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The evolution of checkpoint blockade as a cancer therapy: what's here, what's next?

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Unleashing the immune system to fight cancer has become one of the main treatment modalities since the anti-CTLA-4 antibody, ipilimumab was approved for patients with advanced melanoma in 2011. Pembrolizumab and nivolumab, two anti-PD-1 antibodies recently approved for the treatment of patients with metastatic melanoma, are being actively investigated for the treatment of multiple caners including lung, breast, bladder and renal cancers along with other anti-PD-1/L1 antibodies. Early results of combining of anti-CTLA-4 antibody and anti-PD-1 antibody treatment for advanced melanoma patients are showing impressive response rates with manageable toxicity profiles. There are several other checkpoint molecules that are likely potential inhibitory targets. The outcome of blocking some of these negative immune regulators, such as LAG-3 or TIM-3, is being pursued in the clinic or about to enter clinical development. Blockade of these molecules is demonstrating promising preclinical activity alone or when combined with anti-PD-1/L1. Future studies will define bio-markers of these therapies and how to target them alone or in combination with other immunotherapies, chemotherapy, radiotherapy and small molecule inhibitors.

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Introduction

Our immune system has the capability to identify and eliminate cells expressing tumor antigens [1•,2]. At the same time, it has developed sophisticated ways of controlling its activity to maintain tolerance to self-antigens [3]. Various diseases arise from defects in this system such as autoimmune diseases [4], and cancer cells can exploit this system escaping from immune attack (immune evasion), which has been a major limitation of cancer immunotherapy [5–9]. Efforts made over past decades have allowed for our current understanding about immune escape mechanisms, one of which is the manipulation of immune cell-intrinsic checkpoints that are induced upon T cell activation [10]. First evidence of improved survival with blockade of these checkpoint molecules came from patients with advanced melanoma treated with the anti-CTLA-4 antibody ipilimumab, which demonstrated 10–15% of durable response [11••]. Blockade of the PD-1/PD-L1 pathway demonstrated unprecedented durable response in 30–35% of patients with advanced melanoma [12], which led to the FDA approval of pembrolizumab (anti-PD-1 antibody) in September 2014, and nivolumab in December 2014. This class of immune modulatory antibodies blocking PD-1 or PD-L1 are arguably the most exciting development in current cancer drug development (Figure 1).

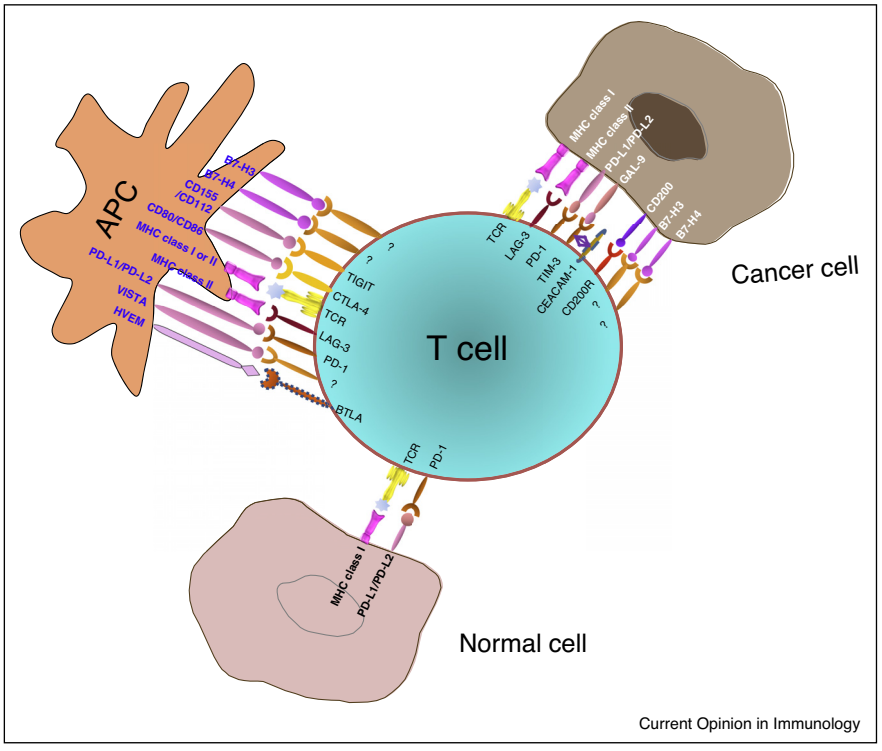
A great deal of research has uncovered the complex interplay of immune regulatory molecules (co-inhibitory and co-stimulatory) that are regulating T cell activity [13••,14–16], and as CTLA-4 and PD-1 inhibitors have taught us, we can actually manipulate these molecules to activate our immune system to fight against cancers. However, many other studies will be required to understand how we can improve anti-tumor activity by combining checkpoint inhibitors or co-stimulatory activators, and possibly combining with conventional oncological therapies. Some of the new checkpoint inhibitors, such as LAG-3 and TIM-3, are close to clinical development, especially combined with PD-1 inhibitors [17–19]. There are many other potential candidates for further development. We currently do not have validated bio-markers to predict the likelihood of response for each patient for these checkpoint inhibitors. However, it is anticipated that in the near future, we will have tools to predict the chance of response of each patient before the initiation of these therapies.

