BTN3A: A Promising Immune Checkpoint for Cancer Prognosis and Treatment

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Abstract: Butyrophilin-3A (BTN3A) subfamily members are a group of immunoglobulins present on the surface of different cell types, including innate and cancer cells. Due to their high similarity with the B7 family members, different studies have been conducted and revealed the involvement of BTN3A molecules in modulating T cell activity within the tumor microenvironment (TME). However, a great part of this research focused on γδ T cells and how BTN3A contributes to their functions. In this review, we will depict the roles and various aspects of BTN3A molecules in distinct tumor microenvironments and review how BTN3A receptors modulate diverse immune effector functions including those of CD4+ (Th1), cytotoxic CD8+ T cells, and NK cells. We will also highlight the potential of BTN3A molecules as therapeutic targets for effective immunotherapy and successful cancer control, which could represent a bright future for patient treatment.

Keywords: BTN3A; T lymphocytes; cancer; immune system; prognostic factor

1. Introduction

Therapeutic strategies for cancer have garnered a large amount of interest in recent years. Along with conventional therapies, such as radiotherapy and chemotherapy, immunotherapy seems to be a promising approach to cancer management [1]. The exhaustion of effector T cell functions via inhibitory signaling pathways triggered by certain immune checkpoints represents the most effective strategy to promote cancer cell escape and growth [2,3]. Therefore, therapies based on immune-checkpoint blockade(s) (ICB), such as ipilimumab and nivolumab, have achieved promising results and offer hope [4–6].

However, tumor microenvironment (TME) heterogeneity and toxicity/resistance occurrences limit the scope of this ICB [7]. Studies have revealed that the success rate does not exceed 15 to 30% in different human cancers, such as melanoma, hepatocellular carcinoma, and non-small cell lung cancer [8–12]. To overcome this issue, different approaches must be considered, including the implementation of personalized treatment, and the identification of additional genetic mutations, biomarkers, or new target molecules involved in tumor mechanisms and immune cell activities [13]. Thus, several studies have initiated investigations on new families of immune checkpoints in order to clarify their specific roles in TME and immune cell modulation [14–16].

Based on previous studies, BTN3A molecules (also termed CD277), are expressed in different types of immune cells, including T cells, natural killer cells, dendritic cells, and monocytes [17–19], and some cancers, such as ovarian cancer, gastric cancer, pancreatic cancer, breast cancer, and colorectal cancer [20–24].
Among all 13 BTNs that are known in humans, only the BTN3A subfamily of butyrophilins are expressed by tumor cells [25] as well as by all cells of the immune system, including T cells, B cells, monocytes, dendritic cells, and natural killer (NK) cells. This broad expression pattern has made this group of butyrophilins among the best-studied subtypes [19,26,27]. Another particularity of the BTN3A molecules is that the biological impacts of the various inhibitor and stimulator antibodies of BTN3 on immune responses are quite different. In addition, the molecules of the BTN3A subfamily are specific for primates and other eutherian species and are not present in rodents [28,29]. This feature of BTN3A molecules has piqued the interest of researchers because their potential biological/immunological roles are characteristic of a well-defined species. Similar to γδ T cells, BTN3A isotypes are found at the border of innate and adaptive immune responses. Highlighting their roles in modulating CD4+, CD8+, monocyte, and NK cell activity will undoubtedly lead to a better understanding of the communication between innate and adaptive immune cells and, as a result, better utilization of potential therapeutic strategies.

The literature on BTN3A primarily focuses on γδ T cells [23,28,30–32], which may lead to an underestimation of this molecule’s potential in modulating the immunological activities of CD4+, CD8+, monocytes, and NK cells. Therefore, it is important to provide a comprehensive overview of the BTN3A molecule’s effect on CD8 T cells, CD4 (Th1) T cells, and NK cells, summarize differential prognoses, clinical implications, therapeutic potential, and downstream signaling cascades based on the cellular contexts of BTN3A molecules in cancers.

2. Genetic Profile and Structural Organization

Butyrophilin genes are encoded in clusters and have been known to share the same Phylogenetic tree with other B7 family members [17]. The genetic profile of BTNs shows a set of genes localized in the telomeric region of chromosome 6p22, close to the major histocompatibility complex (MHC) class I genes. To date, 7 BTNs have been described in humans: BTN1A1, BTN2A1, BTN2A2, BTN2A3, BTN3A1, BTN3A2, BTN3A3, and 6 BTNLs: BTNL2, BTNL3, BTNL8, BTNL9, BTNL10, and SKINT-like [33,34].

It has been reported that BTN1A1 and BTN2A are the only common variants found in both mice and humans. However, BTN3A molecules have generated great interest due to their specificity for humans and have been classified into three isoforms, BTN3A1, BTN3A2, and BTN3A3 [33,34]. The extracellular domains of these paralogous genes display very high degrees of similarity (>95%) [19,27]. The structural organizations of BTN3A1, BTN3A2, and BTN3A3 receptors consist mainly of two extracellular domains (IgV and IgC domains), a transmembrane domain, and the B30.2 intracellular domain. However, BTN3A2 does not share this B30.2 intracellular domain and expresses only the transmembrane and extracellular regions [35] (Figure 1).

The B30.2 domain has been shown to contain the tripartite motif (TRIM) family proteins, which could act as pattern-recognition receptors, such as toll-like receptors (TLRs) or nucleotide-binding oligomerization domain (NOD)-like receptor proteins [36–39]. It is well-known that these abovementioned structures have a high affinity for infection-associated molecules, such as pathogen-associated molecular patterns (PAMPs) or cell damage (DAMP) [40]. Therefore, evidence has highlighted the involvement of the B30.2 domain in the interaction with some endogenous (DAMP) and exogenous (PAMP) molecules. The signaling pathway ensuing from this interaction could play a key role in stimulating immune responses [28,37].
**Figure 1.** Schematic representation of the CD277/BTN3A gene, mRNA, and protein structural domains. (A) The extracellular domain of interaction with the ligand. (B) The transmembrane domain. (C) The intracellular domain. BTN3A1 and BTN3A3 share the B30.2 domain involved in the interaction with the phosphoantigens.
3. Clinical Significance of BTN3A Molecule Expression

The failures of several immunotherapeutic approaches have shifted the focus toward the identification of more relevant prognostic markers for accurate therapy and detection of very early-stage cancers. These prognostic markers could be the expressions of specific genes or transcript alterations or the level of particular proteins in body fluids. Thus, the prediction of treatment response or the evaluation of disease progression is made possible by the measurements of these markers [41].

Studies revealed that BTN3A expression can be detected in the spleen, heart, placenta, pancreas, lymph node, trachea, adrenalin gland, ovary, small intestine, appendix, thymus, and lymphocytes [17,25–27].

In some cases, BTN3A expression, especially in the plasma, could help predict the outcomes of distinct therapies. Thus, depending on the type of cancer, differential prognoses and clinical implications of BTN3A molecules are presented in Table 1.

T cells are considered the leading players in the immune response. Being able to identify the specific roles of immune checkpoint proteins on T cell activities is crucial to enhancing immune responses, preventing cancer progression, and improving patient survival [42,43]. Hence, a prominent role of BTN3A in regulating different cellular and molecular immune actors has been explored in various cancer tissues.

