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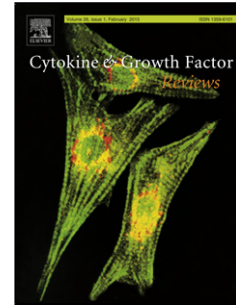
**Reference:**

Van Acker Heleen, Campillo Davó Diana, Roex Gils, Versteven Maarten, Smits Evelien, Van Tendeloo Vigor.- The role of the common gamma-chain family cytokines in  $\gamma\delta$  T cell-based anti-cancer immunotherapy  
Cytokine and growth factor reviews - ISSN 1359-6101 - Oxford, Elsevier sci ltd, 41(2018), p. 54-64  
Full text (Publisher's DOI): <https://doi.org/10.1016/J.CYTOGFR.2018.05.002>  
To cite this reference: <https://hdl.handle.net/10067/1524940151162165141>

## Accepted Manuscript

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PII: S1359-6101(18)30060-1  
DOI: <https://doi.org/10.1016/j.cytogfr.2018.05.002>  
Reference: CGFR 1056

To appear in: *Cytokine & Growth Factor Reviews*

Received date: 21-4-2018  
Revised date: 7-5-2018  
Accepted date: 9-5-2018

Please cite this article as: Van Acker HH, Campillo-Davo D, Roex G, Versteven M, Smits EL, Van Tendeloo VF, The role of the common gamma-chain family cytokines in  $\gamma\delta$  T cell-based anti-cancer immunotherapy, *Cytokine and Growth Factor Reviews* (2018), <https://doi.org/10.1016/j.cytogfr.2018.05.002>

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## The role of the common gamma-chain family cytokines in $\gamma\delta$ T cell-based anti-cancer immunotherapy

Heleen H. Van Acker<sup>a,\*</sup>, Diana Campillo-Davo<sup>a</sup>, Gils Roex<sup>a</sup>, Maarten Versteven<sup>a</sup>, Evelien L. Smits<sup>a,c</sup> and Viggo F. Van Tendeloo<sup>a</sup>

Author affiliations:

<sup>a</sup> Laboratory of Experimental Hematology, Tumor Immunology Group (TIGR), Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Faculty of Medicine and Health Sciences, Antwerp, Belgium

<sup>b</sup> Center for Cell Therapy & Regenerative Medicine, Antwerp University Hospital, Edegem, Belgium

<sup>c</sup> Center for Oncological Research (CORE), Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium

**\*Corresponding author:** Heleen Van Acker

Wilrijkstraat 10, 2650 Edegem, Belgium

Tel +32 3 821 58 57

Fax +32 3 821 44 56

e-mail [heleen.vanacker@uantwerp.be](mailto:heleen.vanacker@uantwerp.be)

### **Highlights**

- The cytokines of the common gamma-chain receptor family all have their unique effects on  $\gamma\delta$  T cells.
- Interleukin-15-activated  $\gamma\delta$  T cells arise as strong candidates for future cancer immunotherapies.

**Abstract**

Cytokines of the common gamma-chain receptor family, comprising interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15 and IL-21, are vital with respect to organizing and sustaining healthy immune cell functions. Supporting the anti-cancer immune response, these cytokines inspire great interest for their use as vaccine adjuvants and cancer immunotherapies. It is against this background that gamma delta ( $\gamma\delta$ ) T cells, as special-force soldiers and natural contributors of the tumor immunosurveillance, also received a lot of attention the last decade. As  $\gamma\delta$  T cell-based cancer trials are coming of age, this present review focusses on the effects of the different cytokines of the common gamma-chain receptor family on  $\gamma\delta$  T cells with respect to boosting  $\gamma\delta$  T cells as a therapeutic target in cancer immunotherapy. This review also gathers data that IL-15 in particular exhibits key features for augmenting the anti-tumor activity of effector killer  $\gamma\delta$  T cells whilst overcoming the myriad of immune escape mechanisms used by cancer cells.

Key words – cancer immunotherapy, common gamma-chain family, gamma delta T cells, IL-2 cytokine family, interleukin-15

Gamma delta ( $\gamma\delta$ ) T cells are a conserved population of innate lymphocytes taking part in numerous immune responses during tissue homeostasis, infectious disease, autoimmune disease, inflammation, transplantation and tumor surveillance. Since their accidental discovery in the 1980s, while characterizing the alpha beta ( $\alpha\beta$ ) T cell receptor (TCR) genes [1],  $\gamma\delta$  T cells remain an enigmatic immune cell subset which has not yet revealed all his secrets. They are very effective in thwarting most attempts at defining their exact role in the immune system. Depending on the particular context,  $\gamma\delta$  T cells share attributes of the adaptive or innate immune system, or both [2-5]. In humans, there are three major subsets of  $\gamma\delta$  T cells identified by their variable (V) $\delta$  chain, namely V $\delta$ 1, V $\delta$ 2 and V $\delta$ 3 T cells. These subsets are largely non-overlapping in function as they differ in their tissue localization and antigen recognition. V $\delta$ 1 T cells recognize various stress-related antigens, mostly uncharacterized, and are predominant in the thymus and peripheral tissues [6]. The most abundant subset of circulating  $\gamma\delta$  T cells is the V $\delta$ 2 subset (preferentially paired to the V $\gamma$ 9 chain), which is mainly activated by phosphoantigens, i.e. metabolic intermediates of the isoprenoid biosynthesis [7]. The two most studied phosphoantigens are (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP), produced by the non-mevalonate pathway of many bacteria and protozoan parasites, and isopentenyl pyrophosphate (IPP), which is produced in eukaryotic cells through the mevalonate pathway. Finally, the V $\delta$ 3 T cell subset is enriched in the liver and is generally more common in patients with some viral infections and leukemia [8]. In the present review, we focus on the effects of the different cytokines of the common gamma-chain receptor family on  $\gamma\delta$  T cells, in view of boosting  $\gamma\delta$  T cells as a therapeutic target in cancer immunotherapy.

### $\gamma\delta$ T cells in cancer

$\gamma\delta$  T cells are an imperative part of the tumor immune microenvironment and are known to affect the anti-tumor immune response in a wide variety of tumors [9]. This is reflected, *inter alia*, by the fact that  $\gamma\delta$  T cells are able to infiltrate many types of tumors, including breast cancer [10], colorectal cancer [11], lung cancer [12] and melanoma [13]. In addition, a recent study analyzing the gene expression profiles of 18,000 samples derived from 39 different cancer types, both solid tumors and hematopoietic malignancies, identified tumor-infiltrating  $\gamma\delta$  T cells as the most significant favorable cancer-wide prognostic signature [14]. Furthermore, patients with a high  $\gamma\delta$  T cell immune reconstitution (after hematopoietic stem cell transplantation [HSCT]) show significantly improved long-term disease-free survival rates as compared to patients with low or normal  $\gamma\delta$  T cell counts [15-17]. This can be attributed to the plethora of protective properties of  $\gamma\delta$  T cells; production of abundant pro-inflammatory cytokines, including interferon (IFN)- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$ , major histocompatibility complex (MHC)-independent recognition of antigens, direct lysis of malignant cells, antibody-

Abbreviations =  $\alpha\beta$ , alpha beta; ADCC, antibody-dependent cellular cytotoxicity; APC, antigen-presenting cell; BrHPP, bromohydrin pyrophosphate; BTLA, B and T lymphocyte attenuator; CAR, chimeric antigen receptor; DC, dendritic cell;  $\gamma_c$ , common gamma;  $\gamma\delta$ , gamma delta; HSCT, hematopoietic stem cell transplantation; HMB-PP, (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate; IL, interleukin; IFN, interferon; IPP, isopentenyl pyrophosphate; MHC, major histocompatibility complex; NFAT, nuclear factor of activated T cells; NK, natural killer; NKG2D, natural killer group 2D; PBMC, peripheral blood mononuclear cells; PD, programmed cell death; TCR, T cell receptor; Tfh, T follicular helper; Th, T helper; TIL, tumor infiltrating lymphocytes; TNF, tumor necrosis factor; Treg, regulatory T cell; V, variable

dependent cellular cytotoxicity (ADCC), establishment of a memory response and even antigen presentation [18]. Unfortunately, cancer patients often have exhausted and/or reduced numbers of  $\gamma\delta$  T cells [19, 20]. On the other hand, evidence is emerging that certain subsets of  $\gamma\delta$  T

