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Immunoregulatory Signal Networks and Tumor Immune Evasion Mechanisms

- **Insights into therapeutic targets and agents in clinical development**

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Abstract

Through activation of immune cells, the immune system is responsible for identifying and destroying infected or otherwise damaged cells including tumorigenic cells that can be recognized as foreign, thus maintaining homeostasis. However, tumor cells have evolved several mechanisms to avoid immune cell detection and killing, resulting in tumor growth and progression. In the tumor microenvironment, tumor infiltrating immune cells are inactivated by soluble factors or tumor promoting conditions and lose their effects on tumor cells. Analysis of signaling and crosstalk between immune cells and tumor cells have helped us to understand in more detail the mechanisms of tumor immune evasion and this forms basis for drug development strategies in the area of cancer immunotherapy. In this review, we will summarize the dominant signaling networks involved in immune escape and describe the status of development of therapeutic strategies to target tumor immune evasion mechanisms with focus on how the tumor microenvironment interacts with T cells.

Introduction to tumor immune evasion mechanisms

Since Burnet [1] and Thomas [2] proposed the tumor immunosurveillance hypothesis in the late 1950s, mechanisms of anti-tumor immunity have been investigated. More than three decades later, studies using mouse tumor models revealed an indispensable role of the immune system in recognizing and destroying cancer cells through both innate and adaptive immune responses (reviewed in [3]). The discovery of endogenous interferon γ (IFN-γ) and perforin from lymphocytes protecting the host against primary tumor development supported the existence of a functional anti-cancer immune system [4-6].

The immune system not only protects against tumor formation, but also sculpts tumor immunogenicity by the so-called" tumor immune editing" mechanism, a progress divided into phases of elimination, equilibrium and escape [7, 8]. At the elimination stage, the immune system successfully deletes transformed tumor cells by the action of innate lymphocytes (NKT cell, NK cell, γδT cells, macrophages and dendritic cells) and through secretion of cytotoxic cytokines or chemokines. Tumor-specific CD4+ and CD8+ T cells are also involved in recognizing and destroying tumor cells that carries antigens not recognized as self (for example mutant proteins with immunogenic neoepitopes, embryonal and other proteins to which the immune system is not normally exposed). In the equilibrium phase, there is a balance between the anti-tumor activity of the host immune system and tumor cell development and immune evasion. The adaptive immune system is mainly involved in keeping tumor dormancy and control outgrowth of occult tumors. However, in the process of maintaining equilibrium, tumor cells which acquire mutations or other changes that avoid immune recognition are selected to survive under the pressure of immune

surveillance. In the escape phase, tumor cells proliferate dramatically due to increased cancer-induced tumor immune suppression or more major events that allows full immune escape.

Tumor immune escape occurs by a variety of different mechanisms, including selection of non-immunogenic cells that are invisible to the immune system [9] and by establishment of an immunosuppressive tumor microenvironment (TME). For an overview of tumor immune evasion mechanisms they can be classified into five classes as follows (Figure 1): i) Loss of MHC class I (MHCI) antigen presentation by tumor cells avoiding recognition by cytolytic CD8+ T cells [10, 11], which may occur through mechanisms that include down-regulation of non-essential MHCI molecules, acquired defects in making and transporting MHCI or in its peptide loading [12, 13], loss of MHC I heavy chain genes or of β2M [14, 15], loss of transcription of MHC I pathway genes [16], or down-regulation of the MHCI pathway by MAPK signaling under extrinsic stimuli from TME [17]. ii) Secretion of tumor-derived soluble inhibitors and immunosuppressive cytokines, such as adenosine [18], PGE2 [19], IL-10 and transforming growth factor β (TGF β) [20-22], or galectin-1 (Gal-1) may diminish the killing activity of effector T cells towards tumor cells [23]. iii) Increased expression of Indoleamine 2, 3-dioxygenase (IDO) alters the metabolism of tryptophan in the TME yielding kynurenic acid that gives T-cell apoptosis and dysfunction [24]. Preference for aerobic glycolysis in the TME can also inhibit immune cell function. iv) Recruitment or peripheral induction of suppressive regulatory T cells (Tregs) in the TME may reduce anti-tumor immune response activity [25]. v) Lastly, immune checkpoint activation induces T cell exhaustion and functional inhibition [26]. In the following sections, we will discuss some of the most relevant signaling mechanisms involved in anti-tumor immune escape in the TME and cancer immunotherapies developed accordingly.

Immuno-regulatory signal pathways in the tumor microenvironment

Adenosine-A_{2A}R signaling pathway

Adenosine pathway effects on immune regulation

Adenosine, a purine nucleoside, is a suppressive metabolite produced at high levels in the TME [27]. Extracellular adenosine is a metabolic product generated from dephosphorylation of ATP via AMP by the ectoenzymes CD39 (ecto-nucleoside triphosphate diphosphohydrolase-1) and CD73 (ecto-5'-nucelotidase) [28]. Extracellular ATP and adenosine are normally kept at very low levels, however, higher ATP levels occur as a result of apoptosis, cell death/necrosis, hypoxia and persistent inflammation in the TME [29]. Hypoxia in the TME as a result of tumor growth leading to increasingly poorer blood supply and reduced oxygen tension induces hypoxia-inducible factor-1α (HIF-1α) which induces expression of CD39 and CD73 that next metabolizes ATP from dying cells leading to accumulation of adenosine [30]. Expression of CD39 and CD73 on tumor infiltrating Tregs also contributes to increased levels of adenosine in the TME [31].

Adenosine signaling proceeds through four G-protein coupled adenosine receptors (A1, A_{2A}, A_{2B}, A3), and their expression on tumor cells promote growth, survival and metastatic dissemination [32, 33]. The immunosuppressive function of adenosine is mediated primarily through A_{2A} receptors (A_{2A}R) with high affinity for adenosine and which predominantly are expressed on immune cells. Upon stimulation of A_{2A}R by extracellular adenosine, Gα directly stimulates the activation of adenylyl cyclase (ACs) resulting in intracellular production and accumulation of cyclic adenosine monophosphate (cAMP) [34, 35]. cAMP levels are balanced by the antagonistic functions of ACs and cAMP-specific phosphodiesterase (PDEs) that hydrolyze cAMP to 5'-AMP [36, 37]. Intracellular cAMP in T cells activates protein kinase A (PKA) and exchange protein activated by cAMP (EPAC). PKA is the dominant effector in the cAMP signaling pathway, and binding of cAMP to a pool of PKA type I

regulatory subunit (RIα) releases and activates the catalytic subunit (C) which inhibits proximal TCR signaling activation through phosphorylation of Csk, negatively regulating Lck and the subsequent activation of ZAP70, a gatekeeper pathway that must be sent off duty when the T cell activates [38, 39].This immunoregulatory pathway with PKA type I and Csk is held by a scaffold of ezrin as the A kinase achoring protein (AKAP) holding PKA, EBP-50 as a linker and PAG as the binding protein for Csk (Figure 2) [40-42]. PKC and MAP kinases critical for effector T cell activation and proliferation are also suppressed by PKA [43]. In addition, PKA induces activation of cAMP responsive element binding (CREB), cAMP responsive element modulator (CREM) and activating transcription factor-1 (ATF-1), resulting in reduced production of IL-2, IFN-γ, TNFα and IL-4 [44, 45].

The role of adenosine in attenuating inflammation and tissue damage by different immune cells has been investigated intensively. Adenosine can block differentiation of monocytes to DCs by binding to A_{2B}R, while diminishing the ability of DCs to prime Th1 immune responses by binding to A_{2A}R [46, 47]. DCs treated with adenosine revealed decreased expression of TNFα and IL-12, while production of suppressive cytokines such as IL-10 and TGFβ increased. Adenosine can also induce antigen presenting cells (APCs) that produce immunosuppressive molecules such as TGF-β and IL10, kynurenic acid or prostaglandin E2 [48]. The adenosine-A_{2A}R pathway can dampen pro-inflammatory macrophages by inhibiting production of IL-12 and TNFα, while promoting M2 polarization [49]. Upon binding to A_{2A}R, adenosine suppresses maturation and proliferation of NK cells, prohibits the stimuli-introduced upregulation of CD69 and production of IFN-γ and TNFα. In addition, the adenosine pathway can promote expansion of regulatory T cells and myeloid-derived suppressor cells (MDSCs), to enhance their suppressive functions on effector T cells [50, 51]. Moreover, the adenosine-A_{2A}R signaling could stabilize FoxP3 expression in Tregs, promoting their suppressive activity on effector T cells. Adenylyl cyclase AC7 is expressed in resting and activated Tregs, inducing elevated levels of cAMP [52, 53]. Increased expression of immune checkpoint proteins such as PD1, CTLA-4 and LAG-3 are also observed following adenosine-A_{2A}R signaling [54-56].

Cancer treatment targeting the adenosine pathway

Considering the elevated adenosine concentration in the TME, targeting the immunosuppressive effects of the adenosine pathway has been a focus in development of new drugs to boost anti-tumor immune responses. There are mainly two approaches to target the adenosine pathway: i) To suppress the production and accumulation of adenosine in the TME; and ii) To antagonize adenosine receptor activation to block the downstream adenosine signaling pathway (See Table 1).

<u>Targeting CD39 and CD73:</u>

As the key enzymatic components in the extracellular generation of adenosine from ATP, the potential for drug targeting of the ectoenzymes CD39 and CD73 has been intensively investigated. In the TME, hypoxia and incessant inflammatory conditions can induce overexpression of CD39 and CD73. CD39 is highly expressed on immune cells in the TME, including Tregs, myeloid cells, as well as tumor endothelium. Tumor infiltrating CD8+CD39+ T cells exhibit exhausted features and higher immune checkpoint receptor expression in solid tumors [57]. Expression of CD39 and CD73 on Tregs stabilize and contribute to the suppressive functions of Tregs [31]. CD73 is considered a prognostic marker, which is correlated with poor clinical outcome in many types of cancer [58, 59]. Elevated CD73 expression and activity were discovered in colorectal cancer associated fibroblasts and shown to contribute to anti-tumor immune evasion through the adenosine-A_{2B} receptor pathway [60]. CD39 and CD73 contribute to clearance of pro-inflammatory ATP in a hypoxic TME, leading to accumulation of adenosine and subsequent inhibition of CD8+ Tcells, NK cells macrophages and DCs [61]. Therefore, CD39 and CD73 are now considered as novel immune checkpoint targets.