### Table 1. Differential prognosis and clinical implication of BTN3A molecules.

| BTN3A Isoforms | Cancer Types                  | Detection Methods                | Clinical Significance                  | Prognosis   | References |
|---------------|-------------------------------|----------------------------------|---------------------------------------|-------------|------------|
| BTN3A1        | Metastatic renal cell carcinoma (MRCC); non-small cell lung cancer (NSCLC) Pancreatic ductal adenocarcinoma (PDAC) Breast cancer/non-small cell lung cancer (NSCLC) Low-grade glioma (LGG) Melanoma | Baseline plasma levels of soluble BTN3A1 mRNA/protein expression profile Plasma levels of soluble BTN3A1 mRNA/protein expression profile Profiling Interactive Analysis datasets GEPIA datasets | Predicting PD-1 (Programmed cell death protein 1) immunotherapy response Prognostic biomarker Biomarkers that reflect the progression and prognosis of PDAC Prognostic biomarker Unfavorable Prognosis marker | Favorable [44] Favorable [45] Unfavorable [22] Favorable [46] Favorable [46] |
| BTN3A2        | Pancreatic ductal adenocarcinoma (PDAC) Epithelial ovarian cancer (EOC) Lung adenocarcinoma (LUAD) Pancreatic ductal adenocarcinoma Breast cancer Brain cancer | Transcriptional level Protein expression mRNA/protein expression profile Culture cell/flow cytometry | Prognosis marker Prognosis marker Prognosis marker Prognosis marker Prognosis marker | Unfavorable [22] Favorable [20] Favorable [46] Favorable [46] Favorable [46] |
| BTN3A3        | Non-small cell lung cancer (NSCLC) Ovarian cancer (OC) Gastric cancer | mRNA/protein expression profile Protein expression Cancer Therapeutics Response Portal (CTRP)/The Cancer Genome Atlas (TCGA) | Prognosis marker Prognosis marker | Favorable [48] Favorable [49] Favorable [50] |

4. BTN3A and Immune-System

The immune system is a large and complex organization that brings together different cells, tissues, organs, proteins, and other biological components involved in the defense of the organism [51,52]. The immune cells interact via a multitude of signaling pathways, and for certain cells, the activation occurs after different co-stimulatory signals [53]. It has been shown that the T cell receptor (TCR) is involved in the first activation signal while the second signal is delivered by certain B7 family molecules [54–56].

Bioinformatic analyses via the TCGA (The Cancer Genome Atlas) database have revealed the involvement of the BTN3A2 co-expression gene in many biological processes, including the activation of T cell receptor signaling pathways, cytokine receptors signaling...
pathways, and immune infiltration of CD8+ T cells, Th1 cells, dendritic cells (DCs) [47]. Strikingly, BTN3A2 was positively correlated with T cell transcription factors, and anti-tumoral mediators, such as TBX21, STAT1, IFNγ, and GZMB, respectively, in triple-negative breast cancer (TNBC), compared with other breast cancer subtypes, which suggests that high expression of BTN3A2 in TNBC patients could significantly increase the number of cytotoxic cells CTLs, Th1, and DC in the tumor microenvironment [47]. In lung adenocarcinoma (LUAD) and epithelial ovarian cancer (EOC), it has been suggested that BTN3A2 expression was positively correlated with CD4+ T cell, neutrophil, B cell, and macrophage infiltration to the tumor area [20,45]. On the other hand, BTN3A3 expression was positively correlated with the density of CD8+ T cells, anti-tumor immune response, and less invasive phenotype in non-small cell lung cancer (NSCLC) patients [48]. The TME is driven by a panoply of inflammatory signals [57]. Research shows that BTN3A expression is increased by pro-inflammatory cytokines, such as IFNγ and TNFα [26,47,58].

The mechanism underlying the involvement of BTN3A molecules in immune cell infiltration and CD4+/CD8+ T cell activities is not yet well understood. However, it might be related to the specificity of the ligand/receptor binding domains expressed on antigen presenting cells (APCs), and T cells, respectively, and the type of signal transmitted as a result of this binding [20]. BTN3A receptors expressed on APCs could promote negative co-stimulatory functions following the binding on the ligand expressed by T cells. As a matter of fact, BTN3A1 expressed on APCs has been shown to restrain human T cell proliferation in ovarian carcinoma microenvironments [19,20,25]. On the other hand, a positive co-stimulatory function could be induced when BTN3A1 is expressed on T cells or epithelial cells as a receptor [20,25].

4.1. BTN3A Co-Inhibitory Function

Interaction with different BTN3A receptors induces distinct effects on T cell functions, depending on both heterogeneity and the binding domain. Yamashiro et al. have generated the 232-5 mAb specific to the variable site of the extracellular region of BTN3A molecules. This variable region includes amino acids 35–139 from the N-terminal position and displays a high affinity to 232-5 mAb.

Significant inhibition of CD4+/CD8+ T cell proliferation and IL-4 and IFNγ production has been reported after treatment of Human peripheral blood mononuclear cells (PBMCs) with BTN3A3-specific inhibitory monoclonal antibody 232.5 [26], suggesting the inhibition property of 232-5 mAb. Interestingly, the same inhibitory effect has been observed on CD25+ Treg cell-depleted PBMCs, suggesting that this suppression is independent of CD25+ Treg cells and involves both 232.5 mAb and the variable region of the extracellular region of the BTN3A molecule [26]. Furthermore, significant expression of BTN3A on MHC-II+ ovarian cancer-associated DC/macrophages was found to be associated with both inhibitions of the anti-tumor T cell response and production of proinflammatory cytokines, such as IL-2, IFNγ, and TNFα [25]. However, elevated BTN3A expression was associated with an increase in IL-17 and IL-6 production [25]. Cellular FLICE-like inhibitory protein (c-FLIP) through interaction with pro-caspase 8, represents one of the key components that could explain the altered function of T cells. The Mitogen activated protein kinase (MAPK)/Extracellular-signal-regulated kinase (ERK) and Phosphatidyl-inositol 3 kinase (PI3K)/serine/threonine protein kinase (AKT) cascade represent two major signaling pathways that regulate the transcription factor Nuclear factor kappa B (NF-κB) [59–62].

It has been shown that this anti-apoptotic mediator c-FLIP promotes T cell maturation and survival via the activation cascade ERK/NF-κB [63–65]. It would seem that BTN3A suppresses c-FLIP expression and, thus, contributes to the inhibition of T cell activities [25]. The negative co-stimulatory function of BTN3A has also been highlighted in a recent study using engineered antigen-presenting cells expressing BTN3A1 (BTN-K32 aAPCs) [66]. CD4+/CD8+ T cell proliferation, activation, and IFNγ production were suppressed after 6 days of co-culture with HLA-A2+ BTN3A1-K32 cells. Furthermore, CD4+/CD8+ T cells have recovered their function after treatment with the CTX-2026 mAb that binds to the
IgV extracellular domain of BTN3A1 [66]. The involvement of the transmembrane PTPase CD45 could be behind this inhibitory effect of BTN3A1. CD45 is well recognized to play a key role in T cell activation through the TCR signaling pathway [67,68]. BTN3A1 has been shown to bind the N-mannosylated residues of CD45 and, thus, could trigger co-inhibitory TCR signaling. Interestingly, CRISPR-mediated deletion of CD45 in CD4+/CD8+ T cells has completely restored their primary function and obviates CTX-2026 mAb utilization [66].

