TIGIT expressing CD4+ T cells represent a tumor-supportive T cell subset in chronic lymphocytic leukemia

Kemal Catakovic, Franz Josef Gassner, Christoph Ratswohl, Nadja Zaborsky, Stefan Rebhandl, Maria Schubert, Markus Steiner, Julia Christine Gutjahr, Lisa Pleyer, Alexander Egle, Tanja Nicole Hartmann, Richard Greil and Roland Geisberger

ABSTRACT

While research on T cell exhaustion in context of cancer particularly focuses on CD8+ cytotoxic T cells, the role of inhibitory receptors on CD4+ T-helper cells have remained largely unexplored. TIGIT is a recently identified inhibitory receptor on T cells and natural killer (NK) cells. In this study, we examined TIGIT expression on T cell subsets from CLL patients. While we did not observe any differences in TIGIT expression in CD8+ T cells of healthy controls and CLL cells, we found an enrichment of TIGIT+ T cells in the CD4+ T cell compartment in CLL. Intriguingly, CLL patients with an advanced disease stage displayed elevated numbers of CD4+ TIGIT+ T cells compared to low risk patients. Autologous CLL-T cell co-culture assays revealed that depleting CD4+ TIGIT+ expressing T cells from co-cultures significantly decreased CLL viability. Accordingly, a supportive effect of TIGIT+CD4+ T cells on CLL cells in vitro could be recapitulated by blocking the interaction of TIGIT with its ligands using TIGIT-Fc molecules, which also impeded the T cell specific production of CLL-prosurvival cytokines. Our data reveal that TIGIT+CD4+ T cells provide a supportive microenvironment for CLL cells, representing a potential therapeutic target for CLL treatment.

Introduction

Chronic lymphocytic leukemia (CLL) is a B cell malignancy, which is associated with substantial T subset skewing and T cell defects.1 In particular, we and others have previously shown that T cells from CLL patients show typical signs of T cell exhaustion, as they exhibit increased expression of inhibitory receptors and defects in proliferation, cytokine expression and synapse formation.2-5 In general, T cell exhaustion is the functional silencing of cytotoxic T cells, the cytotoxic T cells non-functional.8 A key molecule in T cell exhaustion was found to be programmed death-1 (PD-1), which functionally impedes T cell receptor (TCR) mediated signaling on exhausted T cells.9 In addition to PD-1, a number of different inhibitory receptors were recently found to be associated with T cell exhaustion. The concept that the exhausted phenotype could be reversed by simply blocking these inhibitory receptors by monoclonal antibodies led to a renaissance of cancer immune therapy with specific immune checkpoint blockade using PD-1 antibodies being considered as a major breakthrough in cancer treatment.10 Considering the strength of checkpoint blockade, it is important to investigate further inhibitory receptors aside of PD-1 to increase efficacy and minimize side effects of this treatment approach, especially in light of the fact that not all cancer entities respond to PD-1 blockade. For CLL, no clear effects were noticed for single agent anti-PD-1 therapy (pembrolizumab) except for patients with Richter’s syndrome, whereas at least partial responses were reported for CLL patients receiving anti-PD-1 therapy (nivolumab) combined with ibrutinib.11,12

TIGIT (T cell immunoreceptor with Ig and ITIM domains) is a recently identified inhibitory receptor which is expressed on T cells, natural killer (NK) and NKT cells.13,14 TIGIT has a cytoplasmic tail containing an immunoglobulin domain (ITIM)- like phosphorylation motif and an immunoreceptor tyrosine-based inhibitory motif (ITIM) (15). The natural ligands for TIGIT are the poliovirus receptors (PVR) CD155 and CD112. TIGIT signaling involves the recruitment of the phosphatase SHIP1 to the ITIM and downstream inhibition of NF-kB, PI3 K and MAPK pathways. PVRs are widely

CONTACT Roland Geisberger r.geisberger@salk.at SCRIMCR, Muller Hauptstr 48, 5020 Salzburg, Austria.

