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Review Article

Therapeutic targeting of immune checkpoints with small molecule inhibitors

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Abstract: Immune checkpoints are known to contribute to tumor progression by enhancing cancer's ability to evade the immune system and metastasize. Immunotherapies, including monoclonal antibodies, have been developed to target specific immunosuppressive molecules on the membranes of cancer cells and have proven revolutionary in the field of oncology. Recently, small molecule inhibitors (SMIs) have gained increased attention in cancer research with potential applications in immunotherapy. SMIs have desirable benefits over large-molecule inhibitors, such as monoclonal antibodies, including greater cell permeability, organ specificity, longer half-lives, cheaper production costs, and the possibility for oral administration. This paper will review the mechanisms by which noteworthy and novel immune checkpoints contribute to tumor progression, and how they may be targeted by SMIs and epigenetic modifiers to offer possible adjuvants to established therapeutic regimens. SMIs target immune checkpoints in several ways, such as blocking signaling between tumorigenic factors, building immune tolerance, and direct inhibition via epigenetic repression of immune inhibitory molecules. Further investigation into combination therapies utilizing SMIs and conventional cancer therapies will uncover new treatment options that may provide better patient outcomes across a range of cancers.

Keywords: Small molecule inhibitors, immune checkpoints, cancer progression, epigenetics, tumor microenvironment

Introduction

A dynamic crosstalk exists between the immune system and tumor cells which promotes immune evasion in the tumor microenvironment (TME). The transition from a static tumor to an immunosuppressive, malignant tumor involves continuous interaction between various receptors and ligands present on both tumor cells and immune cells. Tumors express factors that help suppress T-cell activation towards their own cells through aberrant signaling via immune checkpoints. This in part accounts for the aggressiveness of many tumor types including solid and hematologic cancers and explains their ability to evade the immune system [1-4]. Immune checkpoints PD-1/PD-L1, CTLA-4, OX40, LAG-3, TIM-3, and B7-H3 are commonly involved with this tumorigenic crosstalk. CTLA-4 and B7-H3 inhibit T-cell function and become overexpressed in most solid cancers such as breast cancer, prostate cancer, renal cell carcinoma, liver cancer and brain cancer [5]. TIM-3 and PD-1/PD-L1 promote tumor cell migration by suppressing normal T-cell activation and function [6, 7]. Binding of tumor-associated PD-1 to either of its ligands (PD-L1 or PD-L2) has been shown to induce T-cell apoptosis and suppress the release of cytokines [8]. LAG-3 is a surface molecule that promotes activation of T-cells. Recently, it has been shown to block antibodies that target breast and renal cancers and therefore inhibit immunotherapeutic drugs from performing their function [9]. Additionally, OX40 is a surface molecule in the tumor necrosis factor receptor family. Studies have found that a crosstalk between OX40 and OX40L downregulates T cell activity in the TME [10].

These discoveries highlight a new path forward for cancer treatments by targeting aberrant signaling in various immune checkpoints. Several notable monoclonal antibodies (mAbs) against inhibitory checkpoints PD-1/PD-L1 and CTLA-4 are currently in clinical use and have been reported on extensively [11, 12]. However, these treatments are still imperfect. For example, 18% of subjects within a randomized control trial for ipilimumab (CTLA-4 mAb) with advanced melanoma survived beyond two years [13]. While results like these are promising, there remains room for improvement. Small molecule inhibitors can cross cell membranes easily, and they have several possible targets and routes for suppressing oncogene expression or products, more so than conventional therapies. Other advantages of SMIs over therapeutic antibodies include lower production cost, higher stability, ability for oral administration, and better tumor penetrance [14]. SMIs offer a promising avenue of cancer treatment since cancer can be targeted from a variety of avenues ranging from direct target inhibition to epigenetic inhibition of gene transcription. More importantly, they can be used to sensitize neoplastic cells towards recognition and destruction by the host's own immune system. Additionally, SMIs may offer promising results when used in combination with other well-established cancer treatments by way of synergism.