Blockade of CTLA-4

Biology

Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4, CD152) was initially discovered through cDNA library screening as one of the members of the immunoglobulin superfamily and found to be a co-inhibitory molecule expressed on T cells evidenced by profound autoimmune

Figure 1



Schematic representation of inhibitory immune checkpoints between antigen presenting cell (APC), T cell, cancer cell and normal cell. Upon T cell activation via T cell receptor (TCR) engagement by MHC-peptide complex between APC, inhibitory checkpoints are interacting with their cognate ligands expressed on APC. When T cell encounters antigen presented by MHC class I molecule on cancer cell, cancer cell expresses cognate ligands to interact with inhibitory checkpoints expressed on T cell. Inhibitory immune checkpoints also play a role in self-tolerance when T cell encounters normal cell. Most of inhibitory immune checkpoint molecules are members of immunoglobulin superfamily (IgSF), except HVEM, which is a member of TNF (Tumor necrosis factor) receptor superfamily. Molecules cited; CTLA-4 (Cytotoxic T lymphocyte-associated antigen-4, CD152); PD-1/L-1 (Programmed cell death-1 or CD279/Programmed cell death ligand-1 or CD274); TIM-3 (T-cell immunoglobulin and mucin containing protein-3); LAG-3 (Lymphocyte activation gene-3 or CD223); B7-H3 (B7 homolog 3, CD276); B7-H4 (B7 homolog 4, B7S1, B7x, VTCN1); VISTA (V-domain Ig suppressor of T cell activation, B7-H5 or PD-1Homolog, differentiation of embryonic stem cells 1; Dies 1); CEACAM1 (Carcinoembryonic antigen-related cell adhesion molecule 1, CD66a); BTLA (B and T lymphocyte attenuator, CD272); CD200R (CD200 receptor), CD200 (OX2); TIGIT (T cell Ig and ITIM doman, Vsig 9, Vstm3 or WUCAM).

phenomenon in knockout mouse models [20–23]. It has approximately 30% of sequence homology with CD28, which is a critical co-stimulatory molecule constitutively expressed on T cells. CTLA-4 is not expressed by naïve $CD4^+$ or $CD8^+$ T cells; however, it is contained intracellularly and TCR engagement induces trafficking to the cell surface where it can become engaged in signaling [24]. CTLA-4 binds to B7-1 (CD80) and B7-2 (CD86) as CD28 does, but with much higher affinity (10–40-fold) so that it arrests CD28 induced T cell activation by competing for ligand engagement, resulting in decreased cytokine production and cell cycle arrest [25,26]. It is also reported that CTLA-4 is constitutively expressed by Tregs, which play an important role in peripheral tolerance via suppressive activity on cytotoxic T cells [27–29].

