Published in final edited form as:

Adv Cancer Res. 2019 ; 143: 145–194. doi:10.1016/bs.acr.2019.03.003.

Co-stimulatory and co-inhibitory pathways in cancer immunotherapy

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Abstract

Cancer remains the leading cause of death worldwide. Traditional treatments such as surgery, radiation, and chemotherapy have had limited efficacy, especially with late stage cancers. Cancer immunotherapy and targeted therapy have revolutionized how cancer is treated, especially in patients with late stage disease. In 2013 cancer immunotherapy was named the breakthrough of the year, partially due to the established efficacy of blockade of CTLA-4 and PD-1, both T cell co-inhibitory molecules involved in tumor-induced immunosuppression. Though early trials promised success, toxicity and tolerance to immunotherapy have hindered long term successes. Optimizing the use of co-stimulatory and co-inhibitory pathways has the potential to increase the effectiveness of T cell-mediated antitumor immune response, leading to increased efficacy of cancer immunotherapy. This review will address major T cell co-stimulatory and co-inhibitory pathways and the role they play in regulating immune responses during cancer development and treatment.

Keywords

Cancer immunotherapy; Co-stimulation; Co-inhibition; combination therapy; autoimmunity

Introduction:

Recent advancements in the field of tumor immunology have shown that harnessing the potential of the immune system is needed to eradicate cancer. Immune responses are modulated by a complex network of checks and balances, which include co-stimulatory and co-inhibitory pathways involved in regulating T cell activation and function. Activation of T cells requires at least two signals delivered by the antigen presenting cells (APC). The first signal is antigen presented in the form of peptides bound to a major histocompatibility complex (MHC). The peptide recognized by the T cell receptor and this interaction provides specificity to the T cell response. The second signal is provided by co-stimulatory ligands on APCs that interact with corresponding receptors on the T cell surface. Without co-stimulation T cells will either die or become anergic [1]. T cells require co-stimulatory signals for optimal proliferation, differentiation, and survival making co-stimulation

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Conflict of Interest

Authors have declared that there is no conflict of interest.

necessary to induce productive immune responses. Antitumor immune responses depend on efficient presentation of tumor antigens and co-stimulatory signals by host antigen presenting cells. Cancer evades the immune system through various mechanisms including the use of regulatory cells, anti-inflammatory cytokines, decreased stimulatory receptors, defective antigen presentation, T cell tolerance, and apoptosis. Traditional therapies including chemotherapy, radiation, and surgery are often accompanied with transient immune suppression. This can increase a patient's risk of infection along with decreased ability of immune responses to cancer [2]. Though these pillars of cancer treatment are still used today new strategies for tumor treatment are developing. New therapies include using cellular targets such as T regulatory cells and myeloid derived suppressor cells, vaccine therapy, and adoptive T cell transfer therapy.

The latest tumor immunotherapy is molecular therapy directed against co-stimulatory and co-inhibitory markers in the tumor microenvironment. The expression of these markers is highly versatile and responsive to changes in the tissue environment, making them ideal candidates for tumor immunotherapy. Co-stimulatory and co-inhibitory signals can be broken down into a few major families, two of which are addressed in this review (Figure 1), the Ig family and the TNF family. Studies into CD28 were the first to explore and identify the Ig superfamily of receptor-ligand pairs. Interactions between receptors and ligands in the Ig family are key for T-B cell interaction and development of some secondary lymphoid organs. This family includes ICOS, PD1, LAG3, CTLA-4 and CD28 [3]. These receptors were first thought to only control the expansion and survival of naïve T cells, but more recently have been shown to have both co-stimulatory and co-inhibitory functions on naïve and activated T cells [4]. The TNF superfamily of ligands and receptors provide key communication between various cell types during development. This family consists of TNFR1 and 2, HVEM, CD30, 4-1BB, OX40, GITR, CD27, and CD40. Many members of the TNFR family share ligands and downstream signaling pathways creating a dense network of co-stimulatory and co-inhibitory pathways [5]. Understanding the mechanism of action of these receptors and ligands will help achieve therapeutic effectiveness in human diseases.

Ig-Superfamily Receptors

CD28 and CTLA-4

Expression and Signaling—CD28 is one of the best characterized co-stimulatory molecules on T cells. Functional CD28 is critical for co-stimulation of naïve T lymphocytes and T regulatory (Treg) cells [6, 7]. Without CD28 co-stimulation T cell receptor (TCR) signaling often induces an anergic state or cell death [8]. CD28 plays a critical role in the survival of both effector T cells and Treg cells, as shown by the rapid expanse of T cells in culture after CD28 ligation [9]. The function of CD28 in activated T cells is counteracted by CTLA-4, which competes for ligand binding at the immune synapse. CD28 and CTLA-4 compete for binding to ligands CD80 and CD86. Since CTLA-4 displays a higher avidity for the ligands it displaces CD28 [10]. CTLA-4 dampens T cell responses, potentially through increasing the threshold for TCR signaling, which can protect against the development of autoimmune diseases, such as lupus and autoimmune thyroid disease [8]. CTLA-4 reduces

IL-2 production and increases apoptosis, whereas CD28 increases IL-2 production and leads to increased cell survival [11]. *In vitro* assays show that CTLA-4 blocks the activation of transcription factors preferentially activated by CD28, including cJun and NFκB [12].

Immunotherapy—As humans age CD28 levels decrease on T cells, especially $CD8^+$ populations, which may decrease the potential use for treatment in aging cancer populations [13]. Previous studies suggest that this decrease may be an adaptation of the immune system against chronic stimulation. It was noted that the change was due to a decrease in the percentage of $CD28^+$ cells, not in the expression of CD28 per cell [14]. Early attempts at manipulating CD28 in disease were unsuccessful partially because of the low avidity of CD28 for its ligands and nonspecific polyclonal T cell activation. In contrast, CTLA-4 was very effective at binding CD80/86. CTLA-4 blocks the engagement of CD28 with CD80/86 and is able to inhibit the progression of cell cycle, differentiation, and survival making it an ideal treatment candidate for long-term organ graft survival [15–17]. Studies show that tumor cells transfected with CD80/86 become more immunogenic and are subsequently rejected, increasing interest in using this pathway for tumor immunotherapy [18, 19]. Early CD28 super-agonist trials were associated with serious toxicities and abandoned in phase I clinical trials [20, 21]. Since then, localized and targeted use of CD28 monoclonal antibodies (mAbs) has been tested for improved effects compared to early super-agonists [22].

Chimeric antigen receptor modified T cells (CAR-T) have been created in the hopes of harnessing the antibody specificity, homing, tissue penetration, and target destruction of T cells to fight B cell lineage malignancies. The chimeric receptor features the extracellular antigen binding domain from a tumor specific monoclonal antibody, typically anti-CD19. The transmembrane and intracellular domains of the receptor are derived from T cell signaling molecules, including CD3 and costimulatory signaling domains. The second generation of CAR-T cells used the CD28 co-stimulatory cytoplasmic domain to further enhance T cell function [23]. Studies have shown a complete response rate of over 90% when treating pediatric or adult acute lymphoblastic leukemia (ALL) with second-generation CAR-T cells. When treating solid tumors, the efficacy of CAR-T therapy is reduced. This may be due to several reasons including immunosuppressive factors present in the tumor microenvironment and T cell access to tumors. This immunosuppressive barrier has prompted further studies into third-generation CAR-T cells, which combine multiple intracellular costimulatory domains to enhance cytotoxicity and durability, and more recently T cells redirected for universal cytokine mediated killing (TRUCKs). TRUCK cells are developed from second-generation CARs with additional genes for cytokine production and release [24].