Abbreviations =  $\alpha\beta$ , alpha beta; ADCC, antibody-dependent cellular cytotoxicity; APC, antigen-presenting cell; BrHPP, bromohydrin pyrophosphate; BTLA, B and T lymphocyte attenuator; CAR, chimeric antigen receptor; DC, dendritic cell;  $\gamma_c$ , common gamma;  $\gamma\delta$ , gamma delta; HSCT, hematopoietic stem cell transplantation; HMB-PP, (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate; IL, interleukin; IFN, interferon; IPP, isopentenyl pyrophosphate; MHC, major histocompatibility complex; NFAT, nuclear factor of activated T cells; NK, natural killer; NKG2D, natural killer group 2D; PBMC, peripheral blood mononuclear cells; PD, programmed cell death; TCR, T cell receptor; Tfh, T follicular helper; Th, T helper; TIL, tumor infiltrating lymphocytes; TNF, tumor necrosis factor; Treg, regulatory T cell; V, variable

cells can perform regulatory undertakings, among others, by means of programmed cell death (PD)-1/PD-L1 immune checkpoint inhibition, regulatory T cell (Treg) and T helper type (Th)2 cell-like activity, and interleukin (IL)-17a production [21-24]. Therefore, in view of the use of  $\gamma\delta$  T cells as an anti-cancer therapeutic, their high functional plasticity is a hurdle to take. Namely, exposure of  $\gamma\delta$  T cells to polarizing factors present in the tumor microenvironment might induce the polarization of  $\gamma\delta$  T cells from a desirable Th1 to an unfavorable Treg and Th17 type [25-27]. On the other hand, the reverse polarization can be seen as well. For example, suppressive  $\gamma\delta$  T cells can revert to a more pro-inflammatory phenotype after toll-like receptor ligand encounter [28, 29]. As a result, in the context of cancer immunotherapy, it will be vital to harness  $\gamma\delta$  T cells (back) into the anti-tumor immune response and to consolidate their functional polarization to effector  $\gamma\delta$  T cells for their use as an immunotherapeutic tool.

### $\gamma\delta$ T cells for cancer immunotherapy

Several biotech companies aiming at bringing  $\gamma\delta$  T cells into the clinic recently saw the light of day, including Gadeta (Utrecht, The Netherlands), GammaDelta Therapeutics (London, UK), Incysus (Hamilton, Bermuda) and Lymphact (Coimbra, Portugal). Indeed, targeting  $\gamma\delta$  T cells for the modulation of anti-tumor immune responses holds many promises and (commercial) benefits. A key feature of  $\gamma\delta$  T cells is that they can perceive cancer as a metabolic disorder, through sensing altered lipid pathways, providing an alternative mechanism for detecting and eliminating malignant cells [30]. Since there is no requirement of specific tumor-associated antigens and co-stimulatory molecules for  $\gamma\delta$  T cell activation, unlike for classic  $\alpha\beta$  T cells,  $\gamma\delta$  T cells are still able to recognize cancer cells with loss of tumor MHC class I expression and other defects in antigen presentation. This offers stirring prospects for novel universal (off-the-shelf) cancer immunotherapies. A further advantage of  $\gamma\delta$  T cells, in addition to their aforementioned robust anti-tumor effects, is their chemoresistancy. This leaves  $\gamma\delta$  T cells ideal candidates for combinatorial chemoimmunotherapy, two approaches long considered mutually exclusive [31-33].

$\gamma\delta$  T cell cancer immunotherapy can be divided into two main approaches; (1) *in vivo* expansion of  $\gamma\delta$  T cells using primarily combinations of aminobisphosphonates and IL-2, and (2) the adoptive transfer of *ex vivo* activated and expanded  $\gamma\delta$  T cells [7, 18]. These approaches are continually further developed and refined, comprising the use of chimeric antigen receptor (CAR) engineered  $\gamma\delta$  T cells [34] and TCR transduced cells [35].  $\gamma\delta$  T cell immunotherapy has already been assessed against a variety of solid and hematological malignancies in early phase clinical trials, reviewed by Fisher *et al.* [36]. Recent clinical trials investigating  $\gamma\delta$  T cells as immunotherapeutic are summarized in table 1-2. These trials established  $\gamma\delta$  T cell immunotherapy as feasible, i.e. they can be easily expanded both *in vivo* and *in vitro*, safe, and able to induce anti-tumor immunity. The results of large phase 3 clinical trials are, however, still awaited.

### The common gamma ( $\gamma_c$ ) chain receptor family cytokines

The common gamma-chain receptor family includes six members, namely IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. These cytokines, also referred to as IL-2 family cytokines, all signal through receptor complexes that contain the common cytokine receptor gamma-chain subunit paired

with a distinctive cytokine-specific subunit (Figure 1). IL-2 and IL-15, however, have an additional common IL-2/IL-15R $\beta$  unit, resulting in a heterotrimeric complex. Signaling after binding to their receptor is channeled to three major pathways, namely (1) the Janus kinase–signal transducer and activator of transcription pathway (JAK/STAT pathway), (2) the phosphoinositide 3-kinase (PI3K)–AKT pathway and (3) the mitogen-activated protein kinase pathway (MAPK). The  $\gamma_c$  cytokines are indispensable in the functioning of the immune system, holding decisive functions in the development, homeostasis, proliferation, survival and functioning of various cell lineages of both the innate and adaptive immune system [37, 38]. From this, it is apparent that the common gamma-chain cytokine family is exploited in many immune-related conditions.

Currently, the use of these cytokines to harness the immune system in the fight against malignant cells is one of the most important areas of cancer immunotherapy research. Consistent with this, the function and proliferative capacity of  $\gamma\delta$  T cells heavily relies on the actions of such cytokines, for which IL-2 is by far the most studied cytokine, albeit with limitations that ultimately might be averted through the use of other cytokines of the same family. Below we will discuss each cytokine of the  $\gamma_c$  receptor family in more detail, especially with regard to its use for  $\gamma\delta$  T cell immunotherapy (Table 3; *vide infra*).

## Interleukin-2

IL-2 is one of the earliest cytokines discovered and was the first immunotherapy approved by the FDA to be used as a primary or stand-alone cancer treatment [39]. Originally designated as a T cell growth factor, it is now well-believed that its role in both the innate and adaptive immune system exceeds by far its original description [40]. IL-2 supports among others the cytotoxic capacity of CD8<sup>+</sup> T cells and natural killer (NK) cells, promotes naive CD4<sup>+</sup> T cell differentiation into Th1 and Th2 cells while inhibiting Th17 and T follicular helper (Tfh) cell differentiation [41], and regulates plasma cell activity. Moreover, IL-2 induces activation-induced cell death and is indispensable for the generation and preservation of Treg [42]. Although desirable outside the context of cancer immunotherapy, limiting inappropriate immune reactions, the latter features represent a caveat for the use of IL-2 by tumor immunologists. A second disadvantage is the very short serum half-life of IL-2, which is in the range of minutes, due to its rapid renal clearance, necessitating frequent administration of high doses. This leads unfortunately to severe toxicities including hyperpyrexia, hypotension, vascular leak syndrome, pulmonary edema, and heart toxicities [39].