ARTICLE HISTORY
Received 31 May 2017
Revised 14 August 2017
Accepted 16 August 2017

KEYWORDS
chronic lymphocytic leukemia; T cell exhaustion; TIGIT; PD-1; microenvironment
expressed on different cell types and were also found to be expressed on a number of cancer cells.\textsuperscript{18-21} In parallel, TIGIT tumor infiltrating CD8\textsuperscript{T} cells could be detected in small-cell lung cancers, colorectal cancers and melanoma.\textsuperscript{22-24} Similar to the situation of CTLA-4 and CD28, which are sharing the same ligands\textsuperscript{25}, TIGIT competes with CD226, a costimulatory T cell molecule for PVR binding and can directly prevent CD226 signaling by impeding its homodimerization.\textsuperscript{26} Hence, agonistic TIGIT antibodies could decrease T cell function similar to CD226 knockdown\textsuperscript{26,27} and TIGIT inhibition was recently shown to increase T cell functions of melanoma specific CD8\textsuperscript{T} cells.\textsuperscript{28}

In this study, we investigated expression and function of TIGIT expressing T cells in CLL patients. We observed an increase in TIGIT expressing CD4\textsuperscript{T} cells in CLL compared to healthy controls and we provide evidence that TIGIT+CD4\textsuperscript{T} cells display a microenvironment important for CLL survival. Our data propose TIGIT to be a potential target for immune therapy in CLL.

Results

\textit{TIGIT expressing CD4\textsuperscript{T} cells are elevated in patients with CLL}

To evaluate TIGIT expression in comparison to other inhibitory receptors (PD-1 and 2B4/CD244) which we previously examined in CLL,\textsuperscript{2} we performed phenotypic characterization of T cells from peripheral blood samples of CLL patients and age-matched healthy controls using flow cytometry (Fig 1a and b). In line with our previous results,\textsuperscript{2} we found that the percentages of CD4\textsuperscript{T} or CD8\textsuperscript{T} cells expressing 2B4 or PD-1 were not significantly different between patients and healthy controls (Fig 1b). However, within the CD4\textsuperscript{T} but not the CD8\textsuperscript{T} cell subset, we observed a significant increase in the percentage of TIGIT+ cells in CLL patients compared to healthy controls (Fig 1b). Most of the TIGIT+CD4\textsuperscript{T} cells were also positive for PD-1 (Fig 1c), reflected in a high correlation of PD-1 and TIGIT expression in this T cell subset (Fig 1d). The percentage of TIGIT+CD4\textsuperscript{T}, PD-1+CD4\textsuperscript{T} and PD-1+CD8\textsuperscript{T} cells was higher in bone marrow compared to peripheral blood of the same patient and we observed a high correlation of TIGIT, PD-1 and 2B4 expression in peripheral blood and bone marrow (supplementary figure S1). Notably, the inhibitory receptor TIM-3 was not detectable on T cells from peripheral blood of CLL patients (supplementary figure S2). Moreover, while TIGIT expression on CD8\textsuperscript{T} cells did not correlate with advanced disease stage or prior treatment, we found significantly increased TIGIT+CD4\textsuperscript{T} cells in patients with unmaturated IgVH status, with Rai stage $\geq 1$ or Binet B and in treated patients. No correlation with other prognostic factors (Zap70, CD38, CD49 d expression or chromosomal aberrations) was observed (Fig 1e and supplementary figure S3).

\textit{TIGIT is preferentially expressed on antigen experienced and Th1 polarized cells in CLL}

To more thoroughly evaluate TIGIT expression on T cells, we analysed expression of TIGIT in combination with T cell subset defining markers. In line with previous reports, we observed a general shift towards effector memory T cell populations as defined by CD62 L/CD45RA expression in CLL (Fig 2a). By analyzing naïve (CD62 L+/CD45RA-), central memory (T\textsubscript{CM};CD62 L+/CD45RA-), effector memory (T\textsubscript{EM};CD62 L-/CD45RA-), and terminally differentiated effector memory (T\textsubscript{EMRA};CD62 L-/CD45RA+) CD4+ and CD8+ T cells with respect to their expression of TIGIT, we found that within the TIGIT+CD4+ T cells, T\textsubscript{EMRA} and naïve cells were significantly enriched whereas the percentage of T\textsubscript{CM} cells was decreased in CLL (Fig 2b). In TIGIT+CD8+ T cells,
TEMRA subsets were expanded (Fig 2b). Counting the absolute number of cells within the respective T cell subsets revealed that TIGIT+CD4+ T cells were enriched within the naive, TEM and TEMRA subsets and CD8+ TIGIT+ T cells within the TEM subset in CLL (Fig 2c). We further characterized Th1 (CD45RA-CXCR3+CCR4-), Th2 (CD45RA-CCR3-CXCR5+PD-1+) cells and found a general skewing towards Th1, Treg and Tfh subsets in CLL compared to healthy controls (Fig 3a). While we observed a significant increase in the percentage of TIGIT+Th1 cells in peripheral blood from CLL patients compared to healthy controls, the absolute cell numbers of TIGIT+Th1, TIGIT+-Treg as well as of TIGIT+Tfh cells were all significantly