SMIs appear to be most promising in their epigenetic targeting of oncogenes and immune checkpoint aberrations for the treatment of cancers with established mutations [15]. This is intuitive considering that halting the production of oncogenic proteins produced by cancer cells is more effective than trying to target proteins once they have already been produced. By promoting the transcription of tumor suppressors or decreasing the expression of oncogenes, epigenetic inhibitors can suppress tumor progression in a variety of ways. There are several protein families implicated in epigenetic regulation of gene expression through post-translational modifications of histones, including histone acetyl transferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), and lysine demethylases (KDMs) [16]. These epigenetic markers are mutated in several cancers and offer unique targets for research and development in cancer therapy [17, 18].

This review will briefly summarize the currently understood mechanisms by which several immune checkpoints interplay with tumor progression. We will then highlight SMIs that are being developed to target these checkpoints and the immunotherapeutic promise of using SMIs in combination with already-established adjuvant therapies to treat cancer.

Targeting immune checkpoints in the TME

PD-1/PDL-1

One of the most extensively studied immune checkpoints regarding cancer is PD-1 and its ligand, PD-L1. PD-1 was first reported in 1992 and is expressed on the surface of T- and B-cells [19]. This protein receptor, which is encoded by the Programed Cell Death 1 gene on chromosome 2 and is a member of the CD28 superfamily, delivers inhibitory signals to adjacent cells upon interaction with its ligand. This checkpoint is critical in the regulation of T-cell activity, but PD-1 is only present on T-cells once they are activated [20]. PD-1, upon binding its ligands PD-L1 (B7-H1) or PD-L2 (B7-DC), helps exert immunoregulatory effects on T-cells by downregulating T-cell activity [21, 25]. Knockdown experiments of PD-1 in mice have demonstrated the development of autoimmune disease [22]. Hence, PD-1 is a critical negative regulator of immunity that serves to identify and preserve "self" tissue.

Due to its usefulness in evasion of T-cell dependent killing, PD-L1 overexpression has been linked to numerous cancers including melanoma, colorectal cancer, and renal cell carcinoma [23]. This allows cancer cells to evade immune detection and destruction via T-cells. This discovery has made PD-1 a promising target for immunotherapies. Blocking PD-1 is thought to enhance the efficacy of T-cells in the TME, thereby offering an alternative cancer treatment in contrast to cytotoxic agents [24]. Anti-PD-1 and anti-PD-L1 antibodies have been used to treat several types of cancers and have been found to significantly increase progression-free survival in patients [24]. Despite their benefits, they still have adverse effects such as pyrexia, hepatotoxicity, pneumonitis, ototoxicity, and nephrotoxicity [25].

Targeting the PD-1/PD-L1 interaction with SMIs has been challenging due to the relatively flat

and hydrophobic surfaces where the two proteins interact, which makes the physical placement of inhibitors on those surfaces extremely difficult [26]. Promising SMIs have come from Bristol-Myers Squibb (BMS) consisting of triaromatic structures: BMS-8, 37, 202, 230, and 242. Specifically, BMS-8, BMS-37, and BMS242 were found to bind to PD-L1 and dissociate the PD-1/PD-L1 complex *in vitro* [27]. Two improved BMS compounds (BMS-1001 and BMS-1166) have since been synthesized and have been shown to restore the activation of effector Jurkat T-cells *in vitro* [28]. Moreover, BMS compounds have been shown to induce the formation of dimers of soluble PD-L1 (sPD-L1) which facilitates the inhibition of the PD-1/PD-L1 interaction since both binding surfaces of the proteins are engaged during the dimerization process [28]. Soluble PD-L1 is known to interfere with the activation of T-cells in the blood, and its presence in the serum of cancer patients is associated with poor prognosis [29, 30]. Therefore, BMS compounds offer a promising route forward in the functional inhibition of the PD-1/PD-L1 interaction as well as the elimination of sPD-L1 in cancer patients to increase the immune competence of circulating T-cells. Investigation into the effects of these compounds has yet to be conducted *in vivo,* which leaves questions about the efficacy and toxicities of these compounds unanswered.