$\gamma\delta$  T cells – Focusing on  $\gamma\delta$  T cells, they are renowned for their distinct expansion through IL-2 signaling, both paracrine and autocrine [43, 44]. Namely, *in vivo*  $\gamma\delta$  T cells produce high levels of IL-2, promoting their own survival and proliferation, as a result of (among others) CD28 co-stimulation [44]. Accruing from this, the clinical manipulation of  $\gamma\delta$  T cells in the field of cancer immunotherapy and beyond largely depends on IL-2 stimulation (in combination with phosphoantigens) [36]. For example, IL-2 reverses dysfunctional  $\gamma\delta$  T cell responses from obese individuals [45] and in non-human primates the combination of IL-2 and phosphoantigens controls *Mycobacterium tuberculosis* infection [46]. However, to date, clinical trials with IL-2 showed only modest efficacy, significant toxicity and even some effects counteracting the anti-tumor benefits, including immunotherapy-induced vascular endothelial



growth factor release [36, 47]. The question therefore arises whether this approach would generate the best results.

While binding of IL-2 to its receptor results in the activation of all three IL-2 signaling pathways in IL-2 stimulated  $\gamma\delta$  T cells, direct translation into  $\gamma\delta$  T cell proliferation or (enhanced) effector functions are not perceived.  $\gamma\delta$  T cell expansion, and IFN- $\gamma$ , TNF- $\alpha$  and CD107a expression are only invigorated by IL-2 in addition to phosphoantigen activation [48-51]. This essential prerequisite of  $\gamma\delta$  TCR contribution results from the activation of the calcium pathway, leading to the translocation of nuclear factor of activated T cells (NFAT)1 and NFAT4 proteins to the nucleus [48]. Moreover, while clinical trials exploring the combination of IL-2 and phosphoantigens as  $\gamma\delta$  T cell therapy demonstrate positive effects on tumor growth, this approach is also associated with  $\gamma\delta$  T cell exhaustion and anergy, conforming to known IL-2 effects [52-54]. Besides, all  $\gamma\delta$  T cells cannot be tarred with the same brush. Although the majority of  $\gamma\delta$  T cells are CD4<sup>+</sup>CD8<sup>-</sup>, positive subsets exist, possibly representing functional distinct subsets [3, 55]. Indeed, whereas the percentage of CD8<sup>-</sup>  $\gamma\delta$  T cells is decreased in peripheral blood mononuclear cells (PBMCs) after IL-2 stimulation, the opposite is seen for CD8 $\alpha\alpha$ <sup>+</sup> and CD8 $\alpha\beta$ <sup>+</sup>  $\gamma\delta$  T cells [56]. Moreover, IL-2 signaling increases IFN- $\gamma$  and TNF- $\alpha$  production in the CD8 $\alpha\beta$ <sup>+</sup>  $\gamma\delta$  T cells, leaves the cytokine production of the CD8 $\alpha\alpha$ <sup>+</sup> subset unchanged and obstructs the pro-inflammatory cytokine response of CD8<sup>-</sup>  $\gamma\delta$  T cells [3]. Hence, the use of IL-2 in this study actually had a detrimental effect on the anti-tumor capacity of the majority of  $\gamma\delta$  T cells [55]. Finally, in line with the immunosuppressive effect of IL-2 on classic  $\alpha\beta$  T cells, IL-2 supports Th17  $\gamma\delta$  T cells, who emerge as an important pro-tumor subset [24, 57-59]. The effect is however not unilateral. Even though IL-2 induces Th17  $\gamma\delta$  T cell proliferation, it also negatively impacts on their survival via downregulation of the IL-7R [59]. Thus, notwithstanding the undeniable qualities of IL-2, the use of this cytokine warrants careful dosing and implementation.

Turning to the other cytokines of the common gamma-chain family cytokines, interesting properties come to light, considering they have both unique and overlapping effects. Different cytokines could therefore be of interest depending on the context and the aspired objectives. By way of example, direct comparison between  $\gamma\delta$  T cells within PBMCs stimulated with HMB-PP and IL-2, IL-4 or IL-21 showed that HMB-PP- and IL-2-stimulated  $\gamma\delta$  T cells displayed a prototypic proinflammatory phenotype, characterized by IFN- $\gamma$  and TNF- $\alpha$  production, but surprisingly also the Th2 cytokines IL-5 and IL-13. Conversely, Th2-like  $\gamma\delta$  T cells obtained by HMB-PP and IL-4 stimulation are found to be deficient in the secretion of IL-5 and IL-13. IL-21 and HMB-PP stimulated  $\gamma\delta$  T cells had a limited proinflammatory phenotype, although they promoted B cell maturation [60]. This is further evidenced by microarray analysis defining 513 and 328 differentially expressed genes between  $\gamma\delta$  T cells stimulated with HMB-PP in combination with IL-2 and IL-4 or IL-21, respectively (Table 3). However, these differences between stimulated  $\gamma\delta$  T cells were far outnumbered by the differences between activated and fresh  $\gamma\delta$  T cells, reflecting the family ties [60].

#### **Interleukin-4**

IL-4 induces differentiation of naive T cells to Th2 and Tfh cells, regulates immunoglobulin class switching, and promotes mast cell survival and proliferation [61]. This results from the binding of IL-4 to IL-4R $\alpha$ , leading to the recruitment of the common gamma-chain and/or IL-

13R $\alpha$ 1, and ultimately the formation of three different types of IL-4R complexes; (I) IL-4R $\alpha$  in combination with the IL-2R $\gamma$ , (II) IL-4R $\alpha$  and IL-13R $\alpha$ 1 and (III) all three chains together, namely IL-4R $\alpha$ , IL-2R $\gamma$  and IL-13R $\alpha$ 1 [62]. In cancer, IL-4 has been ascribed both an anti-tumor and a tumor-promoting role. IL-4 was first used as an immunotherapeutic agent against malignancy [63]. However, more and more evidence is gathered of (direct) tumor-promoting effects of IL-4. Namely, increased IL-4 levels are seen in cancer patients, associated with tumorigenesis, Th2-polarized immune responses, reduced cytotoxic T lymphocyte activity and the differentiation of macrophages into an “M2” phenotype, exhibiting poor antigen-presenting capacity and local anti-inflammatory effects [64-68]. Owing to these knowledge gaps, more research is warranted to unravel the complete extent of the IL-4 signaling, also keeping in mind that not all of the effects are mediated via a common gamma-chain containing receptor.

$\gamma\delta$  T cells – To date, the effect of IL-4 on  $\gamma\delta$  T cells is poorly studied. Potentially, IL-4 assists in the differentiation of Th1-like effector  $\gamma\delta$  T cells in the thymus and periphery, as evinced by the induction of eomesodermin, a T-box transcription factor [69]. Furthermore, although no direct effect of IL-4 on  $\gamma\delta$  T cells was reported, patients with low-grade non-Hodgkin lymphomas had a better prognosis with increased circulating V $\delta$ 1 T cells and high levels of serum IL-4 [70]. Conversely, IL-4 confers an inhibitory effect on the activation of naive  $\gamma\delta$  T cells, namely reduced  $\gamma\delta$  T cell proliferation and seriously suppressed IFN- $\gamma$  and TNF- $\alpha$  production, but induced IL-10 production [71]. This IL-4-mediated inhibition of  $\gamma\delta$  T cell activation, in the presence of TCR stimulation, was attributed to the suppression of among others the Ca<sup>2+</sup> release [70]. Unexpectedly, IL-4 does promote the proliferation of already activated  $\gamma\delta$  T cells, with a predisposition of the V $\delta$ 1 subset [70]. Therefore, IL-4 signaling seems to induce a seemingly conflicted dual effect; inhibition of TCR signaling and promotion of proliferation. Altogether, in view of the immunoregulatory profile of the expanded V $\delta$ 1 T cell subset by IL-4, it can be speculated that IL-4 will weaken the  $\gamma\delta$  T cell-mediated anti-tumor immune response.