Preclinical data

Based on *in vitro* studies suggesting that CTLA-4 is a key checkpoint molecule in adaptive immune responses, its antitumor activity was first tested in various animal tumor models, such as breast [30], prostate [31], lymphoma [32], sarcoma [33], colon [34] and melanoma [35] via anti-CTLA-4 blocking antibody therapy. The first preclinical report was published in 1996 by Alison and co-workers, a study that demonstrated that anti-CTLA-4 antibody effectively enhances immune response to reject tumors [36]. The efficacy was restricted to a few cancer cell lines, and the B16 melanoma model was less sensitive and therapeutic efficacy was only achieved when it was combined with a GM-CSF vaccine [37,38]. These animal studies provided the evidence that the CTLA-4 blockade could result in significant anti-tumor activity by enhancing naturally or vaccine induced T cells, which led to testing it in a clinical setting.

Clinical data

Two anti-CTLA-4 fully human monoclonal antibodies, ipilimumab (IgG1 isotype) [39] and tremelimumab (IgG2

isotype) [40] reached clinical trials, mostly tested in patients with advanced melanoma. A pivotal phase III randomized trial was published for ipilimumab in 2010 [11••]. This trial demonstrated significantly improved overall survival for patients with previously treated unresectable stage III or stage IV melanoma compared to a peptide vaccine. A subsequent phase III randomized trial for treatment-naïve patients with advanced melanoma showed that ipilimumab plus dacarbazine improved overall survival compared with treatment with dacarbazine alone [41••]. These studies led to the FDA approval of ipilimumab for metastatic melanoma patients in 2011. Tremelimumab entered phase III trial for previously untreated patients with promising early phase I and II study results [42,43]. However, phase III data did not show survival benefit. There are several potential reasons for the difference between ipilimumab and tremelimumab: different dosing regimens (every 3 weeks for ipilimumab, every 3 months for tremelimumab), different exclusion criteria (tremelimumab trial excluded patients with LDH > 2X ULN) as well as unintended crossover patients in the control chemotherapy arm to ipilimumab which was easily accessible during the tremelimumab trial [44]. Currently, tremelimumab continues to be evaluated as a treatment in combination with other anticancer agents for melanoma and other tumor types [45,46].

Blockade of PD-1/PD-L1 pathway

Biology

Programmed cell death-1 (PD-1, CD279) is a type I transmembrane receptor member of the immunoglobulin superfamily, expressed by activated T cells, and binds to two ligands, PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273), both of which are part of the B7 immunoglobulin superfamily [47]. PD-L1 is expressed on multiple normal tissues and malignant cells whereas PD-L2 is mainly expressed by antigen presenting cells [48,49]. The critical role of PD-1 in immune regulatory function has been demonstrated by inhibition of the effector phase of T cells primarily within the tumor microenvironment as well as mouse strain-specific development of autoimmune disease with long latency in a PD-1 knockout mouse model [50–54]. Given the selective immune suppressive signals delivered by cancer, it was predicted that the blockade of PD-1/PD-L1 pathway will have greater antitumor activity and fewer side effects compared to CTLA-4 blockade [13••,48].

Preclinical/clinical data

Blockade of PD-1 has been tested in animal models such as murine pancreatic carcinoma [55], B16 melanoma [56], squamous cell carcinoma [57] and CT26 colon carcinoma [58,59], which demonstrated effective anti-tumor T cell responses. A PD-L1 blocking antibody was tested in 4T1 breast carcinoma model which overcame the resistance to immune mediated tumor rejection when treated together with a 4-1BB agonist antibody [60]. These studies confirmed the therapeutic potential of targeting this immune checkpoint and multiple anti-PD-1/PD-L1 antibodies have been evaluated in clinical trials as Table 1 shows. These studies have demonstrated an unprecedented 30–35% durable response in patients with advanced melanoma, which led to a first FDA approval for pembrolizumab (anti-PD-1 antibody) in September of 2014 [12,61••,62,63,64•,65], followed by nivolumab in December 2014. Anti-PD-1/L1 blocking antibodies are currently being evaluated in other malignancies, such as lung [66], breast [67–69], bladder [70,71,72••], renal cancers [73,74••] and chemotherapy-refractory Hodgkin disease [75••] with promising results. Arguably, this is one of the most exciting developments in cancer therapeutics and has illuminated the path to a new era in treatment of patients with cancer [76].