While it seems difficult to target the PD-1/PD-L1 interaction directly, inhibition of proteins important for the expression of these immunoregulatory factors offers an alternative route for disrupting their immunosuppressive effects. The signaling pathways that regulate PD-1 expression on T-cells have been the subject of great interest, yet remain unclear. Researchers have concluded that certain transcription factors, nuclear factor of activated T-cells (NFAT), forkhead box protein O1 (FoxO1), and activator protein 1 (AP1), regulate the transcription of *pdcd1*, the gene that encodes PD-1 [31, 32]. This implies that targeting these proteins could be a useful strategy to downregulate the production of PD-1 and PD-L1. Glycogen synthase kinase 3 (GSK-3) is a serine threonine kinase in resting T-cells that becomes inactivated once T-cells are active and has been found to be of importance in the transcription of the *pdcd1* gene [33, 34]. GSK-3 promotes the exit of NFAT from the nucleus of $CD4^+$ T-cells which inhibits their proliferation [35, 36]. Inhibition of GSK-3 using an SMI, SB415286, has been shown to be as effective as anti-PD-1 and anti-PD-L1 antibody therapies in B16 melanoma and EL-4 lymphoma tumor growth in mice [27]. Additionally, no autoimmune diseases or side effects were noted over the two-year course of this drug treatment in mice, a noteworthy advantage that this SMI holds over traditional anti-PD-1/PD-L1 immunotherapies [27].

Modulation of epigenetic protein expression or function represents another strategy for targeting the expression, rather than the function, of tumor suppressors and oncogenes. Histone deacetylase inhibitors (HDACi's) are one class of epigenetic drugs that have been investigated for their anti-tumor properties. HDACs represent a family of epigenetic proteins that have a wide variety of effects on gene transcription and cell cycle through deacetylation of histones that package DNA, thereby impacting the transcription of specific genes including oncogenes and tumor suppressors. HDACi's have been shown to induce cell cycle arrest and apoptosis in various transformed cells while normal cells are fairly resistant to HDACi's [37], an effect which has been most conclusively studied in melanoma tumors [38]. That HDAC inhibition seems to preferentially affect transformed cells makes it an attractive and potentially useful method for treating cancers. Two HDACi's used to target PD-1/PD-L1, vorinostat and panobinostat, have been shown to upregulate PD-L1 expression in a dose-dependent manner in triple-negative breast cancer (TNBC) by relaxing chromatin at the PD-L1 and PDL-2 promoters, allowing for increased transcription of the genes [38, 39]. Other inhibitors, including azacytidine and decitabine, have also been proven to upregulate PD-L1 and PD-L2 levels in melanoma cells [40]. The desirability of increasing PD-L1 and PD-L2 expression seems counterintuitive, but when HDACi's were used in combination with PD-1 antibody therapy in mice, results showed decreased tumor burden and improved survival [39, 40]. This is consistent with recent literature that reports PD-L1 expression in breast cancer is associated with better responses to therapy and improved survival [41].

Another HDACi that has shown promising *in vitro* and *in vivo* results is entitostat. Entitostat is currently in clinical trials in combination with pembrolizumab (PD-1 mAb) for numerous types of cancers [42]. This drug is known to target

class I and IV HDAC's which helps promote histone hyperacetylation and transcriptional activation of certain genes. It also can lead to an upregulation of genes like *p21* which leads to cell cycle arrest [43]. Most importantly, it has been shown in several studies to enhance the anti-tumor properties of immunotherapy and chemotherapy treatments as well as decrease the immunosuppressive tumor microenvironment of several types of cancers including lung, renal, and lymphoma [43, 44] these responses are promising and show entitostat as a potent adjuvant to current immunotherapies. Entitostat also appears to be well tolerated and has shown relatively few side effects during treatments in the preliminary results from the clinical trials. On the other hand, some preliminary data from clinical trials have shown no improvement in tumor burden or survival for colorectal tumors when using entitostat, indicating that it may not be broadly effective in all tumor types [45]. Further results from the clinical trials of entitostat are necessary to definitively assess the efficacy and safety of the drug in humans.

