RESEARCH ARTICLE

Editorial Process: Submission:01/14/2023 Acceptance:06/22/2023

Relative Expression of BATF and CD112 in PBMC of Patients with Chronic Lymphocytic Leukemia

Ahmad Najafi[1], Matineh Sabaghi[1], Hossein Asgarian-Omran[1], Reza Valadan[1], Ramin Shekarriz[2], Ehsan Zaboli[2], Ghasem Janbabai[2], Mohsen Tehrani[1]*

Abstract

Objective: BATF, as a transcription factor, and CD112, as a receptor for TIGIT, are involved in T-cell exhaustion. We investigated BATF and CD112 gene expression in the peripheral blood mononuclear cells from CLL patients and healthy subjects. **Methods:** In a case-control study, 33 patients with CLL and 20 sex- and age-matched healthy individual were enrolled. Diagnosis and classification of patients was done according to immunophenotyping via flow cytometry and RAI staging system, respectively. Relative mRNA expression of BATF and CD112 was measured using qRT-PCR. **Result:** Our results showed that the expression of BATF and CD112 in CLL samples were significantly decreased in comparison those of the healthy controls (P = 0.0236 and P = 0.0002, respectively). **Conclusion:** These findings suggest the role of BATF and CD112 not only as a role in T cell exhaustion, but in effector differentiation program in CLL, which warrants further studies in future.

Keywords: Chronic lymphocytic leukemia- T-cell- exhaustion CD112- BATF

Asian Pac J Cancer Prev, **24 (6)**, 2171-2176

Introduction

Chronic lymphocytic leukemia (CLL) is a B cell malignancy characterized by the accumulation of CD5+ B cells in the blood, bone marrow, lymph nodes, and spleen (Chiorazzi et al., 2005). CLL is the most common form of leukemia in adults in the Western countries and accounts for 25% of all leukemias, but in Eastern societies it accounts for less than 5% of leukemias (Kalil and Cheson, 1999). CLL is associated T-cell dysfunction, such as high level production of immunomodulatory cytokines, including TGF-β and IL-10, that suppress T cell activation, expansion, and effector functions (Riches et al., 2013). In chronic infections and cancers, the dysfunction of T cells is due to their constant exposure to antigens, which is known as "T-cell exhaustion" (Dolina et al., 2021; Wherry and Kurachi, 2015). Exhausted T cells are deficient in proliferation, cytokine production, and cytotoxicity. Inhibitory signals from some receptors, known as immune checkpoints, have been shown to be associated with the phenomenon of T cell exhaustion, the most important of which are PD-1, CTLA-4, TIM-3, LAG-3, and TIGIT (Belk et al., 2009; McLane et al., 2019).

TIGIT (also called WUCAM, Vstm3, VSIG9) is a receptor of the Ig superfamily and expressed on the surface of T cells, which plays a critical role in limiting adaptive and innate immunity (Hamid et al., 2019; Stanietsky et al., 2009). TIGIT participates in a complex regulatory network involving multiple IRs (e.g., CD96/TACTILE, CD112R/PVRIG), one competing costimulatory receptor (DNAM-1/CD226), and multiple ligands (e.g., CD155 (PVR/NECL-5), CD112 (Nectin-2/PVRL2) (Bottino et al., 2003; Wu et al., 2019; Yu et al., 2009) are expressed on the surface of antigen-presenting cells (APCs) and tumor cells. Binding of the TIGIT molecule to its ligands activates some adaptor molecules, such as Fyn, Lck, and SHP1, which ultimately lead to impaired T cell proliferation and cytotoxicity, as well as decrease in polarization of granules in NK cells (Kim et al., 2020). TIGIT is expressed by activated CD8+ T and CD4+ T cells, natural killer (NK) cells, regulatory T cells (Tregs), and follicular T helper cells in humans (Joller et al., 2011; Ostroumov et al., 2021; Zhu et al., 2016). In sharp contrast with CD8+ T cells, NK cells present at low frequencies in metastatic tumors downregulate both TIGIT and CD226 expression (Chauvin et al., 2020). TIGIT binds two ligands, CD155 and CD112, that are expressed on monocytes, dendritic cells (DCs), and many non-hematopoietic cells including tumor cells of different histological types (Lozano et al., 2012; Pende et al., 2005; Zhang et al., 2018). Surface CD155 triggers CD226 internalization and degradation, resulting in decreased NK cell-mediated tumor reactivity.