Table 1

Checkpoint blockade agents in clinical development

Target	Agent	Class
PD-1	Nivolumab (MDX1106, BMS936558, BMS-ONO)	IgG4 fully human antibody
	Pembrolizumab (MK3475M, lambrolizumab, Merck)[a]	IgG4 engineered humanized antibody
	Pidilizumab (CT-011, Cure Tech)	IgG1 humanized antibody
	AMP-224 (Amplimmune-GSK)	Fc-PD-L2 fusion protein
	AMP-514 (MEDI0680, Amplimmune)[b]	Monoclonal antibody
	AUNP-12 (Pierre Fabre, Aurigene)	Branched 29-amino acid peptide
PD-L1	BMS935559 (MDX-1105, BMS-ONO)	IgG4 fully human antibody
	BMS936559 (BMY)	IgG4 fully human antibody
	MPDL3280A (Genentech)	IgG1 engineered humanized antibody
	MEDI4736 (MedImmune, AZ)	IgG1 engineered humanized antibody
	MSB0010718C (Merck-Serono)	IgG1 antibody
CTLA-4	Tremelimumab (ticilimumab, CP-675,206, AZ)	IgG2 fully human antibody
LAG-3	BMS-986016	Monoclonal antibody
B7-H3	MGA271 (Macrogenics)	IgG1k humanized antibody

[a] FDA approved for patients with advanced melanoma.
[b] NCT02013804. Phase I (clinicaltrials.gov).

Biomarker/patient selection

With the success of the PD-1/PD-L1 blockade, it has become a top priority to identify and characterize the factors in the tumor microenvironment that predict which patients are likely to respond to this therapy. Tumor PD-L1 expression has been pursued as a potential biomarker and studies have demonstrated that PD-L1 baseline expression level showed a strong association with response to anti-PD-1 therapy. However, clinical responses were observed also in patients considered to be negative for PD-L1 expression in the tumor [77]. Therefore, we need to integrate it into a more complex equation. In order for PD-1/PD-L1 blockade therapy to work, tumor antigen specific pre-existing T cells that become disabled by tumor PD-L1 expression are likely required [72••]. PD-L1 expression in the absence of T cells in a tumor microenvironment (constitutive expression by oncogenic process) as well as the presence of T cells without an adaptive PD-L1 expression (inducible PD-L1 expression upon cytokine stimulation mainly by interferons from immune cells) [13••,78–81] is of unknown significance. Many endeavors are taking place to develop highly sensitive and specific assays to detect and quantify PD-L1 expression and yet PD-L1 expression alone will not likely capture the full biology of PD-1/PD-L1 interaction. The presence of cytotoxic $CD8^+$ T cells is a standard IHC protocol. However, it does not provide tumor specificity. An indirect way of measuring tumor specific T cells is the expression of PD-1, PD-L1, CD137, OX40 or TIM3, which are TCR activation markers [74••,82••]. Clonality of TCR can be analyzed by deep sequencing [83] and specific TCR engagement with tumor antigen led to release of cytokines, especially interferons which can be detected by laser capture microscopy, PCR or in situ hybridization methods [84••]. These efforts to characterize each variable in this complex equation will inform oncologists of a patient's predisposition to respond to anti-PD-1/L1 antibody therapy [85].

