenvironment.

Keywords: monitoring, human, γδ T cells, pancreatic ductal adenocarcinoma, bispecific antibodies, phosphorylated antigens, aminobisphosphonate

Abbreviations: BrHPP, bromohydrin-pyrophosphate; γδTc, γδ T cells; mAb, monoclonal antibody; n-BP, nitrogen-containing bisphosphonate; PAg, phosphorylated antigen; PDAC, pancreatic ductal adenocarcinoma; RTCA, real-time cell analyzer; TCR, T cell-antigen receptor.

The success of γδ T cell-based immunotherapy, where the cytotoxic activity of circulating γδ 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’ γδ T cells. A possible influence of radio- or chemotherapy on γδ 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 γδ T cell subpopulations in small volumes of whole blood from cancer patients and determining γδ 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 γδ T cells to tumor-associated antigens, will be discussed. Such strategies induce expansion and enhance γδ T cell cytotoxicity and might possibly avoid their exhaustion and overcome the immunosuppressive tumor microenvironment.

Keywords: monitoring, human, γδ T cells, pancreatic ductal adenocarcinoma, bispecific antibodies, phosphorylated antigens, aminobisphosphonate

Abbreviations: BrHPP, bromohydrin-pyrophosphate; γδTc, γδ T cells; mAb, monoclonal antibody; n-BP, nitrogen-containing bisphosphonate; PAg, phosphorylated antigen; PDAC, pancreatic ductal adenocarcinoma; RTCA, real-time cell analyzer; TCR, T cell-antigen receptor.
Oberg et al. Monitoring to optimize γδ T cell-based immunotherapy

FIGURE 1 | Determination of the absolute cell number of circulating γδ T cells and their subsets in blood of PDAC patients. Fifty microliters whole blood samples from PDAC patients were stained with the indicated mAb in BD Trucount™ Tubes. These mAbs were previously titrated and a final concentration of 2–5 µg/ml was used. The mAb cocktail can be prepared in advance in bulk. The BD Trucount™ tubes contain lyophilized pellets that dissolve after adding liquid, thereby releasing a known number of fluorescent beads. Two hundred microliters of BD Lysing buffer was added to lyse red blood cells. To distinguish lymphocytes and beads from granulocytes and monocytes, an appropriate gate was set on CD45+ 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+ (CD3), TCRγδ+ (γδ), TCRγδ+TCRnon-Vδ2+ (non-Vδ2), and TCRγδ+TCRVδ2+ (Vδ2) within CD45+ 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 µl. Two representative determinations (PDAC-Donor 7 and 2) of 21 are shown, as are the percentages of the different cell populations.

| Parameter | Region | Percent | Value/ Abs. Counts |
|-----------|--------|---------|--------------------|
| Bead Events | Beads |         |                     |
| CD3+      | Q2+Q4  | 80      | 1402               |
| TCRγδ+    | Q2     | 10.8    | 214                |
| TCRVδ2+   | P4     | 10.1    | 191                |
| non-Vδ2   | Q1-2   | 1.2     | 11                 |

representative donors, the absolute numbers of total γδTc as well as Vδ2 and non-Vδ2 subsets are shown (Figure 1). Moreover, cells can be stained with anti-Vδ1 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 Vδ2 γδTc 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 Vδ2 γδTc did not correlate with cancer stage/progression, but rather with patient age (16).