Small molecule inhibitors or monoclonal antibodies against CD39 have been developed and are under evaluation for anti-tumor treatment (Table 1). Preclinical studies using the small molecule inhibitor polyoxotungstate-1 (POM1) on CD39 showed anti-tumor activity in a mouse melanoma model with neglectable toxicity [62]. POM1 treatment exhibited effective anti-tumor activity in a mouse colon adenocarcinoma model and human B-cell lymphoma, by facilitating T cell infiltration, enhancing T cell proliferation and Th1 cytokine production, which also rescued the anti-PD1 treatment resistance [63]. ARL-67156 is a nucleotide analog selectively targeting CD39 and acting as a competitive inhibitor [64]. ARL-67156 abrogates adenosine production from ovarian cancer cells. Blockade of CD39 on M2 macrophages by ARL-67156 was shown to release inhibition of CD4+T cell proliferation in co-culture [65]. In a study in the B16F10 mouse tumor model, ARL-67156 reduced CD39 expression on Tregs which resulted in augmentation of IFN-γ/Granzyme B-produced CD8+ T cells and tumor growth inhibition [66].

Three anti-CD39 monoclonal antibodies (TTX-030, SRF617 and IPH5201) are now found to be safe and with indications of effect in phase 1 clinical trials for cancer treatment, alone or in combination with immune checkpoint blockade or other chemotherapies. TTX-30 was validated by its effect in enhancing tumor immunity and maintaining pro-inflammatory extracellular ATP through specific CD39 inhibition [67]. SRF617 is a fully humanized antibody that enzymatically inhibits CD39. Treatment with SRF617 induced macrophage infiltration in the MOLP-8 myeloma xenograft model, leading to tumor growth inhibition via blocking CD39 enzymatic activity [68]. SPF617 was recently approved by FDA (March 2021) for treatment of patients with pancreatic cancer (based on results from NCT04336098) and is in clinical trial in patients with prostate cancer (NCT05177770). Another anti-CD39 mAb, IPH5201, developed by Innate Pharma revealed efficacy in blocking ATP hydrolysis, thereby promoting DC maturation and macrophage activation, in addition to increased anti-tumor activity in mouse melanoma and fibrosarcoma models. Furthermore, treatment of PBMCs from healthy donors or breast cancer patients with IPH5201 and IPH5301 (anti-CD73) demonstrated abrogation of the suppressive effect by extracellular ATP (through the adenosine pathway) which thereby promoted proliferation of CD4+ and CD8+ T cells [69].

The monoclonal antibody CPI006 from Corvus Pharmaceuticals is a humanized IgG1 FcγR binding-deficient antibody that binds to CD73 and is under evaluation in a phase I study in patients with advanced cancer, alone or in combination with anti-PD1 antibody and the $A_{2A}R$ antagonist ciforadenant (see below). The preliminary clinical results showed decrease of peripheral blood $CD73^+$ B cells and an overall increased CD4:CD8 ratio within 1 hour of drug infusion, suggesting rapid immune modulation on treatment. Monotherapy with CPI006 demonstrated tumor regression in patients with prostate cancer after 5 cycles of administration (Clinicaltrials.gov, NCT03454451) [70]. Other anti-CD73 blocking antibodies, for example NZV930 (SRF373) from Novatis, INCA0186 from InCyte corporation, oleclumab (MEDI9447), BMS-986179 and AB680 are also under evaluation in clinical trials for treatment of cancer patients (see Table 1). In the clinical trial on advanced colorectal cancer or pancreatic cancer, preliminary results revealed that treatment with oleclumab alone could increase CD8+ TILs by down-regulation of CD73 in 5 of 9 patient tumor samples (Clinicaltrials.gov, NCT02503774) [71]. Interestingly, a bi-specific antibody against CD73xEpCAM developed in the Helfrich lab, revealed significant inhibition of the immunosuppressive activity of CD73-exposing extracellular vesicles (EVs), by targeting $CD73^+$ $EpCAM^+$ carcinoma cell lines and patient-derived colorectal cancer cells [72]. Combinations of anti-CD73, anti-CD39 and other immune therapies or standard chemotherapies are considered as promising opportunities to increase the efficiency of immune oncology treatments.

Adenosine receptor antagonists:

Up to now, most of the adenosine antagonist drugs developed are targeting $A_{2A}R$. In multiple murine tumor models, including the MC-38 and CT-26 colon tumors, B16F10 melanoma and the RENCA renal cell cancer model, treatment with $A_{2A}R$ antagonist CPI-444 (ciforadenant) as a single agent induced anti-tumor immune responses, and suppressed tumor growth. It also augmented the efficacy of anti-PD1/anti-PD-L1 and anti-CTLA-4 immune checkpoint inhibitors. In addition, CPI-444 was reported to decrease the expression of multiple checkpoint pathways and to improve T cell infiltration and effector functions in MC-38, CT-26, and B16OVA tumor models [73]. A completed phase 1a/1b study of CPI-444 examining safety in humans and effect on advanced cancer patients alone or in combination with the anti-PD-L1 antibody atezolizumab, showed anti-tumor activity in both arms in refractory renal cell (RCC) and non-small cell lung cancer (NSCLC) patients. Patients resistant to anti-PD1/PD-L1 therapy also benefitted from CPI-444 treatment (Clinicaltrials.gov, NCT02655822) [74]. Another phase I study evaluates CPI-444 in combination with anti-CD73 antibody CPI006 in patients with relapsed solid tumors as mentioned above (Clinicaltrials.gov, NCT03454451).

A novel $A_{2A}R$ inhibitor, AZD4635, reverses adenosine mediated T cell suppression and induces anti-tumor immunity alone, or together with anti-PD-L1 in preclinical models [75]. In a phase 1a/2 study in patients with refractory solid tumors (Clinicaltrials.gov, NCT02740985) treatment with AZD4635 alone or in combination with anti-PD1 treatment led to reduction in adenosine signaling signature scores in 70% of patients (5 of 7) which correlated with higher overall survival, while 4 patients revealed gene expression signatures of cytolytic activity and IFN-γ signaling in T cells [76]. Indeed, metastatic castrate-resistant prostate cancer (mCRPC) patients with positive adenosine gene expression signature showed longer progression-free survival [77].

A phase 1/2 clinical study of the $A_{2A}R$ antagonist NIR178 (PBF-509) in patients with advanced NSCLC (Clinicaltrials.gov, NCT02403193) alone or in combination with anti-PD1 mAb PDR001 showed that the compound was well tolerated. Clinical benefit was observed both in patients on NIR178 alone and in combination with immunotherapy, irrespective of the PD-L1 status. A durable response with tumor shrinkage was observed in 2 ongoing immunotherapy-exposed patients (SD for >44 weeks) [78].

Other $A_{2A}R$ antagonists are also now being tested in clinical trials for cancer treatment, such as AB928, and EOS100850 (inupadenant) (See Table 1). AB928 (etrumadenant) is a dual adenosine receptor $A_{2A}R$ and $A_{2B}R$ antagonist under investigation in several clinical studies in patients with solid tumors. Preliminary results demonstrated that AB928 is well tolerated in combination with chemotherapy or anti-PD1 (Clinicaltrials.gov, NCT03720678, NCT03719326, NCT03629756) [79].

PGE2-EP signaling pathway

Mechanisms of PGE2 pathway on immune regulation

Prostaglandin E_2 (PGE_2) is a key lipid mediator of immune responses in the tumor microenvironment. PGE_2 has pleiotropic roles in inflammation [80] and tumor immunosuppression [19]. Production of PGE_2 follows on tissue injury to promote wound healing [81]. As the most abundant prostaglandin found in many types of human malignancies, elevated levels of PGE_2 are correlated with poor prognosis in breast, colon and lung cancer [82, 83]. In the TME, PGE_2 is produced by tumor cells, monocytes and induced Tregs (iTregs) and promotes tumor proliferation, progression and metastasis, by direct action on tumor cells, induction of angiogenesis and by regulating anti-tumor immune responses (reviewed in [19, 84]. Synthesis of PGE_2 is initiated from arachidonic acid released from cell membrane phospholipids by activation of phospholipase A_2 (PLA_2) and followed by a cascade of

enzymatic catalysis. Two forms of cyclooxygenase, COX1 and COX2 transform arachidonic acid into PGH_2 which is catalyzed into PGE_2 in the following step by PGE synthases, including mPGES-1, mPGES-2, and cPGES that belongs to the membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) family [85]. It was recently discovered that vascular endothelial growth factor (VEGF)-dependent release of thromboxane A_2 (TXA_2) triggers Ca^{2+} transients in tumor cells, which therefore induces cytoplasmic PLA_2 activation and triggers the arachidonic acid cascade, resulting in PGE_2 secretion and subsequent immune evasion in the early stages of tumorigenesis [86].

Functions of PGE_2 in immune cells are mediated through interaction with the PGE_2-specific E prostanoid (EP) receptors EP1, EP2, EP3 and EP4 which are GPCRs [84, 87], and the engagement of the four receptors trigger extensive and distinct signaling networks [88, 89]. Among these, the EP2 and EP4 receptors are coupled to $G_\alpha s$ and trigger signaling through an increase in cAMP levels and activation of the PKA signaling pathway (as for the adenosine pathway described above), which subsequently inhibits TCR signaling activation via the non-receptor tyrosine kinase Csk acting on Lck in the TCR activating pathway (Figure 2) [38, 89]. In addition, EP4 can be coupled to $G_\alpha i$, which inhibits PKA and triggers phosphoinositide 3-kinase (PI3K) signaling [90].

PGE_2 contributes in different ways to immunosuppressive effects in the TME, hereunder inducing a shift from M1 to M2 type macrophages, inducing production of pro-inflammatory chemokines such as CXCL1 and IL6 from macrophages, regulating the recruitment and differentiation of myeloid-derived suppressor cells (MDSCs) and activating their suppressive functions for example by upregulating arginase expression [91]. In addition, PGE_2 signaling inhibits activation and accumulation of DCs by altering the capacity of cytokine secretion and maturation, through inhibiting production of the inflammatory chemokines CCL3 and CCL4, resulting in reduced accumulation of immune cells [92]. PGE_2 also suppresses T cells by introduction of apoptosis and cell death, regulation of differentiation and TCR activation as well as cytokine production. Moreover, PGE_2 promotes FoxP3 expression and Treg suppressive function, thus attenuating the anti-tumor immune responses [93]. PGE2-EP2/EP4 signaling pathway also positively regulates PD1 expression in CD8+ tumor infiltrating lymphocytes (TIL) for immune tolerance [94].