Another method of epigenetic alteration of gene transcription is inhibition of bromodomains that allow proteins to bind to acetylated histones in chromatin and activate transcription [46]. Bromodomain inhibitors bind to proteins to prevent protein-protein interaction between bromodomain and extra-terminal domain (BET) proteins, acetylated histones, and transcription factors. There are two notable bromodomain inhibitors, JQ1 and I-BET151, that have demonstrated antiproliferative activity in pre-clinical studies by decreasing the downstream production of the oncogenes c-MYC and BCL2 [47-49]. Inhibition of bromodomains using JQ1 has also been shown to suppress the PD-L1 gene in ovarian cancer cell lines, which allows T-cell activity to increase in mouse models [50]. The dual activity of bromodomain inhibitors having antiproliferative effects as well as preventing T-cell exhaustion is an extremely promising observation, indicating that they could be useful additions to established immunotherapies to prevent resistance and relapse.

CTLA4 (CD152)

CTLA4 (CD152) is a molecule that delivers inhibitory signals to activated T-cells, making it another molecule important for immune regulation. Normally, CD80 or CD86 on dendritic antigen-presenting cells (APCs) bind to CD28 on neighboring T-cells to activate them and induce cytokine production. However, CTLA4 is a CD28 homolog with a higher affinity for CD80/86 that can be expressed to attenuate T-cell activation. This molecule is extremely important in the immune escape mechanisms of cancer [51]. One theory proposes that CTLA4 inhibits T-cell activation by decreasing the contact time between T-cells and neighboring APCs, limiting the possibility of CD80/86-CD28 interactions occurring [52].

The CTLA4 pathway can be targeted via several routes. Inhibition of CD80 is one avenue that has been explored by Huxley *et al*. in 2004 with several novel molecules that showed promising results in blocking T-cell co-stimulation [53]. This study established 6 potential inhibitors simply named compounds "1-6". These compounds successfully blocked T-cell co-stimulation in cell-based assays as well as blocking CD28 and CTLA4 binding. As a result, the release of cytokines IL-2, IFNγ, and TNF-α was significantly inhibited. This was all accomplished at submicromolar potency, making these compounds promising drugs for use in autoimmune diseases as well as cancers that overproduce CTLA4. Wyeth Research has also reported SMIs that target the B7-1 protein that interacts with CD28 and CTLA4, known as "compounds 8 and 9" [54, 55]. While the authors of this study concluded these compounds targeted the CTLA4 binding site, the compounds seemed to only weakly inhibit the B7-1-CTLA4 interaction. This was evident from the lack of observed inhibition in a cell adhesion assay. Currently, it seems the prospects of CTLA4 SMIs are limited and more investigation remains to be done to identify new compounds and assess their efficacy.

The current immunotherapy antibody used to target CTLA4 in a clinical setting is ipilimumab. Ipilimumab was the first checkpoint-blocking antibody approved for clinical use and showed prolonged survival in advanced melanoma patients [13]. Chiapinelli *et al.* have proposed clinical trials that aim to combine epigenetic inhibitors along with ipilimumab to treat melanoma, NSCLC, and MDS [56]. These inhibitors include entitostat, panobinostat, ACY-241, and azacytidine [56]. As of May 2016, these trials

were in the recruitment phase [56] and results will be forthcoming soon.

OX40

The OX40 immune checkpoint plays a crucial role in the regulation of immune cell activation and sustained inflammatory responses. OX40L is a member of the tumor necrosis factor superfamily and is mainly expressed by professional antigen-presenting cells such as dendritic cells, macrophages, and activated B cells as well as endothelial cells and T-cells [57]. The OX40 receptor (CD134) is a costimulatory protein that is expressed on the surfaces of NK-cells and activated T-cells [58]. The OX40 receptor/ligand connection is crucial for antigen-specific memory for T-cells and allows for anti-tumor immunity [59]. OX40 triggering has also been shown to inhibit the suppressive functions of IL-10 producing regulatory T (Treg) cells and inhibit the immunosuppressive effects of TGF-β on $CD4^+$ naive cells [60, 61]. This effect of inhibiting Treg cells and other immune suppressive functions means OX40 is a crucial link to maintain immune responses, especially within the context of the tumor microenvironment.