Figure 2. TIGIT is particularly expressed on antigen experienced T cells. (a) T cell subsets in peripheral blood samples from CLL patients and age-matched healthy controls (HC) were measured by FACS analysis defined by CD62 L and CD45RA. Plots represent naive (Tnaive: CD62 L+CD45RA+), central memory (TCM: CD62 L+CD45RA+), effector memory (TEM: CD62 L-CD45RA+) and terminally differentiated effector memory (TEMRA: CD62 L-CD45RA+). (b) T cell subset distribution in the TIGIT– and TIGIT+ T cell compartment. (c) Absolute cell counts (cells/µL blood) of CD4+ and CD8+ subsets expressing TIGIT.
increased in peripheral blood from CLL patients compared to healthy controls (Fig 3b and c), with no apparent correlation with tumor load or treatment status (supplementary figure S4). In addition, we found that in CLL, a significantly smaller percentage of TIGIT+CD4+T cells expressed CD226, a costimulatory molecule competing with TIGIT for the same ligands (Fig 3d). Further analysis of the CD8 compartment revealed decreased percentage of CD226 expressing...
cells among TIGIT+T cells compared to TIGIT- CD8+T cells (Fig 3d) and a negative correlation of CD226 with TIGIT expression (Fig 3e).

**TIGIT+CD4+ but not TIGIT+CD8+ T cells affect in vitro survival of autologous CLL cells**

Next, we wanted to assess CLL prosurvival or proapoptotic capacities of TIGIT+ CD4+ and CD8+ T cells. To this end, we performed autologous CLL/T cell co-culture assays using CD3/CD28 activated T cells which were either depleted of TIGIT or PD-1 expressing T cells using flow cytometric cell sorting (as outlined in the methods section; Fig 4a). Thereby we found that absence of TIGIT+ cells from all T cells in our co-culture setting resulted in significant decrease in the percentage of viable CLL cells (Fig 4b). While absence of PD-1+ T cells had no significant effect on CLL cells, absence of both PD-1+ and TIGIT+ T cells again resulted in decreased CLL viability (Fig 4b). To elucidate whether the prosurvival impact of TIGIT+ T cells depends on CD4+ or CD8+ T cells, we further depleted T cells from CD4+TIGIT+ or CD8+TIGIT+ cells prior to co-culture with CLL cells (Fig 4a). Thereby we observed that only absence of TIGIT+CD4+ but not TIGIT+CD8+T cells decreased CLL cell survival (Fig 4b). In these assays, also absence of PD-1+CD4+ T cells resulted in decreased CLL cell survival (Fig 4b). Of note, this effect on CLL survival in these co-culture assays was not based on reduced overall numbers of T cells, as the T/CLL cell ratio was not significantly different in the respective assays (Fig 4c).

**Blocking TIGIT interactions decreases CLL viability and interferes with production of prosurvival cytokines**

To further examine the CLL-supportive function of TIGIT+CD4+T cells, we analyzed the cytokine expression profile of TIGIT+ and TIGIT- CD4+T cells using intracellular cytokine staining (Fig 5a). We observed a significantly higher percentage of IFNγ and IL-10 producing CD4+T cells within the TIGIT+ population while the percentage of IL-21 and IL-4 producing cells was comparable in TIGIT+ and TIGIT- subpopulations (Fig 5b). In addition, low but measurable expression levels of the ligands for TIGIT (CD112 and CD155) could be detected on the surface of primary CLL samples as well as on T cells (Fig 5c).