BTN3A co-inhibitory function encompasses a multitude of structural and biological parameters, including TME complexity, binding domain specificity, location, and different signaling pathways. This suggests the key role of BTN3A molecules in modulating the immune system, which requires further research to better understand the different facets of these receptors.

4.2. BTN3A Co-Stimulatory Function

The involvement of BTN3A molecules in positive co-stimulation of TCR signaling has been investigated by using a newly generated mAb clone, 20.1 [27]. Messal et al. have demonstrated the potential of BTN3A (CD277) molecules to induce a significant increase in IL-2 and IFNγ production by CD4+ T cells after different cell culture conditions. CD4+ T cells were stimulated with CD3 plus CD28 mAbs or CD3 plus CD277 mAbs or CD3 mAb plus IgG1 (control conditions) and, surprisingly, IFNγ production was significant in CD3 plus CD277 stimulation compared to CD3 plus CD28 co-activation [19]. Thus, it would appear that BTN3A receptor stimulation by 20.1 mAb results in T cell proliferation and cytokine production in a dose-dependent manner [19]. PI3K/AKT and MAPK/ERK signaling pathways appear to be involved in the co-stimulatory property of BTN3A1. These pathways regulate cell growth, proliferation, survival, and invasion after phosphorylation cascades [69,70]. Stimulation by CD3 plus CD277 mAbs may have resulted in the phosphorylation of AKT and ERK and subsequent positive stimulation of CD4+ T cells [19]. BTN3A1 and BTN3A2 were stimulated on the KGHYG-1 NK cell line ‘nucleofected’ with constructs encoding for flag epitope which tagged BTN3A1 and BTN3A2. The construction of the flag epitopes was performed by deleting the signal peptide sequences from WT full-length human cDNA of BTN3A1 and BTN3A2. The results suggest that BTN3A1 stimulation increases IFNγ production, whereas, BTN3A2 stimulation decreases the NKP30-induced IFNγ production [19]. These results could be explained by the NK cell that expresses mainly BTN3A2, which lack the B30.2 intracellular domain. BTN3A2 may be considered a putative receptor devoid of co-signaling function in NK cells compared to well-known co-stimulatory (DNAM-1) and co-inhibitory (NKG2A) molecules [19]. In addition, it has been demonstrated that transfection of NSCLC cell line with siRNA to knock-down BTN3A3 as well as patients with low BTN3A3 expression displayed invasion, migration, and proliferation of NSCLC cells [48]. This study underlines the crucial role of BTN3A3 since patients with high BTN3A3 expression have shown increased CD4+/CD8+ T cell infiltration and better clinical outcome [48]. Recently, another monoclonal antibody, ICT01 has been developed with a similar affinity for the three BTN3A isoforms. De Gassart et al. have reported that BTN3A+ Vy9Vδ2 T cell activation by ICT01 induced apoptosis of multiple tumor cell lines and primary tumor cells without affecting normal cell viability. It has been reported in melanoma patients that ICT01 may promote immune cell infiltration within the tumor microenvironment. Moreover, preliminary results of phase 1/2a performed on patients with various types of advanced stages of solid tumors showed that ICT01 was well endured and pharmacodynamically active. In addition, a co-culture of PBMCs with PC3 or HT29 cell lines promoted Vy9Vδ2 T cell expansion and cancer cell mortality [71].
Therefore, the CD277 co-stimulatory pathway may differentially contribute to the regulation of various immune cell response (see Table 2). Thus, the co-stimulatory function of BTN3A could turn out to be a determinant in the therapeutic strategies. It is well established that the percentage of CD8+ T cell infiltration is crucial for the prediction and success of certain therapies including immunotherapy [72–76]. Thus, BTN3A receptors through their involvement in CD4+/CD8+ T cell activation and high pro-inflammatory cytokine production, are considered the main actors in immune cell infiltration and cancer treatment.

Table 2. Therapeutics potential of BTN3A molecules in cancers.

| Cell Subsets Expressing BTN3A | Detection Methods | Impact on Effector Activities | Therapeutics Potential | References |
|-------------------------------|------------------|------------------------------|------------------------|------------|
| CD8 T cell                    | PBMCs/culture cell/flow cytometry | Attenuation of CD8 T cell proliferation and IFN production | Blocking of BTN3 signal transduction or destruction of BTN3 mRNA with small interfering RNA may be applicable for patients with tumors | [26] |
| CD4 T cell                    | PBMCs and lymph nodes/culture cell/flow cytometry | Decreased IFN-γ, IL-2, IL-17, TNF-α production by NK cell upon specific engagement of BTN3A2 | Positive immunomodulators of T cell responses, which may ensure good responses to immunotherapies | [19] |

Based on previous studies, the accurate functions of BTN3A molecules in immunomodulation are quite disparate. A thorough understanding of the different BTN3A signaling pathways through their putative binding partners is necessary to shed light on BTN3A functionality in the immune system.

5. BTN3A and Putative Ligand

To date, several studies have attempted to identify the exact counter-receptor of BTN3A molecules. BTN3 ligand has been found in leukemia and solid tumor cell lines, such as HeLa and MCF-7, Raji, C91, HUT78, and JA16 [27,77]. Using the CD277-Fc fusion protein on C91 cells, a clone population termed C91T3.3 was generated. The binding stability of the CD277-Fc fusion protein with the potential ligand was confirmed by flow cytometry. In addition, the co-immunoprecipitation assay after cross-linking confirmed the presence of the putative ligand of BTN3A molecules. However, the characterization of the exact ligand for BTN3 could not be achieved due to the possible loss of the CD277 counter-receptor heterodimerization during extraction of the corresponding bands from the PAGE-SDS gel for mass spectrometry detection [77]. Detection strategies could not be appropriate and should be optimized for the correct characterization of this counter-receptor. Research is still ongoing concerning the accurate detection of BTN3A ligands [77]. In the same vein, Compte et al. excluded PD-L1, CTLA-4, CD28, ICOS, and BTLA as candidate binding partners of BTN3A on T cells and demonstrated that the IgG-V domain of BTN3A is mainly involved in the interaction with the counter-receptor [27].

Interestingly, more recent studies have shed light on the potential new receptors for BTN3A1 and BTN3A3. It has been shown that the LSECtin protein acts as a co-inhibitory ligand through the interaction with BTN3A1. Indeed, through the immunological ELISA test, interactions between LSECtin, and BTN3A1 have been highlighted. This led to an impairment of T cell activation and proliferation, as well as a decrease in the production of pro-inflammatory cytokines, such as IFN-γ, IL-2, IL-17, and TNF-α. Moreover, anti-BTN3A1 antibody administration has partially restored T cell activity. Therefore, the LSECtin/BTN3A1 axis appears to be a promising therapeutic target [78]. BTN3A3 has been also found to interact with LSECtin on tumor-associated macrophages and contribute to the promotion and survival of breast cancer cells [79].
This interaction was confirmed using HEK293 cells transfected with a commercial human cDNA library and devoid of known LSECtin receptors. cDNA library-expressing HEK293 cells were generated after transfection and BTN3A3 was recognized as the LSECtin-binding receptor after screening of LSECtin-binding cells plasmids. It would seem that the extracellular region (IgC/IgV) of BTN3A3 is imperative for LSECtin interaction while the intracellular domain is involved in the initiation of the The Janus kinase (JAK2)/Signal Transducer And Activator Of Transcription3 (STAT3) signaling pathway and phosphorylation cascades [79]. Research shows that JAK/STAT3 signaling is strongly involved in cancer stem cell (CSC) promotion, epithelial–mesenchymal transition (EMT), and breast cancer cell proliferation [80,81]. Therefore, the interaction between BTN3A3 and LSECtin contributes to breast cancer progression by activating the JAK-STAT pathway [79].