CD28 and CTLA-4 are critical regulators in autoimmune disease and tolerance to solid organ transplants. Animal models using CD28 deficient mice have shown a reduction of disease intensity in some autoimmune diseases [25–27]. In fact, $CD28^-$ T cells have been used in transplants to promote tolerance by tolerizing allogeneic antigen presenting cell (APCs). The interaction of CD28- T cells with allogeneic APCs induced the expression of inhibitory receptors and down-regulation of costimulatory molecules on the APCs. This in turn converted effector T cells into suppressive $FOXP3^+$ T regulatory cells [28]. But, it is unclear to date whether $CD28^-$ T cells are the cause or consequence of infectious and

inflammatory conditions [29, 30]. Studies of mutations in the CTLA-4 locus highlighted the importance of CTLA-4 in immune homeostasis. Patients with CTLA-4 mutations were observed to have decreased suppressive function in Treg cells and extensive $CD4^+$ T cell infiltrate in several organs [31, 32]. Clinical trials using CTLA-4Ig for reversing autoimmune disease and inducing allograft tolerance are currently ongoing in addition to the proven use of CTLA-4Ig in arthritis and prevention of rejection of renal transplants [33–35]. Currently, the long-term benefits of CTLA-4Ig treatment outweigh the potential drawback of lymphoproliferative disorder [36, 37]. The use of CTLA-4 on Treg populations *in vivo* has shown contradictory results in solid organ transplant models. Some studies show no change to circulating Treg cells, but a significant increase in FoxP3/CD3 ratios in graft biopsies after CTLA-4 treatment [38]. Other studies show that CTLA-4 antibodies *in vivo* do not promote the expansion of Treg cells and will not induce tolerance in solid organ transplants [39]. Many clinical trials looking at different disease types are still ongoing for both CD28 and CTLA-4 (Table 1). Most of the completed trials used CTLA-4 blockade as a monotherapy, but ongoing and future clinical trials are increasingly combining this therapy with other traditional or molecular therapies. FDA has approved a monoclonal antibody targeting CTLA-4 (ipilimumab) to treat melanoma and more recently renal cell carcinoma. Unfortunately, treatment has been associated with severe and potentially fatal adverse side effects in about 10–20% of patients. Dermatologic toxicity, enterocolitis, hepatotoxicity, and neurological toxicity are the most commonly reported immune related adverse events following treatment [40, 41].

ICOS and ICOS-L

Expression and Signaling—ICOS was named for being an inducible co-stimulatory molecule found mainly on activated $CD4^+$ T cells. Expression has not been found on resting T cells but is rapidly induced on $CD4^+$ and $CD8^+$ T cells following TCR engagement [42–45]. The ligand for ICOS, ICOS-L, is expressed on professional APCs including B cells, DCs, and macrophages [46]. ICOS has a similar structure and signaling pathway to CD28, promoting T cell proliferation and cytokine secretion. Studies using CD28 knockout mice have shown that ICOS is a more potent activator of the PI3K/Akt pathway than CD28 and can lead to enhanced activation of downstream MAPKs cascade [43, 47–49]. ICOS-dependent $CD4^+$ T cell proliferation is independent of IL-2 [43, 45, 50]. ICOS favors Th2 and Th17 lymphocytes, but is critical for the induction, maintenance, and homing of Tfh cells [42, 51–54]. Studies using ICOS deficient mice have shown that ICOS deficiency on T cells does not affect primary clonal expansion of memory $CD4^+$ T cells, but these cells have defective reactivation *in vivo* [55]. Similarly, ICOS-deficient patients have decreased circulating $CD4^+$ memory T cells [56].

Immunotherapy—ICOS can enhance or dampen Th1 or Th2 responses dependent on the pathogenic challenge [57]. Common variable immunodeficiency (CVID), characterized by low serum gamma globulins, occurs in humans that have a homozygous ICOS deficiency. Patients with ICOS gene mutations had increased susceptibility to infections, mainly due to defects in B cell compartments [58, 59]. Studies involving ICOS have mainly pursued its role in hypersensitivity and autoimmune diseases because of its expression on Th2 and Tfh cells [60]. Lupus patients treated with anti-ICOSL antibodies have diminished antibody

production and decreased disease associated symptoms. Neutralization of ICOSL on innate lymphoid cells has also been shown to decrease release of cytokines central to airway hypersensitivity. Transplantation studies looking at modulating ICOS have had less definitive results. Cardiac allograft survival in rats increased after anti-ICOS treatment, but in kidney transplant models no effect was observed. In cardiac allograft models the combination of an ICOS blockade with a CD28 or a CD40 blockade increased long-term allograft survival [57, 61, 62]. Other studies have shown that a blockade of ICOS lessens GVHD, especially when used in combination with traditional transplant therapies or CD28 blockade [63, 64].

Since ICOS is only expressed on activated T cells, there is interest in using it to identify immunocompetent T cells within tumors [49]. Studies analyzing the peripheral blood from colon cancer patients identified ICOS expression, along with other B7 family members, as significantly decreased compared to control expression, suggesting that ICOS expression profile may serve as an early indicator of colon cancer [65]. ICOSL is highly expressed on human melanomas and may directly drive activation and expansion of Treg cells in the tumor microenvironment. Therefore blockade of ICOSL or ICOS may have a therapeutic benefit by decreasing intratumoral Treg cells [66]. In preclinical models of melanoma and pancreatic cancer, ICOS activation had a synergistic effect with CTLA-4 blockade. The combination therapy was able to reject established melanoma and prostate cancers in mice. The authors hypothesize that this occurs because CTLA-4 blockade enables activation of tumor-reactive T cells with up-regulation of ICOS. Then the engagement of ICOS, through the IVAX vaccine, a whole cell vaccine consisting of ICOS-L expressing B16 melanoma, is able to further enhance T cell proliferation, survival and migration into the tumor [67]. Though few trials using anti-ICOS therapies are ongoing, preclinical tumor data have led to four new trials that were recently proposed (Table 1).

LAG-3 (CD223)

Expression and Signaling—LAG-3 was once identified as a marker of Treg cells [68]. It was discovered to be upregulated on activated $CD4^+$ and $CD8^+$ T cells as well as NK cells [69]. LAG-3 resembles the CD4 co-receptor and binds to MHCII with a higher affinity, but it may also have additional ligands. The binding of CD4 and LAG-3 to MHC II is required for interaction with the APC [70, 71]. LAG-3 functions as a negative regulator of T cell responses, especially expansion, to MHC II restricted antigen presentation [70, 72, 73]. Studies using LAG-3 deficient mice have shown that signaling of LAG-3 decreases homeostatic expansion of both $CD4^+$ and $CD8^+$ T cells. Dysregulation of T cell homeostasis, due to the lack of LAG-3 expression, resulted in the expansion of other cell types including B cells, macrophages and dendritic cells [71]. LAG-3 is co-expressed with PD1 on exhausted $CD8^+$ T cells. Antibody studies have shown that antagonism of LAG-3 and PD1 synergistically reactivated exhausted $CD8^+$ T cells, suggesting a non-overlapping role of LAG-3 and PD1 in regulating immune responses [74, 75].

Immunotherapy—Since LAG-3 was identified on Treg cells, it was also studied in autoimmune disease onset. Though LAG-3 deficiency alone does not result in autoimmunity, when combined with a non-obese diabetic (NOD) mouse background it results in 100%

diabetes onset at a time when other NOD mice exhibit only 15% disease onset. Studies have also shown that blocking LAG-3 can exacerbate type 1 diabetes [76, 77]. Animal models of allergen-specific immunotherapies show a positive correlation between LAG-3 expression and IL-10 production from Treg cells [78]. Together these studies suggest an immunoinhibitory role of LAG-3 on T cell function.

