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# γδ T Cells as Immuno-Oncology Treatments in the Era of Precision Medicine

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## γδ T Cell Biology:

Harnessing the potential of the immune system to treat cancers has been the goal of many scientific investigations in the last few decades. Recent advances in cancer biology and immunology have allowed for cancer immunotherapy to become a reality. The premise of cancer immunotherapy is to stimulate the patient's own immune system to attack and reject the malignant cells, sparing normal surrounding tissues. Strategies that rely on the Dendritic Cells (DC) to Cytotoxic T Lymphocytes (CTL) axis to mount a specific immune response against different types of cancers have met variable success so far due largely to tumor evasion of recognition by the majority of T lymphocytes, known as αβ T lymphocytes, by several mechanisms including reduced expression levels of MHC Class I molecules [1] and secretion of immunosuppressive cytokines, such as Interleukin-10 (IL-10) and Transforming Growth Factor-β (TGF-β) [2, 3, 4] On the other hand, T lymphocytes that express the γδ TCR (γδ T cells) do not require antigen presentation by target cells. Instead, they recognize non-peptidic phospho-antigens (pyro-phospho-antigens), such as (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) and isopentenyl pyrophosphate (IPP), a metabolite of the mevalonate metabolic pathway, which can accumulate in tumor cells with increased metabolic activity of the mevalonate metabolic pathway (Figure 1). These phospho-antigens are usually recognized in complex with Butyrophilin 3A1 (BTN3A1), also known as CD277 [5, 6].

Additionally, the C-type lectin Natural Killer Group 2D (NKG2D) on γδ T cells recognizes stress-induced self-antigens widely expressed on cancer cells, such as the MHC Class I-like stress-associated molecules MIC-A and MIC-B or the UL-16 binding proteins ULBP-1, 2, and 3 (Figure 1) [7, 8, 9] This recognition can mediate direct cytotoxicity and lysis of tumor cells without prior antigen exposure or priming [9] and secretion of anti-tumor cytokines, such as Interferon gamma (IFN-γ) and Tumor Necrosis Factor-α (TNF-α). Thus, giving γδ T cells the advantage of a rapid and uniform response similar to innate immune responses.

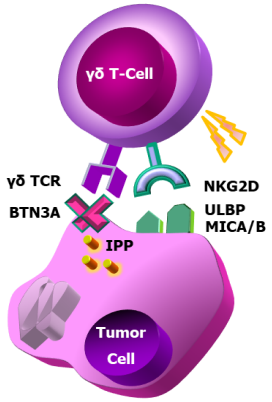


**Figure 1.** schematic representation of the interactions between γδ T cell and tumor cell

Human γδ T cells are divided into two major subsets, Vδ1 or Vδ2, according to their TCR-δ chain [10, 11]. Vδ1 is the dominant phenotype in mucosal areas [12, 13, 14], while Vδ2 is typically paired with Vγ9, making Vγ9Vδ2 the dominant phenotype of γδ T cells in the peripheral blood. Although the variable regions of the γ and δ chains exhibit diversity, circulating Vγ9Vδ2 repertoire has remarkably reduced diversity because of chronic positive selection due to exposure to phospho-antigens produced by host cells or resident microbes [15, 16].

Although γδ T cells normally constitute only 1-5% of peripheral blood T cells, they can be *ex vivo*-expanded and activated when cultured with IL-2 and Zoledronate (or Zoledronic acid) [17, 18, 19]. Zoledronate is an FDA-approved bisphosphonate mainly used

to prevent bone fractures in patients with metastatic cancer or postmenopausal osteoporosis. Bisphosphonates interfere with the mevalonate metabolic pathway by inhibiting the farnesyl diphosphate synthase (FDPS), which leads to accumulation of the isopentenyl pyrophosphate (IPP) in cancer cells and subsequent TCR-dependent activation of γδ T cells, specifically the subpopulation carrying the Vδ2 receptor [20, 21, 22].

This method has been used to expand γδ T cells from cancer patients with hepatocellular [23], colorectal [23], prostate [24], lymphoid [25] and breast cancers [26]. However, γδ T cells from cancer patients are typically diminished in their numbers and have decreased proliferation in response to mitogens [27, 28]. Alternatively, we have demonstrated that γδ T cells can be expanded from healthy donors [29, 30] as these allogeneic donor effector cells are safe to transfer to cancer patients since γδ T cells do not recognize foreign MHC I antigens and are not likely to trigger graft versus host disease (GVHD). Collectively, these biological qualities of γδ T cells make them a promising option for cancer immunotherapy.