Inducing anti-tumor immunity by targeting the PGE2 pathway

Many pre-clinical and clinical studies have targeted the PGE_2 pathway, through inhibition of PGE_2 production or blockade of PGE_2 receptors (EP2 and/or EP4) activation. (See Table 2)

<u>COX2 inhibitors</u>:

Formation of prostacyclin (PGH_2) from arachidonic acid by COX2 is a rate-limiting step in PGE_2 synthesis and COX2 thus serves as a good target to block PGE_2 production and the down-stream signaling pathways. The expression level of COX2 in normal cells is negligible. In contrast, overexpression of COX2 in tumor cells has been implicated in the pathogenesis of several cancers where it impacts oncogenic signaling, invasion and metastasis, survival and angiogenesis. For instance, COX2 is elevated in 85% of human colorectal carcinomas and 40%-75% of invasive breast carcinoma, correlating with poor prognosis [95-97]. COX2 is demonstrated to be an initiator of hepatocellular carcinoma (HCC) [98], squamous cell carcinoma (SCC) [99] and adenocarcinoma [96]. In the TME, overexpression of COX2 is observed in cancer cells, cancer-associated fibroblasts and M2 macrophages, facilitating its use a prognostic marker and highlighting its potential as target for therapy [100]. In addition, expression of COX2 is associated with suppressive function in tumor infiltrating Tregs [93].

The use of aspirin (acetylsalicylic acid) and non-steroidal anti-inflammatory drugs (NSAIDs) that target COX1 and COX2 in cancer primary and secondary prevention as well as therapeutically has been studied extensively. By coupling the randomized clinical trials that documented the effects of aspirin on platelet function preventing cardiovascular disease to national cancer registries years later, it was evident that aspirin taken for 5 years or longer with at least 75mg daily reduced overall cancer deaths (hazard ratio 0.66) and particularly gastrointestinal cancer deaths [101]. By analyzing individual patient data linked to cancer registries, daily treatment with aspirin as low as 75mg for 4 years or longer reduced 20-year risk of mortality in several cancer types, including stomach, colorectal and prostate cancer. Long duration of treatment was associated with increased benefit [102]. In addition, short term treatment with aspirin could also reduce cancer incidence and death, with lower risk of extra-cranial bleeding and fewer case-fatalities [103].

Meta-analysis of data from 118 published studies of patients taking aspirin at least 3 times per week showed around 20% overall reduction in cancer deaths, not restricted to cancer type [104]. However, despite the benefit of aspirin in reducing cancer incidence and mortality, the risks of gastrointestinal bleeding and brain hemorrhage has called for caution and individual assessment when considering primary prevention in healthy individuals in the general population [105-107].

In contrast, the risk/benefit analysis is different in patients that have already had cancer and where the question is whether aspirin can prevent cancer recurrence. Furthermore, as the immune system has now been exposed to the cancer, effects of aspirin on restoring anti-tumor immunity through the PGE_2-cAMP immunoregulatory pathway would kick in [108-110] . Association of aspirin use post-diagnosis in patients with colorectal cancer with cancer-specific and overall survival has been reported in several studies (see for example [111, 112]). In a population-based, retrospective cohort study linking patients diagnosed with colorectal cancer with data on their aspirin use (>23,000 patients of whom > 6,100 were on aspirin after diagnosis), the hazard ratio was 0.85 [113]. Based on such observed associations, the secondary preventive effect of low-dose aspirin in colorectal cancer is now evaluated in several ongoing randomized, placebo-controlled clinical trials (https://www.lumc.nl/org/atcg/) such as ALASSCA, SAKK, EPISODE III [114], ASCOLT, ASPIRIN and ASPIRIN-Belgium in primary cancer and Add-Aspirin, and ASAC in metastatic cancer (Clinicaltrials.gov, NCT03326791, NCT02647099) [115]. As these trials will be reporting in the period 2025-29, we should have more conclusive evidence on use of aspirin in secondary prevention and for what subpopulation of patients there would be benefit, such as, for example, patients with PIK3CA or KRAS mutations [116, 117].

Celecoxib is a drug that selectively inhibits COX2, which has been investigated for its anti-tumor and anti-metastasis effects in pre-clinical models of human cancer. It's shown that COX2 inhibition by celecoxib decreased tumor associated macrophages (TAMs) and cytokine production in mammary tumor progression. Celecoxib treatment reduced colorectal tumor growth in mice, as well as decreased metastatic potential by inducing anti-angiogenesis and apoptosis [118]. An interventional phase 1/2 clinical trial of COX2 inhibitor celecoxib together with radiation therapy in non-small cell lung cancer (NSCLC) patients (Clinicaltrials.gov, NCT00046839) showed a one-year overall survival rate of 44.4% and a two-year overall survival rate of 22.2%, with progression-free one-year survival at 33.3%. In contrast, in the REACT randomized clinical trial in patients with ERBB2-negative breast cancer, two years of treatment with celecoxib as adjuvant did not show survival benefit, which may indicate that longer treatment or higher dose is required and should be examined in further studies (Clinicaltrials.gov, NCT02429427) [119]. Other clinical trials with COX2 inhibitors on cancer patients are shown in Table 2.

EP4 receptor antagonist:

Targeting the EP4 receptor to block the PGE2 immunoregulatory pathway in cancer treatment has been investigated in pre-clinical and clinical studies (Table 2).

MF-766 is a highly specific small molecule inhibitor of EP4, which promoted the infiltration of CD8+ T cell, NK, M1 macrophage into the TME in CT26 and EMT6 syngeneic mouse tumor models. MF-766 reversed PEG2-inhibited IFN-γ production of CD8+ T cells and impaired the MDSC2 suppression. EP4 inhibition by MF-766 worked synergistically with anti- PD1 therapy to improve anti-tumor efficacy [120]. Another highly selective EP4 antagonist, E7046, showed significant increase in tumor infiltrating CD8+ T cells in the mouse CT26 colon cancer model. In pre-clinical studies, E7046 inhibited mouse LL2 Lewis lung carcinoma growth by regulating differentiation of monocytes into M2 macrophage and suppressive functions of MDSCs, while promoting accumulation of CD8+ T cells in TME [121]. A Phase 1 clinical trial with E7046 (Clinicaltrials.gov, NCT02540291) was evaluated in patients with advanced cancer, which demonstrated gene expression changes in the EP4 pathway and enhanced anti-tumor immune responses, by inducing expression of PD-L1/PDL-2 gene responsible for IFN-γ production response and decreasing expression of the IDO1 and EOMES genes. E7046 also increased CXCL10 and CCL5 expression in serum to recruit T cells [122]. AN0025 (previously E7046) from Adlai Nortye is now evaluated in a phase 1/1b trial on advanced tumor patients, in combination with anti-PD1/PD-L1 treatment or a pan-PI3K inhibitor (Clinicaltrials.gov, NCT04975958, NCT04432857). Interestingly, the dual EP2/EP4 antagonist TPST-1495 from Tempest Therapeutics is studied in a phase 1a/1b trial on patients with solid tumors, as a single treatment or in combination with anti-PD1 (Clinicaltrials.gov, NCT04344795).

Grapiprant, a specific EP4 antagonist from Arrays Therapeutics is now investigated in a phase 1 trial in patients with microsatellite stable colorectal cancer and metastatic breast cancer (Clinicaltrials.gov, NCT03658772, and NCT05041101). Evaluation of the EP4 antagonist LY3127760 was completed in healthy people in a phase 1 trial (Clinicaltrials.gov, NCT01968070), showing tolerance and safety with observations of increased PGE2 metabolite and TNFα release, which is promising for the upcoming clinical trial in cancer patients [123]. Other selective EP4 antagonists are also evaluated in pre-clinical tests or clinical trials for cancer treatment, for instance ONO-4578, INV1120 and BMS-986310.

Regulation of cAMP-PKA signal pathway

As adenosine and PGE_2 signaling converge downstream of their cognate receptors, the cAMP-PKA–Csk pathway serves as a key negative regulator of TCR proximal signaling (Figure 2). Disrupting this pathway distal to ligand engagement of GPCRs may have merit in reversing cAMP-mediated immune suppression. Such targeting may be at the level of cAMP action on PKA, interfere with protein-protein interactions in the ezrin-EBP50-PAG (protein associated with glycosphingolipid-enriched microdomains) scaffold that serves to assemble the PKA type I/Csk signal pathway including disrupting PKA or Csk binding [42, 124].

Cyclic AMP antagonists reverse cAMP-mediated immune suppression in infectious diseases and colorectal cancer with elevated cAMP signaling [108, 125-127]. However, while a drug derivatization programme yielded compounds with increased efficacy and specificity for PKA type I, it failed to produce orally bioavailable compounds (K. Taskén, unpublished information). Identification of an RI anchoring disruptor (RIAD) peptide with high affinity to type I PKA efficiently blocked its anchoring and reversed cAMP-mediated inhibition of T cells, by displacing PKA from lipid rafts and diminishing phosphorylation on Lck [128, 129]. It also reversed cAMP-immune dysfunction and boosted CAR-T cell therapies in animal studies [130, 131]. Transgenic mice expressing RIAD fusion protein showed augmented TCR signaling activation and enhanced T cell responses, with reduced sensitivity to cAMP-mediated T cell dysfunction, serving as potential strategy for anti-tumor immune therapy [132].

However, RIAD will disrupt all PKA type I-AKAP interactions. Furthermore, the PKA-ezrin interaction surface is flat, hydrophobic and comparably large for fitting a small molecule. Disrupting the scaffold by a protein-protein interaction inhibitor at the level of the EBP-50 ezrin interaction may be a potential strategy that would lend more specificity to blocking the PKA pathway to restore T cell activation in the TME.

Tumor-derived soluble inhibitors and immunosuppressive cytokines

Tumor-derived soluble factors (TDSFs) are key mediators of anti-immune response in the TME. In addition to PGE2 and adenosine, other soluble factors produced by tumor cells like Galectin-1 and IDO are also critical molecules for suppression on immune cells. Accumulation and activation of IDO and arginase I in the TME could introduce production of ROS in tumor-associated immature DCs and TAMs, which therefore inhibit the maturation of DCs and T cell proliferation by promoting apoptosis [133, 134]. Higher expression of IDO is associated with shorter overall survival and poor clinical outcomes in patients with solid tumors, which serves as a biomarker for prognosis and good drug targets for cancer therapy [135]. Galectin-1 is overexpressed and secreted into the TME by many solid cancers including lung, ovarian, bladder, prostate, colorectal, melanoma, breast, and head and neck cancer (HNC), which is universally associated with poor outcome due to its function on immune suppression [136].

Inflammation is one of the hallmarks of cancer, due to its capability to supply bioactive molecules that promote cancer proliferation, invasion, and metastasis. In the TME, cytokines secreted by tumor cells and tumor infiltrating immune cells work on both inhibition and promotion of tumor growth. IFN-γ upregulates MHCI expression on cancer cells leading to their recognition by cytolytic T cells; perforin (pore-forming protein)/granzymes (serine protease) released from cytotoxic lymphocytes and NK cells have direct killing effects on tumor cells by introducing apoptosis and cytolysis. However, immunosuppressive cytokines such as IL-10 and TGFβ that are secreted directly by tumor cells into the TME inhibit DC maturation and T cell functions and activate Tregs, boosting tumor growth [22].