### **Interleukin-7**

IL-7 is indispensable for the adaptive immune system, being required for optimal T cell-dendritic cell (DC) interaction and promoting proliferation and cell survival of both naive and memory T cells [72]. Furthermore, IL-7 signaling is essential in early thymocyte development, it enhances the function of immune effector cells and is able to antagonize the immunosuppressive milieu created by malignancies [73]. Consequentially, IL-7 raises interest as an immunotherapeutic in view of its potential to reconstitute the immune system [74]. This also includes the use of IL-7 as stimulus for immune cells for adoptive transfer. For example, CAR-modified T-cell therapy is an emerging strategy that has already shown unprecedented results in CD19-expressing hematological malignancies [75]. Whereas IL-2 stimulation for T cell expansion is standard, it has been shown that IL-7 and IL-15 are superior for preserving T memory stem cells in the product, as well as improve anti-tumor activity and survival of CAR-modified T cells after consecutive antigen exposure [76]. Moreover, clinical trials with recombinant IL-7 have shown both its safety and immunostimulatory capacity, manifested by elevated numbers of circulating CD4<sup>+</sup> and CD8<sup>+</sup> T cells, enhanced diversity in TCR repertoire and decreased figures of Treg cells [77-80].

$\gamma\delta$  T cells – It seems that  $\gamma\delta$  T cell propagation and IL-7 signaling are linked as well. For example, in humans a correlation was reported between  $\gamma\delta$  T cell numbers and IL-7 levels in the gastric mucosa in the context of gastritis [81]. Mice with transgenic overexpression of IL-7 showcase a substantial increase in dermal  $\gamma\delta$  T cells [82]. On the other hand, thymi of IL-7<sup>-/-</sup> mice are completely devoid of  $\gamma\delta$  T cells and lack TCR $\gamma\delta^+$  dendritic epidermal T cells [82, 83]. Additionally, intraepithelial  $\gamma\delta$  T cells are considerably decreased in the small intestines of IL-7<sup>f/f</sup> FoxN1-Cre mice [83]. Therefore, the question arises as to which  $\gamma\delta$  T cell subset is promoted by IL-7. It has been shown that IL-7 preferentially enriches IL-17-competent  $\gamma\delta$  T cells, rendering Th17  $\gamma\delta$  T cells the main subset in the thymus at the expense of IFN- $\gamma$ -competent  $\gamma\delta$  T cells [84, 85]. These data are opposed to IL-2 stimulation, supporting all  $\gamma\delta$  T cells (with a predisposition of the IFN- $\gamma$  producing subset) and IL-15 stimulation which only supported the Th1  $\gamma\delta$  T cell subset. The induction of lymph node and skin resident Th17  $\gamma\delta$  T cells seems, however, self-limiting, considering IL-7 signaling induces the expression of the inhibitory receptor B and T lymphocyte attenuator (BTLA), which in turn limits IL-7-dependent responses [86]. In mice, the induction of Th17  $\gamma\delta$  T cells has been therapeutically investigated with a recombinant *Mycobacterium bovis* bacillus Calmette–Guérin expressing antigen 85B-IL-7 fusion protein [87]. Interestingly, here the increase in Th17  $\gamma\delta$  T cells led to an elevated Th1 response as a reaction to mycobacterial infection.[87] The latter might explain why in a mouse model of disseminated neuroblastoma, the addition of IL-7 as a combination therapy with  $\gamma\delta$  T cells and anti-GD2 antibodies offered a supplementary survival benefit on top of a protective effect on  $\gamma\delta$  T cell survival [88].

### Interleukin-9

Similar to the other members of common gamma-chain family cytokines, IL-9 has pleiotropic functions, inducing the proliferation, differentiation and effector functions of numerous immune cell subsets, among others T and B cells, eosinophils, neutrophils and mast cells [89]. Herewith, IL-9 is suggested to be involved in the anti-tumor immune response of which most evidence exists in the melanoma setting [90-92]. The general research interest in the characteristics of IL-9 is, nevertheless, fairly recent, making the available knowledge on the effects of IL-9 on  $\gamma\delta$  T cells scarce. The only report of Guggino *et al.* investigated IL-9 signaling in  $\gamma\delta$  T cells of psoriatic arthritis patients, showing an increase in IFN- $\gamma^+$  IL-17<sup>+</sup>  $\gamma\delta$  T cells of patients after stimulation with IL-9, but not in healthy controls in accordance with their reduced expression of IL-9R [93].

### Interleukin-15

The IL-15R is a heterodimeric receptor consisting of the common cytokine receptor gamma-chain and the IL-2/IL-15R $\beta$ -chain, also known as CD122. Similar activities as IL-2 are therefore expected of IL-15. However, IL-15 also binds to a specific receptor part, namely IL-15R $\alpha$ , forming a heterotrimeric receptor complex with IL-2/IL-15R $\beta$  and  $\gamma_c$  on neighboring cells (trans-presentation) or the same cell (cis-presentation, using IL-15 derived from autocrine or paracrine sources). Although binding with IL-15R $\alpha$  increases the bioavailability of IL-15, signaling by membrane-anchored IL-15 and (high concentrations of) soluble IL-15 as such, is possible as well [94].

IL-15 plays a central part in stimulation of both innate and adaptive immune cells. This includes the development, proliferation and activation of classic  $\alpha\beta$  T cells,  $\gamma\delta$  T cells, NK cells, NKT cells and B cells, the reactivation of memory T cells and the induction of DC maturation [95-97]. Furthermore, IL-15 endorses the survival of immune cells by averting apoptosis through the upregulation of anti-apoptotic and downregulation of pro-apoptotic factors. An absolute plus for cancer immunotherapy is the fact that IL-15, in stark contrast to IL-2, does not provoke activation-induced cell death and Treg induction, and it can actually protect effector immune cells from the immunosuppressive activities of Treg cells [98, 99]. Because of the drawbacks of IL-2 therapy and the additional advantageous properties of IL-15, interest has grown in IL-15 as stimulator of the immune system over IL-2. The latter observation is reinforced by the prognostic value of IL-15 as a biomarker in cancer [100]. It may therefore come as no surprise that IL-15 was ranked third on the CITN priority list of immunotherapy agents after anti-PD-1 and anti-CD40 [101].

The first-in-human clinical trial was, moreover, very promising, demonstrating the safety of IL-15 administration in metastatic cancer patients as well as NK cell, monocyte,  $\gamma\delta$  T cell and memory CD8<sup>+</sup> T cell activation [102]. In analogy with this, the first clinical trial with the IL-15 superagonist ALT-803 showed that the therapy was well-tolerated and even resulted in clinical responses in acute myeloid leukemia and myelodysplastic syndrome patients who relapsed following HSCT [103]. Additionally ALT-803 stimulated NK cell, CD8<sup>+</sup> T cell and  $\gamma\delta$  T cell expansion [103, 104]. Proceeding to immune cell therapy for adoptive transfer, CAR T cells stably co-expressing IL-15/IL-15R $\alpha$  countered leukemia engraftment and exhibited the promise of sustained resistance by displaying a memory stem cell phenotype [105].