Basic leucine zipper transcription factor ATF-like (BATF; also known as SFA2), BATF2 (also known as

*[1]Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran. [2]Department of Hematology and Oncology, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran. *For Correspondence: drmtehrani@gmail.com*

SARI) and BATF3 (also known as JDP1 and p21SNFT) comprise the BATF family, which is a subgroup of the larger family of basic leucine zipper (bZIP) transcription factors that plays an important role in regulating differentiation and function in many lymphocyte lineages (Boi et al., 2021; Finn et al., 2010). Members of this family have an α helical bZIP domain, which contains a basic DNA-binding region and regularly spaced leucine residues known as the leucine zipper motif (Landschulz et al.,1988; O'Shea et al., 1989) that is expressed in blood cells, especially B cells, dendritic cells, as well as in CD4+ (Murphy et al., 2013) and CD8+ T cells (Quigley et al., 2010). The BATF gene is located on mouse chromosome 12q (human chromosome 14q), 87 kilobases (kb) from the gene locus that encodes JDP2, which is another bZIP family member. BATF, along with IRF4, was recently proposed as a 'pioneer factor' in T cells (Ciofani et al., 2012; Jiang et al., 2022). Also, via binding to c-jun, BATF can inhibit the AP-1 transcription. Also, the function of PD-1 in depleted CD8+ T cells is dependent on the BATF transcription factor (Quigley et al., 2010; Seo et al., 2021).

Although increased expression of both TIGIT ligands, including CD155 and CD112, has been observed in many tumors, such as colorectal cancer (Masson et al., 2001), gastric cancer (Tahara-Hanaoka et al., 2006), and neuroblastoma (Castriconi et al., 2004), expression of CD112 and BATF in hematopoietic cancers has not been considered so far. Since the T cell exhaustion in CLL has been demonstrated, the results of this study provide a deeper understanding of the role of the TIGIT/CD155/CD112 pathway as well as the PD-1/PD-L1 pathway in the T cell exhaustion as a result of inhibition. This pathway will lead to the goal of treating CLL patients. Accordingly, the aim of this study was to evaluate the relative expression of CD112 and BATF gene in malignant B cells in patients with CLL and his comparison with healthy controls.

Materials and Methods

Patients and controls

This study was carried out as a case-control study on two groups. Group I consisted of 33 CLL patients who were admitted to the Imam Khomeini Hospital (affiliated to Mazandaran University of Medical Sciences Sari, Mazandaran, Iran). CLL was diagnosed by clinical examination, blood cell count, examination of blood cell morphology in peripheral blood or bone marrow samples, and finally immunophenotyping according to WHO criteria (Swerdlow et al., 2016). Based on the RAI staging system (Scarfo et al., 2016) patients were classified into stages 0-IV, which can be divided into two groups: primary (stage 0-I) and final (stage II-IV). Group II consisted of 20 healthy volunteers who were age- and sex-matched to the patient group and had no history of malignant diseases, chronic inflammatory diseases, autoimmune diseases, or inherited or acquired immunodeficiencies. Eight to ten milliliter of heparinized peripheral blood samples were taken from each study subject after obtaining written informed consents. The study protocol was approved by the Ethical Committee of Mazandaran University of Medical Sciences.

RNA isolation and cDNA synthesis

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh peripheral blood using Ficoll-Histopaque (Biosera, Nuaille, France) density-gradient centrifugation. Total RNA was isolated from 1×10^6 PBMCs using the FavorPrep Blood/ Cultured Cell Total RNA Mini Kit (Favorgen, Taiwan) based on the manufacturer's protocol. The quantity and purity of the extracted RNA were assessed via measuring absorbance at 260 nm and the ratio A260/A280 in a nano-spectrophotometer (Thermo Fisher Scientific Inc., USA) and agarose gel electrophoresis. Next, a total of 1 µg of RNA for use in complementary DNA (cDNA) synthesis was performed using the Thermo Scientific RevertAid first strand cDNA synthesis kit (Thermo Scientific, Massachusetts, USA) based on the manufacturer's instructions.