We next analyzed whether cytokine production could be modulated by blocking TIGIT/ligand interactions using recombinant TIGIT-Fc molecules. As shown in Fig 6a, presence of TIGIT-Fc resulted in impaired IFNγ and IL-10 production in T cells (Fig 6a). Notably, this effect was dependent on the presence of CLL cells, as in T cell solo cultures, cytokine production was not significantly affected by addition of TIGIT-Fc (Fig 6b).

Finally, we tested whether the CLL-supportive function of TIGIT+T cells could also be recapitulated by blocking TIGIT/ligand interactions using recombinant TIGIT-Fc molecules. Therefore, we assessed CLL viability in co-culture assays in

---

**Figure 4.** CD4+ TIGIT+ cells provide a supportive microenvironment for CLL cells. (a) Representative dot plots showing gating strategy for flow cytometric cell sorting. (b) PBMCs have been depleted of TIGIT+, PD-1+ or TIGIT+PD-1+ CD4+ or CD8+ cells followed by incubation with CD3/CD28 activating beads. After 5 days in culture, CLL viability was measured and corresponding T/CLL ratios were analysed (n = 6) (c).
response to 5 day anti-CD3/CD28 stimulation in presence or absence of recombinant TIGIT-Fc. In experiments on 12 CLL samples, we did not observe a significant impact on CLL survival, however, dividing samples in high and low expressing TIGIT CD4+ T cells based on ROC curve analysis using a cut-off of 52.6% TIGIT cells within the CD4+ T cell subset (Fig 6c), we found a significant impact on CLL viability in the TIGIThigh sample group (Fig 6c).

**Discussion**

In CLL, many alterations within the T cell compartment, such as skewing towards effector and Treg subsets, abnormal CD4/CD8 ratios, increased sensitivity of CD4+ T cells towards Fas-mediated apoptosis and the occurrence of expanded monoclonal CD4+ T cell populations have been observed. Also, an aberrant expression profile of inhibitory receptors associated with T cell exhaustion has been noticed in CD4+ and CD8+ T cells from CLL patients and within the CD8+ T cells, the expansion of PD-1 expressing cells featuring defects in proliferation and cytotoxicity have been described. Furthermore, in contrast to many aggressive tumors, CLL cells are strongly depending on the microenvironment for proliferation and survival. In this regard, T cells were previously shown to execute a substantial CLL-supportive function. Hence, a more precise characterization of the CLL supportive T cell subset would allow to specifically target this CLL/T cell interaction, which would not only directly affect CLL viability but likely also render CLL cells more vulnerable to conventional treatment options. In this study, we found that particularly CD4+ T cells expressing the inhibitory receptor TIGIT were not only increased in patients with an advanced disease stage but also potent in increasing viability of autologous CLL cells in vitro co-culture assays. Moreover, we could show that this supportive impact on CLL viability of TIGIT expressing T cells is dependent on TIGIT/ligand interactions, as this effect was targetable by recombinant TIGIT-Fc molecules. In line with this, we observed low but measurable levels of CD112 and CD155 on the surface of CLL cells, the ligands for TIGIT. Moreover, we demonstrated that IFNγ and IL-10 producing T cells were enriched in the TIGIT+ compartment and blocking TIGIT/ligand interactions suppressed the production of both IL-10 and IFNγ. This effect was dependent on the presence of CLL cells, further supporting a direct TIGIT mediated CLL/T cell crosstalk, consistent with recent observations that expression of these cytokines in T cells was increased upon TIGIT dependent interactions.
interaction with antigen-presenting cells. The prosurvival effect of TIGIT T cells could well be based on secretion of these cytokines. Although the proinflammatory cytokine IFNγ has been associated with cytotoxic and antitumor mechanisms, IFNγ can also exert protumorigenic effects. IFNγ attenuates programmed cell death in CLL cells in vitro, and CLL T cells produce increased levels of IFNγ, while CLL cells show high expression of IFNγ-receptors. These data support an ambiguous role for IFNγ, which depends on the cellular, microenvironmental and molecular context.