$\gamma\delta$  T cells – From the above, it stands to reason to have high expectations of IL-15-mediated  $\gamma\delta$  T cell activation. In celiac disease intestine, the presence of IL-15 induces  $\gamma\delta$  T cell recruitment into the intraepithelial compartment, which is not seen with IL-2 and IL-4, and only to a lesser extent with IL-7, suggesting that IL-15 may be a potent chemotactic for  $\gamma\delta$  T cells [106, 107]. In addition, IL-15 controls the generation of the restricted TCR repertoire of  $\gamma\delta$  intestinal intraepithelial lymphocytes and is critical for their effector functions [108, 109]. Next, the redundant role of IL-15 regarding  $\gamma\delta$  T cell homeostatic expansion has been shown in murine models, demonstrating the dependency of both classic  $\alpha\beta$  T cells and  $\gamma\delta$  T cells on IL-15, and to a lesser degree on IL-7, for their proliferation. This to such an extent that competition for IL-15, trans-presented by DCs, dictates the interplay between  $\gamma\delta$  T cells and  $\alpha\beta$  T cells [110-112]. Furthermore, in line with the characteristics typical of a survival factor, apoptosis-resistant  $\gamma\delta$  T cells, but not their susceptible counterparts, exhibit enhanced sensitivity to IL-15, through enhanced expression of the IL-15R $\alpha$  [113]. The same is seen with *ex vivo* cultures of  $\gamma\delta$  T cells, showing a prolonged survival with IL-15, as compared to IL-2, associated with improved effector functions [114, 115]. Herewith, isolated  $\gamma\delta$  T cells are able to proliferate in response to IL-15 and IPP, but not to IL-2 + IPP [116], and the combination of IL-15 and bromohydrin pyrophosphate (BrHPP) was able to expand  $\gamma\delta$  tumor infiltrating lymphocytes (TILs) from renal cell carcinoma patients, whereas BrHPP and IL-2 did not [117]. In the context of adoptive cell transfer, IL-15 boosts IL-2/zoledronate-mediated expansion and activation of  $\gamma\delta$  T cells of healthy donors and of acute myeloid leukemia patients [116]. The latter observation alludes to cellular immunotherapy benefiting of the unique characteristics of IL-15 stimulation.

It has been shown that IL-2/IL-15 signaling is required for the differentiation of immature  $\gamma\delta$  thymocytes into Th1 cytotoxic  $\gamma\delta$  T cells [118]. In general, opportune  $\gamma\delta$  T cell subsets are supported by IL-15, namely CD122<sup>+</sup> Th1  $\gamma\delta$  T cells are maintained by IL-15 whereas CD25<sup>+</sup> Th17  $\gamma\delta$  T cells are sustained by IL-2 [116, 119-121]. With regard to the influence of IL-15 on  $\gamma\delta$  T cell cytotoxicity,  $\gamma\delta$  T cells expanded from cord blood mononuclear cells using IL-15 and alendronate produce the cytotoxic mediators perforin, granzyme B and TNF- $\alpha$  [114]. Interestingly, this production was associated with CD56 expression, which defines  $\gamma\delta$  T cells with increased antitumor activity [114, 122]. The same CD56 upregulation was seen with isolated  $\gamma\delta$  T cells stimulated with IPP and IL-15, concomitant with improved tumor cell killing [116]. Expanded  $\gamma\delta$  TILs from rectal tumors have also been shown to demonstrate improved killing capacity against the rectal carcinoma cell line HR-8348 and the Burkitt's lymphoma cell line Daudi [123]. The low affinity type III Fc $\gamma$  receptor CD16, involved in, among others, phagocytosis, ADCC, and the acquisition of potent antigen-presenting cell (APC) function, is as well upregulated by IL-15 [124, 125]. The combination of TCR triggering and IL-15 generates  $\gamma\delta$  T APCs that are able to process different antigens for presentation and to stimulate other immune cells [126, 127]. In turn, the IL-15 DC vaccine has been shown to induce  $\gamma\delta$  T cell activation through *in situ* IL-15 secretion [128]. From this we can conclude that, thus far, IL-15 seems the most preferable cytokine from the common gamma-chain cytokine family to harness  $\gamma\delta$  T cells in the anti-tumor immune response, improving clinical responses of human  $\gamma\delta$  T cell-based immunotherapy.

### **Interleukin-21**

IL-21, mainly produced by CD4<sup>+</sup> T cells and NKT cells, is the most recently described cytokine belonging to the common gamma-chain cytokine receptor family. Also, this cytokine has been ascribed pleiotropic properties, including, but not limited to, enhancing NK cell and CD8<sup>+</sup> T cell cytotoxicity, modulating plasma cell differentiation and inhibiting Treg cells. On the other hand, IL-21 seems to sustain Th17 development, induces IL-10 secretion from T cells and NK cells, and prevents GM-CSF-mediated activation and maturation of DCs [129, 130]. Although careful evaluation of the breadth of actions of IL-21 is necessary to optimize its favorable anti-cancer effect, the results of the first clinical trial with IL-21 are promising [114].

$\gamma\delta$  T cells – IL-21 induces  $\gamma\delta$  T cell division to some extent, but is unable to sustain long-term antigen-induced proliferation of *ex vivo*  $\gamma\delta$  T cells as opposed to for example IL-2 and IL-15. Therefore, it will not be likely to design  $\gamma\delta$  T cell adoptive transfer protocols solely based on IL-21. Moreover, there is no contribution of IL-21 to IL-2-mediated  $\gamma\delta$  T cell expansion [131]. Advantage could, however, be attained by IL-21 as an enhancer of the expression of lytic effector molecules, including granzyme A/B, perforin and CD56 [131], and the suppression of IL-17 producing  $\gamma\delta$  T cells through induction of apoptosis [132, 133]. On the flip side, increased expression levels of inhibitory NK receptors and lower levels of natural killer group 2D (NKG2D) receptor were seen as well with the addition of IL-21 [131]. Yet, the net effect of IL-21 stimulation on  $\gamma\delta$  T cell killing capacity seems positive, albeit reversible, fading away rapidly upon IL-21 removal [131].

### Conclusion

As discussed in this review, the use of  $\gamma\delta$  T cells for (cellular) immunotherapy is highly promising. Today  $\gamma\delta$  T cell-based immunotherapies are still early stage and have likely not yet reached their full potential. More insight in  $\gamma\delta$  T cell biology is needed as to why only certain  $\gamma\delta$  T cell subsets hold robust anti-tumor effects, whereas others possess immunoregulatory properties. The use and/or activation of the appropriate  $\gamma\delta$  T cell subset, with preservation of their functionality during treatment, is therefore of key importance. This is the first comprehensive review analyzing our current understanding of the effects that cytokines pertaining to the common gamma-chain receptor family exert on  $\gamma\delta$  T cells. From the presented data the pivotal role played by these cytokines concerning  $\gamma\delta$  T cell functionality emerges. In particular, the role and effect of IL-15 seems to outcompete the other members of this family (Figure 2). This cytokine provides the necessary stimulatory signals for  $\gamma\delta$  T cell expansion, survival, unbiased Th1 promotion and cytotoxicity, with little evidence of potential downsides so far, holding potential as a protective agent against immunosuppressive cells and milieus. Hence, future advances in the field are expected with the implementation of IL-15 in adoptive transfer protocols or its use *in vivo* as general immune cell activator. Therefore, the importance of potential combinatory treatments is warranted; not only to increase the anti-tumor activity of effector killer cells such as  $\gamma\delta$  T cells, but also to overcome the myriad of immune escape mechanisms used by cancer cells. From this review, we conclude that IL-15-activated  $\gamma\delta$  T cells will arise as strong candidates for future cancer immunotherapies, holding the key to unlock cancer immune escape and resistance in both active and passive strategies.

**Funding**

This work was supported by grant G.0399.14N and G.0535.18N from the Fund for Scientific Research Flanders (FWO Vlaanderen), by a grant of the Fund Baillet-Latour, a grant of the Belgian Foundation against Cancer (Stichting tegen Kanker) [2016-138 (FAF-C/2016/764)] and by a Methusalem grant from the University of Antwerp. HHVA holds a PhD fellowship of the FWO (grant 11ZL518N), DCD is supported by a DOC-PRO PhD grant of the Special Research Fund (BOF) of the University of Antwerp and MV holds a Doctoral (PhD) Grant Strategic Basic Research of the FWO (grant 1S24517N).

**Competing interests** - The authors have no conflict of interest to declare.

**Acknowledgements**

The authors would like to thank dr. Sébastien Anguille for granting us the right to use his artwork in the current publication.