When studied in models of cancer and chronic viral diseases, LAG-3 is highly expressed on dysfunctional and exhausted T cells. These T cells have defects in proliferation and effector function [79]. Using LCMV clone 13 to model chronic infection, LAG-3 correlated strongly with severity of infection. Virus specific $CD8^+$ T cells co-expressed PD1 and LAG-3. The blockade of LAG-3 alone had little effect on T cell activity, but when combined with PDL1 blockade T cell response was dramatically improved [74]. In the setting of chronic malaria infection, combined blockade improved parasite clearance mediated by $CD4^+$ T cells. The combined blockade of PD-1 and LAG-3 was able to clear blood stage malaria as well as prevent chronic infection. This is believed to be due to the combination therapy increasing Tfh and plasma cells, which clear the infection [80]. Patients with melanoma and colorectal cancer often have high expression levels of LAG-3 on Treg cells expanded in PBMC, lymph nodes, and tumor tissue. These LAG-3 expressing Treg cells produce high levels of IL-10 and TGF-β [81]. Tumor specific T cells that were LAG-3^+PD1^+ had impaired IFNɣ and TNFα production compared to single positive cells. LAG-3 and PD1 are often co-expressed on tumor infiltrating lymphocytes (TILs), and the blockade has significantly improved anti-tumor T cell responses. Combination LAG-3 and PD1 blockade during T cell priming augmented the proliferation and cytokine production of tumor specific $CD8^+$ T cells [75, 82]. In addition, increased LAG-3 expression on tumor infiltrating T cells is associated with poor prognosis of colorectal cancer as well as ovarian cancer [83]. Blockade of LAG-3 synergizes with anti-tumor vaccination to improve $CD8^+$ T cell activation. This was shown to be a due to a direct role of LAG-3 on $CD8^+$ T cells and independent of its role on $CD4^+$ T cells [84]. Together, these promising preclinical data with viral and tumor models have led to increased interest in new clinical trials using LAG-3 blockade as a monotherapy and in combination with other existing immunotherapies (Table 1). However, future studies on LAG-3 still need to look into its function in modulating effector T cells, Treg cells, and NK cells in different diseases and disease stages.

PD1 and PDL1/PDL2

Expression and Signaling—After the immunosuppressive function of PD1 and its ligands was discovered, immense interest and investment have been placed into studies of the receptor/ligand pair. PD1 is expressed on T lymphocytes during thymic development. The expression of PD1 on early thymocytes weakens the interaction of autoreactive T cells with DCs presenting self-antigen [85, 86]. PD1 is also expressed on activated T cells, B cells, NKT cells, monocytes and some DC subsets. The expression of PD1 is upregulated upon antigenic stimulation of T cells, both $CD4^+$ and $CD8^+$, and B cells [87]. One ligand, PDL1, is expressed on resting T cells, B cells, DCs, macrophages, parenchymal cells, endothelial cells, and pancreatic islets. PDL1 can be expressed on hematologic and non-hematologic tissues. Antigen presenting cells often co-express CD80/86 with PDL1 [88]. The second ligand, PDL2, is more selectively expressed on activated DCs, macrophages, and

B cell subsets [85, 87]. PDL2 has a 3-fold higher affinity than PDL1 for binding with PD1 [86]. Interaction of PD1 and its ligands leads to inhibition of T cell receptor mediated proliferation and cytokine secretion, thereby acting as a major mechanism of peripheral tolerance. The interaction can also decrease the cytolytic function of T cells and NKT cells [85, 87, 88]. PD1 is a member of the CD28 family of T cell regulators. The trans-membrane domain of PD1 resembles the Ig region of the CD28 family. Phosphorylation of PD1's immunoreceptor tyrosine-based switch motif (ITSM) results in the direct dephosphorylation of signaling molecules directly downstream of the TCR. Ligation of PD1 augments PTEN inhibition of PI3K activity to decrease T cell proliferation, survival, protein synthesis, and IL-2 release [86].

Immunotherapy—PD1 expression and signaling have been shown to be important in many aspects of immune dysregulation such as autoimmunity, infectious disease, and cancer. PD1 helps regulate the balance between stimulatory and inhibitory signals needed for the maintenance of T cell homeostasis and immune responses. The complete knockdown of PD1 in animal models results in a breakdown in peripheral tolerance in hosts [88]. The high expression of PD1 and PDL1 on Treg cells enhances the expansion of these cells, which control the development, maintenance, and function of peripheral responses [87]. In viral models, PD1 is rapidly upregulated following viral infection, but downregulated as the virus is cleared. In cases of chronic infection, $CD8^+$ T cells express PD1 constitutively, leading to the "exhausted" T cell phenotype [85]. Studies of HIV were among the first to show that a PD1/PDL1 blockade could regain effective immunity [89]. In HIV studies, it was discovered that PDL1 could be upregulated on neutrophils, and expression was induced by IFNα, TLR7/8, and LPS. The upregulation of PDL1 on neutrophils in HIV is correlated to T cell exhaustion and expression of PD1 on T cells [90]. Animal studies using mouse hepatitis virus (MHV) in PD1 deficient mice showed significant tissue damage, especially of the liver. Blockade of IFNɣ and TNFα in the infected mice led to reduced mortality, suggesting that a major role of PD1 in viral infection is controlling the release of pro-inflammatory cytokines [91]. A similar role for PD1 control of cytokine secretion was observed in models of autoimmune disease. In diabetes models, ligation of PD1 leads to the reduction of cytokine production through the truncation of TCR signaling. The blockade of PD1 or PDL1 led to increased onset of diabetes [92]. The role of PD1 in other autoimmune diseases is not as well defined. PD1 deficient mice often display lupus-like disease, but the blockade of PD1 in established lupus delays disease progression. In animal models of lupus, the expression of PDL1 on Treg cells suppresses autoreactive B cells that express PD1 [87, 93].

Many of the major breakthroughs using PD1 therapy have been in studies of cancer. Tumors often use this pathway to silence the immune system. Tumor cells can evade T cell killing by upregulating PDL1 and PDL2 following T cell infiltration and release of IFNɣ [85, 86]. Functional PDL1 expression on tumors can also suppress IL-2 production by T cells to further inhibit T cell proliferation and survival within the tumor microenvironment [94, 95]. In most tumors the expression of PDL1 is a poor prognosis for overall survival and tumor control, and early studies using an antibody blockade of PDL1 have had promising outcomes with higher response rates and durable responses [96, 97]. Blockade of this pathway in late stage cancers can significantly reduce tumor burden and promote a durable tumor regression

as seen in studies of advanced melanoma and renal cell cancer [98, 99]. The blockade of this pathway has recently been used in many studies looking at the combination with co-stimulatory agonists on T cells. Though PD1 can limit proliferation of T cells, it is dependent on strength of signal delivered via the TCR and CD28. A strong enough TCR +CD28 signal can overcome PD1 mediated inhibition [88]. The use of PD1 and PDL-1/-2 therapy in clinical trials has been growing rapidly over the years. These treatments have been used in multiple cancer types, autoimmune diseases, viral infections, and much more. The excitement over this pathway can be seen in the dramatic increase in clinical trials that are currently recruiting or recently proposed (Table 1). Recently two anti-PD1 (pembrolizumab and nivolumab) and one anti-PDL1 (atezolizumab) antibodies have been approved by the FDA for use in various solid and hematological cancers, which include melanoma, non-small cell lung cancer (NSCLC), urothelial carcinoma and Hodgkin's lymphoma [100]. Unfortunately, PD1 and PDL1 are also expressed on cardiomyocytes which have recently led to substantial adverse effects. Some patients treated with PD1 or PDL1 inhibitors have developed severe and sometimes lethal myocarditis and heart failure [101, 102].