Given the structural organization of BTN3A, the intracellular domain represents a potential binding site, particularly for BTN3A1 and BTN3A3. Indeed, this domain distinguishes the three isoforms and is involved in the integrity of cell cytoskeleton via the interaction with the plakin family proteins such as periplakin. However, the di-leucine motif is only found in the cytoplasmic tail of BTN3A1, which is crucial for interaction with the plakin family members. Therefore, BTN3A2 or BTN3A3 do not share this functionality ensuing from this binding [23,82]. It has been shown that phosphoantigens, such as IPP (isopentenyl-pyrophosphate), DMAPP (dimethylallyl-pyrophosphate), or HMB-PP((E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate), as well as biochemical intermediates of isoprenoid biosynthesis, promote the co-stimulatory activity of BTN3A through the B30.2 intracellular domain [28,37].

6. BTN3A Signaling Pathways

T cell activation by butyrophilins 3 may occur through the antigen-presenting model, the release of phospho-antigen to the extracellular region, and the formation of the BTN3A1-phospho-antigen complex that binds to \(\gamma\delta\) TCR activation [32]. This could also involve an inside-out signaling model, through which the phospho-antigen attaches to the N-terminal part of the intracellular B30.2 domain of BTN3A1 with high affinity to a positively charged pocket to elicit a (\(\beta\)-\(\alpha\)) conformational transition of H351 residue, then the BTN3A1-phospho-antigen complex displaces to juxtamembrane region. This complex immobilization increases the attraction between the extracellular domain of BTN3A1 and the \(\gamma\delta\) TCR which induces \(\gamma\delta\) T cell activation [36,83,84].

It has been recently shown that 350 and 391 are the two crucial residues responsible for H351 transition in the B30.2 domain, and the mutation of w391 decreases phospho-Antigen-B30.2 domain binding. Further, the residues of positively charged pocket are highly conserved in the BTN3A1 B30.2 domain compared to other proteins [83,84]. The requirement of BTN3A2 and BTN3A3 in T cell activation was also explored. Results indicated that these two isotypes optimize the effect of BTN3A1 by controlling the suitable routing, dynamic, and stability of BTN3A1 [85].

Recognizing cell expression of the phosphor-antigen-BTN3A1-B30.2 domain complex by T cells induces an immunological synapse [86] establishment, which leads to signal transduction and activation of different signaling pathways. Anti-CD277mAb provokes the phosphorylation of phosphoinositide-specific phospholipase (PLCY2) in TCR V\(\gamma\)9\(\gamma\delta\) cells, and the activation of AKT by the phosphorylation of its residues by MTORC2 and PDK1. Thereby, modulation of metabolism and cell survival upon NF-κB activation. On the other hand, TCR activation elicits equally the MAPK pathway by increasing intracellular T cell phosphorylation of ERK1 and P38 after anti-CD277mAb treatment [87] (Figure 2).
Figure 2. BTN3A interaction patterns and signaling pathways. BTN3A-dependent co-stimulatory mechanisms: (1) engagement of BTN3A with its putative ligand on APC and/or (2) treatment of BTN3A with CD277 mAb 20.1 provides a co-stimulatory T cell activation signal that induces production of IL-2 and IFNγ as well as T cell proliferation via the PI3K/AKT signaling pathway. BTN3A-dependent co-inhibitory mechanisms; (3) interaction of BTN3A with a specific mAb 232.5 suppresses c-FLIP expression. The cellular inhibitory protein type FLICE-like (c-FLIP) is well-known to promote the maturation and survival of T cells through interaction with pro-caspase, which activates the activation cascade ERK/NF-κB. The inhibition of c-FLIP expression by BTN3A triggers an inhibiting signal that interferes with the production of IFNγ, IL-4, and the proliferation of T cells. (4) BTN3A1 binds the N-mannosylated residues of CD45 and, thus, elicits the inhibition of the TCR signal on T cells. CD45 is well recognized to play a key role in T cell activation through the TCR signaling pathway. The BTN3A1 and BTN3A2 provide differential regulation of Nkp30-induced IFNγ production; (5) The co-engagement of BTN3A1 with Nkp30 after treatment with anti-Nkp30 mAb modulates the Nkp30-induced IFN-γ production, (6) whereas the lack of B30.2 domain in the BTN3A2 structure impairs the co-engagement of BTN3A2 with Nkp30 and thus lead to the attenuation of the Nkp30-induced IFN-γ production. LSECtin on tumor-associated macrophages improves the stemness of tumor cells through interaction with BTN3A receptor; (7) BTN3A1/2 Interaction with LSECtin in tumor cells activates the JAK2/STAT3 signaling pathway, which promotes cancer cell survival, invasion, and proliferation.

7. Perspectives and Conclusions

In light of the preceding, we can state that BTN3A molecules play a significant role in modulating CD4+, CD8+, monocytes, and NK cell activity. Their roles as biomarkers in different types of cancer make them critical players in facilitating the prognosis of patients.

However, many questions remain unanswered about BTN3A receptors, particularly their specific ligands that would induce a co-stimulatory/inhibitory effect on T lymphocyte activities.

Do T cell immunomodulatory effects (upon the interaction of BTN3A with potential ligands or different antibodies) occur under specific physiological conditions? Which IgV
extracellular-binding domains are involved in this interaction? What is the distinguishing feature of each antibody?

BTN3A receptors are classified into two major domains: the extracellular domain and the B30.2 domain (primarily for BTN3A1/3). So, which part of the BTN3A receptor (blocking or enhancing a co-stimulatory function) would be more advantageous to target?

What role does the B30.2 domain play in the signaling pathways and cascades triggered by the putative ligand’s interaction with the extracellular region of BTN3A receptors on T cells? How do agonistic or antagonistic BTN3A-targeting molecules affect adaptive immunity in vivo, and is combination immunotherapy with checkpoint blockade therapies, such as anti-PD-1 blocking antibodies and BTN3A agonists or antagonists, beneficial?

In summary, several pieces of evidence pinpoint the crucial role of BTN3A molecules on T cell functions within the tumor microenvironment in solid tumors. Current research on the signaling pathways by which BTN3A expression affects CD4+, CD8+, monocytes, and NK cell functions, is limited. Further studies should be pursued to shed light on the exact activating and/or inhibiting roles of BTN3A molecules. Answering these critical questions would elucidate how BTN3A proteins could be used in immunotherapy to improve the anti-tumor immune response.