While determination of the absolute γδ 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 γδTc 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 V82 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 V82 T cell exhaustion through repetitive n-BP stimulation and overcome the immunosuppressive activity of PDAC stromal cells on cytotoxic γδ T cells, novel bispecific antibodies such as [Her2xCD3] and [(Her2)xVγ9] were designed. [(Her2)xVγ9] is specific for Vγ9 on γδTc (associated with V82) and for human epidermal growth factor receptor HER2/neu overexpressed on PDAC, breast, and prostate cancer cells. The [(Her2)xVγ9] triobody design allows monovalent binding to γδTc and bivalent HER2-targeting, which enhances avidity to the tumor cell and thereby increases cytolytic activity. Both bispecific antibodies selectively target γδTc to tumor antigens, thereby enhancing the cytotoxic activity of γδTc 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 γδ T cell lines or freshly isolated γδ Tc. Aiming to simplify handling of cells from patients with a low γδ T cell number in the following experiments, we investigated the functional capacity of cytotoxic γδTc within PBMC. We observed that the functional cytotoxic activity of circulating γδTc from patients can be determined in as few as of 1–2 × 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 γδTc within freshly isolated PBMC, the real-time cell analyzer (RTCA) 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 γδ 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 γδTc 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 bromohydridin-phosphate (BrHPP), or (iii) [(Her2)xVγ9]. During the extended time course, we observed that γδTc 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)xVγ9] 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 γδTc demonstrated that cytotoxic activity of γδTc is not induced by IL-2 alone (16).

Regarding the absolute V82 T cell numbers presented in Figure 2B (table), we correlated the unresponsiveness of the majority of the tested patient samples [negative [(Her2)xVγ9] reactivity] with their low initial V82 T cell number. PBMC from patients with more than 30V82+ γδTc/µl blood were responsive (responder in Figure 2A and “positive” in Figure 2B), whereas in samples with <30V82+ γδTc/µl blood, no induction of cytotoxic activity to PAg or [(Her2)xVγ9] stimulation was observed (non-responder in Figure 2A and “negative” in Figure 2B).

The weak capacity of bispecific antibodies to induce γδ T cell proliferation could explain the observed unresponsiveness to [(Her2)xVγ9]. 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% γδTc 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)xVγ9], 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?**

γδ T cell monitoring can provide an estimate for a potential treatment of cancer patients. Although knowledge of the functional capacity of γδTc within PBMC does not provide information about their migration and infiltration into the tumor, characterization of these circulating γδTc is useful since they are activated by intravenous n-BP or PAg administration (8, 10). In clinical trials where γδTc 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 γδTc 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 triobody [(Her2)xVγ9]. Adoptive transfer of γδTc with [(Her2)xVγ9] and IL-2 significantly reduced growth of pancreatic tumors grafted into SCID-Beige mice in comparison to adoptively transferred γδTc together with n-BP and IL-2 (16).

V82 γδTc 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 V82 T cell expansion, while [(Her2)xVγ9] does not induce strong proliferation of γδTc (unpublished data). Independently of the proliferative response of γδ Tc, the cytotoxic activity of PAg or n-BP expanded V82 T cell lines can be significantly enhanced after re-stimulation with [(Her2)xVγ9]. Moreover, the addition of [(Her2)xVγ9] did not induce cell death of V82 T cells, in contrast to restimulation of V82 T cell lines with PAg (unpublished data). Thus, [(Her2)xVγ9] provides a tool to further enhance cytotoxic activity of adoptively transferred γδTc, whereas PAg or
FIGURE 2 | Correlation between absolute cell number and functional capacity of Vι2 T cells.

(Continued)
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 γδTc hampers the expansion of autologous γδTc required for adoptive transfer. Considering these challenges, one might suggest adoptively transferring allogeneic or haploidentical γδTc from (younger) healthy donors or activating γδTc within PBMC in vivo with bispecific antibodies (21–23). To investigate the effect of bispecific antibodies on unstimulated γδTc, we monitored whether [(Her2)xVγ9] can induce cytotoxic activity in γδTc within PBMC. As described above, no or weak responses to [(Her2)xVγ9] were obtained with PBMC from PBMC donors with a lower frequency of Vδ2 γδTc (non-responder), whereas PBMC with a higher Vδ2 γδTc frequency responded to [(Her2)xVγ9] resulting in enhanced cytotoxicity (responder) (Figure 2A). Interestingly, n-BP- or PAg-mediated enrichment of non-responder γδTc within PBMC for 7–14 days led to enhanced cytotoxic activity after restimulating the cells with [(Her2)xVγ9] (Figure 2C).