Blockade of TIM-3, LAG-3, B7-H3, B7-H4, VISTA, CEACAM1, BTLA, CD200-CD200R and TGIT

TIM-3

T-cell immunoglobulin and mucin containing protein-3 (TIM-3) was discovered a decade ago as a molecule expressed on IFN-γ producing $CD4^+$ T helper 1 (Th1) and $CD8^+$ T cytotoxic 1 (Tc1) cells [85]. The functional significance of TIM-3 was studied in the setting of autoimmunity initially [85–87], and its function as a T cell inhibitory receptor became clear as galectin-9 (C-type lectin) was identified for its ligand, which is mainly expressed on Treg [88]. TIM-3 is believed to play a critical role in inhibiting Th1 responses through Treg expressing galectin-9, evidenced by TIM-3 blockade administration at the time of allogeneic transplantation which resulted in loss of tolerogenic effects of donor-derived Treg cell transfusion [87]. Its critical role in tumor-induced immune suppression at the immune checkpoint came from a study showing that TIM-3 marks most suppressed or dysfunctional populations of $CD8^+$ T cells in animal models of solid and hematologic malignancies [89•,90–92]. TIM-3 blockade in these animal models has successfully demonstrated similar anti-tumor activity compared to PD-1 pathway blockade [93], with greater efficacy with the combined use of TIM-3 and PD-1 blockade [19]. Increasing data support the relevance of TIM-3 blockade in human cancer as TIM-3 positive NY-ESO-1 specific $CD8^+$ T cells in patients with melanoma showed dysfunctional phenotypes and TIM-3 blockade restored IFN-γ and TNF-α production as well as the proliferation of NY-ESO-1-specific $CD8^+$ T cells in response to antigenic stimulation [89•]. TIM-3 has now emerged as an immune checkpoint receptor with its selective expression in tumor tissue as well as its critical role in multiple immune suppressive mechanisms, which strongly supports TIM-3 targeted immunotherapies as single or combined modalities. Monoclonal antibodies blocking TIM-3 are under preclinical development by several companies; however detailed information about these is not yet available.

LAG-3

Lymphocyte activation gene-3 (LAG-3, CD223) is another surface molecule of the immunoglobulin superfamily, expressed on activated T cells, NK cells, B cells and plasmacytoid dendritic cells, that plays an important role in negative regulation of T cell proliferation via binding to MHC class II with high affinity [94–96]. LAG-3 is also required for optimal function of Treg. Its co-expression with PD-1 in tumor infiltrating lymphocytes in certain malignancies correlates with impaired $CD8^+$ effector T cell function [97,98]. LAG-3-Ig (Immutep, IMP321, Paris), a fusion protein (antigen presenting cell activator), has been tested in animal models as well as in human clinical trials. Early trial with standard influenza vaccine or hepatitis B vaccine showed high tolerability and Th1 CD4 T cell response was observed in several participants [99,100]. This led to a clinical trial in patients with renal cell carcinoma as a single agent, dose escalation trial [101]. This agent was well tolerated and it appeared to be correlated to development of effector phenotype of $CD8^+$ T cells. There were no objective responses, although several patients showed stabilization of disease. It was then tested in patients with metastatic breast cancer in combination with first line paclitaxel [102,103], which demonstrated an objective response rate of 50% compared to an historical response rate of 25%. Phase III of the clinical trial is expected to be launched in 2015. As eluded to above, previous studies have suggested that dual inhibition of LAG-3 and PD-1 could enhance T effector activity and antitumor activity [17,104] and a clinical trial is actively recruiting patients to assess the safety and tolerability as well as dose limiting toxicity of BMS-986016 alone and in combination with nivolumab in

subjects with advanced solid malignancies (metastatic and/or unresectable; NCT01968109, clinicaltrials.gov). Based on the concurrent expression of both PD-1 and LAG-3 being largely limited to tumor infiltrating lymphocytes, it is expected to have less toxicity than CTLA-4, likely similar to anti-PD-1 antibody therapy.