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**Abbreviations**

| Acronym | Full Form |
|---------|-----------|
| APCs    | antigen-presenting cells. |
| BTLA    | B and T lymphocyte attenuator. |
| BTN3A   | butyrophilin subfamily 3 member A. |
| BTNs    | butyrophilins. |
| c-FLIP  | cellular FLICE-like inhibitory protein. |
| CSC     | cancer stem cell. |
| CTLA-4  | cytotoxic T-lymphocyte associated protein 4. |
| DAMP    | damage-associated molecular patterns. |
| DMAPP   | dimethylallyl-pyrophosphate. |
| ELISA   | the enzyme-linked immunosorbent assay. |
| GEPIA dataset | gene expression profiling interactive analysis. |
| GZMB    | granzyme B. |
| HMB-PP  | (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate |
| ICB     | immune-checkpoint blockade ICB. |
| ICOS    | inducible T cell co-stimulator. |
| IFNγ    | interferon gamma. |
| JAK/STAT3 | the Janus kinase (JAK)-signal transducer and activator of transcription. |
| mAb     | monoclonal antibodies. |
| MAPK/ERK| mitogen activated protein kinase/extracellular-signal-regulated kinase. |
| MHC     | major histocompatibility complex. |
| NF-κB   | nuclear factor kappa B. |
| NK cells | natural killer cells. |
| DLB     | Dementia with Lewy bodies |
PAMPs: pathogen-associated molecular patterns.

PBMCs: human peripheral blood mononuclear cells.

PD-1: programmed cell death protein 1.

PI3K/AKT: phosphatidylinositol 3 kinase/signal transducer and activator of transcription.

STAT1: signal transducer and activator of transcription.

TBX21: T-box transcription factor 21.

TCGA database: The Cancer Genome Atlas.

TCR: T-cell receptor.

Th1 cells: T helper 1.

TLRs: toll-like receptors.

TME: tumor microenvironment.

TNBC: triple-negative breast cancer.

TNFa: tumor necrosis factor alpha.

TRIM: tripartite motif family protein.

References

1. Liu, M.; Guo, F. Recent updates on cancer immunotherapy. *Precis. Clin. Med.* 2018, 1, 65–74. [CrossRef] [PubMed]

2. Wherry, E.J. T cell exhaustion. *Nat. Immunol.* 2011, 12, 492–499. [CrossRef] [PubMed]

3. Jiang, W.; He, Y.; He, W.; Wu, G.; Zhou, X.; Sheng, Q.; Zhong, W.; Lu, Y.; Ding, Y.; Lu, Q.; et al. Exhausted CD8+ T Cells in the Tumor Immune Microenvironment: New Pathways to Therapy. *Front. Immunol.* 2020, 11, 622509. [CrossRef] [PubMed]

4. Borghaei, H.; Paz-Ares, L.; Horn, L.; Spigel, D.R.; Steins, M.; Ready, N.E.; Chow, L.Q.; Vokes, E.E.; Felip, E.; Holgado, E.; et al. Nivolumab versus Docetaxel in Advanced Non-Squamous Non-Small-Cell Lung Cancer. *N. Engl. J. Med.* 2015, 373, 1627–1639. [CrossRef]

5. Seidel, J.A.; Otsuka, A.; Kabashima, K. Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, Efficacy, and Limitations. *Front. Oncol.* 2018, 8, 86. [CrossRef]

6. Jiang, W.; He, Y.; He, W.; Wu, G.; Zhou, X.; Sheng, Q.; Zhong, W.; Lu, Y.; Ding, Y.; Lu, Q.; et al. Exhausted CD8+ T Cells in the Tumor Immune Microenvironment: New Pathways to Therapy. *Front. Immunol.* 2020, 11, 622509. [CrossRef] [PubMed]

7. Park, J.A.; Cheung, N.-K.V. Limitations and opportunities for immune checkpoint inhibitors in pediatric malignancies. *Cancer Treat. Rev.* 2017, 58, 22–33. [CrossRef]

8. Haslam, A.; Prasad, V. Estimation of the Percentage of US Patients With Cancer Who Are Eligible for and Respond to Checkpoint Inhibitor Immunotherapy Drugs. *JAMA Netw. Open* 2019, 2, e192535. [CrossRef]

9. Sharma, P.; Hu-Lieskovan, S.; Wargo, J.A.; Ribas, A. Primary, Adaptive and Acquired Resistance to Cancer Immunotherapy. *Cell 2017, 168, 707–723. [CrossRef]

10. Schadendorf, D.; Hodi, F.S.; Robert, C.; Weber, J.S.; Margolin, K.; Hamid, O.; Chen, T.-T.; Berman, D.M.; Wolchok, J.D. Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. *J. Clin. Oncol.* 2015, 33, 1889–1894. [CrossRef]

11. Lu, M.; Su, Y. Immunotherapy in non-small cell lung cancer: The past, the present, and the future. *Thorac. Cancer 2019, 10, 585–586. [CrossRef] [PubMed]

12. Massarelli, E.; Papadimitrakopoulou, V.; Welsh, J.; Tang, C.; Tsao, A.S. Immunotherapy in lung cancer. *Transl. Lung Cancer Res.* 2014, 3, 53–63. [CrossRef] [PubMed]

13. Ventola, C.L. Cancer Immunotherapy, Part 3: Challenges and Future Trends. *Pharm. Ther.* 2017, 42, 514–521. [CrossRef]

14. Kong, X. Discovery of New Immune Checkpoints: Family Grows Up. *Front. Immunol.* 2017, 8, 65–74. [CrossRef]

15. Park, J.A.; Cheung, N.-K.V. Limitations and opportunities for immune checkpoint inhibitors in pediatric malignancies. *Cancer Treat. Rev.* 2017, 58, 22–33. [CrossRef]

16. Qin, S.; Xu, L.; Yi, M.; Yu, S.; Wu, K.; Luo, S. Novel immune checkpoint targets: Moving beyond PD-1 and CTLA-4. *Mol. Cancer Ther.* 2017, 16, 1203–1211. [CrossRef]

17. Qi, M.; Su, Y. Immunotherapy in non-small cell lung cancer: The past, the present, and the future. *Thorac. Cancer 2019, 10, 585–586. [CrossRef] [PubMed]

18. Massarelli, E.; Papadimitrakopoulou, V.; Welsh, J.; Tang, C.; Tsao, A.S. Immunotherapy in lung cancer. *Transl. Lung Cancer Res.* 2014, 3, 53–63. [CrossRef] [PubMed]

19. Ventola, C.L. Cancer Immunotherapy, Part 3: Challenges and Future Trends. *Pharm. Ther.* 2017, 42, 514–521. [CrossRef]

20. Kong, X. Discovery of New Immune Checkpoints: Family Grows Up. *Front. Immunol.* 2017, 8, 65–74. [CrossRef]

21. Qi, M.; Su, Y. Immunotherapy in non-small cell lung cancer: The past, the present, and the future. *Thorac. Cancer 2019, 10, 585–586. [CrossRef] [PubMed]
22. Benyamine, A.; Loncle, C.; Foucheur, E.; Blazquez, J.-L.; Castanier, C.; Chrétien, A.-S.; Modesti, M.; Secq, V.; Chouaib, S.; Gironella, M.; et al. BTN3A3 is a prognosis marker and a promising target for Vγ9Vδ2 T cells-based-immunotherapy in pancreatic ductal adenocarcinoma (PDAC). *Oncoimmunology* 2017, 7, e1372080. [CrossRef] [PubMed]