Recently, Joller et al. reported that TIGIT+ Tregs exhibit superior immunosuppressive functions compared to the TIGIT- counterpart. In this study, we observed a general increase of Tregs and we found that more Tregs were expressing TIGIT in CLL patients compared to healthy controls. This fits to the substantial IL-10 production in TIGIT+ CD4+ T cells from CLL patients as IL-10 not only dampens anti-tumor responses in vivo, but also has a direct protective and prosurvival effect on CLL cells. In line with our data, it has been recently reported that a subset of TIGIT+ circulating Tfh cells have a strong B cell-supportive effect due to protective cytokine secretion and high expression of costimulatory molecules. Additionally, the occurrence of Tfh cells is significantly increased in CLL, particularly in high risk patients.

Our results are of particular interest in light of recent approaches for anticancer immune therapies aiming at blocking TIGIT to reinvigorate anticancer immune responses (NCT02794571, NCT02913313; clinicaltrials.gov). Similar to therapeutic antibodies for CTLA-4 (ipilimumab) and PD-1 (nivolumab and pembrolizumab) or its ligand PD-L1 (atezolizumab, durvalumab), which have already been approved for multiple hematological malignancies, antibodies blocking TIGIT were developed to impede inhibitory signals on cytotoxic T cells in order to regain anti-tumor immunity.

In this regard, our own results suggest that anti-TIGIT antibodies could specifically target the CLL-supportive TIGIT+CD4+T cell compartment, defining an alternative rationale for using these antibodies especially in combination with other treatment options.

**Materials and methods**

**Patients**

Peripheral blood samples were obtained from patients with confirmed diagnosis of CLL (Department of Haematology and Oncology, University Hospital Salzburg, Austria) on basis of >5000 light chain restricted B cells and CD5, CD19, CD20low and CD79blow co-expression per μl blood and twenty-nine age-matched healthy control samples (Stroke Prevention Center, Department of Neurology). Monoclonal antibodies for flow cytometric analysis were provided by Beckman coulter:
CD5-PC7 (A21690), CD19-APC. Alexa Fluor 750 (A94681), CD23-APC (A69964), CD20-Pacific Blue (B49208) and CD79b-PE (IM1612). Of 114 measured patients, 36 received treatment and 78 were chemotherapy naïve. Analysis of the mutational status of immunoglobulin heavy chain variable region (IgVH), expression of CD38, ZAP70, CD49d and assessment of genomic aberrations (Tri12, delChrl1q, delChrl7p and delChrl3q) was performed as described previously(2). Overall 73.7% of patients showed genomic aberrations. For detailed patient information and treatment status see supplementary table 1 (samples used for respective experiments are given on individual excel spreadsheets within supplementary table 1). Full informed consent was obtained from all CLL patients and healthy volunteers and the study was conducted in accordance with institutional Guidelines and the Declaration of Helsinki and under approval of the Salzburg Ethics committee (no. 415-E/1287/13). Peripheral blood mononuclear cells (PBMCs) were collected in heparinized or EDTA-coated tubes during routine examinations and separated by density centrifugation using Biocoll (Biochrom AG).

Immunofluorescence staining and flow cytometric analysis

PBMCs were separated by density centrifugation of fresh CLL blood samples. PBMCs were incubated with directly conjugated mAbs for 20 minutes at room temperature. Flow cytometric analysis was performed after erythrolysis with IOTest mAbs for 20 minutes at room temperature. Flow cytometric blood samples. PBMCs were incubated with directly conjugated antibodies for cytokines were stained with anti-human IFN-γ-FITC (ThermoFisher, 11-0199-42), II-21-PE (ThermoFisher, 12-7219-42), IL-10-PerCP-eFluor710 (ThermoFisher, 46-7108-42) and IL-4-APC (ThermoFisher, 17-7049-42). For surface marker expression TIGIT-PE-Cyanine7 (ThermoFisher, 25-9500-42), CD3-AF700 (ThermoFisher, 56-0038-42) and CD4-Brilliant Violet 650 (Biolegend, 300536) were used.