The validity of this monitoring system to determine γδT cell-reactivity within PBMC needs to be confirmed in patients undergoing γδ 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 Vδ2 γδTc followed by treatment with bispecific antibodies engaging γδTc plus IL-2 in order to avoid the Vδ2 T cell exhaustion observed in patients mediated by repetitive application of n-BP plus IL-2.

**WHAT ARE THE BENEFITS OF COMBINING γδ T CELL-BASED IMMUNOTHERAPY WITH BISPESIFIC 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 γδ T cell-based immunotherapy seems very promising. Rituximab enhanced cytotoxic activity of ex vivo expanded CD16+ (FcγRIII) γδTc against CD20+ chronic lymphocytic leukemia, while Trastuzumab increased γδ T cell cytotoxicity against HER2+ 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 Vγ9 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 γδTc 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 γδ 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+ αβ T cells (21, 22, 29, 30). A successful anti-tumor activity was described for patients with refractory hematological malignancies after adoptive transfer of haploidentical γδTc (23). Labeling ex vivo expanded haploidentical γδTc 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 γδTc to phagocytose and present antigens to αβ 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 γδTc, 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 γδ as well γδ T cells. However, under certain circumstances, it would be desirable to activate only γδ Tc rather than a polyclonal population of Tc cells. For instance, CD8+ γδ Tc were presented at low frequency but at higher number than CD8+ γδ T cells in ductal epithelium and nearby stroma in PDAC tissues. This γδ Tc accumulation suggests an important role of γδ 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 triobody [(Her2) × γδ Tc], which selectively targets γδ Tc and enhances their cytotoxic activity, provides a tool to determine the functional capacity of γδ Tc within the blood or within tumor-infiltrating T lymphocytes isolated from fresh tumor tissue of tumor patients. Whether bispecific antibodies targeting γδ Tc have the capacity to overcome the immunosuppressive stroma in PDAC patients, has yet to be investigated in further in vivo studies.

ACKNOWLEDGMENTS
We gratefully acknowledge Prof. Dr. Ilka Vogel and Elfi Jerg for organizing and providing blood from PDAC patients. The authors thank Sandra Usat, T. T. Hoa Ly, and Kyoung-A. Yoo- Ott for technical assistance. We also thank Prof. Dr. Holger Kalthoff and Dr. Christian Röder for providing PDAC cell lines. BrHPP was kindly provided by Innate Pharma (Marseille, France). Many thanks to Dr. Gabrielle Siegers for helpful comments on this article. All authors declare no competing financial interests. Financial support: This work was supported by the Medical Faculty of Kiel University (DW), and the DFG Pancreatic Cancer Consortium Kiel (DK, DW; WE 3559/2-1; SS; SE 1831/4-1).