TIM family Receptors

TIM3 (HAVCR2)

Expression and Signaling—TIM3 was initially discovered as a cell surface molecule that was selectively expressed on IFNγ producing Th1 and $CD8^+$ T cells. More recently TIM3 has been identified on DCs, NK cells, and monocytes [103]. TIM3 is upregulated on mature and functional NK cells, serving as a marker of IFNγ producing NK cells [104, 105]. There are currently several ligands known to interact with TIM3 such as Gal9, HMGB1, and CEACAM1 [106–108]. When co-expressed with PD1 on $CD8^+$ T cells, TIM3 identifies highly dysfunctional cells that are unable to respond to antigen stimulation [109]. These T cells are typically referred to as exhausted T cells. A role for TIM3 in regulating the function of Treg cells has recently been proposed. Studies have shown that $TIM3^+$ Treg cells have superior suppressive function compared to $TIM3^-$ Treg cells [110]. $TIM3^+$ Treg cells have been identified at tumor sites as well as within allografts [111–115]. TIM3 has also recently been shown to indirectly suppress immune function through MDSCs. Overexpression of TIM3 on T cells promoted the expansion of MDSCs [116]. Therefore, when considering a TIM3 blockade on human tumors, it is important to remember that a blockade would affect multiple target cell types. This includes $CD4^+$, $CD8^+$, Treg cells, NK cells, DCs, and MDSCs [117].

Immunotherapy—In autoimmunity and chronic viral infections, TIM3 is a prognostic indicator of disease course and a negative regulator of type 1 immunity. Mouse models have shown that TIM3 blockade can lead to spontaneous autoimmunity. This is due at least partially to dampening the immunosuppressive function of Treg cells. Mice treated with anti-TIM3 antibody showed hyperproliferation of Th1 cells and increased Th1 cytokine release. These studies suggest that TIM3 might act as an inhibitory molecule that serves to reduce cytokine driven inflammation [118, 119]. Treatment with anti-TIM3 antibody in mice also led to development of hyper-acute EAE, which was accompanied by uncontrolled macrophage activation [103]. TIM3 in both humans and mice has a role in regulating

monocyte and macrophage function. Downregulation of TIM3 increases macrophage production of IL-1B, IL-6, IL-10, IL-12, and TNFα, which suggest a role of TIM3 as a regulator of pro- and anti-inflammatory immune responses [112, 120]. TIM3 can also inhibit DC activation by limiting expression of pro-inflammatory cytokines, which leads to reduced inflammation. Studies of mouse and human tumors show that TIM3 is highly expressed on tumor associated DCs. When tumors were treated with a DNA vaccine, TIM3 diminished the efficacy of the vaccine by interfering with the recruitment of nucleic acids into the DC endosomes. This decreased the immunogenicity of tumor cells [121]. Altogether, TIM3 appears to be protective in autoimmune disorders although its expression is poorly defined; in contrast in cancer models TIM3 is highly expressed but contributes to the dampening of protective immunity.

TIM3 marks dysfunctional T cells in cancer [122]. The frequency of $TIM3^+$ T cells correlates with cancer severity and poor prognosis in several cancer types, including NSCLC, and follicular lymphoma (FL) [111, 112]. In models of melanoma, NSCLC, and FL, TIM3 blockade improves T cell function [122, 123]. The effect of TIM3 blockade was further enhanced when used in combination with a 4-1BB agonist [124]. The increased efficacy of the combination treatment may be due to the interaction of TIM3 and Gal9 on tumor cells [125, 126]. The cells that are $TIM3^+$ and $PD1^+$ are often the most dysfunctional in tumor studies [122]. Studies of chronic viral infections have offered insights to how TIM3 blockade may enhance tumor clearance. Using a model of chronic LCMV, TIM3 marks virus specific $CD8^+$ T cells that have the worst defects in pro-inflammatory cytokine production. These virus specific T cells co-express PD1 and TIM3. It was observed that a co-blockade of PD1 and TIM3 was most effective in chronic LCMV models [109]. Blockade of TIM3 in HIV was shown to restore proliferative potential to T cells in response to HIV peptides [127]. The restoration of T cell function after using TIM3 blockade has been repeated in several other models of chronic viral infections [128–131]. In HIV TIM3 can be found on T cells that lack PD1, suggesting that TIM3 and PD1 have non-redundant and synergistic functions in inhibiting T cell responses [127]. This finding has driven studies into using combination TIM3 and PD1 therapy to treat advanced cancers. Though only a few clinical trials have been completed using TIM3 blockade many more have been proposed and are now recruiting patients (Table 1).

TNF-Superfamily Receptors

4-1BB and 4-1BBL

Expression and Signaling—4-1BB (CD137) was first discovered on activated T lymphocytes [132]. 4-1BB has been found on all subsets of T cells including Treg cells. It has more recently been discovered on NK cells, B cell, neutrophils, and cells within the myeloid lineage [133, 134]. Ligation with 4-1BBL was found to induce T cell activation, survival and effector functions [133]. 4-1BB and 4-1BBL ligation especially favored proliferation through IL-2 production [135, 136]. 4-1BB is expressed rapidly after T cell activation and expression remains detectable on activated T cells [137]. Like many of members of the tumor necrosis factor receptor superfamily (TNFRSF), 4-1BB uses TRAF

adapter proteins, namely TRAF2 and TRAF5, that result in increased NFκB and MAP-kinase signaling [138–140]

Immunotherapy—The use of a 4-1BB agonist has shown a role in maintaining immune cell homeostasis. Studies using knockout mice to look at precursor cell turnover in primary and secondary lymphoid organs have shown that 4-1BB also plays a role in myelopoiesis [133]. Agonizing antibodies to 4-1BB in mouse studies have been shown to increase graft versus host disease (GVHD), accelerate rejection of cardiac allografts, and eradicate established tumors. In tumor models, studies suggest that this is due to 4-1BB preferentially stimulating $CD8^+$ T cells, but largely not affecting $CD4^+$ T cells [141, 142]. Agonists to 4-1BB have recently entered phase 1 trials. Phase 1 studies looking at serum samples from patients with solid tumors and B cell non-Hodgkin's lymphoma showed that with agonist 4-1BB treatment tumor infiltrating $CD8^+$ T cells and NK cells significantly increased while effector $CD4^+$ and $CD4^+FoxP3^+$ Treg cells decreased. An increase in pro-inflammatory cytokines was also noted after agonist treatment. The specific agonistic activity increased T cell and NK proliferation and activity to eradicate remaining tumor cells [22, 143]. Targeted therapies, such as cetuximab (EGFR- targeting mAb), can lead to upregulation of 4-1BB on NK cells. NK mediated cytotoxicity of tumor cells following cetuximab therapy was further enhanced when combined with a 4-1BB agonist. This combination therapy is dependent on NK and $CD8^+$ T cells, as depletion of either cell type abrogated therapy efficacy [144]. The role of NK cells in 4-1BB function remains controversial: some murine tumor models show the requirement of NK cells for the functionality of the 4-1BB agonists, while studies using different tumor models show no reliance on NK cells [142, 145]. Combination studies involving radiotherapy and chemotherapy have reported synergism with 4-1BB agonists. When looking at poorly immunogenic tumors, including murine lung carcinomas, spinal tumors, and melanomas, they often became refractory to 4-1BB treatment alone. Yet tumor regression could be achieved even in established tumors.by 'educating' cytotoxic T lymphocytes (CTLs) with a tumor antigen vaccine in combination with 4-1BB agonism. Tumor regression of established tumors was also observed when 4-1BB therapy was combined with an IL-12 secreting vaccine. In studies combining chemotherapy and 4-1BB agonism, mice also rejected a re-challenge with the original tumor, suggesting a long-lasting tumor-specific memory [146–150]. However, treatment with the agonists does result in adverse effects including mild liver inflammation, which is dependent on the presence of 4-1BB, IFNγ, and TNFα [151].

The cytoplasmic tail of 4-1BB has also recently been used in CAR-T cell therapy development. This addition of the 4-1BB tail is critical to support the persistence and expansion of the modified CAR-T lymphocytes. The addition of the cytoplasmic domain of 4-1BB also reprogrammed the CAR-T cells for increased cytokine secretion of predominantly Th1 cytokines, with low or undetectable levels of anti-inflammatory cytokines [152]. Second generation CAR-T therapy generally combined CD28 or 4-1BB cytoplasmic domains with a CD3 intracellular domain in order in increase intratumoral persistence and function, but third generation CARs are now combining more T cell co-stimulatory domains to further enhance persistence. Recent studies including ICOS domain along with a 4-1BB domain saw increased anti-tumor effects and increased persistence *in*

vivo compared to second generation CARs. This study also determined that the placement of each domain in the CAR-T cells determines the *in vivo* functions, only when ICOS was proximal to the cell surface was the third generation CAR most effective [153].