B7-H3

B7-H3 (B7 homolog 3, CD276) is a type I transmembrane protein which shares 31% of sequence homology with B7-H1 [105,106]. Its transcript has a ubiquitous expression pattern, however, protein expression is limited. It is also found to be highly expressed in a variety of cancer types, including breast, pancreatic, prostate, ovarian and renal cell carcinoma [107–112]. The function of B7-H3 is controversial since both co-stimulatory and co-inhibitory properties have been described [105,113,114], and its expression in cancer is associated with either prolonged survival or poor prognosis. These studies indicate the complex role of B7-H3 in tumor immunity and it appears to be dependent on the contexts of tumor specific micro-environmental factors [115,116]. MGA271, a fully human monoclonal antibody, has been developed to target B7-H3, which demonstrated potent anti-tumor activity in xenograft models of renal cell and bladder carcinoma [117]. Clinical trials with MG271 for multiple refractory cancers that express B7-H3 are ongoing and the result will provide further insights on role of B7-H3 on tumor immunity.

B7-H4

B7-H4 (B7 homolog 4) is a member of the B7 family transmembrane protein identified in 2003, also known as B7S1, B7x and V-set domain containing T cell activation inhibitor (Vtcn1), which binds to unknown receptors on activated T cells and results in inhibition of effector function [118–120]. Its protein expression appeared to be limited to APC as well as cancer cells, although mRNA is detected in most nonhematopoietic tissues [116,121]. Studies have demonstrated the association of high expression of B7-H4 with poor prognosis in human cancers [122–128], inverse correlation with its expression by cancer cells as well as tumor associated macrophages (TAM) and tumor infiltrating lymphocytes, which had been tested in xenograft models by blocking B7-H4 expression [129,130]. Although it has not been yet tested in the clinical setting, B7-H4 may be an attractive target based on its limited expression on tumors as well as TAM and its important roles in immune evasion and tumorigenesis.

VISTA

V-domain Ig suppressor of T cell activation (VISTA, also known as B7-H5 or PD-1Homolog and differentiation of embryonic stem cells 1; Dies 1) is a negative checkpoint regulator and shares homology with PD-L1 [131•,132,133]. It is mainly expressed on hematopoietic compartment and highly expressed on myeloid lineage, especially APCs. VISTA induces forkhead box p3 (FOXP3) expression in T cells, and VISTA-expressing APCs or VISTA-Ig fusion protein inhibits T cell proliferation and *in vitro* cytokine production. [134]. In mouse models, its expression is positively correlated with CD11b, also found on tumor infiltrating lymphocytes, and the blockade of VISTA enhances antitumor immunity [135•]. Its expression on cancer cells enhances tumor-invasion by augmenting type I matrix metalloproteinase (MMP) [136,137] and it may offer therapeutic application for human cancer.

CEACAM1

Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1, CD66a) is a transmembrane glycoprotein that belongs to the carcinoembryonic antigen family and is also a member of the immunoglobulin superfamily [138–140]. It is composed of sequentially ordered immunoglobulin-like domains, expressed on a variety of cells, and has multiple biological functions, especially cell to cell adhesion [141]. It has been reported that CEACAM1 is an independent poor prognostic factor for patients with melanoma and it strongly predicted the development of metastatic disease [142]. CEACAM1 acts as an inhibitory molecule that negatively regulates cytotoxic T cell proliferation, which is activity evidenced by direct T cell receptor cross linking [143], and its homotypic interaction inhibits natural killer cell mediated killing as well [144,145]. Interestingly, a recent study by Huang *et al.* showed that the presence of CEACAM1 endows TIM-3 inhibitory function. CEACAM1 facilitates the maturation and surface expression of TIM-3 by forming a heterodimeric interaction, and co-blockade of CEACAM1 and TIM-3 enhanced the anti-tumor immune response [146••]. Monoclonal antibody targeting CEACAM1 was developed and showed that it efficiently blocks CEACAM1 homophilic interactions and renders melanoma cells susceptible to T cell mediated cytotoxicity both *in vitro* and *in vivo* [147]. Other than melanoma, several other malignancies, such as NSCLC have a prognostic association between CEACAM1 expression. Therefore, CEACAM1 targeted immunotherapy will be developed clinically soon.