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Vitae

**Heleen Van Acker** graduated as a pharmacist from the University of Antwerp, Belgium in 2013. She is currently a PhD student at the Tumor Immunology Group (TIGr) of the Laboratory of Experimental Hematology of the University of Antwerp and holds a competitive grant of the Research Foundation - Flanders (FWO). Her doctoral research is focused on the topic of 'harnessing the expression of interleukin-15 and CD56 in immunotherapeutic strategies combating leukemia', with a special emphasis on  $\gamma\delta$  T cells. In 2017 she joined John Anderson's Research group at University College London (United Kingdom) for three months, for which she received a FWO Grant for a long stay abroad. The work focused on the (antigen-presentation) properties of chimeric antigen receptor-engineered  $\gamma\delta$  T cells (CAR  $\gamma\delta$  T cells).



**Diana Campillo-Davo** (MSc) is a PhD student at the Tumor Immunology Group (TIGR), which is a part of the Laboratory of Experimental Hematology (Vaccine & Infectious Disease Institute, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium). She obtained her Bachelor's Degree in Biology in 2011 from the University of Alicante (Spain) and continued her studies at the University of Granada (Spain), where she received her Master's Degree in Research and Progress in Molecular and Cellular Immunology in 2012, obtaining Full Marks on her Master's thesis. She then worked as a laboratory technician in Bioarray, SL, a Spanish technology-based company specialized in genetic analysis. In 2013, she was awarded a predoctoral fellowship from the Spanish Ministry of Economy and Competitiveness to study the Genetic basis of autoimmune diseases at the Institute of Parasitology and Biomedicine "López-Neyra" (IPBLN) of the Spanish National Research Council. In 2015, she was awarded a DOCPRO grant from the University Research Fund (BOF) of the University of Antwerp to start her doctoral studies under the supervision of Prof. Viggo Van Tendeloo and Dr. Eva Lion. Her doctoral project focuses on the development of new tools and strategies to improve cancer immunotherapy, in particular, using gene-engineered T lymphocytes.



**Gils Roex** is a graduate student in ‘Bioscience Engineering: cell and gene technology’ from the KU Leuven, Belgium. He is currently working on his master thesis at the Laboratory of Experimental Hematology. His research is centered around engineered CD4<sup>+</sup> T cells targeting WT1 epitope bearing tumor cells in an MHC class-I restricted manner.



**Maarten Versteven** graduated as a biomedical scientist at the University of Antwerp in 2016. He joined the Tumor Immunology Group (TIGr) as a PhD student in 2017, where he is currently holder of a strategic basic research mandate granted by the Research Foundation – Flanders (FWO). His research focusses on developing a novel dendritic cell based immunotherapy by implementing interleukin 15 and blockade of the immune inhibitory molecules programmed death ligand 1 and -2.



Professor **Evelien Smits** obtained her PhD in Medical Sciences from the University of Antwerp, Belgium in 2008. She is team-leader and co-head of the Center for Oncological Research (CORE) at the University of Antwerp. Her team, the Solid Tumor Immunology Group (STIG), focuses on developing novel immunotherapeutic combination strategies to treat solid tumors

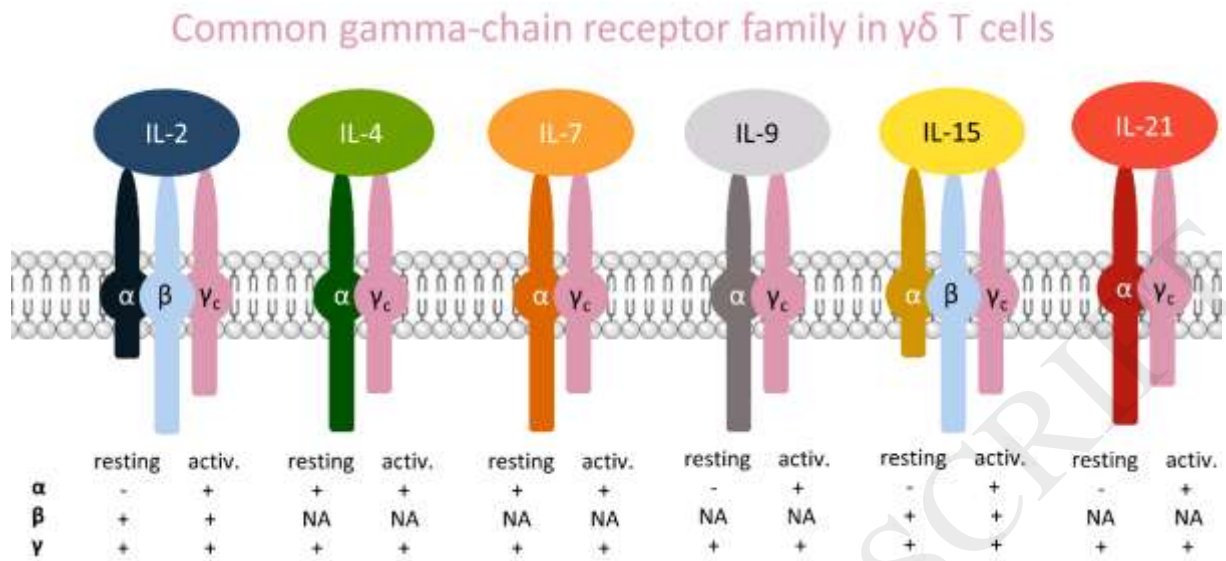
and on unraveling the tumor immune microenvironment to gain deeper insights in immune escape mechanisms. In addition to basic and translational research, she is co-principal investigator of three investigator-driven academic clinical studies testing cellular immune therapy at the Center for Cell Therapy and Regenerative Medicine (Antwerp University Hospital) in acute myeloid leukemia, glioblastoma multiforme and malignant pleural mesothelioma. She is also supervising translational research to further optimize the cell therapy protocols and to monitor vaccine responses, both for cancer and infectious diseases. Her experience with cancer immunotherapy development was broadened during her research stays at Cardiff University, University of Southampton, King's College London and Radboud Universiteit Nijmegen. She is board member of the Belgian Young Academy and elected member of the European Cancer Immunotherapy Association. Furthermore, she is an appointed expert in the Belgian Superior Health Council.