Few clinical trials have been completed using 4-1BB and 4-1BBL therapies, but many more have recently begun recruiting patients. The ongoing or recently completed trials have used 4-1BB as an important component in CAR-T therapy in both hematological tumors and solid tumors (Table 2). Despite initial successes with 4-1BB expressing CAR-T cells in hematological tumor, these cells still face a barrier overcoming the immunosuppressive microenvironment in solid tumors [154].

OX40 and OX40L

Expression and Signaling—OX40 was first discovered on activated $CD4^+$ T cells [155, 156]. Transiently expressed, OX40 appears 12 hours after activation and decreases by 4 days [157]. OX40 expression is important to the survival, expansion and memory formation of T cells [157, 158]. It is also important for the reactivation of $CD4^+$ and $CD8^+$ memory populations [159]. More recently expression of OX40 has been seen on NK cells, NKT cells and neutrophils [160–162]. OX40L has been identified on activated APCs, hematopoietic, non-hematopoietic cells, and activated endothelium cells [163–167]. The ligand is the limiting factor in OX40 signaling [168]. Ligation of OX40 recruits TRAF2 and TRAF3, which activate both the canonical and non-canonical NFκB pathways. This leads to enhanced T cell expansion, differentiation, and generation of long lived memory cells [169, 170].

Immunotherapy—Stimulation of OX40 can cause pro-inflammatory or pro-survival effects dependent on disease type and expression pattern. In animal models of sepsis, anti-OX40L treatment was able to decrease disease severity and improve survival. In this context OX40L activation was macrophage dependent and T cell independent. Patients with sepsis have elevated OX40L on neutrophils and monocytes that correlates to disease severity [171]. Several disease models have been used to determine the role of OX40 in disease progression including asthma, allergies, colitis, GVHD and diabetes. In each of these models, blockade of OX40 has played an important role in reducing disease severity [172–175].

In cancer immunotherapy, agonism of OX40 is thought to play an important role in the reduction of Treg-mediated immunosuppression. In animal models of colon cancer, it was observed that intratumoral injection of an OX40 agonist was able to induce tumor rejection in a $CD8^+$ T cell-dependent manner. The authors show that both Treg cells and effector T cells must be triggered by OX40 for tumor rejection [176]. It has also been shown that OX40 agonist treatment can reactivate memory T cell population, therefore playing an important role in tumor antigen recall and anti-tumor activity. Studies show that in both new and established tumors, OX40 was critical for early priming of $CD8^+$ T cells by APCs and recall response of previously primed $CD8^+$ T cells. OX40 plays a role in the survival and accumulation of these primed $CD8^+$ T cells at the tumor site [177–179]. The agonism of OX40 promotes the proliferation and survival of activated T cells and generates a $CD8^+$ T cell response with increased secretion of IFNγ and granzyme B [180, 181]. Preclinical

models using OX40 agonist have shown effectiveness in sarcomas, CT26 colon carcinomas, breast cancer, and melanoma. Many of these models study the use of OX40 agonists in combination with other tumor treatments. When combined with chemotherapy agents, OX40 agonism was able to provide potent antitumor immunity and decrease the size of established animal tumor models. This combination therapy resulted in a significant decrease in tumor-infiltrated Treg cells, but interestingly an increase in peripheral Treg cells. When used in combination with radiation, OX40 agonists worked by increasing proliferation of tumor antigen specific $CD8^+$ T cells. The combination with radiation significantly increased disease-free survival [182, 183]. Preclinical ovarian cancer models using checkpoint inhibitor combinations have proven successful by engaging multiple immune subsets to combat the tumor challenge. A murine model combining TIM3 blockade and OX40 agonism showed that the combination treatment was able to inhibit ovarian cancer growth significantly better than either mono-treatment. A similar study looking at the combination with anti-PD1 saw tumor growth inhibited in the majority of host mice. This is due to the increase in $CD4^+$ and $CD8^+$ effector T cells combined with a decrease in Treg and myeloid derived suppressor cell (MDSC) populations [124, 184]. From these preclinical models, two humanized anti-OX40 mAbs are currently on the verge of clinical development. Ongoing clinical trials using OX40 monoclonal or combination trials have mainly focused on use in advanced stages of cancers (Table 2).

GITR and GITR-L

Expression and Signaling—GITR was first discovered as a TNF family receptor induced by glucocorticoids [185]. GITR is unique in that it is constitutively expressed on T regulatory cells, but only expressed on effector $CD4^+$ and $CD8^+$ T cells after TCR engagement [186–190]. Expression of GITR has also been reported on dendritic cells, monocytes, and NK cells [188, 191]. The ligand for GITR, GITRL, is highly expressed on activated DCs, B cells, macrophages, and endothelial cells at the site of inflammation [192, 193]. The ligation of GITR recruits TRAF2 and TRAF5 to its cytoplasmic tail, activating MAP-kinase and NFκB signaling pathways [194]. GITR is important in CD28-driven activation of $CD8^+$ T cells by lowering the threshold of activation. GITR is crucial for CD28-mediated NFκB activation; activated $GITR^{-/-}$ T cells had impaired nuclear translocation of NFκB compared to $GITR^{+/+}$ T cells when stimulated with anti-CD3 and anti-CD28 [195].

Immunotherapy—GITR has a critical role in supporting effector T cell activity by inducing T cell proliferation, effector function, and survival. Agonizing GITR on T cells increased effector function by increasing cytotoxic killing of a mouse mastocytoma cell line [195, 196]. In animal models of melanoma, agonism of GITR at early timepoints in tumor growth was able to decrease tumor burden. This is believed to be due to the decreased suppressive function of Treg cells after ligation of GITR [197]. The mechanism of increased effector T cells in the tumor microenvironment is due to direct targeting of GITR on Treg cells. This may work through directly depleting intratumoral Treg cells or via targeting GITRL on suppressive myeloid cells. Agonizing GITR was shown to decrease the stability of intratumoral Treg cells and result in the loss of FoxP3 expression as well as decreased infiltration into the tumor [198, 199]. Agonists to GITR have shown synergism when

combined with vaccines, TLR agonists, and other immunostimulatory mAbs. Combined GITR agonism with a DNA vaccination was able to offer increased protection from a lethal challenge with melanoma. This combination treatment led to prolonged persistence of the antigen-specific $CD8^+$ T cells and enhanced recall response to a booster vaccine. These findings are supported by additional tumor models, including myeloma [200, 201]. When downregulation of Treg activity is critical for success, this pathway has recently been shown to be effective when combined with other therapies, such as PD-1 and CTLA-4 blockade. The combined treatments have shown a durable effect associated with $CD4^+$ and $CD8^+$ memory response. PD-1 combined with GITR therapy resulted in increased frequency of IFNγ producing cells and decreased MDSCs. The tumor microenvironment shifted from immunosuppressive to immunostimulatory [202, 203]. Launched from these preclinical data, a few clinical trials studying the role of GITR have been completed. A number of new trials have been proposed to use GITR therapy on solid and hematological cancers (Table 2).