As opposed to PD-1 and CTLA4, OX40 is an immune stimulator rather than a suppressor [57]. A 2009 study used an agonistic human mAb against the OX40 receptor in combination with cyclophosphamide and showed that this treatment induces Treg cell deletion and leads to the regression of established tumors *in vitro* [62]. This laid the groundwork for the OX40 antibody to be used in monotherapy and in combination with radiation therapy and cyclophosphamide. It has shown to reduce tumor volume for patients with metastatic prostate cancer who have failed first-line therapies [63]. Another study conducted *in vitro* has shown increased efficacy of this agonistic OX40 mAb when tumors are also treated with a cytosine-phosphorylated guanine (CpG) oligodeoxynucleotide ligand for TLR9, stimulating increased expression of OX40 on intratumoral $CD4^+$ cells [64]. This synergism has been effective against metastatic *de novo* breast cancers, making it a promising method of stimulating anti-tumor immune responses in patients. Combination treatment using this OX40 mAb with a TGF-β inhibitor, SM16, yielded regression of established mammary tumors and reduced spontaneous metastasis in mice [65]. Therefore, evidence suggests that the use of this antibody in combination with either established chemotherapy agents or small molecule inhibitors could show improved clinical outcomes for patients.

Several small molecules, DB36, DB71, DB15, CVN, have been found to directly interrupt the OX40-OX40L interaction and activate OX40 signaling downstream *in vitro* [66]. Notably, CVN was found to have the best effect of inhibiting Treg generation as well as stimulating helper T cell (Th9) generation, ideal for maintaining an anti-tumor immune response. These drugs achieved their effect at low micromolar potency and were found to be similarly efficacious to the OX40 human mAb, a promising sign for their clinical application. To date, these compounds remain the only examples that have produced such results. Further development and clinical testing remains to be done on these compounds to ensure their high effectiveness and minimal adverse outcomes.

Attempts to epigenetically influence the expression of OX40 and its ligand have been undertaken most notably using HDACi's. MGCD0103 and SNDX-275 are class I and IV HDACi's that have been shown to upregulate OX40L surface expression and inhibit the production of IL-10 producing Treg cells [67]. These effects have been hypothesized to derive primarily from the essential role that HDAC11 plays in the regulation of OX40L expression. Inhibition of this HDAC plays a crucial role in promoting the transcription of the OX40L gene, making it a promising target for drug development to be administered alongside cancer vaccines or other established immunotherapies. Although the exact mechanisms of this OX40L upregulation are unclear, it may be a beneficial addition to currently implemented therapies to further prevent T-cell exhaustion and relapses.

LAG3 (CD233)

Lymphocyte activation gene 3 (LAG3) is an inhibitory ligand expressed on the surfaces of activated CD4 and CD8 T-cells as well as Treg cells. There has also been evidence showing that LAG3 can be expressed on B-cells, resulting in stimulation of T-cells following soluble LAG3 exposure [68]. LAG3 is a part of the Ig superfamily and shares structural similarity to the CD4 molecule. MHC-II is the only known

ligand for LAG3 and it binds at a unique site different from CD4 [69]. Its inhibitory effects are mainly to stop CD4 and CD8 cell proliferation as well as allow Treg-mediated suppression of other immune cells [70]. LAG3's function in immune suppression has important consequences for antitumor immunity. Interestingly, LAG3 seems to be commonly co-expressed with PD-1, which plays a synergistic role in tumor immune system evasion [71]. This has been highlighted by studies that show LAG3-knockout mice experience slowed tumor growth while *Pd1/Lag3*-double knockout mice show complete tumor rejection [71]. A soluble form of LAG3 also exists and is known to bind to MHC-II on only a small subset of antigen presenting cells (APCs) [72]. This binding seems to disrupt the interactions between membrane bound LAG3 and MHC-II molecules which has been shown to enhance antitumor T-cell function in mice models [73]. Early clinical trials have begun with a soluble LAG immunoglobulin named IMP321 (Immutep) in combination with cancer vaccines and/or chemotherapy in attempts to stimulate T-cell activity towards tumors.