Galectin

Tumor-derived galectins are soluble glycan-binding proteins, which are a family of endogenous lectins abundantly expressed in the TME. Expression of 11 galectins have been identified in human cells, among which Galecin-1, 3 and 9 have been subject tto most studies due to their effects on tumor growth, migration and suppression of T-cell mediated immune responses.

Overexpression of Galectin-1 is observed in various types of cancer, including thyroid malignancies, head and neck squamous cell carcinoma (HNSCC), adenocarcinoma, ovarian and bladder cancer [137]. Tumor-secreted Galectin-1 mediates immune evasion by introducing T cell apoptosis and preventing T cell migration into the TME, through binding to surface glycoproteins such as CD2, CD29, CD45, CD43 and CD7 and regulation of down-stream signaling pathways such as activation of AP1 and downregulation of Bcl-2 [138], activation of the JNK/c-Jun/AP-1 pathway [139] and promoting TCRζ phosphorylation [140, 141]. Galectin-1 also triggers differentiation of DCs and M2 macrophages which inhibit T cell adhesion and migration. Importantly, Galectin-1 is overexpressed and upregulated upon TCR activation in Tregs, promoting Treg expansion and increase expression of T-cell regulatory molecule LAT [142]. Galectin-1 in the TME remodels tumor endothelium to suppress T cell infiltration through upregulation of immune checkpoint ligands such as PD-L1 [143]. Moreover, binding of Galectin-1 to N-glycans on CTLA4 enhances retention of CTLA4 at the T cell surface, resulting in T cell exhaustion and growth arrest [144].

Similarly, higher expression of Galectin-3 in many cancer types was reported to contribute to tumor progression and metastasis in colorectal cancer, ovarian cancer, NSCLC and hepatocellular carcinoma [145-147]. Galecin-3 induces T cell apoptosis through direct binding to CD45 and CD71 [148]. Expression of Galectin-3 in tumor-infiltrating CD8+ T cells is associated with loss of TCR, resulting in inhibition of TCR-mediated activation of T cells [149]. In addition, binding of Galectin-3 to LAG-3 on CD8+ T cells in the TME mediates suppression of CD8+ T cell function [150]. Tumor-derived Galectin-3 inhibits IFN-γ permeation and reduces CXCL9 content to block recruitment of CD8+ T cells in TME [151, 152].

Galectin-9 is also investigated in many studies, demonstrating positive connections to tumor cell adhesion or metastasis in melanoma, HCC and breast cancer [153]. Interestingly, Galectin-9 has a dual function, by specific binding to T cell immunoglobulin mucin 3 (TIM-3) expressed on Th1 cells or dendritic cells, which induces apoptosis or inflammatory responses respectively [154].

IL-10

IL-10 has pleiotropic functions with both pro- and antitumor effects, preventing inflammatory environment created by cancer. lL-10 was originally identified as a suppressor of CD4+ T cell proliferation and secretion of the Th1 cytokine IFN-γ. IL-10 is produced by Th cells, mast cells, Tregs, B cells, macrophages and NK cells in the TME. Production of IL-10 involves Toll-like receptor or Fc receptor-dependent stimulation of ERK1/2, p38 MAPK and NF-kB signaling. Then the function of IL-10 is fulfilled through binding to IL10 receptor complex consisting IL-10 receptor 1 and 2, which subsequently activates JAK1 (IL10Rα) /Tyk2 (IL10Rβ)-STAT3 signaling pathway for downstream transcriptional regulation [155].

Many studies support immunosuppressive functions of IL-10. High expression of IL-10 in serum leads to poor survival and poorer outcomes in patients with advanced or metastatic melanoma [156], gastric cancer [157] and pancreatic tumors [158]. Elevated levels of IL-10 are associated with increased tumor growth and drug resistance in both solid tumors and hematological malignancies in a meta-analysis [159]. Mechanisms of IL-10 activation includes STAT3-induced IL-2 regulated (NFIL3) transcriptional repressor nuclear factor and suppressor of cytokine signaling 3 (SOCS3) blocking the production of pro-inflammatory cytokines such as IL-6, IL-12 and IL-23 in LPS-induced T cells [160]. IL-10 signaling suppresses IFN-γ dependent expression of MHC II and CD86 on monocytes/macrophages, thereby down-regulating antigen presentation to CD4+ T cells [161]. In addition, IL-10 from MDSCs decreases IL-12 production from macrophages to impair anti-tumor immunity.

In contrast, IL-10 is reported to mediate stimulation of cytotoxicity, IFN-γ secretion, and IL-2 activated expansion of CD8+ T cells, which thereby reduce tumor growth [162]. Overexpression of IL-10 in human cancer models or treatment with a pegylated IL-10 (PEG-IL-10) led to tumor rejection and long-lasting tumor immunity. In summary, IL-10-induced tumor rejection is dependent on the expression of IFN-γ and granzymes in tumor-resident CD8+ T cells and the upregulation of MHC molecules [163].

TGFβ

TGFβ plays dual function in tumors: it blocks tumor cell cycle progression at an early stage, whilst promoting tumor growth in later stages of the disease. TGFβ was discovered to be responsible for immune cell exclusion in colorectal cancer and for blocking of development of a Th1-effector cell phenotype in progressed metastasis disease [164]. In addition, high TGFβ levels in breast, colon, lung and gastric cancer patients are linked to poor clinical outcome, progression, metastasis and poor overall survival.

The TGFβ signal is transmitted by ligand binding to the type I and type II serine/threonine kinase receptors TβR1/TβRII, which elicits SMAD-dependent and independent canonical and non-canonical pathways. In the canonical pathway, TGFβ receptor-specific phosphorylation of SMAD2 and SMAD3 leads to formation of a complex with SMAD4 that translocate to the nucleus and regulate downstream gene expression via SMAD-binding element. In the non-canonical pathway, there is crosstalk between TGFβ signaling and other signal pathways at the level of the TNF receptor associated factor 4, TAK1, p38 MAPK, PI3K-AKT, ERK, JNK or NF-kB, Ras/RAF pathways [165].

TGFβ may work directly on cytotoxic lymphocytes to block expression of perforin, granzyme A/B, Fas ligand and IFN-γ responsible for the killing activity. Moreover, TGFβ inhibits cytotoxic T lymphocyte (CTL) development in the early stages of CD8+ T cell differentiation and TCR-driven activation of autoreactive and high-affinity T cells [166]. Differentiation of T cells into Th1 and Th2 cytokine producing cells and activation are also inhibited by TGFβ. In the TME, TGFβ promotes release of VEGF and recruitment of Tregs, MDSCs and other suppressive immune cells, in addition to inhibition of CD8+ T cells and NK cells. TGFβ can promote differentiation of Tregs, thereby inhibiting CD4+ and CD8+ T cell proliferation and effective function (reviewed in [167]. In addition, TGFβ inhibits DCs maturation and modifies chemokine receptor expression, and directly suppresses NK cell-mediated production of IFN-γ through SMAD3 regulation on IFN-γ promoter thus inhibiting cytotoxicity of NK cells [168]. Moreover, TGFβ inhibits CTL development in early stages of CD8+ T cell differentiation and TCR-driven activation of autoreactive or high-affinity T cells. Indeed, TGFβ blocks IL-2 production through inhibiting IL-2 promoter activity required for T cell proliferation and survival, upregulates cell cycle inhibitors such as p15, p21, p27 and down regulates c-myc, cyclin D2, CDK2 and cyclin E required for cell cycle progression [169].

Therapeutic strategies targeting soluble factors in the TME

-Targeting Galectins

Inhibition of Galectins can be achieved by different approaches, such as glycan-based inhibitors, allosteric antagonists or peptidomimetics, natural or modified polysaccharides and anti-galectin-specific neutralizing antibodies discussed below. Drugs inhibiting Galectin-1, Galectin-3 and Galectin-9 have been developed for cancer therapies (see Table 3).

Several Galectin-1 inhibitors are investigated in pre-clinical studies. Anginex (βpep-25) is a peptide-based galectin-1 inhibitor of 33 amino acids with anti-angiogenic and anti-tumor effects [170]. Anginex treatment promotes leukocyte-endothelium interactions and infiltration of leukocytes including CD8+ T cells in tumor-bearing mice with human LS174T colon carcinoma and mouse B16F10 melanoma [171]. The disruption of ligand binding to Galectin-1 using peracetylated 4-fluoro-glucosamine (4-F-GlcNAc), a metabolic inhibitor of N-acetyl-lactosamine biosynthesis, decreased tumor growth in melanoma by boosting antitumor immunity [172]. Galectin-1 inhibitor TDG (thiodigalactoside) was tested in mice with B16F10 melanoma and 4T1 orthotropic breast cancer, which revealed reduced tumor growth with increase of tumor infiltrating CD8+ T and CD4+ T cells and reduction of CD31 endothelial cell content [173]. In addition, nanobodies (single domain antibodies) developed by Ablynx Inc. were patented to target multiscavenger receptors including Galectin-1, which not only overcomes cancer immunosuppression but can also circumvent resistance to anti-vasculo-endothelial growth factor (VEGF) treatment.

Moreover, GM-CT-01, a galactomannan obtained from guar gum, is a Galectin-1 antagonist reported to be safe in cancer patients, effectively boosting the cytotoxic activity and IFN-γ production of tumor infiltrating CD8+ T cells [174]. A phase 1 clinical trial is completed in solid tumor patients, in single treatment with GM-CT-01 or in combination with 5-FU (Clinicaltrials.gov, NCT00054977). OXT008 is a

peptidomimetic targeting Galectin-1 with significant anti-cancer effects both *in vitro and in vivo* in Gal-1 expressing thyroid cancer cell lines in pre-clinical studies [175]. OXT008 is now in a first-in-man phase 1 clinical trial in patients with advanced solid tumor (Clinicaltrials.gov, NCT01724320).

Drugs targeting other galectins are also evaluated in clinical trials for cancer treatment. GR-MD-02 is a Galectin-3 inhibitor evaluated in phase 1 clinical trial on patients with metastatic melanoma, NSLC and HNSCC, in single treatment or in combination with anti-PD1 or anti-CTLA4 (Clinicaltrials.gov, NCT02117362, NCT02575404). LYT-200 is a monoclonal antibody against Galectin-9 which is tested in solid tumor patients in phase 1/2 clinical trial in combination with chemotherapy and anti-PD1 treatment (Clinicaltrials.gov, NCT04666688).

-Targeting IL-10

Although IL-10 is defined as an immunosuppressive cytokine, pre-clinical and clinical studies have demonstrated anti-tumor effects of IL-10 or recombinant human IL-10 (rhIL-10). High concentrations of IL-10 extended the life span and activated proliferation and cytotoxicity of CD8+ T cells in mouse tumor models [162, 176].