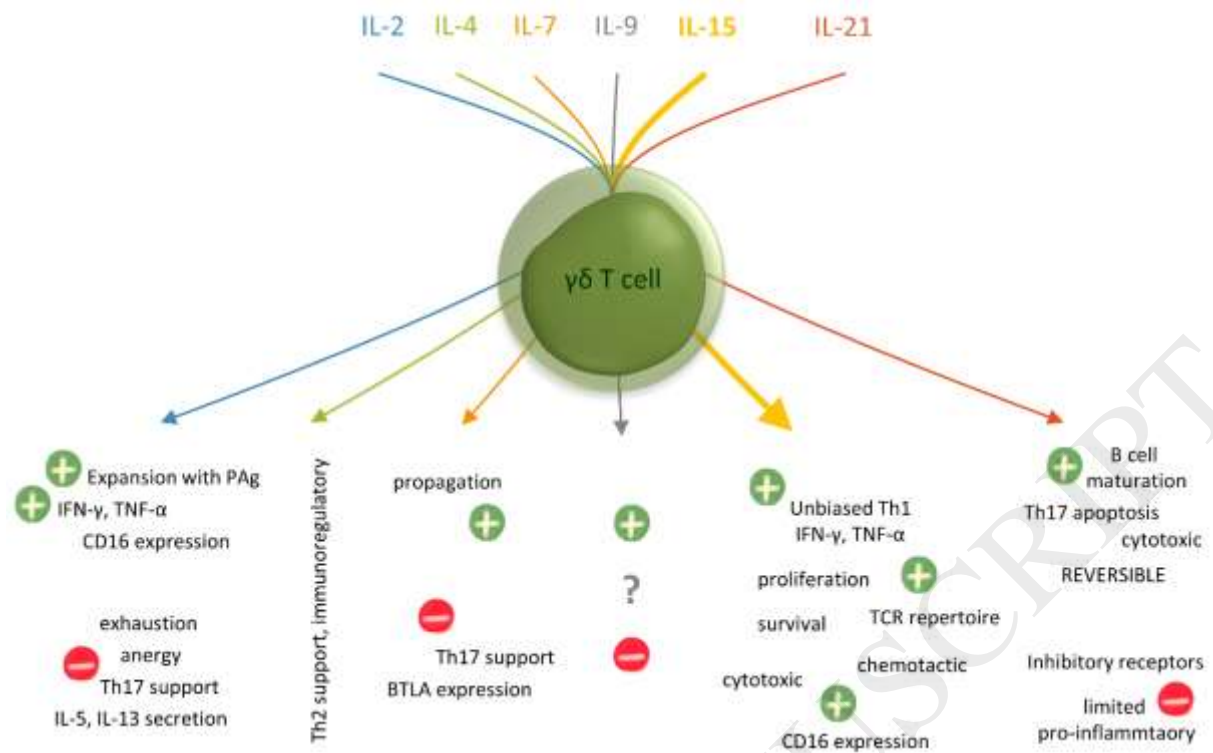
**Viggo Van Tendeloo** is Associate Professor at the Vaccine & Infectious Disease Institute of the Faculty of Medicine and Health Sciences of the University of Antwerp. He received his PhD in Biochemistry in 2000 from the University of Antwerp and worked as a postdoc and senior scientist in the Laboratory of Experimental Hematology. In 2007 he became appointed as a full-time research professor in Cellular Immunotherapy at the University of Antwerp. In 2005 he founded together with his mentor Zwi Berneman the Center for Cell Therapy and Regenerative Medicine (CCRG) at the Antwerp University Hospital of which he was the scientific director until 2012. Currently, he heads the Tumor Immunology Group (TIGR) within the Laboratory of Experimental Hematology. He is inventor of a US patent on mRNA electroporation as an efficient transfection method of hematopoietic cells and stem cells. Dr. Van Tendeloo's research is focused on immunobiology and modulation of human myeloid dendritic cells in order to induce T cell immunity and to enhance their interaction with innate effector cells such as natural killer cells and  $\gamma\delta$  T cells. He is a member of the scientific advisory board of the Belgian Foundation against Cancer and provides expert consultancy to Federal Agency of Medicine and Health Products and to spin-off biotech companies in the field of therapeutic cancer vaccination. He was the principal investigator for a number of completed early-phase clinical trials on personalized cancer and HIV therapeutic vaccines using antigen mRNA-transfected dendritic cells and is currently involved in the immunomonitoring of tumor-specific immune responses in cancer patients after therapeutic vaccination. To date, he has authored more than 120 peer-reviewed papers and has an h-index of 41.

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Figures captions



**Figure 1. Overview of the expression of common gamma-chain receptors on the  $\gamma\delta$  T cell surface. Interpretation:** -, not expressed; +, expressed; active, activated; NA, not applicable



**Figure 2. Graphical summary.**

Abbreviations. IFN- $\gamma$ , interferon- $\gamma$ ; IL, interleukin; PAg, phosphoantigen; TCR, T cell receptor; Th, T helper, TNF- $\alpha$ , tumor necrosis factor alpha

## Tables

Table 1. Clinical trials aimed at the *in vivo* expansion of  $\gamma\delta$  T cells

Paper	Disease	n	Ph	IL-2	Schedule	aBP or PAg	Schedule	Courses	Additional treatments
Pressey JG. <i>et al.</i> 2016 [134]	Refractory neuroblastoma	4	1	3 or $6 \times 10^6$ units s.c.	Day 1-5 and 15-19 / 28 days	4 mg/m <sup>2</sup> ZOL i.v.	Day 1	1-3	-
Wilhelm M. <i>et al.</i> 2014 [135]	AHM	4	pilot	1.0x10 <sup>6</sup> IU/m <sup>2</sup> s.c.	Day 1 and day 6	4 mg/m <sup>2</sup> ZOL i.v.	Day 0	1	DILI and Hi-Cy/Flu

Abbreviations: aBP, aminobisphosphonate; AHM, advanced hematological malignancies; DILI, donor innate lymphocyte

infusion (day 0); Hi-Cy/Flu, 'fludarabine 25 mg/m<sup>2</sup> day -6 until day -2 and cyclophosphamide day -6 and -5'; Pag, phosphoantigen; Ph, phase; s.c. subcutaneous; ZOL, zoledronate.

Table 2. Clinical trials using adoptively transferred  $\gamma\delta$  T cells

Paper	Disease	n	Ph	Expansion condition		Cycle length (d)	No. of cells	Schedule	Courses	Additional treatments
				IL-2 (IU/mL)	aBP or PAg					
Aoki T. <i>et al.</i> 2017 [136]	Pancreatic cancer	56	1	1000	5 $\mu$ mo I/L ZOL	14	$>1 \times 10^9$	day 4 and 18 / 4 weeks	6	1000 mg/m <sup>2</sup> gemcitabine on day 1,8 and 14
Wada I. <i>et al.</i> 2014 [137]	Malignant ascites	7	pilot	1000	5 $\mu$ mo I/L ZOL	14	Median $5.9 \times 10^9$ (0.06- $6.98 \times 10^9$ )	weekly	1-4	1 mg ZOL; day 0 i.v., Day 7/14/21 i.p.
Izumi T. <i>et al.</i> 2013 [115]	Colorectal cancer	6	pilot	1000	5 $\mu$ mo I/L ZOL	14	Mean $6.8 \times 10^9$	weekly	8	-

Abbreviations: aBP, aminobisphosphonate; Pag, phosphoantigen; Ph, phase

Table 3. Effects of cytokines of the common gamma-chain family on  $\gamma\delta$  T cells

	Conc.	Effect	Condition	Comparison with other cytokines or remarks	Ref
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IL-2	100 IU/mL	↑ CD69	PBMC (cynomolgus monkeys)	(-) proliferation, ↑ In combination with phosphoantigens	[43]
	100 IU/mL	↑ CD25, CD69, CD94, CD152, CD244, ICAM-1, KLRG-1, LFA-1 and NKG2D and ↓ CD27 expression	HMB-PP stimulated PBMC	CD25/CD152/KLRG-1: ↑↑ than IL-4, ↑ than IL-21 CD69: (-) than IL-4, ↓ than IL-21 CD244: ↑ than IL-4, ↓ IL-21 ICAM-1: (-) than IL-4, ↑↑ than IL-21 LFA-1: ↑ than IL-4, (-) IL-21 CD27: ↓↓ than IL-4, ↓ than IL-21	[60]
	100 IU/mL	↑ CD16	Expanded and isolated $\gamma\delta$ T cells	healthy donors and MS patients ↓ than IL-15	[124]
	1x10 <sup>9</sup> IU	(-) proliferation	cynomolgus macaques	↑ in combination with HMB-PP injection	[49]
	10 IU/mL	(-) proliferation	PBMC of healthy donors and MM patients	↑ in combination with zoledronate	[51]
	?	(-) proliferation	Isolated $\gamma\delta$ T cells	↑ + IPP, ↑↑ + IPP + (im)mature dendritic cells	[49]
	100 IU/mL	↑ proliferation	HMB-PP stimulated PBMC	↑↑ than IL-4 ↑ than IL-21	[60]
	50 ng/mL	IFN- $\gamma$ , TNF- $\alpha$	PBMC and isolated $\gamma\delta$ T cells	↑ CD8 $\alpha\beta$ <sup>+</sup> , (-) CD8 $\alpha\alpha$ <sup>+</sup> and ↓ CD8 <sup>-</sup> $\gamma\delta$ T cell subsets	[56]
	100 IU/mL	(-) IFN- $\gamma$ , IL-18	PBMC	-	[138]
	100 IU/mL	(-) IFN- $\gamma$ , TNF- $\alpha$	PBMC	↑ In combination with phosphoantigens	[48]
	100 IU/mL	↑ IFN- $\gamma$ , TNF- $\alpha$ , GM-CSF, IL-13, CXCL10	HMB-PP stimulated PBMC	CXCL10: ↑↑ than IL-4, (-) than IL-21 Rest: ↑↑ than IL-4 and IL-21	[60]
	50 IU/mL	↑ IFN- $\gamma$	PBMC (influenza infection)	IL-2 restores IFN- $\gamma$ production in $\gamma\delta$ T cells from obese donors	[45]
	100 IU/mL	(-) CD107a	PBMC	↑ In combination with phosphoantigens	[48]
	10 ng/mL	↑ cytotoxicity	Expanded $\gamma\delta$ TILs	(-) IL-4 and IL-15	[123]
100 IU/mL	$\gamma\delta$ IL-2 T-APC generation	purified $\gamma\delta$ T cells + monocytes + HMB-PP	Limited and no effect of IL-21 and IL-7	[126]	