REFERENCES
1. Himoudi N, Morgenstern DA, Yan M, Vernay B, Saraiva L, Wu Y, et al. Human γδ T lymphocytes are licensed for professional antigen presentation by interaction with opsonized target cells. J Immunol (2012) 188:1708–16. doi:10.4049/jimmunol.1102654
2. Meuter S, Eberl M, Moser B. Prolonged antigen survival and cytosolic export in γδ T lymphocytes, in combination with IL-2, in patients with metastatic renal cell carcinoma. Cancer Immunol Immunother (2009) 57:136–25. doi:10.1007/s00262-007-0491-8
3. Devilder MC, Allain S, Doussot C, Bonneville M, Scotet E. Early triggering of exclusive IFN-γ responses of human Vγ9Vδ2 T cells by TLR-activated myeloid and plasmacytid dendritic cells. J Immunol (2009) 183:3625–33. doi:10.4049/jimmunol.0901571
4. Kabelitz D, Kalyan S, Oberh HH, Wesch D. Human Vδ2 versus non-Vδ2 γδ T cells in antimyotum immunity. Oncoimmunology (2013) 2:e23304. doi:10.4161/onci.23304
5. Fournière JJ, Sicard H, Poupart M, Bezzombes C, Blanc A, Romagne F, et al. What lessons can be learned from γδ T cell-based cancer immunotherapy trials? Cell Med Immunol (2012) 10:35–41. doi:10.1038/cmium.2012-39
6. Wu YL, Ding YP, Tanaka Y, Shen LW, Wei CH, Minato N, et al. γδ T cells and their potential for immunotherapy. Int J Biol Sci (2014) 10:119–35. doi:10.7150/ijbs.7823
7. Bouet-Toussaint F, Cabillic F, Toutrias O, Le GM, Thomas DLP, Daniel P, et al. Vγ9Vδ2 T cell-mediated recognition of human solid tumors. Potential for immunotherapy of hepatocellular and colorectal carcinomas, Cancer Immunol Immunother (2008) 57:531–9. doi:10.1007/s00262-007-0391-3
8. Dieli F, Vermijden D, Fulford F, Caccamo N, Mervaglia A, Cicero G, et al. Targeting human γδ T cells with zolodele and interleukin-2 for immunotherapy of hormone-refractory prostate cancer. Cancer Res (2007) 67:7450–7. doi:10.1158/0008-5472.CAN-07-1999
9. Kobayashi H, Tanaka Y, Yagi J, Osaka Y, Nakazawa H, Uchiyama T, et al. Safety profile and anti-tumor effects of adoptive immunotherapy using γδ T cells against advanced renal cell carcinoma: a pilot study. Cancer Immunol Immunother (2007) 56:469–76. doi:10.1007/s00262-006-0199-6
10. Mervaglia A, Eberl M, Vermijden D, Todaro M, Buccheri S, Cicero G, et al. In vivo manipulation of Vγ9Vδ2 T cells with zolodele and low-dose interleukin-2 for immunotherapy of advanced breast cancer patients. Clin Exp Immunol (2010) 161:290–7. doi:10.1111/j.1365-2249.2010.04167.x
11. Braza MS, Klein B. Anti-tumour immunotherapy with Vγ9Vδ2 T lymphocytes: from the bench to the bedside. Br J Haematol (2012) 160:123–32. doi:10.1111/bjh.12090
12. Sicard H, Ingouwe S, Luciani B, Serraz C, Fournie J, Bonneville M, et al. In vivo immunomanipulation of Vγ9Vδ2 T cells with a synthetic phosphoantigen in a preclinical nonhuman primate model. J Immunol (2005) 175:5471–80. doi:10.4049/jimmunol.175.8.5471
13. Coscia M, Vitale C, Peola S, Foglietta M, Rigoni M, Grigorio V, et al. Dysfunctional Vγ9Vδ2 T cells are negative prognosticators and markers of deregulated mevalonate pathway activity in chronic lymphocytic leukemia cells. Blood (2012) 120:3271–9. doi:10.1182/blood-2012-03-417519
14. Wilhelm M, Kunzmann V, Eickstein S, Reimer P, Weissinger F, Ruediger T, et al. γδ T cells for immune therapy of patients with lymphoid malignancies. Blood (2003) 102:200–6. doi:10.1182/blood-2002-12-3665
15. Mariani S, Muraro M, Pantaleoni F, Fiore F, Nuschkal B, Peola S, et al. Effector γδ T cells and tumor cells as immune targets of zolodele acid in multiple myeloma. Leukemia (2005) 19:664–70. doi:10.1038/leu.2005.249