Pegilodecakin (LY3500518), a PEGylated IL-10, induced lasting, elevated serum concentrations of IL-10 which restored cytotoxicity and led to expansion of tumor-specific CD8+ T cells which express high levels of the IL-10 receptor. Furthermore, it also induced expression of intratumoral antigen presentation through enhanced IFN-γ secretion from CD8+ T cells. Together, treatment with pegilodecakin induced amplification of tumor-specific activated CD8+ T cells and CD8+ T cell-mediated rejection of tumors in a mouse PDV6 squamous carcinoma model [177]. Moreover, pegilodecakin treatment in patients with intermediate-to-poor-risk renal cell cancer showed promising anti-tumor responses, by inducing CD8+ T cell activation and elevated production of IFN-γ and Granzyme B, as well as reduced activation of Tregs. In addition, combined treatment with pegilodecakin and the immune checkpoint inhibitor anti-PD1 increased expansion of exhausted LAG-3+PD1+CD8+Tcells infiltrated in the TME [178]. Pegilodecakin in combination with anti-PD1 treatment was studied in clinical trials in patients with advanced solid tumors (Clinicaltrials.gov, NCT02923921, NCT02009449). Preliminary results from renal cell carcinoma patients revealed an overall response (ORR) of 43% (15 of 35) in the pegilodecakin plus anti-PD1 group, compared to ORR of 20% with anti-PD1 inhibitors alone, indicating a possible efficacy of IL-10 on improvement of anti-tumor immune therapy (Table 3) [179].

In addition, a novel study of a cetuximab-based IL-10 (CmAb-$(IL10)_2$) in mice demonstrated significant anti-tumor effects. Again, regulation of IFN-γ production from T cells through IL-10R signaling prevented DC-mediated apoptosis of tumor specific CD8+ T cells. Moreover, CmAb-$(IL10)_2$ and immune checkpoint blockade (anti-PD-L1/anti-CTLA4) could significantly improve anti-tumor effects in a mouse melanoma model by overcoming resistance to immune checkpoint inhibitors (ICIs) [180].

-Targeting TGFβ

Small molecules, antibodies, peptides and antisense oligonucleotides have been developed to block the TGFβ pathway, by targeting either TGFβ or TGFβ receptors [181].

Fresolimumab, GC1008 and SAR-439459 are humanized monoclonal antibodies against TGFβ now studied in clinical trials on solid tumor patients (Table 3). TASO001 is anti-sense oligonucleotide targeting TGF-β2 which is under evaluation in clinical studies on patients with advanced solid tumor (Clinicaltrials.gov, NCT04862767). AVID200 is a TGFβ1/ TGFβ3 protein trap which blocks TGFβ signaling, revealed by decrease of phosphorylation on SMAD2. A first-in-class clinical study is ongoing

to evaluate the effect of AVID200 in patients with advanced solid tumors and reveals modulation of TGFβ targets and immune activation. This provides support for further studies in combination with anti-PD1 treatment (Clinicaltrials.gov, NCT03834662) [182].

Galunisertib is a small molecule inhibitor targeting the TGFβ type I receptor to specifically down-regulate SMAD2/3 phosphorylation which can reduce tumor burden and metastasis in mice 4T1-LP tumor models [183]. Clinical trials on patients with several types of solid tumors are evaluating the effect of galunisertib in single treatment or in combination with chemotherapy or immune therapy (Clinicaltrials.gov, NCT02423343, NCT02452008, NCT02906397, NCT01373164, NCT02688712, NCT02734160).

The small molecule SM16 is a kinase inhibitor that binds to the ATP-binding pocket of the kinase domain and serves as an antagonist of the TGFβ receptor 1. Blockade of TGFβ by SM16 prohibited primary and metastatic tumor growth in mice with 4T1 breast cancer implanted, as TGFβ-induced generation of Tregs was abrogated, diminishing their suppressive function on effector T cells [184]. Treatment of mice with malignant mesothelioma AB12 by SM16 significantly inhibited tumor growth through suppression on SAMD2/3, which increased cytotoxic activity of CD8+ T cells [185].

IMC-TRI (also called, LY3022859) is a TβRII-blocking monoclonal antibody which attenuated TGF - mediated downstream signaling and thereby inhibited tumor growth by enhancing NK cell and CTL activity in a pre-clinical study [186]. IMC-TRI completed phase 1 clinical trials in patients with advanced solid tumors (Clinicaltrials.gov, NCT01646203), however the results did not determine a safe dose without infusion-related reactions [187].

Recently, several bi-specific drugs targeting both TGFβ and other immune targets have been developed and are under investigation in clinical trials on cancer patients. SHR1701 is a bi-functional fusion protein composed of a mAb against PD-L1 and extracellular domain of TGF-β receptor II, which revealed safety and promising anti-tumor activity in advanced NSCLC patients (Clinicaltrials.gov, NCT03774979) [188].

TST005 is another bi-functional anti-PD-L1/TGF-β fusion protein, consisting of a PD-L1 antibody fused with the C-terminal of the TGF-β Receptor type II protein. TST005 displayed potent activity in vitro in reversing TGF-β induced T-cell suppression and enhancing IFN-γ production. In multiple syngeneic tumor models of MC38 and EMT6, treatment with TST005 induced significant increase of CD8+ T-cell infiltration into PD-L1 expressing tumors and displayed dose-dependent tumor growth inhibition [189]. A first-in-human phase 1 study of TS005 is now in progress in patients with locally advanced or metastatic solid tumors (Clinicaltrials.gov, NCT04958434).

Recruitment and activation of Tregs in TME

Tregs are suppressive subsets of CD4+ T cells, maintaining homeostasis of immune responses by suppressing effector T cells and preventing immunological overshoot. High frequencies of Tregs in the TME are related to poor prognosis and survival in many different types of solid tumors [190, 191], including breast [192] and ovarian carcinoma [193], renal cell carcinoma (RCC) [194], cervix [195] and prostate carcinoma [196], urinary bladder cancer [197], non-small cell lung carcinoma (NSCLC) [198], hepatocellular carcinoma (HCC) [199], pancreatic adenocarcinoma (PDAC) [200], glioblastomas [201] and gastric cancer [202].

The recruitment of Tregs to the TME is driven by chemokines interacting with cognate receptors, including CCL22/CCL17/CCL2 interacting with CCR4, CCL1 interacting with CCR8, CCL5 with CCR5, CCL28 with CCR10 and CXCL12 with CXCR4 [203]. CCR4 on Tregs serves as receptor for CCL22 and

CCL17 which are highly expressed on ovarian, gastric, esophageal, breast, lung and hand and neck cancer cells [204-206]. Elevated production of CCL2 in a human malignant glioma microenvironment also facilities recruitment of Treg via CCR4 [207]. nTregs have high CCR5 expression and migrate into the TME when CCL5 is overexpressed on tumor cells [208]. CCL5 and CCR5- dependent recruitment of Tregs into TME is confirmed in many cancer models, including pancreatic, breast, colorectal, prostate, lung cancer and skin squamous cell carcinoma [209-212]. CXCL12 can also attract Tregs into the tumor TME through interaction with CXCR4 expressed on Tregs [213, 214]. CCR6+ positive Tregs are enriched in breast cancer and HCC patients' TME, by the affinity to CCL20 expression on tumor cells [215]. CXCR3+ Treg recruitment to ovarian cancer and HCC TME is mediated by interaction with CCL9/10/11 [216]. Interestingly, hypoxia in the TME also favors homing of Tregs. CCL28 is upregulated in human ovarian cancer by HIF-1α, resulting increased migration of Tregs by interaction with CCR10 [217].

The inhibitory function of Tregs on effector CD4+ and CD8+ T cells has been shown to be important in facilitating tumor immune escape in the TME. The suppressive function of Tregs is exerted through different mechanisms (Figure 3) [218]. Firstly, expression of CD25, the high affinity IL-2 receptor α chain (IL-2Rα) on Tregs compete for and quench IL-2 availability to effector T cells, resulting in their apoptosis. Secondly, Tregs produce perforin and granzyme B for direct killing and secrete suppressive cytokines such as IL-10, IL-35 and TGFβ to inhibit effector T cell proliferation and function. Thirdly, Tregs express inhibitory receptors such as PD1, LAG3, CTLA4 and TIM3 to block co-stimulation leading to effector T cell exhaustion. Treg immune checkpoint receptors also inhibit maturation of DCs and convert DCs into tolerogenic partners. Lastly, high expression of CD39 and CD73 on Tregs facilitate effective conversion of ATP into adenosine which subsequently inhibits effector T cell activity via the adenosine-A2AR receptor pathway. In addition, high expression of COX2 in Tregs induces elevated level of PGE2 to suppress effector T cells via the PGE2-EP2/4 receptor-PKA pathway by blocking TCR activation.

FoxP3 is the lineage-defining transcription factor of Tregs, regulating gene expression of key molecules such as CD25, CTLA4 essential for inhibitory functions and stability. In the TME, TGFβ and IL-10 contribute to the conversion of conventional CD4+ T cells into peripherally induced Tregs. TGFβ and IL-10 increases FoxP3 and CTLA4 expression in Tregs, facilitating Treg differentiation and expansion. Indeed, TGFβ upregulates FoxP3 expression through enhancing SMAD-2/3-induced binding of the transcription factor E2A to the FoxP3 promoter, as well as inhibiting suppressive transcriptional regulation by GATA-3 [219]. Notably, it was recently discovered that TGFβ is essential for maintaining expression of CD103 on Tregs that is required to retain and accumulate Tregs in the colon [220]. Tregs are also a main source of IL-10 in the TME, which serves as a loop to increase the Treg population and induces production of the suppressive cytokine IL-10 [221]. Moreover, high expression of CTLA4 and ICOS on Tregs also contribute to the development of tumor resistance to immunotherapy such as immune checkpoint inhibitors [222, 223].

In addition, high expression of IDO, released from tumor cells, tumor associated macrophages, or MDSCs and DCs can also promote activation of Tregs and thereby inhibition of CD4+ and CD8+ T cell proliferation by metabolic depletion. Mechanistically, IDO inhibits mTORC2 and Akt signaling pathways to upregulate Foxo3a and PD1 which are critical for Tregs suppressive activity [224].

Interestingly, recent studies demonstrated that Neurophilin 1 (Nrp1) expressed in Treg cells also contributes to immune suppression in the TME, through activation of PTEN and inhibition of Akt to preserve FoxO3 expression thereby stabilizing Treg suppressive activity [225]. Therefore, depletion of Nrp1 reduced FoxP3 expression and enhanced production of IFN-γ to increase intratumoral CD8+ T cells [226]. Patients with solid tumors such as head and neck squamous cell carcinoma, ovarian

cancer, NSCLC, colorectal cancer and pancreatic cancer show quite high numbers of intratumoral $Nrp1^+$ Tregs, which correlated with poor prognosis [227]. Additionally, intratumoral $Nrp1^+$ Tregs could upregulate expression of inhibitory proteins, such as of TIGIT, CCR8 and TNF receptor super family members.