Quantitative RT-PCR

Quantitative Reverse Transcriptase PCR (qRT-PCR) was performed on the Applied Biosystems Real-Time PCR instrument (ABI, Thermo Fisher Sci) using Real Q Plus 2x Master Mix (High Rox, Ampliqon, Denmark) and specific primers for BATF, CD112, and β-actin, as a housekeeping gene (Table 2). Primer efficiencies were defined as 98.7%, 101%, and 99.4% for BATF, CD112, and β-actin, respectively. PCR was carried out at 95°C for initial denaturation followed by 40 cycles at 95°C for 15 seconds, 60°C for 30 seconds, and extension at 72°C for 30 seconds. All samples were done in duplicate and the relative expression level of these genes were carried out with $2^{-\Delta\Delta Ct}$ value using β-actin as an internal housekeeping gene (Livak and Schmittgen, 2001).

Statistical analysis

For the statistical study, Graph Pad Prism statistical software, version 8.0 (Graph Pad, San Diego, CA, USA) was used. The normality was evaluated by the Kolmogorov–Smirnov test. Mann-Whitney U test was considered to compare the mean differences between two groups. Data are represented as median (interquartile range) and p-values less than 0.05 were considered significant.

Results

A total of 33 CLL patients (19 males and 14 females, mean age: 61.5 years) and 20 age- and sex-matched control subjects (14 males and 6 females, mean age: 56.8 years) participated in the study. According the Rai staging system, 28 patients were in early stages and 5 in advanced stages. Serum LDH concentration and CD38 percentage were not significant between patients with early and advanced stages of CLL (p=0.590 and p=0.722, respectively). Major clinical and laboratory characteristics patients and controls are summarized in Table 1.

All sample showed detectable expression of BATF and CD112 mRNA. The mRNA level of BATF and CD112 in CLL patients were significantly lower than the control group (p= 0.0236 and 0.0002, respectively, Figure 1A, 1B). Further analysis showed that BATF expression level

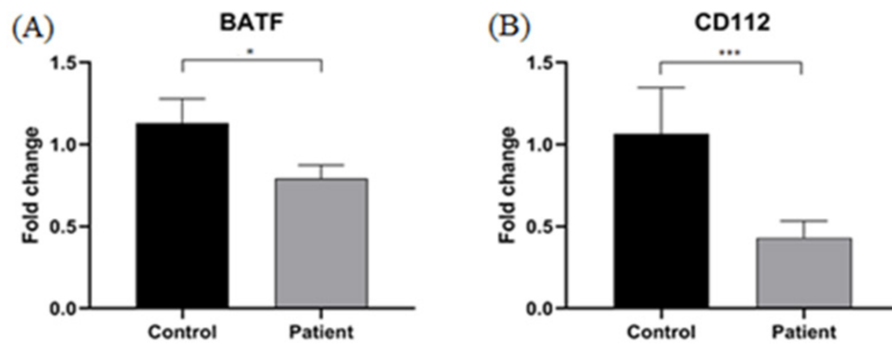

Figure 1. Expressions of BATF and CD112 in CLL Patients Compared with Controls. The results are represented as median ± interquartile range (IQR). *, P < 0.05; ***, P < 0.0005

was not significantly different between CLL patients with early and advanced stages (p=0.15, Figure 2A), while CD112 expression level was significantly lower between CLL patients with early and advanced stages (p=0.04,