Depletion of T cells for in vitro co-culture experiments

The impact of specific T cells on the viability of autologous CLL cells was assessed in in vitro experiments. To deplete PBMCs from TIGIT+ and/or PD1+ cells, we stained PBMCs using TIGIT or PD1 antibodies and depleted positive cells by flow cytometric cell sorting (FACS Aria III, Becton Dickinson). To deplete PBMCs from all CD8+ T cells together with TIGIT+ or PD-1+ cells (to keep CD4+TIGIT- or PD-1- T cells in co-culture with CLL cells) we stained PBMCs using TIGIT or PD-1 and CD8 antibodies and depleted positive cells by flow cytometric cell sorting. To deplete PBMCs from all CD4+ T cells together with TIGIT+ or PD-1+ cells (to keep CD8+TIGIT+ or PD-1- T cells in co-culture with CLL cells) we stained PBMCs using TIGIT and CD4 antibodies and depleted positive cells by flow cytometric cell sorting. Purity of sorted cells was >92% for all experiments. Similar to Chauvin et al.,24 sorted PBMCs were stimulated in vitro with anti-CD3/CD28 coated beads (ThermoFisher). After five days CLL and T cells were stained with rAnnexin V-FITC and 7-AAD Viability Staining Solution (ThermoFisher) and analyzed on a Gallios™ Flow Cytometer research system.

Blockade of TIGIT/PVRp

PBMCs were activated for five days with CD3/CD28 coated beads (ThermoFisher) in presence of recombinant human TIGIT Fc chimera protein or recombinant human IgG1 Fc control (100 μg/mL, R&D System). Antibodies used for viability stain were anti-CD5-AF700 (Beckman Coulter, A78835), CD19-PE, rAnnexin V-FITC and 7-AAD Viability Staining Solution (ThermoFisher).

Statistical analysis

Statistical analyses were performed using Graph Pad Prism Version 5.02 (GraphPad Software, Inc.). Data are presented either as dot plots or as box plots showing 25th and 75th percentile and median inside the box. Whiskers represent minimum to maximum of all data.

Correlations were performed using the parametric pearson or nonparametric spearman test

Data was compared depending on Gaussian distribution (unpaired t test/ paired t test or Mann-Whitney test/ Wilcoxon matched pairs test). P values of less than 0.05 were considered statistically significant (’p < 0.05, **p < 0.01, ***p < 0.001).

Conflict of interest

The authors declare no conflict of interest.
Acknowledgments

The authors thank the stroke prevention center of the Christian-Doppler-Klinik Salzburg and the team of the Paracelsus 10,000 study for providing blood samples from healthy volunteers upon consent. This work was supported by the SCRI-LIMCR, the Province of Salzburg, the City of Salzburg, and grants from the Austrian Science Fund FWF (Projects P24100 to R.G., P28201 to R.G., I 2795-B28 to A.E., P25015 to T.N.H. and T 516-B13 to N.Z.).