Attempts to directly target LAG3 have been undertaken mainly with the use of mAb's named BMS-986016 (BMS-ONO) and GSK28-31781 in early clinical trials with others remaining in pre-clinical development [74]. These antibodies are primarily used in combination with PD-1 mAb due to the co-expression of these two immune checkpoints and the synergistic effect that dual inhibition shows. However, in terms of direct inhibition using small molecules, there are currently no promising developments. The current literature heavily focuses on antibody immunotherapy as well as LAG3-immunoglobulin administrations *in vitro* and clinically.

Even so, there appears to be promising avenues for targeting LAG3 via epigenetic mechanisms. Analysis of breast cancer tumors revealed an epigenetic signature at the promoters of the immunosuppressive checkpoints PD-1, CTLA4, and LAG3 that would promote gene expression. Further analysis of the promoter regions of these genes identified significant hypomethylation of the CpG islands in breast tumors when compared to normal tissues [75]. Hypomethylation of these promoters could explain their upregulation since their genes are more accessible to transcription factors and RNA polymerases. Additionally, the histone repressors H3K9me3 and H3K27me3, which normally bind chromatin at promoters and inhibit transcription, were found to bind more weakly to LAG3 promoters in breast cancer tissues when compared to normal tissues [75]. This further explains the observed upregulation of LAG3 in breast cancer tissues and indicates that H3K27me3 or H3K9me3 could be important mediators of LAG3 expression.

Polycomb repressive complex 2 (PRC2) is a chromatin remodeling complex that actively methylates lysine 27 on histone 3 (H3K27me3), leading to transcriptional repression. H3K27-me3 has been the subject of intense investigation in recent years as it is known to transcriptionally repress genes and lead to immune resistance in cancers [76]. Additionally, the enzymatic subunit of PRC2 called enhancer of zeste homolog 2 (EZH2) has been the focus of research due to its inhibitory impact on cell proliferation and immune resistance [77]. Expression of EZH2 has been shown to control methods of immune resistance to immunotherapy as well as suppress antigen presentation in melanoma tumors. In the context of LAG3, inhibition of EZH2 using a compound called GSK503 has been shown to decrease the expression of LAG3 as well as PD-1 and TIM-3 [77]. EZH2 inhibition in the same study was also found to reverse melanoma immune resistance mechanisms as well as synergize with anti-CTLA-4 and IL-2 therapy. Therefore, LAG3 remains a viable target through epigenetic mechanisms dependent upon tumor type.

TIM-3

T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) is an inhibitory immune checkpoint that reduces the activity of $CD4^+$ T-helper 1 (Th1) and $CD8^+$ T cytotoxic 1 cells [78]. This inhibitory effect makes it a useful tool for cancer cells to evade the immune system. TIM-3 has been shown to play a role in tumor immunity by being upregulated on $CD4^+$ and $CD8^+$ tumor infiltrating leukocytes from a variety of lung tumors which facilitates immune evasion [79]. Several inhibitory mAbs, including RMT3-23, ATIK2a, and TSR-022, have been generated towards TIM-3 to mainly target colon cancer and melanoma and have shown promising results that include decreased tumor size and increased IFN-γ production in murine models [80-82]. These mAbs work by binding TIM-3 on