CD27 and CD70

Expression and Signaling—CD27 is unique as a T cell co-stimulatory molecule in that it is constitutively expressed at significant levels on the majority of T cells, including naïve T cells and Treg cells. CD27 expression is lost on T cells following differentiation and activation but is retained on memory cells [204–206]. CD27 is also expressed on early hematopoietic cells, memory B cells, plasma cells and a subset of NK cells [206–208]. CD27 has recently been shown to drive the cytolytic activity of some subsets of NK cells [209]. Expression of CD27 on naïve T cells helps T cells with low affinity TCRs enter the cell cycle [210]. This pathway is important for sustained effector functions, T cell survival, and development of memory T cells [211, 212]. CD70, the ligand for CD27, regulates the interaction with CD27 because it is only transiently expressed on activated APCs, T cells, NK cells [208, 213, 214]. During chronic inflammation it has been observed that CD70 can become constitutively expressed causing dysregulation of this pathway [215]. The chronic signaling of CD27 has been shown to lead to T cell exhaustion [216]. The ligation of CD27 recruits TRAF2 and TRAF5, which activate the NFκB and c-Jun pathways to promote cell survival, enhance expansion, and increase effector functions [217, 218]. As a co-stimulatory molecule, CD27 counteracts apoptosis by increasing anti-apoptotic Bcl-xl and decreasing FasL on $CD4^+$ and $CD8^+$ T cells [219, 220]. Signaling through CD27 also rapidly induces the expression of Pim-1, which is known to increase aerobic glycolysis and protein translation [221]. SIVA1 can also be recruited to the cytoplasmic tail of CD27. This protein is known to promote caspase dependent apoptosis [222]. Together these findings suggest that under certain circumstances CD27 can act as either a co-stimulatory or a co-inhibitory receptor.

Immunotherapy—Therapies involving the CD27-CD70 pathway must appreciate the pathways inhibitory and stimulatory effects under different immune contexts. During acute challenges CD27 has thus far been regarded as important for the formation and function of effector and memory T cells. Studies analyzing influenza and acute viral challenges are examples of the co-stimulatory role CD27-CD70 have on primary T cell responses. $CD27^+$ and $CD70^+$ cells often secrete higher levels of IFNγ and are therefore more effective at inhibiting viral replication [220]. This is in contrast to studies looking at CD27 in the

context of strong and persistent immune challenges, such as chronic infections or GVHD, where CD27-CD70 have been shown to play an inhibitory role on T cell responses [216, 223–225]. The role of CD27 in diseases is also dependent on tissue context and duration of expression [22]. Use of antagonists or agonists will require precise triggering in specific tissues and cell types.

The CD27-CD70 pathway has been studied in several different autoimmune diseases. In models of lupus high levels of soluble CD27 and $CD27^+$ plasma cells correlate to an increased disease index. Patients also have increased $CD70^+CD4^+$ T cells with a memory-like phenotype. In rheumatoid arthritis patients, CD70 is found in higher levels on naïve and memory $CD4^+$ T cells compared with healthy patients. These T cells also secrete higher levels of IFNγ and IL-17. In mouse models, treatment with an anti-CD70 antibody was able to ameliorate autoimmune disease. Animal studies using an anti-CD70 treatment in multiple sclerosis decreased TNFα production and prevented disease. But in similar models of multiple sclerosis CD27 or CD70 deficiency was seen to exacerbate disease. Increased sCD27 in cerebrospinal fluid has been validated as a biomarker of intrathecal T cell activation in neuro-immunological diseases [226].

Solid and hematological tumors have been reported to have overexpression of CD70 at high levels [227, 228]. CD70 has been identified as a biomarker for renal cell cancers as well as several hematological malignances [229]. Using an anti-CD70 antibody against the malignant T and B cells has been shown to regulate their expansion. Neutralization of CD70 has also been shown to inhibit the signaling of CD27, therefore blocking the activation of and proliferation of Treg cells [228, 230]. In some models of lymphoma expression of CD70 on tumor cells and APCs improves anti-tumor immunity. Unfortunately, in other tumor models intact CD27/CD70 signaling has been associated with decreased anti-tumor immune responses and increased intratumoral Treg cells through the reduction of Treg apoptosis and increased production of IL-2 necessary for Treg survival [231]. Recently, the use of a CD27 agonist has shown protection against intravenous injection of two lymphoma cell lines. The agonism of CD27 induced proliferation and cytokine production from T cells [232]. Since this discovery, ongoing clinical trials for use of a CD27 agonist in combination with PD1 antibodies have been approved for non-Hodgkin lymphoma and some solid tumors [233]. Prostate cancer bearing mice treated with a combination of a DC vaccine and CD27 agonist showed decreased tumor growth and increased T cell proliferation and effector function when compared to controls. A CD27 agonist is also being used to treat B-cell lymphomas and melanomas [232, 234]. Combined results from these studies suggest that the agonism of CD27 can lead to improved anti-tumor immunity and favors long term persistence of TILs [232, 235–237].

Recently preclinical models using CD70 CAR-T cell therapies to treat cancers expressing CD70 have been tested, and this therapeutic strategy has been approved for phase I/II clinical trials. In normal tissue, CD70 is expressed only on activated lymphocytes, therefore targeting this pathway with CD70 expressing tumors may spare toxicity to other normal tissues. Creating CARs with the extracellular binding portion of CD27 along with intracellular co-stimulatory domains of CD28 and 4-1BB in animal models was able to cure tumor bearing mice with limited toxicities [229]. The use of CD70 targeting CAR-T cells

has also recently been tested in preclinical models of gliomas. These preclinical studies show that CD70 expression is associated with poor prognosis in patients with recurrent tumors. This led authors to identify CD70 as an immunosuppressive mediator in part because of its production of pro-tumor chemokines and inducing $CD8^+$ T cell death [238].

Several clinical trials using anti-CD27 as a monotherapy in hematological tumors and autoimmune disease treatment have recently been completed. After early successes, new trials using CD27 and CD70 therapies are actively recruiting or recently proposed (Table 2).

CD40 and CD40L

Expression and Signaling—CD40 was initially characterized on B cells. Expression of CD40 has since been discovered on DCs, monocytes, platelets, macrophages and some non-hematopoietic cells such as myofibroblasts, epithelial and endothelial cells [239, 240]. Depending on cell type and context CD40 can be constitutively or inducibly expressed. CD40L (CD154) is primarily expressed on activated $CD4^+$ T cells. CD40L expression has also been seen on B cells and platelets. In inflammatory settings CD40L can be expressed on monocytic cells, NK cells, mast cells, and basophils [241, 242]. The CD40 receptor has no enzymatic activity and therefore must directly bind TRAF family proteins for signal transduction [243]. TRAF2, TRAF3, TRAF5 and TRAF6 have been shown to directly interact with CD40 receptor. This interaction results in the activation of MAPK signaling, cytokine secretion, proliferation and differentiation [240]. Ligation of CD40 on dendritic cells promotes the induction of other co-stimulatory molecules and facilitates cross-presentation of antigens to the T cells [244]. Signaling of CD40 on DCs results in the heightened expression and increased stability of the MHC-antigen complex [245] and production of pro-inflammatory cytokines. On B cells, CD40 signaling promotes isotype switching, formation of germinal centers, and formation of long lived plasma cells [246]. The CD40-CD40L pathway plays a critical role in the survival of germinal center B cell, DCs, and endothelial cells in both normal and inflammatory conditions.