16. Oberg HH, Peipp M, Kellner C, Sebens C, Krause S, Petrick D, et al. Novel bispecific antibodies increase γδ T-cell cytotoxicity against pancreatic cancer cells. Cancer Res (2014) 74:1349–60. doi:10.1158/0008-5472.CAN-13-0675
17. Nicholson JK, Stein D, Mui T, Mack R, Hubbard M, Denny T. Evaluation of a method for counting absolute numbers of cells with a flow cytometer. Clin Diag Lab Immunol (1997) 4:309–13.
18. Hensley-McBain T, Heit A, De Rosa SC, McElrath MJ, Andersen-Nissen E. enumeration of major peripheral blood leukocyte populations for multicenter clinical trials using a whole blood phenotyping assay. J Vis Exp (2012) 411:23–36. doi:10.3791/4302
19. Sole CV, Calvo FA, Atahualpa F, Berlin A, Herranz R, Gonzalez-Bayon L, et al. Role of radiotherapy in the chemotherapy-containing multidisciplinary management of patients with resected pancreatic adenocarcinoma. Strahlenther Onkol (2014). doi:10.1007/s00269-014-0759-1
20. Benonoua J, Bompas E, Neirhardt EM, Rolland F, Philip I, Galea C, et al. Phase-1 study of Innacell γδ, an autologous cell-therapy product highly enriched in γδ T lymphocytes, in combination with IL-2, in patients with metastatic renal cell carcinoma. Cancer Immunol Immunother (2008) 57:1599–609. doi:10.1007/s00262-008-0491-8
21. Lamb LS Jr, Lopez RD. γδ T cells: a new frontier for immunotherapy? Biol Blood Marrow Transplant (2005) 11:161–8. doi:10.1016/j.bbmt.2004.11.015
22. Godder KT, Hensley-Downey PJ, Mehta J, Park BS, Chiang KY, Abhyankar S, et al. Successful adoptive transfer and in vivo expansion of haploidentical γδ T cells. J Transl Med (2014) 12:45–50. doi:10.1186/1479-5876-12-45
23. Singh JC, [hasveri K, Esteva FJ. HER2-positive advanced breast cancer: optimizing patient outcomes and opportunities for drug development. Br J Cancer (2014) 111:1888–98. doi:10.1038/bjc.2014.388
24. Wilson WH. Treatment strategies for aggressive lymphomas: what works? Hematology Am Soc Hematol Educ Program (2013) 2013:584–90. doi:10.1182/ashedducation.2013.1.584
25. Tokuyama H, Hagi T, Mattarollo SR, Morley J, Wang Q, So HE, et al. Vγ9 Vδ2 T cell cytotoxicity against tumor cells is enhanced by monocular anti-body drugs – rituximab and trastuzumab. Int J Cancer (2008) 122:526–34. doi:10.1002/ijc.23365
26. Nagorsen D, Kuper P, Baeuerle PA, Bargou R. Blinatumomab: a historical perspective. Pharmacol Ther (2012) 136:334–42. doi:10.1016/j.pharmthera.2012.07.013
27. Klinger M, Brandl C, Zugmaier G, Hijazi Y, Bargou RC, Topp MS, et al. Immunopharmacologic response of patients with B-lineage acute lymphoblastic
leukemia to continuous infusion of T cell-engaging CD19/CD3-bispecific BiTE antibody blinatumomab. Blood (2012) 119:6226–33. doi:10.1182/blood-2012-01-400315

29. Bertaina A, Merli P, Rutella S, Pagliara D, Bernardo ME, Masetti R, et al. HLA-haploidentical stem cell transplantation after removal of αβ+ T and B cells in children with nonmalignant disorders. Blood (2014) 124:822–6. doi:10.1182/blood-2014-03-563817

30. Drobyski WR, Majewski D, Hanson G. Graft-facilitating doses of ex vivo activated γδ T cells do not cause lethal murine graft-vs.-host disease. Biol Blood Marrow Transplant (1999) 5:222–30. doi:10.1053/bbmt.1999.v5.pm10465102

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 October 2014; accepted: 03 December 2014; published online: 17 December 2014.

Citation: Oberg HH, Kellner C, Peipp M, Sebens S, Adam-Klages S, Gramatzki M, Kabelitz D and Wesch D (2014) Monitoring circulating γδ T cells in cancer patients to optimize γδ T cell-based immunotherapy. Front. Immunol. 5:643. doi: 10.3389/fimmu.2014.00643

This article was submitted to T Cell Biology, a section of the journal Frontiers in Immunology.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.