23. Rhodes, D.A.; Chen, H.-C.; Price, A.J.; Keeble, A.H.; Davey, M.S.; James, L.C.; Eberl, M.; Trowsdale, J. Activation of Human γδ T Cells by Cytosolic Interactions of BTN3A1 with Soluble Phosphoantigens and the Cytoskeletal Adaptor Periplakin. *J. Immunol. Author Choice* 2015, 194, 2390–2398. [CrossRef] [PubMed]

24. Zocchi, M.R.; Costa, D.; Venè, R.; Tosetti, F.; Ferrari, N.; Minghelli, S.; Benelli, R.; Scabini, S.; Romaine, E.; Catellani, S.; et al. Zoledronate can induce colorectal cancer microenvironment expressing BTN3A1 to stimulate effector γδ T cells with antitumor activity. *Oncoimmunology* 2017, 6, e1278099. [CrossRef] [PubMed]

25. Cubillos-Ruiz, J.R.; Martínez, D.; Scarlett, U.K.; Rutkowski, M.R.; Nesbeth, Y.C.; Camposeco-Jacobs, A.L.; Conejo-Garcia, J.R. CD277 is a Negative Co-stimulatory Molecule Universally Expressed by Ovarian Cancer Microenvironmental Cells. *Oncotarget* 2010, 1, 329. Available online: https://www.semanticscholar.org/paper/CD277-is-a-Negative-Co-stimulatory-Molecule-by-Cubillos-Ruiz-Martinez/6f18b017abc1533c7de969d5d67591ad566b543c (accessed on 4 September 2022). [CrossRef]

26. Yamashiro, H.; Costa, D.; Venè, R.; Tosetti, F.; Ferrari, N.; Minghelli, S.; Benelli, R.; Scabini, S.; Romaine, E.; Catellani, S.; et al. Zoledronate can induce colorectal cancer microenvironment expressing BTN3A1 to stimulate effector γδ T cells with antitumor activity. *Oncoimmunology* 2017, 6, e1278099. [CrossRef] [PubMed]

27. Compte, E.; Pontarotti, P.; Collette, Y.; Lopez, M.; Olive, D. Frontline: Characterization of BT3 molecules belonging to the B7 family expressed on immune cells. *Eur. J. Immunol.* 2020, 50, 632–642. [CrossRef] [PubMed]

28. Harly, C.; Guillaume, Y.; Nedellec, S.; Peigné, C.M.; Mönkkönen, H.; Mönkkönen, J.; Li, J.; Kuball, J.; Adams, E.J.; Netzer, S.; et al. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human γδ T-cell subset. *Blood J. Am. Soc. Hematol.* 2012, 120, 2269–2279. Available online: https://ashpublications.org/blood/article/120/11/2269/30207/Key-implication-of-CD277-butyrophilin-3-BTN3A-in (accessed on 4 September 2022). [CrossRef]

29. Rhodes, D.A.; Stammers, M.; Malcherek, G.; Beck, S.; Trowsdale, J. The Cluster of BTN Genes in the Extended Major Histocompatibility Complex. *Genomics* 2001, 71, 351–362. [CrossRef] [PubMed]

30. Laplagne, C.; Ligat, L.; Foote, J.; Lopez, F.; Fournié, J.-J.; Laurent, C.; Valitutti, S.; Poupot, M. Self-activation of Vγ9Vδ2 T cells by exogenous phosphoantigens involves TCR and butyrophilins. *Cell. Mol. Immunol.* 2021, 18, 1861–1870. [CrossRef]

31. Herrmann, T.; Fichtner, A.S.; Karunakaran, M.M. An Update on the Molecular Basis of Phosphoantigen Recognition by Vγ9Vδ2 T Cells. *Cells* 2020, 9, 1433. [CrossRef] [PubMed]

32. Vavassori, S.; Kumar, A.; Wan, G.S.; Ramanjaneyulu, G.S.; Cavallari, M.; El Daker, S.; Beddoe, T.; Theodossis, A.; Williams, N.K.; Gostick, E.; et al. Butyrophilin 3A1 binds phosphorylated antigens and stimulates human γδ T cells. *Nat. Immunol.* 2013, 14, 908–916. [CrossRef] [PubMed]

33. Abele-Dörner, L.; Swamy, M.; Williams, G.; Hayday, A.C.; Bas, A. Butyrophilins: An emerging family of immune regulators. *Trends Immunol.* 2012, 33, 34–41. [CrossRef] [PubMed]

34. Afrache, H.; Gouret, P.; Ainouche, S.; Pontarotti, P.; Olive, D. The butyrophilin (BTN) gene family: From milk fat to the regulation of the immune response. *Immunogenetics* 2012, 64, 781–794. [CrossRef] [PubMed]

35. Boutin, L.; Scotet, E. Towards Deciphering the Hidden Mechanisms That Contribute to the Antigenic Activation Process of Human Vγ9Vδ2 T Cells. *Front. Immunol.* 2018, 9, 828. [CrossRef]

36. Gu, S.; Nawrocka, W.; Adams, E.J. Sensing of Pyrophosphate Metabolites by Vγ9Vδ2 T Cells. *Front. Immunol.* 2015, 5, 688. [CrossRef]

37. Wang, H.; Henry, O.; Distefano, M.D.; Wang, Y.-C.; Rääkkönen, J.; Mönkkönen, J.; Tanaka, Y.; Morita, C.T. Butyrophilin 3A1 Plays an Essential Role in Preyl Pyrophosphate Stimulation of Human Vγ2Vδ2 T Cells. *J. Immunol.* 2013, 191, 1029–1042. [CrossRef]

38. Künkele, K.-P.; Wesch, D.; Oberg, H.-H.; Aichinger, M.; Supper, V.; Baumann, C. Vγ9Vδ2 T Cells: Can We Re-Purpose a Potent Anti-Infection Mechanism for Cancer Therapies? *Cells* 2020, 9, 829. [CrossRef] [PubMed]

39. Rhodes, D.A.; Reith, W.; Trowsdale, J. Regulation of Immunity by Butyrophilins. *Annu. Rev. Immunol.* 2016, 34, 151–172. [CrossRef]

40. Caruso, R.; Warner, N.; Inohara, N.; Nuñez, G. NOD1 and NOD2: Signaling, host defense, and inflammatory disease. *Immunity* 2014, 41, 898–908. [CrossRef]

41. Riley, R.D.; Sauerbrei, W.; Altman, D.G. Prognostic markers in cancer: The evolution of evidence from single studies to meta-analysis, and beyond. *Br. J. Cancer* 2009, 100, 1219–1229. [CrossRef] [PubMed]

42. Postow, M.A.; Callahan, M.K.; Wolchok, J.D. Immune Checkpoint Blockade in Cancer Therapy. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* 2015, 33, 1974–1982. [CrossRef] [PubMed]