Immunotherapy—CD40 plays a major role in the initiation of T cell dependent autoimmune disease. CD40 functions during T cell selection in the thymus by promoting medullary thymic epithelial cells (mTECs), which leads to the development of self-tolerance [247]. A disruption of CD40 in the thymus can lead to failure of central tolerance. Signaling of the CD40-CD40L pathway results in the production of proinflammatory cytokines including IL-6. This signaling pathway can also skew differentiation towards Th17 cells [248]. The aberrant expression of CD40 in tissues where it is normally undetectable is a major contributing factor in autoimmune disease initiation [249]. In Grave's disease, an autoimmune disease of the thyroid, CD40 is abnormally expressed on thyroid epithelial cells. This leads to the presentation of autoantigens to T cells [250]. Blocking with anti-CD40L antibodies was able to prevent experimental thyroiditis in mouse models [251]. Antagonistic anti-CD40L antibodies have also been tested in the models of inflammatory bowel disease (IBD). Given at the time of colitis induction, blocking CD40L prevented disease onset, blocked lymphocytic infiltration of the gut, and decreased IFNγ production by gut T cells [252, 253]. Production of IFNγ in the gut can cause upregulation of CD40 on normal colonic fibroblasts. $CD40^+$ cells including DCs, B cells and macrophages are found

within the intestinal mucosa of colitis patients [254, 255]. CD40 signaling in these cell types leads to the production of IL-6, IL-12, and IL-23, which contribute to disease initiation [248]. The CD40-CD40L pathway has been shown to be critical for IBD induction but not required for ongoing inflammatory responses [252]. Patients with active Lupus disease have overexpression of CD40L on $CD4^+$ and $CD8^+$ T cells [256]. Disease activity can be correlated to serum levels of CD40L [257]. Mouse models of lupus have shown that blocking CD40L prior to disease onset can prolong survival and ameliorate kidney disease [258, 259]; If treatment of these mice was stopped disease symptoms returned. Autoimmune models have shown that blocking CD40L prevents the relapse of ongoing disease or halts pathogenic progression in rheumatoid arthriris, lupus, multiple sclerosis, IBD and diabetes [260]. However, the blocking antibody has been ineffective in treatment of some established diseases. Ruplizumab was the first CD40L blockade used in clinical trials [261]. Patients with Lupus and Crohn's disease showed partial therapeutic responses, but trials had to be halted due to development of thromboembolism [260]. Studies of allograft rejection sought to exploit the CD40 pathways because it was believed that the blockade of CD40L would limit APC maturation and decrease CD28 signaling resulting in T cell anergy [262]. Unfortunately, anti-CD40L blocking antibodies failed as a monotherapy in inducing allograft tolerance [263, 264]. This was due to the inability of the therapy to block rejection mediated by $CD8^+$ T cells. Nonetheless, the combination of CD40L blockade and immunosuppressive drugs, such as rapamycin, promotes long term graft acceptance [265].

CD40-CD40L is critical for the development of protective anti-tumor immunity. Since CD40 is expressed on a wide variety of normal tissue it has also been discovered on many tumor types. CD40 was first discovered on bladder carcinoma [266, 267]. More recently it has been discovered on melanoma, prostate cancer, lung cancer, cervical, lymphoma, leukemia, and myeloma [268–271]. Human $CD40^+$ breast tumors have been shown to co-express CD40L that may increase proliferation, motility and invasion of tumor cells [266]. CD40 ligation in B cell malignancies has been shown to cause an increase in expression of anti-apoptotic factors such as Bcl-XL [272]. This protects the malignant cells from apoptosis. *In vitro* studies of non-Hodgkin's lymphoma show that low level constitutive signaling of CD40 leads to increased malignant cell proliferation [273]. Whereas *in vitro* and *in vivo* studies of Burkitt's lymphoma show that treatment with sCD40L resulted in reduced proliferation of tumor cells [270]. Soluble CD40L alone or in combination with chemotherapy in models of breast and ovarian cancer can significantly inhibit the growth of the tumors and increase overall survival [274]. When used in combination with tumor vaccines, anti-CD40L blocking treatment inhibited the generation of protective immune responses and decreased potency of the vaccine [275]. This led to an increased interest in studying agonistic CD40 antibodies, which act as powerful adjuvants for inducing tumor immunity. Agonizing CD40 enhances anti-tumor immune responses by promoting DC maturation, survival, and proinflammatory cytokine secretion [276, 277]. CD40 stimulation also induces the upregulation of other co-stimulatory molecules that promote antigen presentation, priming, and cross-priming of T cells. In mouse tumor models CD40 agonism lead to increased survival of mice to primary tumor challenges, but decreased secondary responses [276]. However, the antibody therapy did induce liver toxicity after prolonged treatment. In addition, combination therapies with other co-stimulatory and co-inhibitory molecules and

cytokines have been performed in mouse disease models with promising results that encourage clinical trials in patients [278–281].

Phase I clinical trials in patients with non-Hodgkin's lymphoma treated with recombinant CD40L showed partial to complete responses three months after treatment [282]. Also, promising results from early clinical trials in chromic lymphatic leukemia and multiple myeloma encouraged development of CD40 mAbs to be used in combination therapy.

Concluding remarks

In the past 30 years, studies of co-stimulatory and co-inhibitory pathways have led to several breakthroughs in understanding of human diseases and development of immunotherapies. These co-stimulatory and co-inhibitory molecules equip immune cells a mechanism to sense environmental conditions and respond appropriately. These molecules create complex interactions due the vast number of receptor/ligand pairs and downstream signaling pathways. As more co-signaling molecules are discovered, many with unique and non-overlapping functions, the simplistic "signal two" model will likely be replaced by a complex tidal model of co-signaling. Therefore, designing combination therapies that target multiple co-signaling pathways as well as the tumor microenvironment may offer the greatest chance of successful treatment of cancer.

Acknowledgement

This work attempts to comprehend most studies on the co-stimulatory/co-inhibitory pathways relevant to cancer immunotherapy. However, we apologize for not being able to be comprehensive in including all publications on this topic due to limitation of space.

Funding

This work was supported by NIH Grant R01 HL135325 (to XC)

Abbreviations:

TCR	T cell receptor
MHC	major histocompatibility complex
APC	antigen presenting cell
mAb	monoclonal antibodies
Treg	T regulatory cell
MDSC	Myeloid derived suppressor cell
CAR	chimeric antigen receptor
DC	dendritic cell

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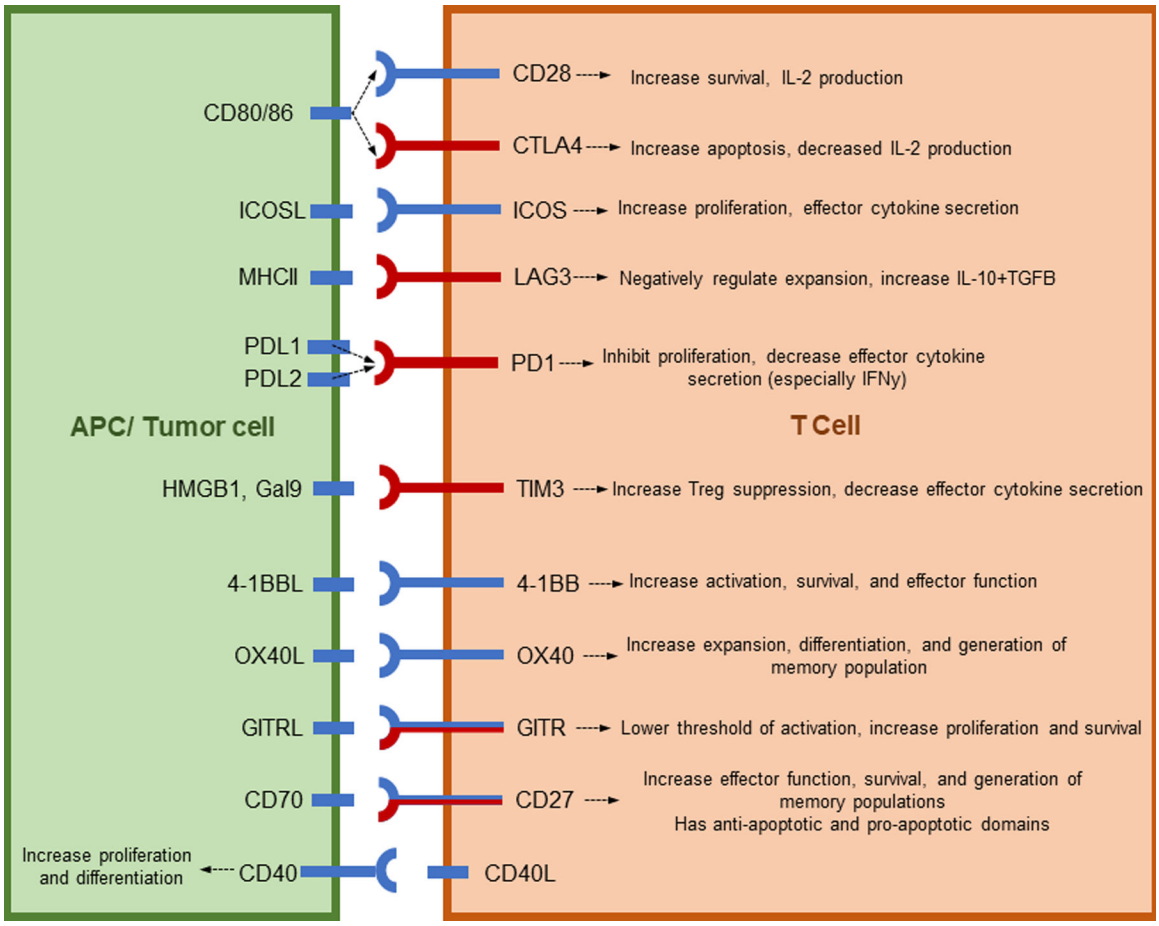