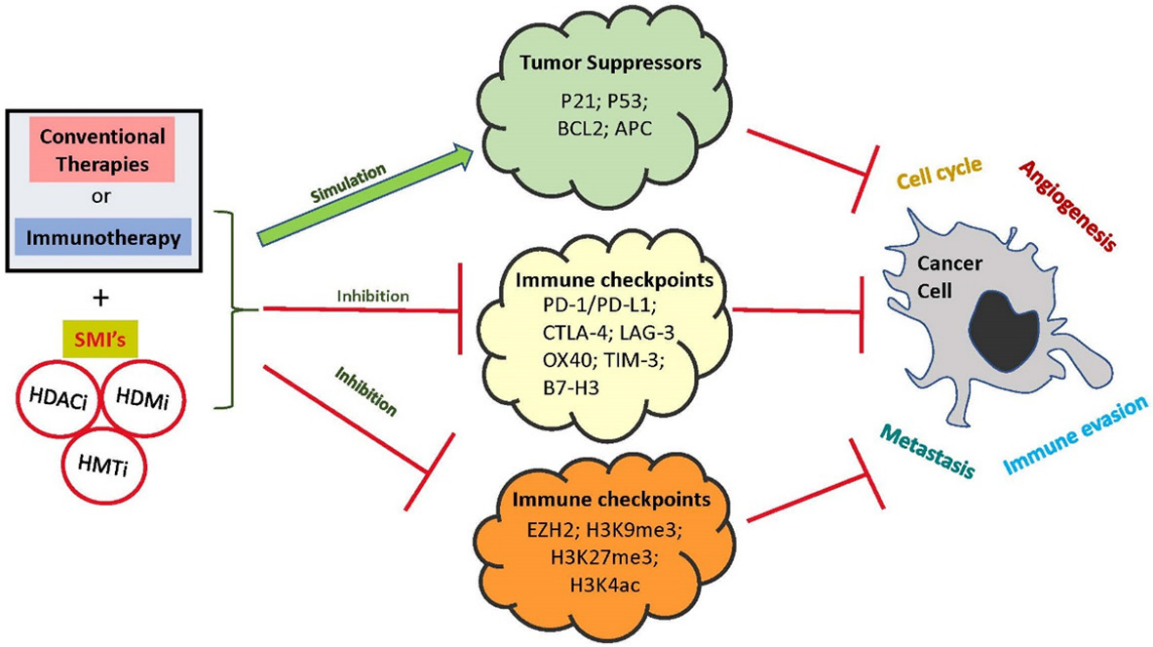

Figure 1. A graphic representation of the potential benefits that SMIs (including epigenetic inhibitors) may yield in addition to conventional therapies or immunotherapies. Upregulation of tumor suppressors may cause cell cycle arrest and halting of various oncogenic properties such as angiogenesis, immune evasion, and metastasis. SMIs directed towards immune checkpoints such as PD-1/PD-L1, CTLA-4, LAG-3, OX40, TIM-3, and B7-H3 may also produce this same effect. Additionally, SMIs can target epigenetic proteins such as EZH2 to inhibit the methylation of specific histones towards specific states such as H3K9me3, H3K27me3, and H3K4ac. Inhibition of these pathways result in cell cycle arrest and inhibition of angiogenesis, immune evasion, and metastasis.

the outside of lymphocytes and inhibiting the ligands galectin-9, phosphatidylserine, high mobility group protein B1 (HMGB1), and carcinoembryonic antigen cell adhesion molecule from binding and suppressing immune activation [83]. Specifically, the TIM-3/Galectin-9 signaling pathway has been shown to induce $CD8^+$ T cells to undergo apoptosis which may explain the immune inhibitory role that TIM-3 plays in cancer progression [84]. Currently, there is only one structure for the mouse form of TIM-3 [85]. This poses a difficult barrier towards targeting TIM-3 with an SMI. Currently, there are no direct SMIs that have been discovered for TIM-3.

From an epigenetic standpoint, inhibition of EZH2 provides a promising route towards targeting TIM-3 in that EZH2 has been found to facilitate galectin-9 expression through trimethylation of H3K27 [86]. Since galectin-9 is a known ligand for TIM-3, inhibition of EZH2 could be a worthwhile avenue to explore in order to impact TIM-3 signaling and activate immune cells towards tumors. Numerous EZH2 inhibitors such as EPZ005687 and DZNep have been under investigation in cancer research and may provide a promising avenue of investigation for their effectiveness in targeting TIM-3 as an immune checkpoint in cancer treatment [87].