Therapeutic targets in Tregs:

Reduction or depletion of Tregs has been considered a potential strategy for cancer treatment. Several drugs targeting surface markers or signature proteins in Tregs were developed and are under evaluation in pre-clinical and clinical studies (See Table 4).

The anti-CD25 mAb daclizumab (anti-Tac) provides a well-known strategy to successfully deplete Tregs, developed in 1981 [228] and first approved in 1997 by FDA for prevention of renal allograft rejection [229]. Daclizumab was evaluated in subsequent studies in CD25-expressing leukemic malignancies, such as HTL-I associated adult T-cell leukemia and Hodgkin's disease, revealing partial responses [230]. LMB2 is a fusion of anti-CD25 mAb and a truncated immunotoxin [231], which demonstrated response in clinical studies on patients with $CD25^+$ hematologic malignancies [232]. Subsequently, *in vitro* studies demonstrated that selective depletion of LMB2 on Tregs in human PBMCs is possible without impairing other lymphocytes [233]. However, in the following clinical studies on patients with metastatic melanoma, administration of LMB2 introduced a significant but transient reduction of $CD4^+CD25^+$ Tregs, but without augmenting responses to cancer vaccination [234]. Considering the liver toxicity induced by treatment of LMB2 in pre-clinical studies and the fact that CD25 is also expressed on activated conventional effector T cells for expansion upon IL-2 stimulation, targeting CD25 for cancer therapy has been approached skeptically.

Recently, an anti-CD25 antibody with enhanced binding to activated FcγR was developed and evaluated in mice tumor models, demonstrating significant depletion of tumor-infiltrating Tregs, which in combination with anti-PD1 treatment promoted tumor rejection synergistically [235]. Further investigation validated the selective activity of this anti-CD25 antibody (RG6292) on depletion of Tregs, without blocking IL-2 mediated STAT5 phosphorylation in effector T cells, and which therefore boosted anti-tumor responses significantly [236]. In addition, treatment of RG6292 did not introduce autoimmune toxicities in cynomolgus monkeys, supporting its use in combination with immunotherapy in clinical trials on patients with solid tumors (Clinicaltrials.gov, NCT04158583, NCT04642365).

Targeting the chemokine-receptor pathways required for recruitment of Tregs into the TME is an alternative approach in drug development. Several CCR4 antagonists have been developed and evaluated in pre-clinical and clinical studies for cancer treatment. AZD-2098 and AZD-1678 are two potent small molecule CCR4 antagonists developed by high-throughput screening by AstraZeneca [237]. FLX475 is another small molecule, non-depleting CCR4 antagonist developed by RAPT Therapeutics, which demonstrated blockade of Treg migration into the TME but not their migration into healthy tissues in preclinical studies. In an open-label, phase1/2 clinical trial in patients with advanced cancer, treatment with FLX475 alone or in combination with anti-PD1 therapy showed an increase in the ratio of effector T cells to Tregs [238] (Clinicaltrials.gov, NCT03674567). FLX475 is also evaluated in combination with the anti-CTLA4 antibody ipilimumab in patients with advanced melanoma (Clinicaltriasl.gov, NCT04894994), or in combination with the anti-PD1 antibody pembrolizumab in patients with advanced gastric cancer (Clinicaltrials.gov, NCT04768686).

Mogamulizumab (KW-0671) is an anti-CCR4 monoclonal antibody, first approved in 2012 in Japan for treatment of CCR4+ adult T-cell leukemia/lymphoma. Pre-clinical studies revealed selective depletion of tumor-infiltrating Tregs by mogamulizumab and significant induction of tumor-antigen specific

CD4+ and CD8+ T cells, thereby enhancing anti-tumor immunity [239]. A phase II study of mogamulizumab in patients with CCR4+ ATLL following a phase 1 dose-finding study demonstrated a 50% objective response, with 8 of 13 patients showing complete response due to enhanced anti-tumor activity [240]. In another phase II study in patients with PTCL or CTCL, treatment with mogamulizumab revealed 34% ORR in PTCL and 50% ORR in CTCL patients, demonstrating promising efficacy in cancer treatment [241]. However, clinical studies of mogamulizumab on solid tumors did not reveal enhanced antitumor efficacy, when in combination with immune checkpoint inhibitor therapy (Clinicaltrials.gov, NCT02301130 and NCT02705105) [242, 243]. No clear correlation of clinical response with reduction of $CCR4^+$ Tregs was discovered.

Drugs targeting other Treg recruitment mechanisms related to chemokine-receptors have also been developed for anti-tumor treatment. For instance, BMS-813160 is a dual antagonist targeting both CCR2 and CCR5 [244], which is now being evaluated in a phase 1 study in patients with advanced solid tumors in combination with chemotherapy or nivolumab. GS-1811 (JTX-1811) is a monoclonal antibody targeting CCR8, developed for selective depletion of tumor-infiltrating Tregs. A phase 1 clinical study use GS-1811 as monotherapy or in combination with anti-PD1 therapy is now recruiting patients with advanced solid tumors (Clinicaltrials.gov, NCT05007782). Furthermore, several drugs, such as BL-8040[245], BKT140[246], BMS-936564 (ulocuplumab) [247, 248] and plerixafor (AMD3100) [249] have been developed that target CXCR4 and the interaction with CXCL12 also involved in controlling Treg migration into the TME, and are now under investigation in clinical studies on patients with solid tumors in single treatment arms or in combination with immune checkpoint inhibitors (See Table 4).

The Treg lineage-defining transcription factor FoxP3 is also considered as a potential target for anti-tumor therapy. A TCR mimicking antibody specific for FoxP3 epitopes (Foxp3-#32) was developed to target an intracellular FoxP3 epitope in the context of HLA-A*02:01, which could selectively recognize $FoxP3^+$Tregs, and induce Treg depletion in xenografts [250]. AZD-8701 is a FoxP3 antisense oligonucleotide designed to inhibit FoxP3, and thereby target expression of genes such as CTLA4, ICOS, CCR8 and GITR. Administration of AZD-8701 in mice with A20 and ID8-VEGF tumor models revealed significant attenuation of tumor growth and regression, as well as enhanced effects when combined with blockade of immune checkpoints [251]. A phase 1 clinical trial is now evaluating effect of AZD8701 in patients with advanced solid tumors, in single treatment or in combination with durvalumab (Clinicaltrials.gov, NCT04504669).

The NRP1 antagonist [Fc(AAG)-TPP11] inhibits the intratumoral stability and function of $Nrp1^+$ Tregs by reducing FoxP3 expression and introducing IFN-γ production, which attenuates tumor growth in mouse colon cancer and melanoma tumor models [226]. The small molecular compound EG01377 is designed as a selective antagonist of Nrp1 that can block TGFβ production from $Nrp1^+$ Tregs, providing basis for future *in vivo* studies [252]. In addition, ASP1948 (also known as PTZ 329) is an anti-Nrp1 monoclonal antibody targeting the SEMA4A binding domain, which is now in a phase 1 clinical trial on patients with advanced solid tumors (Clinicaltrials.gov, NCT04094506, NCT03565445). Notably, anti-CTLA4 antibody could also deplete tumor infiltrating Tregs due to elevated expression of CTLA4 on Tregs in the TME. For instance, it is reported that Fc-engineered ipilimumab can selectively deplete intratumoral Tregs and the Treg:CD4 ratio to evoke anti-tumor immunity [253]. The combination of anti-PD1 and anti-CTLA-4 mAb treatments restores the Teff cell to Treg ratio in a pre-established B16 melanoma model [254]. Therefore, the combination of general drugs targeting Treg together with immune checkpoint inhibitors could benefit patients in cancer treatment.

Immune checkpoint activation in the TME

Immune checkpoints (ICs) are inhibitory pathways essential in the immune system to maintain balanced self-tolerance and to keep the collateral tissue damage in peripheral tissues to a minimum. It is now well accepted that tumor cells highjack the immune checkpoint pathways as a key mechanism for immune evasion and anti-tumor immune tolerance. T-cell mediated immunity is regulated by balanced ligand-receptor interaction inducing both stimulatory and inhibitory signals, a fine-tuned response. Immune checkpoint receptors are inhibitory molecules expressed on T cells, which mainly use monotyrosine signaling motifs such as immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM) to deliver inhibitory signals (see Figure 4). Interaction between ICs and their ligands on tumor cells thereby lead to T cell exhaustion and inactivation [255]. Overexpression of inhibitory ligands of checkpoint receptors in various types of cancer cells are observed in response to cytokine stimulation in the TME and in oncogenic signaling, favoring tumor immune evasion and tumor growth [256].

CTLA4 (Cytotoxic T-lymphocyte-associated protein 4) was the first identified co-inhibitory immune checkpoint inhibitor and binds to the B7 ligands CD80 and CD86 that competes for CD28 binding, attenuating TCR activation in T cells [257]. Upon interaction with the ligand, phosphorylated CTLA-4 leads to dephosphorylation of the CD3ζ chain and attenuation of TCR activation through PI3K pathway, as well as suppression of the IL-2 gene transcription and the cell cycle machinery that is required for T cell proliferation and expansion [258-260]. Moreover, constitutive expression of CTLA4 on Tregs can enhance its immunosuppressive activity on effector T cells [261]. Hence, blockade of CTLA4 could result in increased T cell activity against tumor. Anti-CTLA4 monoclonal antibodies have therefore been developed and evaluated clinically on their effect on restoring anti-tumor immunity. Ipilimumab is an FDA-approved anti-CTLA4 mAb for late-stage melanoma treatment since 2011, which is now broadly evaluated in clinical trials on patients with colorectal cancer, cutaneous melanoma and renal cell carcinoma [262]. Zalifrelimab is another anti-CTLA4 mAb evaluated in clinical trials with promising anti-tumor effects in metastatic cervical cancer treatment [263].