## Cancer Immunotherapies Based on γδ T Cells:

Vγ9Vδ2 T cells were identified as tumor-infiltrating T lymphocytes (TILs) in a majority of colorectal cancer patients [31] where they seemed to correlate with favorable prognosis [32, 33]. Subsequent studies have shown that the *ex vivo* expansion of Vγ9Vδ2 T cells using Bromohydrin pyrophosphate (BrHPP) or Zoledronate yields effector memory cells with the phenotype $CD45RA^{-}$ CD45RO high $CD27^{-}$. These expanded cells exhibited strong lytic activity toward colorectal carcinoma cell lines. The cytotoxicity was mainly dependent on the TCR receptor and on the NKG2D receptor as a costimulatory signal [23]. Similarly, BrHPP- or Zoledronate-stimulated γδ T cells were able to lyse tumor cells freshly isolated from hepatocellular carcinoma but not normal counterpart tissues [23]. Studies have shown that cytotoxicity was dependent on interactions between the DNAX Accessory Molecule-1 (DNAM-1) on γδ T cells and the Nectin-like molecule-5 (Necl-5) expressed on hepatocellular carcinoma cells, in addition to the recognition of MICA/B or ULBP 1-3 by the NKG2D receptor [34].

*Ex vivo* expansion of Vγ9Vδ2 T cells in the presence of Interleukin-12 resulted in large-scale expansion of human γδ T lymphocytes that are resistant to mitogen-induced apoptosis [35]. These apoptosis-resistant γδ T cells have proven effective against prostate cancer cells, such as DU145 and PC-3 cell lines [36]. Prostate cancer cell killing seemed to be dependent on γδ TCR and interactions between Integrin Beta Chain-2 (CD18) and Intercellular Adhesion Molecule-1 (ICAM-1) and is mediated by the perforin/granzyme pathway [36]. A modified protocol for *ex vivo* expansion of Vγ9Vδ2 T cells used pulse Zoledronate stimulation to avoid the toxic effects of farnesyl diphosphate synthase (FDPS) inhibition by continuous exposure to Zoledronate [37]. This has enhanced γδ T cell purity and numbers compared with continuous Zoledronate stimulation. Moreover, the expanded Vγ9Vδ2 T cells produced higher levels of perforin and degranulated in large numbers when exposed to PC-3 prostate cancer cells as evident by the increased expression of the CD107a, which is a lysosomal-associated membrane protein (LAMP-1) that is mobilized to the surface when cytotoxic T and NK cells degranulate for killing [38]. This resulted in a 2.5-fold increase in their anti-tumor cytolytic activity. Adoptive transfer of these effector cells halted the growth of PC-3 tumor cells xenotransplanted into the highly immunodeficient NSG mice, reducing tumor volume by 50% compared with those expanded using continuous Zoledronate stimulation [39].

Vδ2+ γδ T cells were identified in non-cancerous mammary ductal epithelial organoids, perhaps indicating their role in immunosurveillance and subsequent elimination of neoplastically-transformed breast ductal epithelial cells [40], given their ability to detect the changes associated with malignant transformation and stress-induced molecules, such as with MIC-A/B. Moreover, Vδ2+ γδ T cells derived from breast ductal organoids produced the anti-tumor cytokine, IFN-γ, while they efficiently killed the bisphosphonate-pulsed human breast carcinoma cell line, MDA-MB-468, which is triple negative for estrogen receptor, progesterone receptor, and HER2/neu [41]. Similarly, studies involving the peripherally-derived γδ T lymphocytes demonstrated cytotoxic activity against a variety of breast cancer cell lines both *in vitro* and in murine models. This anti-tumor activity seemed to be dependent on breast cancer subtype, TCR engagement, and MICA/B and ICAM1 expression levels [42, 43, 44]. However, it is worth mentioning that Vδ1+ T lymphocytes are the dominant subtype found in tumor-infiltrating T lymphocytes (TILs) of breast cancers, where they exert immune-suppressing effects, such as the suppression of naïve T cell proliferation and DC maturation and the secretion of immunosuppressant cytokines. This pro-tumorigenic activity seems to be mediated by interferon gamma-induced protein 10 (IP-10) secreted by breast tumor cells. IP-10 recruits Vδ1+ T cells to the tumor microenvironment where they promote tumor growth and spread [45].