IL-4	100 ng/mL	↑ transcription factor Eomesodermin	Mice isolated $\gamma\delta$ T cells	-	[69]
	10 ng/mL	↑ CD69, CD62L, CD94, ICAM-1, LFA-1 and NKG2D expression	HMB-PP stimulated PBMC	CD62L: ↑↑ IL-2, ↓ than IL-21 CD69: (-) IL-2, ↓ than IL-21 ICAM-1: (-) IL-2, ↑↑ than IL-21 LFA-1: ↓ than IL-2, ↓ than IL-21	[60]
	4 ng/mL	↓ NKG2D	V $\delta$ 1 T cells	Both in activation and proliferative stages	[71]
	10 ng/mL	↑ proliferation	HMB-PP stimulated PBMC	↓↓ than IL-2 ↓↓ than IL-21	[60]
	4 ng/mL	↓ proliferation	PBMC + anti-TCR V $\delta$ 1 mAb or zol + 100 IU/mL IL-2	But IL-4 promotes the proliferation of activated $\gamma\delta$ T cells, ↑ V $\delta$ 1/V $\delta$ 2 T cell ration	[71]
	4 ng/mL	↓ IFN- $\gamma$ , TNF- $\alpha$ , IL-6, IL-17a and ↑ IL-10	PBMC + anti-TCR V $\delta$ 1 mAb or zol + 100 IU/mL IL-2	-	[71]
	10 ng/mL	↑ cytotoxicity	Expanded $\gamma\delta$ TILs	(-) IL-2 and IL-15	[123]
IL-7	10 ng/mL	↑ BTLA expression	Mouse lymphocytes enriched for $\gamma\delta$ T cells	-	[86]
	50 ng/mL	↑ survival	Isolated $\gamma\delta$ T cells	-	[88]
	20 ng/mL	↑ proliferation	purified splenic mouse $\gamma\delta$ T cells	↑ IL-17 production only in the presence of IL-1 $\beta$ , TNF- $\alpha$ or TCR stimulation	[139]
	20 ng/mL	Expansion of IL-17 producing $\gamma\delta$ T cells	Mouse <i>ex vivo</i> experiments	Comparable results <i>in vivo</i> with i.p. injections of 5 $\mu$ g IL-7/mouse every 2 days for 1 week ↔ IL-2 (↑ all $\gamma\delta$ T cells), IL-15 (↑ Th1 $\gamma\delta$ T cells) and IL-21 stimulation	[84]
IL-9	2 ng/mL	↑ IFN- $\gamma$ , IL-17	Isolated $\gamma\delta$ T cells	In psoriatic arthritis patients, but not in healthy individuals	[93]
IL-15	10 ng/mL	↑ survival	CBMC (+alendronate)	Decline in survival of $\gamma\delta$ T cells with IL-2	[114]
	2 IU/mL	↑ survival	Expanded $\gamma\delta$ T cells	Neither 10 IU/mL IL-2 nor 40 IU/mL IL-7 supports survival and proliferation	[115]

	10 ng/mL	↑ growth and survival	Expanded $\gamma\delta$ T cells	Apoptosis-resistant, but not apoptosis-sensitive $\gamma\delta$ T cells show augmented susceptibility to IL-15	[113]
	10 ng/mL	↑ CD25, CD27, NKG2D	CBMC (+alendronate)	↑ than IL-2	[114]
	10 ng/mL	↑ CD16	Expanded and isolated $\gamma\delta$ T cells	Healthy donors and MS patients ↑ than IL-2	[124]
	12.5 ng/mL	↑ proliferation, CD69, HLA-DR, CD56	Isolated $\gamma\delta$ T cells (+IPP)	IL-2 failed to induce $\gamma\delta$ T cell proliferation	[116]
	20 ng/mL	↑ expansion	TIL (+BrHPP)	IL-2 was inefficient	[117]
	10 ng/mL	↑ IFN- $\gamma$	PBMC (+IPP or +BrHPP)	↑ than IL-2 No IL-10, IL-13 or IL-17	[140]
	12.5 ng/mL	↑ IFN- $\gamma$ , TNF- $\alpha$	isolated $\gamma\delta$ T cells (+IPP)	↑ than IL-2 No IL-10, TGF- $\beta$	[116]
	5 IU/mL	↑ IFN- $\gamma$ , CD107a	Expanded $\gamma\delta$ T cells + zol treated Daudi	↑ than IL-2 and IL-7	[115]
	10 ng/mL	↑ IFN- $\gamma$ , TNF- $\alpha$ , CD107a	PBMC	IL-15 improves innate immunity after allo-HSCT	[141]
	10 ng/mL	↑ granzyme, perforin, CD107a, TNF- $\alpha$	CBMC (+alendronate)	↑ than IL-2	[114]
	12.5 ng/mL	↑ cytotoxicity	isolated $\gamma\delta$ T cells (+IPP)	↑ than IL-2	[116]
	10 ng/mL	↑ cytotoxicity	Expanded $\gamma\delta$ T cells	Against zol pre-treated neuroblastoma	[142]
	10 ng/mL	↑ cytotoxicity	Expanded $\gamma\delta$ TILs	(-) IL-2 and IL-4	[123]
	20 IU/mL	$\gamma\delta$ IL-15 T-APC generation	purified $\gamma\delta$ T cells + monocytes + HMB-PP	Limited and no effect of IL-21 and IL-7	[126]
IL-21	10 ng/mL	↑ CD25, CD27, CD62L, CD69, CD94, CD152, CD244, ICAM-1, KLRG-1, LFA-1 and NKG2D expression	HMB-PP stimulated PBMC	CD25/CD152/KLRG-1: ↓ than IL-2, ↑↑ IL-4 CD27/CD244: ↑↑ than IL-2 and IL-4 CD62L: ↑↑ than IL-2, ↑ than IL-4 CD69: ↑ than IL-2, ↑ than IL-4 ICAM-1: ↓↓ IL-2, ↓↓ than IL-4 LFA-1: (-) IL-2, ↑ than IL-4	[60]
	10 ng/mL	↑ inhibitory receptors ↓ NKG2D	PBMC (+C-HMB-PP)	-	[131]

10 ng/mL	(-) proliferation	PBMC (+HMB-PP)	↓↓ than IL-2 No synergism/interference with IL-2	[131]
10 ng/mL	↑ proliferation	HMB-PP stimulated PBMC	↑↑ than IL-4 ↓ than IL-21	[60]
10 ng/mL	↑ CXCL10	HMB-PP stimulated PBMC	CXCL10: (-) than IL-2, ↑↑ than IL-4,	[60]
10 ng/mL	↑ granzyme A/B, perforin, CD56, cytotoxicity	PBMC (+HMB-PP)	Reversible; nullified with the removal of IL-21	[131]

Abbreviations: (-) same/no effect; CBMC, cord blood mononuclear cells; Conc., concentration; i.p., intraperitoneal; mAb, monoclonal antibody; MM, multiple myeloma; MS, multiple sclerosis; ref, reference; zol, zoledronate