43. Shibru, B.; Fey, K.; Fricke, S.; Blaudszun, A.-R.; Fürst, F.; Weise, M.; Seiffert, S.; Weyh, M.K.; Köhl, U.; Sack, U.; et al. Detection of Immune Checkpoint Receptors—A Current Challenge in Clinical Flow Cytometry. *Front. Immunol.* 2021, 12, 694055. [CrossRef] [PubMed]

44. Incorvaia, L.; Fanale, D.; Badalamenti, G.; Porta, C.; Olive, D.; Luca, I.; Brando, C.; Rizzo, M.; Messina, C.; Rediti, M.; et al. Baseline plasma levels of soluble PD-1, PD-L1, and BTN3A1 predict response to nivolumab treatment in patients with metastatic renal cell carcinoma: A step toward a biomarker for therapeutic decisions. *Oncoimmunology* 2020, 9, 183248. [CrossRef] [PubMed]

45. Zhou, H.; Lin, Y. BTN3A2 Expression Is Connected with Favorable Prognosis and High Infiltrating Immune in Lung Adenocarcinoma. *Front. Genet.* 2022, 13, 848476.
46. Liang, F.; Zhang, C.; Guo, H.; Gao, S.; Yang, F.; Zhou, G.; Wang, G. Comprehensive analysis of BTN3A1 in cancers: Mining of omics data and validation in patient samples and cellular models. FEBS Open Bio 2021, 11, 2586–2599. [CrossRef]

47. Cai, P.; Lu, Z.; Wu, J.; Qin, X.; Wang, Z.; Zhang, Z.; Zheng, L.; Zhao, J. BTN3A2 serves as a prognostic marker and favors immune infiltration in triple-negative breast cancer. J. Cell. Biochem. 2019, 121, 2643–2654. Available online: https://onlinelibrary.wiley.com/doi/abs/10.1002/jcb.29485 (accessed on 30 August 2022). [CrossRef]

48. Cheng, X.; Ma, T.; Yi, L.; Su, C.; Wang, X.; Wen, T.; Wang, B.; Wang, Y.; Zhang, H.; Liu, Z. Low expression of BTN3A3 indicates poor prognosis and promotes cell migration and invasion in non-small cell lung cancer. Ann. Transl. Med. 2021, 9, 478. [CrossRef]

49. Chen, S.; Li, Z.; Wang, Y.; Fan, S. BTN3A3 inhibits the proliferation, migration and invasion of ovarian cancer cells by regulating ERK1/2 phosphorylation. Front. Oncol. 2022, 12, 952425. [CrossRef]

50. Pan, J.; Dai, Q.; Xiang, Z.; Liu, B.; Li, C. Three Biomarkers Predict Gastric Cancer Patients’ Susceptibility To Fluourouracil-based Chemotherapy. J. Cancer 2019, 10, 2953. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6590025/ (accessed on 30 August 2022). [CrossRef]

51. Nicholson, L.B. The immune system. Essays Biochem. 2016, 60, 275–301. [CrossRef] [PubMed]

52. Chaplin, D.D. Overview of the Immune Response. J. Allergy Clin. Immunol. 2010, 125, S3–S23. [CrossRef] [PubMed]

53. Chen, L.; Flies, D.B. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat. Rev. Immunol. 2013, 13, 227–242. [CrossRef] [PubMed]

54. Tai, Y.; Wang, Q.; Korner, H.; Zhang, L.; Wei, W. Molecular Mechanisms of T Cells Activation by Dendritic Cells in Autoimmune Diseases. Front. Pharmacol. 2018, 9, 642. [CrossRef]

55. Mak, T.W.; Saunders, M.E. 14—T Cell Activation. In The Immune Response; Burlington, ON, Canada, 2006; pp. 373–401. ISBN 978-0-12-088451-3.

56. Adaptive Immunity—ClinicalKey. Available online: https://www.clinicalkey.com/#!/content/book/3-s2.0-B9780323655873000167 (accessed on 17 April 2022).

57. Greten, F.R.; Grivennikov, S.I. Inflammation and Cancer: Triggers, Mechanisms, and Consequences. Immunity 2019, 51, 27–41. [CrossRef] [PubMed]

58. Palakodeti, A.; Sandstrom, A.; Sundaresan, L.; Harly, C.; Nedellec, S.; Olive, D.; Scotet, E.; Bonneville, M.; Adams, E.J. The molecular basis for modulation of human Vγ9Vδ2 T cell responses by CD277/butyrophilin-3 (BTN3A)-specific antibodies. J. Biol. Chem. 2012, 287, 32780–32790. [CrossRef] [PubMed]

59. Micheau, O.; Lens, S.; Gaide, O.; Alevizopoulos, K.; Tschopp, J. NF-kappaB signals induce the expression of c-FLIP. J. Exp. Med. 2001, 21, 5299–5305. [CrossRef]

60. Kreuz, S.; Siegmund, D.; Scheurich, P.; Wajant, H. NF-kappaB inducers upregulate cFLIP, a cycloheximide-sensitive inhibitor of death receptor signaling. Mol. Cell. Biol. 2001, 21, 3964–3973. [CrossRef]

61. Kyläniemi, M.K.; Kaukonen, R.; Myllyviita, J.; Rasool, O.; Lahesmaa, R. The Regulation and Role of c-FLIP in Human Th Cell Differentiation. PLoS ONE 2014, 9, e102022. [CrossRef]

62. Yeh, J.H.; Hsu, S.C.; Han, S.H.; Lai, M.Z. Mitogen-activated protein kinase kinase antagonized fas-associated death domain protein-mediated apoptosis by induced FLICE-inhibitory protein expression. J. Exp. Med. 1998, 188, 1795–1802. [CrossRef]

63. Tai, T.-S.; Fang, L.-W.; Lai, M.-Z. c-FLICE inhibitory protein expression inhibits T-cell activation. Cell Death Differ. 2004, 11, 69–79. [CrossRef] [PubMed]

64. Tseveleki, V.; Bauer, J.; Taoufik, E.; Ruan, C.; Leondiadis, L.; Haralambous, S.; Lassmann, H.; Probert, L. Cellular FLIP (Long Isoform) Overexpression in T Cells Drives Th2 Effector Responses and Promotes Immunoregulation in Experimental Autoimmune Encephalomyelitis. J. Immunol. 2004, 173, 6619–6626. [CrossRef] [PubMed]

65. Zhang, N.; Hopkins, K.; He, Y.-W. c-FLIP Protects Mature T Lymphocytes from TCR-mediated Killing. J. Immunol. 2008, 181, 5368–5373. [CrossRef]

66. Payne, K.K.; Mine, J.A.; Biswas, S.; Chaurio, R.A.; Perales-Puchalt, A.; Anadon, C.M.; Costich, T.L.; Harro, C.M.; Walrath, J.; Ming, Q.; et al. BTN3A1 governs anti-tumor responses by coordinating alpha-beta and gamma-delta T cells. Science 2020, 369, 942–949. [CrossRef] [PubMed]

67. Irie-Sasaki, J.; Sasaki, T.; Matsumoto, W.; Opavsky, A.; Cheng, M.; Welstead, G.; Griffiths, E.; Krawczuk, C.; Richardson, C.D.; Aitken, K.; et al. CD45 is a JAK phosphatase and negatively regulates cytokine receptor signalling. Nature 2001, 409, 349–354. [CrossRef]