References

1. Pleyer L, Egle A, Hartmann TN, Greil R. Molecular and cellular mechanisms of CLL: novel therapeutic approaches. Nat Rev Clin Oncol. 2009;6(7):405-18. doi:10.1038/nrclinonc.2009.72. PMID:19488676
2. Gassner FJ, Zaborsky N, Neu reiter D, Huemer M, Melchardt T, Egle A, Rehbandl S, Catakovic K, Hartmann TN, Greil R. Chemotherapy-induced augmentation of T cells expressing inhibitory receptors is reversed by treatment with lenalidomide in chronic lymphocytic leukemia. Haematologica. 2014;99(5):67-9. doi:10.3324/haematol.2013.098459. PMID:24561794
3. Riches JC, Davies JK, McClanahan F, Fatah R, Agrawal S, Ramsay AG, Gribben JG. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood. 2013;121(9):1612-21. doi:10.1182/blood-2012-09-457531. PMID:23247726
4. Ramsay AG, Johnson AJ, Lee AM, Gorgun G, Le Dieu R, Blum W, Byrd JC, Gribben JG. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. J Clin Invest. 2008;118(7):2427-37. PMID:18551193
5. Ramsay AG, Clear AJ, Fatah R, Grenni G, Iqbal S, Agrawal S, Ramirez AG, Gribben JG. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood. 2013;121(9):1612-21. doi:10.1182/blood-2012-09-457531. PMID:23247726
6. Frebel H, Nindl V, Schuepbach RA, Braunschweiler T, Richter K, Vogel J, Wagner CA, Lofing-Cueni D, Kurrer M, Ludewig B. Programmed death 1 protects from fatal circulatory failure during systemic virus infection of mice. J Exp Med. 2012;209(13):2485-99. doi:10.1084/jem.20121005. PMID:23230000
7. Brooks DG, MagC cavagner DB, Oldstone MB. Reprogramming of antiviral T cells prevents inactivation and restores T cell activity during persistent viral infection in mice. J Exp Med. 2006;206(16):1675-85. doi:10.1084/jem.200612856. PMID:16710479
8. Catakovic K, Klieser E, Neureiter D, Geisberger R. T cell exhaustion: defined and undefined-immune checkpoint inhibitor (ICITI) receptor expression, role, and relevance. J Immunother Cancer. 2015;3(1):2. doi:10.1186/s40428-015-0041-7. PMID:26138782
9. Chauvin JM, Pagliano O, Fourcade J, Sun Z, Wang H, Sander C, Kirkwood JM, Chen TH, Maurer M, Korman AJ. TIGIT and PD-1 impair tumor antigen-specific CD8+ T cell effector function. Cancer Cell. 2014;26(6):923-7. doi:10.1016/j.ccell.2014.10.018. PMID:25465800
10. Kurtulus S, Sakuishi K, Ngiow SF, Joller N, Tan DJ, Teng MW, Teng ren HG, Kiessling R, Anderson AC. TIGIT predominantly regulates the immune response via regulatory T cells. J Clin Invest. 2015;125(11):4053-62. doi:10.1172/JCI81187. PMID:26413872
11. Chauvin JM, Pagliano O, Fourcade J, Sun Z, Wang H, Sander C, Kirkwood JM, Chen TH, Maurer M, Korman AJ. TIGIT and PD-1 impair tumor antigen-specific CD8+ T cells in melanoma patients. J Clin Invest. 2015;125(5):2046-58. doi:10.1172/JCI80445. PMID:25866972
12. Sansom DM, CD28, CTLA-4 and their ligands: what does who to and whom? Immunology. 2000;101(2):169-77. doi:10.1046/j.1365-2567.2000.01217.x. PMID:11012769
13. Rembser KM, Hawkins ED, Shimoni R, McGrath M, Chan CJ, Russell SM, Smyth MJ, Oliaro J. Cutting edge: DNAX accessory molecule a 1-deficient CD8+ T cells display immunological synapse defects that impair antitumor immunity. J Immunol. 2014;192(2):553-7. doi:10.4049/jimmunol.1302197. PMID:24337740
14. Zhang T, Wang J, Zhou X, Li X, Wang L, Lu H, Gao G, Dong B, Zhu H. Increased expression of TIGIT on CD4+ T cells ameliorates immune-mediated bone marrow failure of aplastic anemia. J Cell Biochem. 2014;115(19):181-27. PMID:24905442
15. Inozume T, Yaguchi T, Furuta J, Harada K, Kawakami Y, Shimada S. Melanoma Cells Control Antimelanoma CTL Responses via Interaction between TIGIT and CD155 in the Effector Phase. J Exp Med. 2016;213(16):255-63.
16. Tinhofer I, Weiss L, Gassner F, Rubenzer G, Holler C, Greil R. Difference in the relative distribution of CD4+ T-cell subsets in B-CLL with mutated and unmutated immunoglobulin (Ig) VH genes: implication for the course of disease. J Immunother. 2009;32(3):302-9. doi:10.1097/CJI.0b013e318197b5e4. PMID:19242370
17. Weiss L, Melchardt T, Egle A, Grabner M, Greil R, Tinhofer I. Regulatory T cells predict the time to initial treatment in early stage chronic lymphocytic leukemia. Cancer. 2011;117(10):2163-9. doi:10.1002/cncr.25752. PMID:21523729
31. Piper KP, Karanth M, McLarnon A, Kalk E, Khan N, Murray J, Pratt G, Moss PA. Chronic lymphocytic leukaemia cells drive the global CD4+ T cell repertoire towards a regulatory phenotype and leads to the accumulation of CD4+ forkhead box P3+ T cells. Clin Exp Immunol. 2011;166(2):154-63. doi:10.1111/j.1365-2249.2011.04466.x. PMID:21985361