Another well-studied immune checkpoint, PD1 (programmed cell death protein 1), is expressed on the surface of T cells upon TCR stimulation and next interacts with PD-L1 and PD-L2 on antigen presenting cells or tumor cells. Interaction of PD1 with its ligand mediates the recruitment of phosphatase SHP2 to dephosphorylate the TCR-proximal ZAP70 molecule. This perturbs the effect of the co-stimulatory molecule CD28 and consequently blocks T cell activation and inflammatory cytokine production [264]. The interaction also induces cell cycle arrest of T cells by transcriptional inhibition of Bcl-XL that is required for cell survival [265]. Thus, anti-PD1 therapies have been shown to restore effector T cell function and boost anti-tumor immune responses. Nivolumab is an anti-PD1 monoclonal antibody, first approved by FDA in 2014 for treatment of melanoma patients, and later shown in clinical trials to benefit patients with different cancer types, including malignant melanoma, non-small cell lung cancer, renal cancer, gastric cancer, cervical cancer, hepatocellular carcinoma, and MSI positive colorectal cancer [266, 267]. Pembrolizumb is another anti-PD1 monoclonal antibody approved for treatment of cervical cancer [268], esophageal cancer [269], gastric cancer [270], non-small cell lung cancer [271], colorectal cancer [272] and other types of cancer [273]. Recently, a novel anti-PD1 antibody, cemiplimab, was approved for treatment of metastatic cutaneous squamous cell carcinoma in 2018 [274]. As the ligand of PD1, PD-L1 inhibitors are also developed to disrupt the interaction between PD1 and PD-L1 in the TME. Atezolizumab was the first approved anti-PD-L1 antibody in 2018 for treating metastatic urothelial carcinoma [275], and was subsequently approved for NSCLC [276], TNBC [277] and SCLC treatment [278] in combinations with other chemo- or targeted therapies.

In addition to CTLA4 and PD1, many novel ICIs have been discovered and investigated, such as Lymphocyte-activation gene 3 (LAG3), T cell immunoglobulin-3 (TIM3), T cell immunoreceptor with Ig and ITIM domains (TIGIT) and V-domain Ig suppressor of T cell activation (VISTA) (see Figure 4 for their signalling) [256]. Mechanistically, LAG3 (CD223) binds to MHCII and to liver and lymph node sinusoidal endothelial cell C-type lectin LSECtin to inhibit IFN-γ release from Teff [279]. TIM3 interacts with galectin-9 and carcinoembryonic antigen-related cell adhesion molecule (CEACAM) on tumor cells to promote immune escape. Interaction of TIM3 and Galectin-9 promotes dissociation of HLA-B-associated transcript 3 (Bat3) which decreases IFN-γ production and inhibits T cell proliferation, triggering cell death in Th1 cells [280]. The interaction of TIM-3 with high mobility group box 1 (HMGB1) helps the recruitment of nucleic acids into endosomes to promote tumor immune escape [281]. In addition, binding of TIM3 to CEACAM1 (carcinoembryonic antigen cell adhesion molecule 1) induces TIM-3 maturation and functions on exhausted T cells [282]. VISTA interacts with its ligand VSIG-3, elevated in many cancers, to suppress T cell activation and proliferation [283]. TIGIT is expressed on T cells and NK cells, binding to CD155 (PVR) and CD112 (PVRL2, nectin-2) ligands expressed on tumor cells and antigen-presenting cells in TME [284]. Inhibitors targeting these novel ICs have also been developed and are being evaluated in clinical trials for anti-tumor treatment (See Table 5). However, many cancer patients do not respond or develop resistance to treatment with single ICIs. This may be due to their tumor type generally or that the individual patient's tumor specifically does not trigger the immune system ("cold"), either because it is not recognized as foreign (no effective neoepitopes, low tumor mutational burden) or because the tumor uses other tumor immune evasion mechanisms than through ICs. For that reason, the combined effect of ICIs with cytostatic drugs, radiation (for abscopal effects) and targeted therapies, have also been extensively evaluated in numerous clinical trials releasing additional effects. Moreover, as additional immune checkpoint receptors such as TIGIT, PD1, LAG3 and TIM3 may be co-expressed and acting in parallel or other tumor immune evasion mechanisms as discussed here are could be active instead or in parallel with ICs in the same tumor there may be potential for enhancing efficacy by combining different ICIs or adding drugs that perturb other immune evasion mechanisms [285] (See Table 5).

Signaling network crosstalk in the TME and potential combined cancer immunotherapies

Modification of tumor stroma induces immune evasion

In the TME, the interaction of cancer cells with tumor stroma can modify anti-tumor immune responses to create a supportive environment for tumor progression. Stroma cells, such as cancer associated fibroblasts (CAFs) are generally considered to promote an immune-suppressive TME by affecting recruitment of immune cells [286]. Tumor-derived extracellular vesicles (TDEVs) are lipoproteic structures carrying bio-molecules such as mRNA, miRNA, DNA and proteins. TDEVs released into the TME function in reshaping the anti-tumor immune responses. Indeed, TDEVs promote expansion of suppressive immune cells such as MDSCs and Tregs, inhibit differentiation of myeloid cells and DCs, suppress NK cells and induce apoptosis of CD8+ T cells [287]. Importantly, TEVs can induce PD-L1 expression in myeloid cells, enhancing anti-tumor immune suppressive functions [288]. Moreover, circulating levels of PD-L1 in TEVs from HNSCC patients was shown to correlate with tumor stage [289] and is considered as a putative diagnostic and prognostic marker in pancreatic ductal cancer patients [290].

New vessel formation or angiogenesis is essential for tumor cell growth and disease progression. Dysregulation of tumor vessels serves as a physical barrier to hinder anti-tumor immune cell infiltration. As the key driver of angiogenesis, VEGF is also considered as a potent

immunosuppressive factor. Indeed, VEGF interferes with DCs maturation and suppresses Tcell priming [291], while promoting Treg accumulation [292] and introducing CD8+ Tcell exhaustion [293]. As the most-studied VEGF family member, VEGF-A produced by tumors is demonstrated to induce T cell exhaustion by increasing expression of PD1, CTLA4, TIM3 and LAG3 [294]. In colorectal cancer, VEGF-A is involved in promoting Treg proliferation in a VEGF receptor (VEGFR)-2 dependent manner [295].

Drugs targeting VEGF/VEGFR are under clinical development and subject to evaluation of efficacy in cancer treatment. There are mainly two types of drugs targeting VEGF/VEGFR: tyrosine kinase inhibitors with activity to VEGFR, including sunitinib, sorafenib and axitinib and monoclonal antibodies such as bevacizumab targeting VEGF-A and ramucirumab targeting VEGFR-2 [296]. In detail, sunitinib treatment in RCC patients significantly decreased the abundance of Tregs and increased IFN-γ producing T cells [297]; Sorafenib significantly decreased Treg levels in patients with HCC [298]; Axitinib treatment in patients with recurrent glioblastoma demonstrated increase of CD8+ Tcells and reduction of TIM3 expression [299]; Bevacizumab reduced the percentage of Tregs in PBMCs from patients with recurrent ovarian cancer and mCRC [295, 300]; Ramucirumab treatment in patients with advanced gastric cancer showed reduction of tumor-infiltrated Tregs and increase of CD8+ T cell infiltration [301],

In addition, combination of anti-angiogenic therapy and ICIs have been approved by FDA for treatment in patients with HCC, RCC, lung or uterine cancer [302]. In the IMBrave150 clinical trial on patients with HCC, combination of bevacizumab and atezolizumab (anti-PD-L1) revealed higher overall survival compared to sorafenib single treatment (clinicaltrials.gov, NCT03434379) [303]. Resukts from the phase 3 clinical trials in patients with advanced renal cell carcinoma (KEYNOTE-426 and JAVELIN Renal 101 trial) evaluate combining anti-PD1 or anti-PDL1 with axitinib or sunitinib single treatment and with preliminary results showing significantly longer progression-free survival in combined therapy (clinicaltrials.gov, NCT02853331 and NCT02684006) [304, 305]. A phase II clinical trial evaluating combination of anti PD-L1 (atezolizumab) and bevacizumab is recruiting patients with advanced melanoma (clinicaltrials.gov, NCT04356729). Moreover, a bi-specific antibody AK112 against PD-1 and VEGF is approved for clinical trials in patients with advanced non-small cell lung cancer and advanced gynecologic tumors (clinicaltrial.gov, NCT05499390, NCT04900363 and NCT04870177).

Hypoxia-induced tumor immune escape

Hypoxia is common in the TME as a result of tumor growth, aberrant vascularization and poor blood supply causing restricted access to oxygen and nutrients. The hypoxic TME is universally correlated to poor prognosis and survival in many types of cancer. Indeed, hypoxia in the TME not only enhances tumor cell heterogeneity and resistance to therapy allowing tumor cell survival, but also induces immune tolerance and immune escape through different regulatory pathways [306].

Importantly, a hypoxic TME facilitates recruitment of Tregs and promotes the conversion of monocytes to MDSCs and tumor-associated macrophages [307]. For instance, hypoxia-induced expression of CCL28 in ovarian cancer led to a preferred recruitment of $CCR10^+$ Tregs, which in turn increased tumor immune tolerance and promoted angiogenesis [217]. Moreover, hypoxia-inducible factor (HIF)-1α binds to the FoxP3 promoter and induces FoxP3 gene expression in a TGFβ-dependent manner [308]. Furthermore, HIF-2α is a positive regulator of COX2 expression [309], and higher expression of CD39 [310] and CD73 [311] can also be induced by hypoxia, which in turn elevates PGE_2 and adenosine levels in TME, respectively, favoring peripheral induction and differentiation of Tregs that next exert suppressive functions on effector T cells. The hypoxic TME also plays a critical role in

driving IFN-γ-mediated Treg fragility which is required for response to anti-PD1, regulated by HIF-1α [312]. In addition, expression of Galectin-1 is regulated by HIF1α. Galectin-1 level is a prognostic marker in HNSCC inversely correlated with T cell infiltration in tumor samples [313]. Finally, hypoxia also induces expression of ICs, such as PD-L1, CD47 and VISTA [314].

Therapeutic targeting of hypoxia is a strategy that has potential to augment anti-tumor immunity. The drugs targeting hypoxia that are most developed are hypoxia-activated prodrugs, small molecules interfering with HIF signaling or inhibiting the downstream UPR and mTOR pathways as well as metabolic intervention. For instance, evofosfamide (TH-302) is an effective hypoxia-activated bio-reductive prodrug that can reduce and eliminate hypoxia in the TME in prostate cancer [315]. In addition, a phase 2 clinical trial with TH-302 in patients with recurrent bevacizumab-refractory glioblastoma revealed significant improvement of progression-free survival [316]. Combination of TH-302 and blockade of PD1 and CTLA4 cooperatively cures more than 80% of tumors in a prostate cancer mouse model by reducing MDSC-density and promoting T cell infiltration and activation [317]. In addition, TH-302 in a phase II study on patients with HNSCC, showed synergistic anti-tumor effect in PDX models when in combination with CTLA4 blockade [318]. A clinical trial using TH-302 in combination with ipilimumab is aiming to evaluate efficacy in different solid tumors (Clinicaltrials.gov, NCT03098160).