68. Shah, K.; Al-Haidari, A.; Sun, J.; Kazi, J.U. T cell receptor (TCR) signaling in health and disease. Signal Transduct. Target. Ther. 2021, 6, 412. [CrossRef]

69. Cao, Z.; Liao, Q.; Su, M.; Huang, K.; Jin, J.; Cao, D. AKT and ERK dual inhibitors: The way forward? Cancer Lett. 2019, 459, 30–40. [CrossRef]

70. Adlung, L.; Kar, S.; Wagner, M.; She, B.; Chakraborty, S.; Bao, J.; Lattermann, S.; Boerries, M.; Busch, H.; Wuchter, P.; et al. Protein abundance of AKT and ERK pathway components governs cell type-specific regulation of proliferation. Mol. Syst. Biol. 2017, 13, 904. [CrossRef]

71. De Gassart, A.; Le, K.-S.; Brune, P.; Agaugué, S.; Sims, J.; Goubard, A.; Castellano, R.; Joalland, N.; Scotet, E.; Collette, Y.; et al. Development of ICT01, a first-in-class, anti-BTN3A antibody for activating Vγ9Vδ2 T cell-mediated antitumor immune response. Sci. Transl. Med. 2021, 13, eabj0835. [CrossRef]
72. Ait Ssi, S.; Chraa, D.; El Azhary, K.; Sahraoui, S.; Olive, D.; Badou, A. Prognostic Gene Expression Signature in Patients With Distinct Glioma Grades. *Front. Immunol.* 2021, 12, 685213. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8448281/ (accessed on 16 September 2022). [CrossRef]

73. Li, F.; Li, C.; Cai, X.; Xie, Z.; Zhou, L.; Cheng, B.; Zhong, R.; Xiong, S.; Li, J.; Chen, Z.; et al. The association between CD8+ tumor-infiltrating lymphocytes and the clinical outcome of cancer immunotherapy: A systematic review and meta-analysis. *eClinicalMedicine* 2021, 41, 101134. [CrossRef] [PubMed]

74. So, Y.K.; Byeon, S.-J.; Ku, B.M.; Ko, Y.H.; Ahn, M.-J.; Son, Y.-I.; Chung, M.K. An increase of CD8+ T cell infiltration following recurrence is a good prognosticator in HNSCC. *Sci. Rep.* 2020, 10, 20059. [CrossRef]

75. Raskov, H.; Orhan, A.; Christensen, J.P.; Gögenur, I. Cytotoxic CD8+ T cells in cancer and cancer immunotherapy. *Br. J. Cancer* 2021, 124, 359–367. [CrossRef]

76. Tian, S.; Wang, F.; Zhang, R.; Chen, G. Global Pattern of CD8+ T-Cell Infiltration and Exhaustion in Colorectal Cancer Predicts Cancer Immunotherapy Response. *Front. Pharmacol.* 2021, 12, 715721. [CrossRef] [PubMed]

77. Zambrano-Zaragoza, J.; Messal, N.; Pastor, S.; Scotet, E.; Bonneville, M.; Bagnasco, M.; Harly, C.; Guillaume, Y.; Nuñes, J.; et al. CD277 an Immune Regulator of T Cell Function and Tumor Cell Recognition. In *Advances in Cancer Therapy*; IntechOpen Limited: London, UK, 2011; ISBN 978-953-307-703-1.

78. Wang, J.; Manick, B.; Renelt, M.; Gerassenkov, T.; Bi, M.; Kalabokis, V.; Person, A.; Wu, G. LSECtin interacts with BTN3A1 to inhibit T cell activation. *J. Immunol.* 2020, 204, 78-6. Available online: https://www.jimmunol.org/content/204/1_Supplement/78.6 (accessed on 17 April 2022).

79. Liu, D.; Lu, Q.; Wang, X.; Wang, J.; Lu, N.; Jiang, Z.; Hao, X.; Li, J.; Liu, J.; Cao, P.; et al. LSECtin on tumor-associated macrophages enhances breast cancer stemness via interaction with its receptor BTN3A3. *Cell Res.* 2019, 29, 365–378. [CrossRef] [PubMed]

80. Ma, J.; Qin, L.; Li, X. Role of STAT3 signaling pathway in breast cancer. *Cell Commun. Signal.* 2020, 18, 33. [CrossRef]

81. Jin, W. Role of JAK/STAT3 Signaling in the Regulation of Metastasis, the Transition of Cancer Stem Cells, and Chemoresistance of Cancer by Epithelial–Mesenchymal Transition. *Cells* 2020, 9, 217. [CrossRef]

82. Rhodes, D.A.; Chen, H.-C.; Williamson, J.C.; Hill, A.; Yuan, J.; Smith, S.; Rhodes, H.; Trowsdale, J.; Lehner, P.J.; Herrmann, T.; et al. Regulation of Human γδ T Cells by BTN3A1 Protein Stability and ATP-Binding Cassette Transporters. *Front. Immunol.* 2018, 9, 662. [CrossRef]

83. Sandstrom, A.; Peigné, C.-M.; Léger, A.; Crooks, J.E.; Konczak, F.; Gesnel, M.-C.; Breathnach, R.; Bonneville, M.; Scotet, E.; Adams, E.J. The intracellular B30.2 domain of Butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vγ9Vδ2 T cells. *Immunity* 2014, 40, 490–500. [CrossRef]

84. Yang, Y.; Li, L.; Yuan, L.; Zhou, X.; Duan, J.; Xiao, H.; Cai, N.; Han, S.; Ma, X.; Liu, W.; et al. A Structural Change in Butyrophilin upon Phosphoantigen Binding Underlies Phosphoantigen-Mediated Vγ9Vδ2 T Cell Activation—ScienceDirect. *Immunity* 2019, 50, 1043–1053. Available online: https://www.sciencedirect.com/science/article/pii/S1074761319300834 (accessed on 17 April 2022). [CrossRef] [PubMed]

85. Vantourout, P.; Laing, A.; Woodward, M.J.; Zlatareva, I.; Apolon, A.; Jones, A.W.; Snijders, A.P.; Malim, M.H.; Hayday, A.C. Heteromeric interactions regulate butyrophilin (BTN) and BTN-like molecules governing γδ T cell biology. *Proc. Natl. Acad. Sci. USA* 2018, 115, 1039–1044. Available online: https://www.pnas.org/doi/abs/10.1073/pnas.1701237115 (accessed on 17 April 2022). [CrossRef] [PubMed]

86. Kilcollins, A.M.; Li, J.; Hsiao, C.-H.C.; Wiemer, A.J. HMBPP Analog Prodrugs Bypass Energy-Dependent Uptake To Promote Efficient BTN3A1-Mediated Malignant Cell Lysis by Vγ9Vδ2 T Lymphocyte Effectors. *J. Immunol.* 2016, 197, 419–428. [CrossRef] [PubMed]

87. Decaup, E.; Duault, C.; Bezombes, C.; Poupot, M.; Savina, A.; Olive, D.; Fournié, J.-J. Phosphoantigens and butyrophilin 3A1 induce similar intracellular activation signaling in human TCRVγ9+ γδ T lymphocytes. *Immunol. Lett.* 2014, 161, 133–137. [CrossRef] [PubMed]