Table 1. Major Clinical and Laboratory Characteristics of CLL Patients

No	Age (y)	Sex	WBC×10³/ mm³	Lymph (%)	PLT×10³/ mm³	Hb (g/dL)	LDH (IU/L)	Rai stage	CD38 (%)	Organomegaly
1	50	F	4.2	87.7	157	11.6	308	0	NA	Negative
2	64	M	17.85	79	111	12.5	401	I	NA	LAP+
3	61	M	104.67	90.1	226	13.3	354	0	NA	Negative
4	46	M	10.03	57.08	134	16.6	281	0	1.1	Negative
5	54	F	170.09	90.4	42	8.4	709	I	6	LAP+
6	80	M	60.7	92.2	71	8.1	566	IV	NA	LAP+
7	63	F	17.15	60.3	176	13.1	534	0	NA	Negative
8	64	M	18.1	75	16	14.1	630	0	NA	Negative
9	58	M	46.2	76.56	160	12.7	796	0	NA	Negative
10	80	M	71	89	119	11.9	NA	0	1.4	Negative
11	78	M	49.15	92	164	10.5	1008	0	NA	Negative
12	78	M	42.16	85.8	231	13.1	348	0	0.6	Negative
13	75	F	46.56	55.9	191	9.1	356	0	3.57	Negative
14	52	F	84.5	88.15	173	12	232	II	23.01	LAP+SPM+
15	72	F	53.1	84.82	147	10.6	284	0	NA	Negative
16	77	M	49.4	88.15	180	8.5	562	III	6.7	Negative
17	61	F	17.2	70.88	127	12.7	NA	0	NA	Negative
18	78	M	36	84	104	15	NA	0	21	Negative
19	60	F	82	56.9	139	14	510	0	1.1	Negative
20	76	F	78.2	91.2	139	11.9	599	II	0.58	LAP+HSP+
21	84	F	51.3	85.1	231	11	892	II	1.16	SPM+
22	61	M	55.06	89.8	148	12.6	333	0	4.5	Negative
23	70	M	17	72	216	13.5	450	0	5.84	Negative
24	62	F	17.3	65	220	14.5	NA	0	5	Negative
25	65	M	35.4	80	150	13.7	422	0	2.54	Negative
26	64	F	21.38	76.5	220	12.6	387	0	4.72	Negative
27	57	M	25.46	76	188	14.4	272	0	6.94	Negative
28	61	M	28.5	86	126	14	339	I	4.01	LAP+
29	62	M	43.6	88	91	13.6	1380	1	4.7	LAP+
30	53	M	28.8	78.8	223	11.8	452	1	20	LAP+
31	77	M	94.7	90.5	209	12.9	384	0	2.46	Negative
32	44	F	99.5	93.27	139	10.5	600	0	4.9	Negative
33	63	F	47.5	85	188	13.3	548	0	9.44	Negative

CLL, chronic lymphocytic leukemia; M, male; F, female; LAP, lymphadenopathy; SPM, splenomegaly; HPM, hepatomegaly; WBC, white blood cell count; Lym, lymphocytes percent in peripheral blood; PLT, platelet count; Hb, hemoglobin and; LDH, serum lactate dehydrogenase; LAP, lymphadenopathy; SPM, splenomegaly; HPM, hepatomegaly; NA, not available.

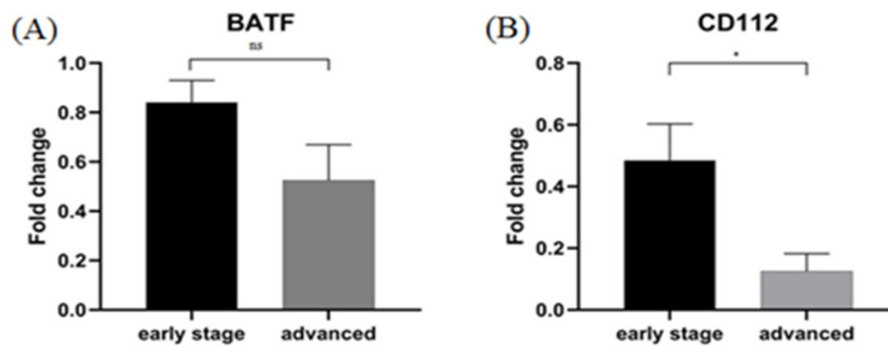

Figure 2. Expressions of BATF and CD112 in CLL Patients with Different Clinical Stages. The results are represented as median ± interquartile range (IQR). *, $P < 0.05$; ns, $P > 0.05$

Table 2. Primer Sequences and Amplicon Lengths for the Specific qRT-PCR of BATF, CD112 and β-actin

Target	Primer sequence	Amplicon size
BATF	Forward, 5'- ATCTGATGATGTGAGAAGAGTTC -3'	152 bp
	Reverse, 5'- GCTTGATCTCCTTGCGTAG -3'	
CD112	Forward, 5'- GACGAGGGCAACTACACTTG -3'	198 bp
	Reverse, 5'- GGATGAGAGCCAGGAGATCC -3'	
β-actin	Forward, 5'- CCTTCCTGGGCATGGAGTCCT -3'	176 bp
	Reverse, 5'- TGGGTGCCAGGGCAGTGAT -3'	

Figure 2B). Evaluation the specificity of the amplification products by dissociation/melting curve did not show any amplification of nonspecific products.