B7-H3 (CD276)

B7-H3 (CD276) is a glycoprotein that is expressed on the surface of antigen-presenting cells that are known to inhibit T-cells [88]. Outside of its immunosuppressive role, B7-H3 also promotes cancer progression via migration, angiogenesis, and gene modification [89-91]. As a result, B7-H3 is upregulated in numerous types of cancers including medulloblastoma, breast cancer, renal cell carcinoma, and prostate cancer [5]. Although there seems to be conflicting evidence regarding whether B7-H3 is a costimulatory or co-inhibitory molecule, its role in cancer progression is apparent. This makes it a potentially important tumor marker as well as a target for therapy.

Relatively little is known about the receptor for B7-H3 on T-cells, limiting the direct targeting of this signaling pathway. Currently, a monoclonal antibody, 8H9, binds directly to B7-H3 protein inducing immune-related tumor death [92].

Table 1. Current small molecule inhibitors targeting immune checkpoints and cellular pathways in the tumor microenvironment

Immune checkpoint	Small molecule inhibitors	Cell pathways	References
PD-1/PD-L1	BMS-8, 37, 202, 230, 242, 1001, 1166, SB415286*, vorinostat*, panobinostat*, azacitidine*, decitabine*, entitostat*, JQ1*, I-BET151*, GSK503*	Invasion Angiogenesis T-Cell suppression Immunoregulation	[27, 39, 40, 42, 50, 77, 94]
CTLA4	Compounds "8 and 9", entitostat*, panobinostat*, ACY-241*, azacytidine*	Angiogenesis Immune control T-Cell activation Cell signaling	[42, 54, 55, 95]
OX40	DB36, DB71, DB15, CVN, MGCD0103*, SNDX-275*, azacytidine*	Angiogenesis Cell activation Immunoregulation Tumor necrosis	[66, 67, 96]
LAG-3	IMP32, BMS986016	T-Cell proliferation Cell maturation & activation	[77]
TIM-3	TSR-022, Sym023, ATIK2a	Immune response Tumorigenesis Cell invasion & activation Cell regulation	[77, 78, 84, 97-99]
B7-H3	c-MYC SMIs, vorinostat*, DZNep*	Migration Angiogenesis T-Cell response Immune response Mediated signaling Promotes the cell cycle	[89]

*, Epigenetic SMI.

This has shown promising results in patients with metastatic solid tumors to the central nervous system by decreasing tumor size and growth [93]. It is known that the receptor(s) on T-cells for B7-H3 engage the FG loop of the IgV domain, indicating a possible location that SMIs could target [88]. Unfortunately, there are no reports of any SMIs that have been developed to directly or epigenetically target B7-H3 as of 2018.

Conclusion

In summary, recent studies have shown that SMIs can restore anti-tumor immunity by targeting immune checkpoints, either directly or epigenetically. **Figure 1** illustrates the impact that combining SMIs with conventional therapies may have. Additionally, **Table 1** outlines SMIs currently under investigation and their targets. These inhibitors help restore T-cell function, forcing cancer cells into the apoptotic phase. The potential for SMIs to target intracellular proteins, including epigenetic modifiers, give SMIs a distinct advantage in combating the mechanisms by which cancer cells evade immune detection. Studies have shown that combining SMIs with conventional therapies can improve survival outcomes tremendously in aggressive cancers including medulloblastoma, glioblastoma, breast cancer, lung cancer, pancreatic cancer, liver cancer, leukemia, and lymphoma. These factors make the targeting of immune checkpoints with SMIs and epigenetic inhibitors crucial additions to standard immunotherapy and chemotherapy regimens. Much clinical progress remains to be seen with SMIs, but in the coming years these compounds may be revolutionary in the way cancer therapy is approached.

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Disclosure of conflict of interest

None.

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