Since Vγ9Vδ2 T cells can be *ex vivo*-expanded and are usually well-tolerated by recipients, highly enriched clinical-grade autologous Vγ9Vδ2 T cells were prepared using Zoledronate and re-infused into cancer patients in a number of adoptive transfer clinical studies to evaluate the safety and potential therapeutic effects. For instance, in two early phase clinical trials of Non-Small Cell Lung Cancer (NSCLC), stable disease was achieved in three out of ten patients in one study and six out of fifteen patients in the other NSCLC study [46, 47]. In advanced renal cell carcinoma (RCC), adoptive transfer of γδ T cells into eleven patients resulted in a prolonged tumor doubling time (DT) as measured by computed tomography (CT), which subsequently led to one complete remission and stable disease in five patients based on Evaluation Criteria in Solid Tumors (RECIST) [48]. Similar outcomes were

achieved for metastatic RCC, where six out of ten patients showed stable disease [49]. Separately, intraperitoneal injection of *ex vivo*-expanded Vγ9Vδ2 T cells yielded a significant reduction in volume of malignant ascites, caused by peritoneal dissemination of gastric cancer, in two of the seven patients enrolled in this study [50]. In another pilot study, four patients with advanced refractory hematological malignancies (T-NHL, AML, secondary plasma cell leukemia, and multiple myeloma), who were not eligible for allogeneic transplantation, received γδ T cells from half-matched (haploidentical) family donors, resulting in three complete responses [51].

Alternatively, Vγ9Vδ2 T cells can be expanded *in vivo* by administration of FDA-approved amino-bisphosphonates and low dose IL-2 into the patients. Phase I/II of clinical trials demonstrated the safety and feasibility of this approach. *In vivo* stimulation of γδ T cells in breast cancer patients showed one case of partial remission and two cases of stable disease amongst three patients that sustained robust Vγ9Vδ2 T cell numbers over twelve months, which also correlated with declining levels of Cancer Antigen 15-3 (CA 15-3), a surrogate breast cancer biomarker [52]. γδ T cells *in vivo* stimulation with Zoledronate and IL-2 in nine hormone-refractory prostate cancer patients resulted in three instances of partial remission and five of stable disease [23]. Finally, in a study focused on lymphoid malignancies, which included a cohort of nine patients with relapsed/refractory Non-Hodgkin's Lymphoma (NHL) or multiple myeloma (MM), partial remissions were achieved in three patients [53].

Although clinical studies have demonstrated the safety and some efficacy of γδ T cells immunotherapy, the overall response in patients has been less than ideal, which can possibly be explained by lymphocyte exhaustion or activation-induced cell death (AICD) upon repeated stimulation. As we usher in the age of precision medicine, efforts are being invested in developing Chimeric Antigen Receptor (CAR)-engineered γδ T Cells, as autologous or allogeneic, off-the-shelf cell therapy products (Figure 2) since these innate immune cells recognize their antigens in an MHC-independent manner. As a proof of concept, γδ T cells were successfully transduced with a second-generation CAR targeting GD2 and containing CD3-ζ and CD28 signaling domains and displayed GD2-specific anti-tumor cytotoxicity [54]. Combined with their capacity for migration to the tumor microenvironment and for uptake of tumor antigens and cross presentation, these CAR-transduced γδ T cells might offer significant advantages as personalized cancer treatments over the conventional αβ CAR-T cells, especially for patients with solid malignant tumors.

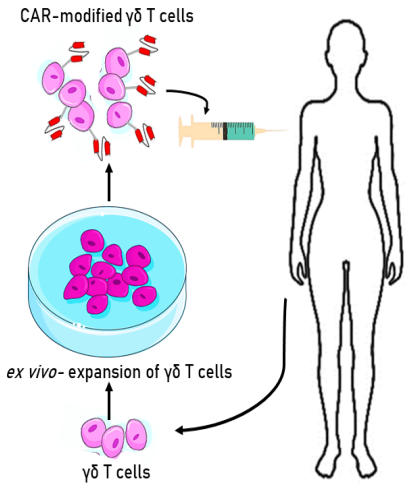


**Figure 2.** γδ T cells extracted from patients can be expanded and genetically modified into chimeric antigen receptor-T cells (CAR-T) and transfused back into the patient as personalized cancer treatment.

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