Discussion

Following chronic antigen stimulation, T cells become exhausted and upregulate several inhibitory receptors (IRs), including programmed cell death receptor 1 (PD-1) and T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT). IR ligands are also expressed by tumor cells and antigen-presenting cells (APCs) in the tumor microenvironment (TME). Targeting IRs with monoclonal antibodies (mAbs) has been beneficial in mouse tumor models and humans, and immune checkpoint blockade (ICB) with anticytotoxic T lymphocyte-associated antigen 4 (CTLA-4), anti-PD-1, or both mAbs are standard treatments for several solid tumors (Chauvin and Zarour, 2020; Wolchok et al., 2013; Zarour, 2016). Further, multiple lines of evidence support that TIGIT plays a critical role in limiting adaptive immunity against tumors (Boles et al., 2009; Stanietsky et al., 2009; Yu et al., 2009).

A role for BATF in T cell exhaustion during chronic viral infections was recently proposed (Quigley et al., 2010). This hypothesis was based on the premise that BATF inhibits conven-tional AP 1 activity, but it did not take into account the positive transcriptional actions of BATF. BATF is one of three transcription factors that are induced by the treatment of Jurkat cells with a PD-1 specific agonist, and expressed by CD8+ T cells isolated from individuals who are infected with HIV (Quigley et al., 2010). The expression of BATF and PD-1 were shown to positively correlated with each other during chronic, but not acute, lymphocytic choriomeningitis virus (LCMV) infection. The forced overexpression of BATF reduced the proliferation of primary human T cells (Quigley et al., 2010), whereas knockdown of BATF increased IL 2 production in Jurkat cells and in HIV-specific CD4+ T cells, and increased IFN-γ production in HIV-specific CD4+ and CD8+ T cells; however, none of these effects required PD1 activation (Quigley et al., 2010).

In the present study, we focused on BATF, as a transcription factor, and CD112, as a TIGIT ligand, to provide a deeper understanding of the role of the TIGIT/CD155/CD112 pathway as well as the PD-1/PD-L1 pathway in the T cell exhaustion as a result of inhibition. Our results showed that relative expressions of BATF and CD112 were lower in CLL patients compared to healthy subjects. Previously, we showed that both CD4+ and CD8+ T-cells are functionally exhausted in CLL and that co-expression of PD-1 and Tim-3 on both cells were higher in advanced stages of CLL compared to early stages (Allahmoradi et al., 2017; Taghiloo et al., 2017). However, more recently, BATF has been reported to play a dual role of the BATF in regulation of T cell functions. BATF is a central regulator of early effector CD8+ T cell differentiation with CD8+ T cells lacking BATF showed impaired proliferation and effector functions which was associated with major defects in cell metabolism, proliferation, and survival (Kurachi et al., 2014). BATF represses effector function in exhausted CD8+ T cells (Quigley et al., 2010). In this setting, the primary functions of BATF seems to be repressing effector genes, such as IFN-γ, regulation of metabolism and/or epigenetics in CD8+ T cells via Sirt1 (Kurachi et al., 2014), and its role as a checkpoint in a transcriptional circuit that ensures proper coordination of effector CD8+ T cell

differentiation. Such an interpretation would suggest that once CD8+ T cells progress past the point of initial effector differentiation, BATF tunes functionality depending on the strength and/or duration of TCR stimulation (Kurachi et al., 2014).

CD112 with CD226 is crucially important for NK cell–mediated tumor cell lysis (Pende et al., 2005). Tahara-Hanaoka et al., (2006) have demonstrated that the interaction between CD226 and CD112 is partially responsible for elimination of virus-induced tumors in vivo, suggesting that NK cells might be directly activated by CD112 on tumor cells. Therefore, the expression level of CD112 might significantly affect the lysis of tumor cells by NK cells. Huang et al., (2014) demonstrated that CD112 expression is significantly down-regulated in HCC cells and that low CD112 expression is associated with poor overall survival of patients. Down-regulation of NK cell–activating ligands such as NKG2D ligands and CD112 is an important strategy by which tumor cells evade the immune attack.

In conclusion, the present study showed that PBMCs in CLL have low level expression of BATF and CD112. These findings suggest that beside the role of BATF and CD112 in T cell exhaustion, they might play a role in T cell effector differentiation program in CLL. However, further studies are needed to explore the exact role of BATF and CD112 in immune response in CLL.