32. Bartik MM, Welker D, Kay NE. Impairments in immune cell function in B cell chronic lymphocytic leukemia. Semin Oncol. 1998;25(1):27-33. PMID:9482524

33. Tinhofer I, Marschitz I, Kos M, Henn T, Egle A, Villunger A, Greil R. Differential sensitivity of CD4+ and CD8+ T lymphocytes to the killing efficacy of Fas (Apo-1/CD95) ligand+ tumor cells in B chronic lymphocytic leukemia. Blood. 1998;91(11):4273-81. PMID:9596676

34. Zaborsky N, Holler C, Geisberger R, Asslaber D, Gassner FJ, Egger V, Piñón-Hofbauer J, Kocher T, Hartmann TN, Greil R. B-cell receptor usage correlates with the sensitivity to CD40 stimulation and the occurrence of CD4+ T-cell clonality in chronic lymphocytic leukemia. Haematologica. 2015;100(8):e307-10. PMID:25911550

35. Rezvany MR, Jeddi-Tehrani M, Wigzell H, Osterborg A, Mellstedt H. Leukemia-associated monoclonal and oligoclonal TCR-BV use in patients with B-cell chronic lymphocytic leukemia. Blood. 2003;101(3):1063-70. doi:10.1182/blood-2002-03-0746. PMID:12393705

36. Os A, Burgler S, Ribes AP, Funderud A, Wang D, Thompson KM, Tjønnfjord GE, Bogen B, Munthe LA. Chronic lymphocytic leukemia cells are activated and proliferate in response to specific T helper cells. Cell Rep. 2013;3(3):566-77. doi:10.1016/j.celrep.2013.07.011. PMID:23933259

37. Zhao W, Dong Y, Wu C, Ma Y, Jin Y, Ji Y. TIGIT overexpression diminishes the function of CD4 T cells and ameliorates the severity of rheumatoid arthritis in mouse models. Exp Cell Res. 2016;340(1):132-8. doi:10.1016/j.yexcr.2015.12.002. PMID:26683997

38. Zaidi MR, Merlino G. The two faces of interferon-gamma in cancer. Clin Cancer Res. 2011;17(19):6118-24. doi:10.1158/1078-0432.CCR-11-0482. PMID:21705455

39. Buschle M, Campana D, Carding SR, Richard C, Hoffbrand AV, Brenner MK. Interferon gamma inhibits apoptotic cell death in B cell chronic lymphocytic leukemia. J Exp Med. 1993;177(1):213-8. doi:10.1084/jem.177.1.213. PMID:7678114

40. Zaki M, Douglas R, Patten N, Bachinsky M, Lamb R, Nowell P, Nowell P, Moore J. Disruption of the IFN-gamma cytokine network in chronic lymphocytic leukemia contributes to resistance of leukemic B cells to apoptosis. Leuk Res. 2000;24(7):611-21. doi:10.1016/S0145-2126(00)00022-9. PMID:10867137

41. Joller N, Lozano E, Burkett PR, Patel B, Xiao S, Zhu C, Xia J, Tan TG, Seifik E, Yajnik V. Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses. Immunity. 2014;40(4):569-81. doi:10.1016/j.immuni.2014.02.012. PMID:24745333

42. Fiorcari S, Maffei R, Audrito V, Martinelli S, Ten Hacken E, Zucchini P, Grisendi L, Potenza L, Luppi M, Burger JA. Ibrutinib modifies the function of monocyte/macrophage population in chronic lymphocytic leukemia. OncoTarget. 2016;7(40):65966-81. PMID:27602755

43. Godefroy E, Zhong H, Pham P, Friedman D, Yazdanbakhsh K. TIGIT-positive circulating follicular helper T cells display robust B-cell help functions: potential role in sickle cell alloimmunization. Haematologica. 2015;100(11):1415-25. doi:10.3324/haematol.2015.132738. PMID:26250578

44. Cha Z, Zang Y, Guo H, Rechlic JR, Olasnova LM, Gu H, Tu X, Song H, Qian B. Association of peripheral CD4+ CXCR5+ T cells with chronic lymphocytic leukemia. Tumour Biol. 2013;34(6):3579-85. doi:10.1007/s13277-013-0937-2. PMID:23807677