BTLA

B and T lymphocyte attenuator (BTLA, CD272) is a type I membrane glycoprotein identified as an inhibitory receptor, which has structural similarities with CTLA-4 and PD-1 and is mainly expressed by immune cells [148,149]. Contrary to other B7 family members (immunoglobulin superfamily), its ligand, Herpesvirus entry mediator (HVEM) is a member of TNF superfamily [150,151]. HVEM has additional partners in addition to BTLA, such as CD160, LTα and LIGHT. Depending on its partner, CD160, BTLA, LIGHT/HVEM pathway delivers bidirectional signaling [152–154]. BTLA over-expression has been reported in hematological malignancies as well as

melanoma and it appears to be associated with impaired tumor-specific T cell activity, especially associated with PD-1 expression [155–158]. In one study, coupled with PD-1 and TIM-3 blockade, BTLA inhibition enhanced the expansion, proliferation and cytokine production of tumor specific $CD8^+$ T cells [159] and this suggests that these three checkpoint molecules play an important role in immune evasion in patients with melanoma.

CD200–CD200R

CD200R is a type I membrane glycoprotein, which belongs to the immunoglobulin superfamily, expressed on myeloid cell as well as lymphoid cells, such as NK cells, $CD4^+$ and $CD8^+$ T cells [160,161]. In contrast to CD200R, CD200 (OX2) is widely expressed on immune and non-immune cells [162], CD200-CD200R pathway has an important role in regulating the strength of the initial response to infectious challenge in order to maintain homeostasis [163,164] and the role of anti-cancer immunity emerged from these studies have shown poor prognostic implication of CD200 expression on multiple myeloma, acute myeloid leukemia, B cell chronic leukemia, ovarian cancer and melanoma [165–167]. Studies have revealed that suppression of anti-tumor activity comes from direct CD200–CD200R inhibitory signaling on NK and myeloid cells [168] and indirect effect on T cells via DCs or macrophages [168]. CD200 expression on non-malignant cells appears to play a significant role as evidenced by CD200 transgenic mice, with larger primary tumor formation as well as lymph node metastases [169]. This implies a systemic effect of CD200-CD200R pathway on anti-tumor immunity and it can be targeted even with CD200 negative tumors. A monoclonal antibody blocking CD200 (ALXN6000: samalizumab) has been tested in patients with B-CLL and multiple myeloma (Clinical trials.gov Identifier: NCT00648739). The study showed 36% (8/22) of evaluable patients demonstrated 10% reduction in bulky disease. One patient had confirmed partial response (71% reduction in bulky disease). One thing to pay attention to block this pathway is that CD200–CD200R is an important regulator of inflammation that may promote cancer growth [170]. Increased inflammation from CD200–CD200R blockade, therefore, may result in unwanted effect of tumor growth or progression [171]. Thus, it would be important to find the fine balance in order to block this pathway for further clinical development [172•].

TIGIT

TIGIT (T cell Ig and ITIM doman, also called Vsig 9, Vstm3 or WUCAM) is a CD28 family receptor protein expressed on T cells and NK cells upon activation, binds to two ligands, CD155 (PVR: poliovirus receptor) and CD112 (PVRL2, nectin-2) on antigen presenting cells that leads to tolerogenic phenotype [173,174]. It shares ligands with CD226 (DNAM-1) and TIGIT/CD226 forms a network that regulates human T cell function as CD28/CTLA-4–CD80/CD86 pathway does. CD226 delivers positive signal, and TIGIT delivers inhibitory signal [175,176]. This pathway appears to be involved in multiple autoimmune diseases, such as type I diabetes and multiple sclerosis [177,178]. Transgenic mouse studies have shown that TIGIT deficiency per se does not cause spontaneous autoimmunity. However, these mice do have augmented immunity upon immunization [179]. Inhibitory role in CNS autoimmunity was observed in a series of studies which showed increased experimental autoimmune encephalitis (EAE) severity upon TIGIT blockade [180]. These studies suggest that TIGIT plays a role in regulating peripheral immune tolerance and future studies will define its role in anti-tumor activity as well as potential therapeutic application.