Author Contribution Statement

All authors contributed to the study. Mohsen Tehrani and Hossein Asgarian-Omran designed and conducted the research. Ahmad Najafi and Matine Sabbaghi Rostami carried out the assays. Ahmad Najafi contributed to data collection and analysis. Ramin Shekarriz, Ghasem Janbabaei, and Ehsan Zaboli provided the samples. Reza Valadan helped in PCR optimization and analysis of PCR data. Ahmad Najafi, Hossein Asgarian-Omran, and Mohsen Tehrani prepared the manuscript. All authors read and approved the final manuscript..

Acknowledgements

General: The authors thank the patients and their families for their support, cooperation, and patience. We would like to thank the staff of the departments associated with care and management of the patients.

Funding Statement

This study was financially supported by Mazandaran University of Medical Sciences; grant number MCBRC-MAZUMS-2178.

Ethical Declaration

All of the authors have indicated they have no potential conflicts of interest to disclose (professional, financial and direct or indirect benefits). This study was financially supported by Mazandaran University of Medical Sciences; grant number MCBRC-MAZUMS-2178.

Conflict of Interest

All of the authors have indicated they have no potential conflicts of interest to disclose.

References

Allahmoradi E, Taghiloo S, Tehrani M, et al (2017). CD4+ T Cells are Exhausted and Show Functional Defects in Chronic Lymphocytic Leukemia. *Iran J Immunol*, **14**, 257-69.

Belk JA, Daniel B, Satpathy AT (2022). Epigenetic regulation of T cell exhaustion. *Nat Immunol*, **23**, 848-60.

Blackburn SD, Shin H, Haining WN, et al (2009). Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol*, **10**, 29-37.

Boi SK, Lan X, Youngblood B (2021). BATF targets T cell exhaustion for termination. *Nat Immunol*, **22**, 936-8.

Boles KS, Vermi W, Facchetti F, et al (2009). A novel molecular interaction for the adhesion of follicular CD4 T cells to follicular DC. *Eur J Immunol*, **39**, 695-703.

Bottino C, Castriconi R, Pende D, et al (2003). Identification of PVR (CD155) and Nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule. *J Exp Med*, **198**, 557-67.

Castriconi R, Dondero A, Corrias MV, et al (2004). Natural killer cell-mediated killing of freshly isolated neuroblastoma cells: critical role of DNAX accessory molecule-1-poliovirus receptor interaction. *Cancer Res*, **64**, 9180-4.

Chauvin JM, Ka M, Pagliano O, et al (2020). IL15 Stimulation with TIGIT Blockade Reverses CD155-mediated NK-Cell Dysfunction in Melanoma. *Clin Cancer Res*, **26**, 5520-33.

Chauvin JM, Zarour HM (2020). TIGIT in cancer immunotherapy. *J Immunother Cancer*, **8**.

Chiorazzi N, Rai KR, Ferrarini M (2005). Chronic lymphocytic leukemia. *N Engl J Med*, **352**, 804-15.

Ciofani M, Madar A, Galan C, et al (2012). A validated regulatory network for Th17 cell specification. *Cell*, **151**, 289-303.

Dolina JS, Van Braeckel-Budimir N, Thomas GD, Salek-Ardakani S (2021). CD8(+) T Cell Exhaustion in Cancer. *Front Immunol*, **12**, 715234.

Finn RD, Mistry J, Tate J, et al (2010). The Pfam protein families database. *Nucleic Acids Res*, **38**, 211-22.

Hamid O, Robert C, Daud A, et al (2019). Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. *Ann Oncol*, **30**, 582-8.

Huang X, Qu P, Chen Y, et al (2014). Low expression of CD112 is associated with poor overall survival in patients with hepatocellular carcinoma. *Hum Pathol*, **45**, 1944-50.

Jiang P, Zhang Z, Hu Y, et al (2022). Single-cell ATAC-seq maps the comprehensive and dynamic chromatin accessibility landscape of CAR-T cell dysfunction. *Leukemia*, **36**, 2656-68.

Joller N, Hafler JP, Brynedal B, et al (2011). Cutting edge: TIGIT has T cell-intrinsic inhibitory functions. *J Immunol*, **186**, 1338-42.

Kalil N, Cheson BD (1999). Chronic lymphocytic leukemia. *Oncologist*, **4**, 352-69.

Kim K, Park S, Park SY, et al (2020). Single-cell transcriptome analysis reveals TOX as a promoting factor for T cell exhaustion and a predictor for anti-PD-1 responses in human cancer. *Genome Med*, **12**, 22.