Figure 1:
Costimulatory and coinhibitory molecules discussed in this review.

Table 1:

Ig Superfamily completed, ongoing, and upcoming clinical trials.

Co-stimulatory Molecule	Number	Status	Main Condition Treated		Treatment	Early Phase 1	Phase 1	Phase 2	Phase 3	Phase 4	N/A:
CD28	14	Active, not-recruiting	Hematological tumors Glioblastoma	Hepatitis Other	CAR-T therapy: 10 Anti-viral: 1 No interventions: 3	-	10	2	-	-	1
	33	Completed	Hematological tumors Kidney HIV/viral Autoimmune/ Allergy	Ovarian/ Breast Lung Other	Ex vivo T cells: 9 Anti-viral: 7 Other: 10 No intervention: 7	-	7	10	3	2	8
	50	Moving forward: Not yet recruiting/Recruiting									
CTLA-4	54	Active, not-recruiting	Melanoma Renal Liver Head Neck Lung	Pancreatic Autoimmune/ Allergy Breast/ Ovarian Hepatitis Solid tumor	Monotherapy: 14 Combo-therapy: 37 No interventions: 3	2	18	24	9	1	1
	79	Completed	Melanoma Renal Liver Hematological tumors Lung Prostate	Autoimmune/ Allergy Breast/ Ovarian Hepatitis Pancreatic Solid tumor	Monotherapy: 50 Combo-therapy: 25 No interventions: 4	1	27	32	21	-	2
	182	Moving forward: Not yet recruiting/Recruiting									
ICOS	4	Moving forward: Not yet recruiting/Recruiting									
LAG3	2	Active, not-recruiting	Melanoma Colorectal caner		Combo-therapy: 2	-	1	1	-	-	
	6	Completed	Renal Cell Carcinoma Autoimmune/ Allergy Breast	Hepatitis Other	Monotherapy: 5 Combo-therapy: 1	-	6	-	-	-	-
	39	Moving forward: Not yet recruiting/Recruiting									

Co-stimulatory Molecule	Number	Status	Main Condition Treated		Treatment	Early Phase 1	Phase 1	Phase 2	Phase 3	Phase 4	N/A:
TIM3	6	Completed	Autoimmune/ Allergy Sepsis Glomerulonephritis	Breast Lung	Monotherapy: 2 Combo-therapy: 1 No interventions: 3	-	1	1	1	-	2
	23	Moving forward: Not yet recruiting/Recruiting									
PD1	176	Active, not-recruiting	Hematological tumors Breast/Ovarian HIV/viral Autoimmune/ Allergy Renal	Liver Melanoma Lung Brain Other	Monotherapy: 60 Combo-therapy: 38 No interventions: 2	2	74	78	36	1	2
	65	Completed	Hematological tumors Breast/Ovariaon HIV/viral Autoimmune/ Allergy	Colorectal Melanoma Lung Other	Monotherapy: 30 Combo-therapy: 17 No interventions: 18	-	28	16	3	1	7
	847	Moving forward: Not yet recruiting/Recruiting									
PDL1	98	Active, not-recruiting	Breast/Ovarian Hematological tumors Renal Colorectal caner Liver	Lung Melanoma HIV/viral Other	Monotherapy: 54 Combo-therapy: 41 No interventions: 3	-	29	30	42	-	-
	21	Completed	Breast/Ovarian HIV/viral Autoimmune/ Allergy Brain	Lung Pancreatic Melanoma Other	Monotherapy: 11 Combo-therapy: 5 No interventions: 3	-	9	6	2	-	-
	227	Moving forward: Not yet recruiting/Recruiting									

Table 2:

TNF Superfamily completed, ongoing, and upcoming clinical trials.

Co-stimulatory Molecule	Number	Status	Condition		Treatment	Early Phase 1	Phase 1	Phase 2	Phase 3	Phase 4	N/A:
41BB	2	Active, not-recruiting	Hematological cancer Pancreatic Cancer		CAR-T therapy	1	-	1	-	-	-
	1	Completed	Multiple Myeloma		CAR-T therapy	1	-	-	-	-	-
	17	Moving forward: Not yet recruiting/Recruiting									
41BBL	3	Moving forward: Not yet recruiting/Recruiting									
Ox40	9	Active, not-recruiting	Advanced cancer Autoimmune/ Allergy Head and Neck	Colon Breast Other	Monotherapy: 5 Combo-therapy: 2 CAR-T therapy: 2	-	8	1	-	-	-
	10	Completed	Advanced cancers Autoimmune/ Allergy Glomerulonephritis	Melanoma Hepatitis Prostate	Monotherapy: 4 Combo-therapy: 1 CAR-T therapy: 1 No interventions: 4	-	5	1	-	-	3
	18	Moving forward: Not yet recruiting/Recruiting									
Ox40L	3	Completed	Autoimmune/ Allergy		Monotherapy: 2 No interventions: 1	-	1	1	-	-	1
	2	Moving forward: Not yet recruiting/Recruiting									
GITR	2	Active, not-recruiting	Metastatic/ Advanced tumors		Monotherapy: 1	-	2	-	-	-	-
	3	Completed	Hepatitis Glomerulonephritis Uveitis		No interventions: 3	-	-	-	-	-	3
	7	Moving forward: Not yet recruiting/Recruiting									
CD27	2	Active, not-recruiting	Refractory solid tumors Hepatitis		Monotherapy: 1 Combo-therapy: 1	-	1	-	-	-	1
	25	Completed	Hematological tumors	Kidney	Monotherapy: 15	1	3	10	3	1	2

Co-stimulatory Molecule	Number	Status	Condition		Treatment	Early Phase 1	Phase 1	Phase 2	Phase 3	Phase 4	N/A:
			Autoimmune/ Allergy Colon Cancer Ovarian/Breast	HIV/viral Other	Combo-therapy: 5 Vaccination: 2 No interventions: 3						
	22		Moving forward: Not yet recruiting/Recruiting								
CD70	1	Active, not-recruiting	Advanced cancers		Monotherapy	-	1	-	-	-	-
	5	Completed	Renal Cancer Melanoma Nasopharyngeal Carcinoma		Monotherapy: 5	-	5	-	-	-	-
	4		Moving forward: Not yet recruiting/Recruiting								
CD40	11	Active, not-recruiting	Lymphoma Non-Small Cell Lung Cancer	Melanoma Respiratory disorder	Monotherapy: 8 Combo-therapy: 2 No interventions: 1	-	5	4	1	-	-
	153	Completed	Melanoma Kidney Solid Tumors	Diabetes GVHD HIV/viral	Monotherapy: 116 Combo-therapy: 22 No interventions: 15	1	48	61	18	14	19
	46		Moving forward: Not yet recruiting/Recruiting								