Combined checkpoint blockade

Preclinical studies combining CTLA-4 and PD-1 blocking antibodies demonstrated superior antitumor activity [70,181••,182] with similar toxicity profile as anti-CTLA-4 monotherapy. The first phase I clinical trial combining ipilimumab and nivolumab [183••] was updated in 2014 ASCO annual meeting [184] with an impressive 2 year survival of 79% (objective response rate of 43%) among patients with advanced melanoma, although it did show increased toxicity compared to either agents alone (62% of grade 3/4 toxicities). A phase III study has completed the accrual, and this combinatorial treatment is being tested in other cancers. This combination strategy was also studied with a tumor vaccine, which showed effective restoration of T cell rejection function in animal models [185].

Combination treatment with checkpoint blockade and small molecule inhibitors is an attractive option given the potential for increased tumor antigen presentation, a larger window period for immunotherapy and the feasibility to deliver full doses of each agent due to different mechanisms of action [186,187•,188–190]. Combination treatment of ipilimumab and vemurafenib was studied for patients with advanced melanoma; however, the trial was terminated prematurely due to significant hepatotoxicity [191]. The etiology of hepatotoxicity is not clear, but likely related to paradoxical activation of the MAPK pathway. Early clinical studies has been launched with anti-PD-1 or anti-PD-L1 antibodies combined with BRAF plus MEK inhibitors for patients with BRAF mutated melanoma given the potential benefit of mitigating paradoxical activation of MAPK pathway although there was a concern for immune cell inhibition [192]. These studies will address the feasibility of a triple combination, toxicity profile as well as the effect on immune cell that is highly relevant to anti-tumor activity. Another attractive strategy is to combine checkpoint blockade with B cell receptor signaling inhibitors (ibrutinib and idelalisib), which were approved for B cell lymphoid malignancies. Recent studies demonstrated

the effects of these agents on T cell activities, especially on Tregs [193••,194,195••,196] and future studies will need to define clinical activity of this combinatorial treatment.

Other checkpoint blockade combinations are in the early clinical developmental stage, combining anti-PD-1 with antibodies blocking LAG-3 or TIM-3. LAG-3 and TIM-3 are being considered as T cell exhaustion markers and can be targeted with PD-1/L1 pathway blockade supported by pre-clinical models demonstrated promising anti-tumor activity [17,19,92,97]. Phase I study comparing LAG-3 vs. LAG-3 plus PD-1 blockade among patients with advanced solid tumors has just launched (NCT01968109) and future studies will tell us whether these combinations are feasible with manageable toxicity and improved responses compared to anti-PD-1/L1 monotherapy.

Future direction and concluding remarks

It is exciting time to witness how immunotherapy, specifically treatment with checkpoint blockade monoclonal antibodies, is becoming one of the main armamentarium for cancer therapy. Unprecedented durable response among patients with advanced melanoma, Hodgkin disease, renal cell carcinoma, lung and bladder cancers, among others, with anti-PD-1 or anti-PD-L1 antibody monotherapy has set the stage to revolutionize treatment approaches for patients with advanced cancer. However, a lot of further work needs to be done in order to define biomarkers to predict the response to anti-PD-1 therapies and identify ideal combinations among checkpoint blockade as well as small molecule inhibitors, chemotherapy and radiotherapy through rational design of the study based on scientific evidence. There are several emerging checkpoint molecules that can be targeted. We have mainly discussed checkpoint blockade, however, immune receptor stimulators are also in active investigation along with others in pre-clinical development, such as ICOS (CD278) agonistic antibody which is being evaluated in combination with ipilimumab. Another promising strategy is the combination of agonistic antibodies targeting CD137 (41BB) (BMS-66513 or PF-05082566) have been tested in phase I and II studies for patients with solid tumors, including melanoma, renal carcinoma, and ovarian cancer. The utilization of these agents to modulate our immune system to against cancer is eagerly anticipated, in particular in combination with checkpoint blockade therapy.

Conflict of interest

A.R. has served as a compensated consultant for Amgen, Compugen, Daiichi-Sankyo, Genentech-Roche, GSK, Merck and Novartis with the honoraria paid to the institution.

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