Kurachi M, Barnitz RA, Yosef N, et al (2014). The transcription factor BATF operates as an essential differentiation checkpoint in early effector CD8+ T cells. *Nat Immunol*, **15**, 373-83.

Landschulz WH, Johnson PF, McKnight SL (1988). The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science*, **240**, 1759-64.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, **25**, 402-8.

Lozano E, Dominguez-Villar M, Kuchroo V, Hafler DA (2012). The TIGIT/CD226 axis regulates human T cell function. *J Immunol*, **188**, 3869-75.

Masson D, Jarry A, Baury B, et al (2001). Overexpression of the CD155 gene in human colorectal carcinoma. *Gut*, **49**, 236-40.

McLane LM, Abdel-Hakeem MS, Wherry EJ (2019). CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. *Annu Rev Immunol*, **37**, 457-95.

Murphy TL, Tussiwand R, Murphy KM (2013). Specificity through cooperation: BATF-IRF interactions control immune-regulatory networks. *Nat Rev Immunol*, **13**, 499-509.

O'Shea EK, Rutkowski R, Stafford WF, Kim PS (1989). Preferential heterodimer formation by isolated leucine zippers from fos and jun. *Science*, **245**, 646-8.

Ostroumov D, Duong S, Wingerath J, et al (2021). Transcriptome Profiling Identifies TIGIT as a Marker of T-Cell Exhaustion in Liver Cancer. *Hepatology*, **73**, 1399-418.

Pende D, Bottino C, Castriconi R, et al (2005). PVR (CD155) and Nectin-2 (CD112) as ligands of the human DNAM-1 (CD226) activating receptor: involvement in tumor cell lysis. *Mol Immunol*, **42**, 463-9.

Pende D, Spaggiari GM, Marcenaro S, et al (2005). Analysis of the receptor-ligand interactions in the natural killer-mediated lysis of freshly isolated myeloid or lymphoblastic leukemias: evidence for the involvement of the Poliovirus receptor (CD155) and Nectin-2 (CD112). *Blood*, **105**, 2066-73.

Quigley M, Pereyra F, Nilsson B, et al (2010). Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. *Nat Med*, **16**, 1147-51.

Riches JC, Davies JK, McClanahan F, et al (2013). T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. *Blood*, **121**, 1612-21.

Scarfo L, Ferreri AJ, Ghia P (2016). Chronic lymphocytic leukaemia. *Crit Rev Oncol Hematol*, **104**, 169-82.

Seo H, Gonzalez-Avalos E, Zhang W, et al (2021). BATF and IRF4 cooperate to counter exhaustion in tumor-infiltrating CAR T cells. *Nat Immunol*, **22**, 983-95.

Stanietsky N, Simic H, Arapovic J, et al (2009). The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc Natl Acad Sci U S A*, **106**, 17858-63.

Swerdlow SH, Campo E, Pileri SA, et al (2016). The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*, **127**, 2375-90.

Taghiloo S, Allahmoradi E, Tehrani M, et al (2017). Frequency and functional characterization of exhausted CD8(+) T cells in chronic lymphocytic leukemia. *Eur J Haematol*, **98**, 622-31.

Tahara-Hanaoka S, Shibuya K, Kai H, et al (2006). Tumor rejection by the poliovirus receptor family ligands of the DNAM-1 (CD226) receptor. *Blood*, **107**, 1491-6.

Wherry EJ, Kurachi M (2015). Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol*, **15**, 486-99.

Wolchok JD, Kluger H, Callahan MK, et al (2013). Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med*, **369**, 122-33.

Wu L, Mao L, Liu JF, et al (2019). Blockade of TIGIT/CD155 Signaling Reverses T-cell Exhaustion and Enhances Antitumor Capability in Head and Neck Squamous Cell Carcinoma. *Cancer Immunol Res*, **7**, 1700-13.

Yu X, Harden K, Gonzalez LC, et al (2009). The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nat Immunol*, **10**, 48-57.

Zarour HM (2016). Reversing T-cell Dysfunction and Exhaustion in Cancer. *Clin Cancer Res*, **22**, 1856-64.

Zhang Q, Bi J, Zheng X, et al (2018). Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. *Nat Immunol*, **19**, 723-32.

Zhu Y, Paniccia A, Schulick A C, et al (2016). Identification of CD112R as a novel checkpoint for human T cells. *J Exp Med*, **213**, 167-76.

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.