Combined therapies with immune checkpoint inhibitors and other immune modulators

As discussed above, a few immune checkpoint inhibitors have been approved by FDA and EMA, including anti-CTLA4 (ipilimumab or tremelimumab), anti-PD1/PD-L1 (nivolumab, pembrolizumab, cemiplimab, avelumab, durvalumab and atezolizumab) in treatment for metastatic melanoma, advanced NSCLC, Hodgkin's lymphoma, squamous cell cancer of the head and neck, Merkel cell carcinoma, hepatocellular carcinoma and gastric cancer. However, only a fraction of patients receiving ICIs reveal long-term responses. In addition, immune-related adverse events (irAEs) are observed. As monotherapies, ICIs fail to achieve durable clinical responses in 60% of cancer patients or more. Relapses and drug-resistance are also observed in patients receiving ICIs treatments [319].

The differential response to ICIs in patients may be due to distinct expression patterns of ICs and variable properties of the tumor infiltrating lymphocytes. Combination of ICIs, or combinations of ICIs with other immune therapeutic strategies such as TME modulating reagents, or cell therapies (CAR-T or TCR engineered cells) are therefore emerging and may potentially produce synergistic effects with ICIs on tumor treatment (see Table 5). For instance, the combination of anti-CTLA4 and anti-PD1 treatment (nivolumab and ipilimumab) demonstrated complementary activity of combined therapy in metastatic melanoma with long-term benefits. The 5-year overall survival in the combined group (52%) was higher than in the nivolumab (44%) or ipilimumab(26%) groups alone [320]. Combination of anti-TIM3, anti-LAG3, anti-VISTA, anti-GITR or anti-TIGT treatment with anti-PD1 or anti-CTLA4 are also currently evaluated in clinical trials on patients with different types of tumors (see Table 5). In addition, as shown in the previous tables, drugs targeting adenosine signaling, the PGE_2 pathway, other soluble factors or Tregs in TME are generally investigated both as single therapy and in combinations with ICIs for potential synergistic effects on cancer treatment.

Conclusion

Anti-tumor immune responses are dampened in the TME due to multiple mechanisms that re-shape tumor cells and tumor infiltrating lymphocytes for immune escape. The mechanisms involved in immune evasion may be more complicated and interconnected than recognized so far and discussed here. Therefore, further mapping of the crosstalk between tumor and immune cells as well as of

immunoregulatory signaling networks in different tumors and individual patients may be necessary as a basis for developing more tailored anti-tumor immunoregulatory treatments in the future.

The gene expression/mutation profile and protein modifications of both tumor and immune cells can be altered distinctly in the TME of individual patients which may affect both neoepitope expression and tumor immune evasion mechanisms. For instance, exhausted T cells are shown to lack epigenetic plasticity. As precision medicine approaches progress, differential gene expression and modification patterns in patients may have to be investigated more deeply, for example by single-cell sequencing and immune profiling of the TME of individual patients providing opportunities to choose specific and individualized drug combinations to optimize anti-tumor immune responses.

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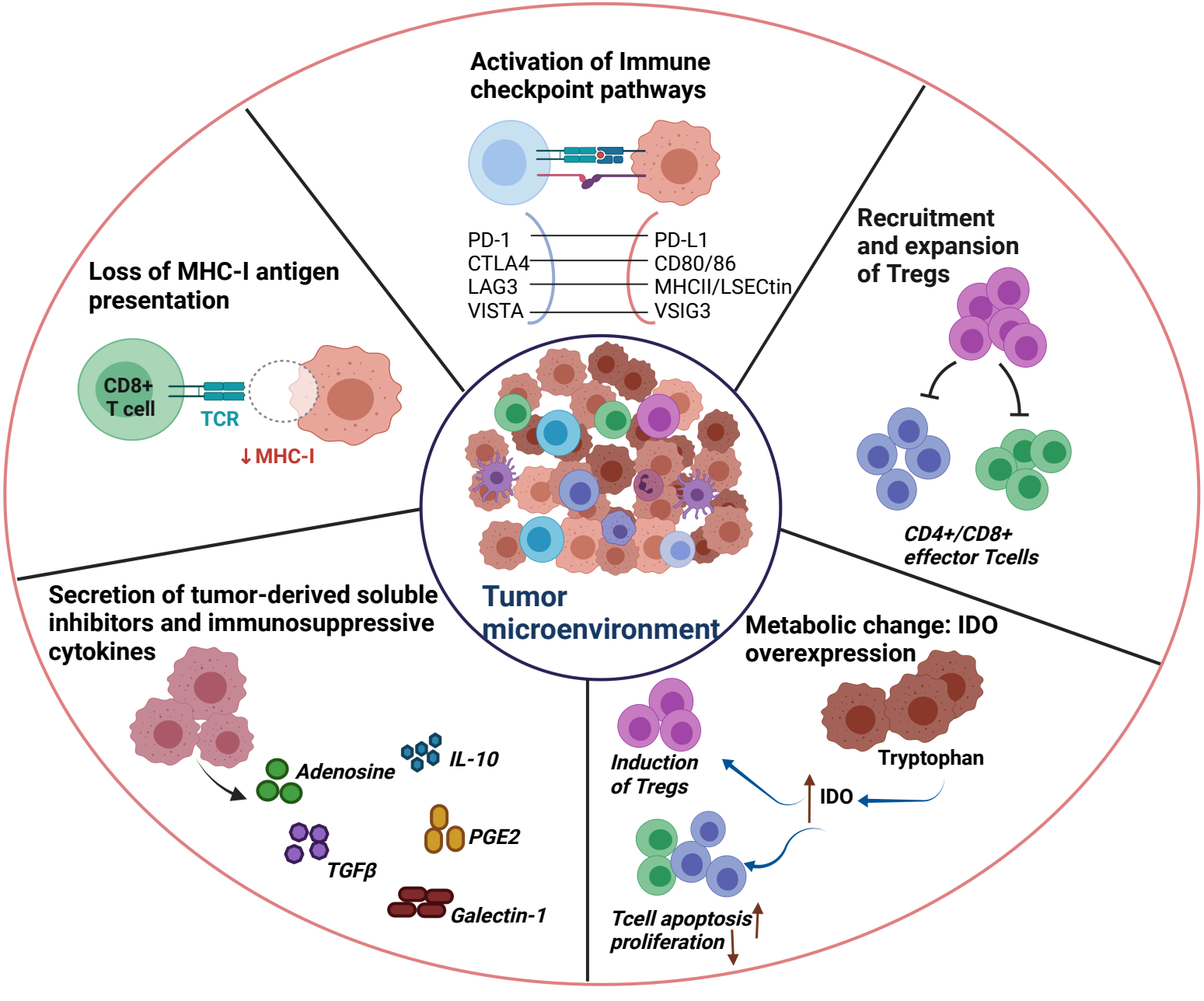
Figure legends

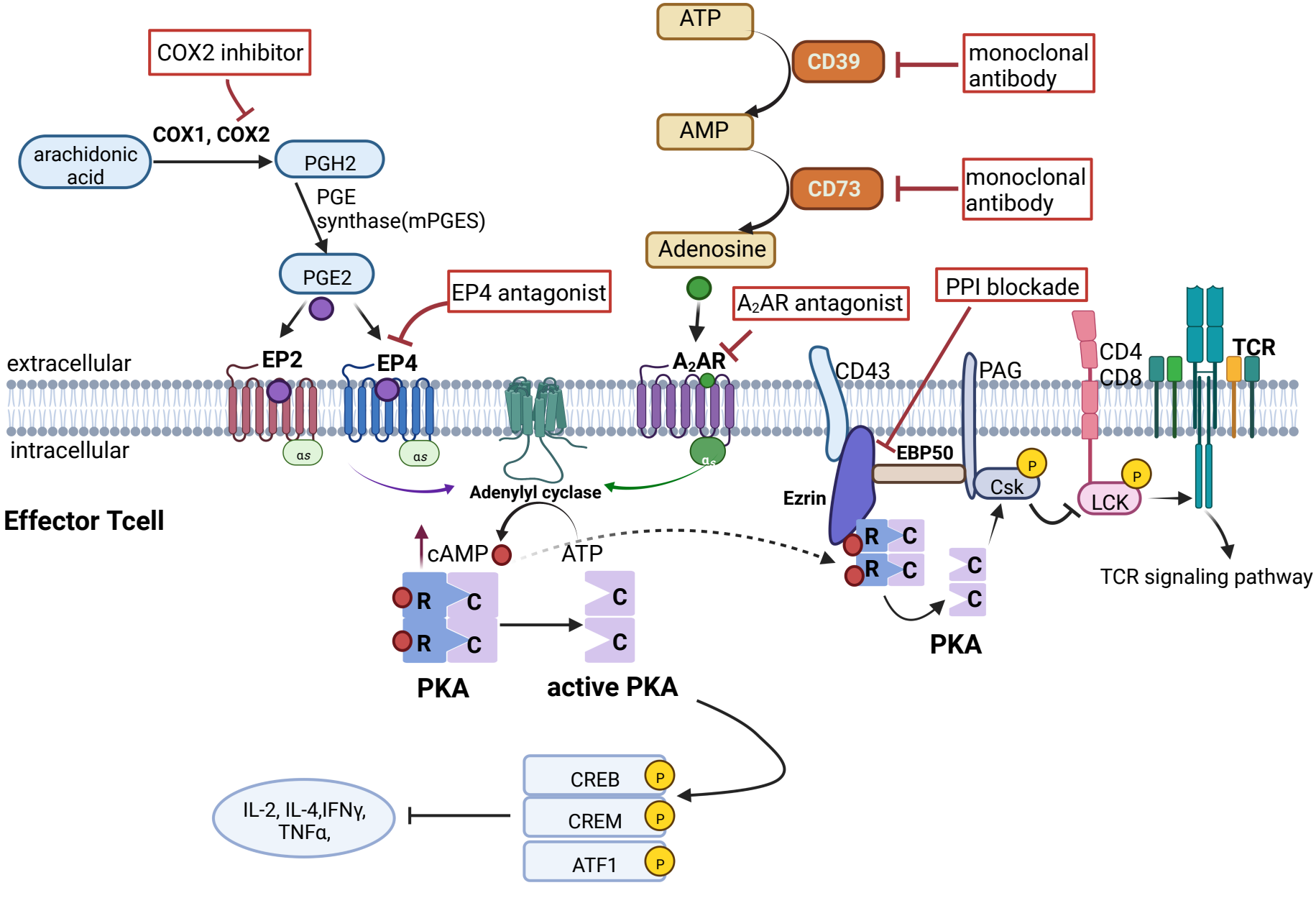
Figure 1. Overview of tumor immune evasion mechanisms focusing on T cell-tumor interaction. In the TME, tumor immune evasion mechanisms can be classified into five classes. These are activation of immune checkpoint pathways and recruitment and expansion of suppressive Tregs which induces exhaustion or functional inhibition of effector T cells (top and top right panels). Furthermore, metabolic changes in tumor cells resulting from increased expression of IDO, secretion of tumor-derived soluble inhibitors and immunosuppressive cytokines and loss of MHC I antigen presentation on tumor cells also inhibit or perturb effector T cell functions (bottom right and left panels).

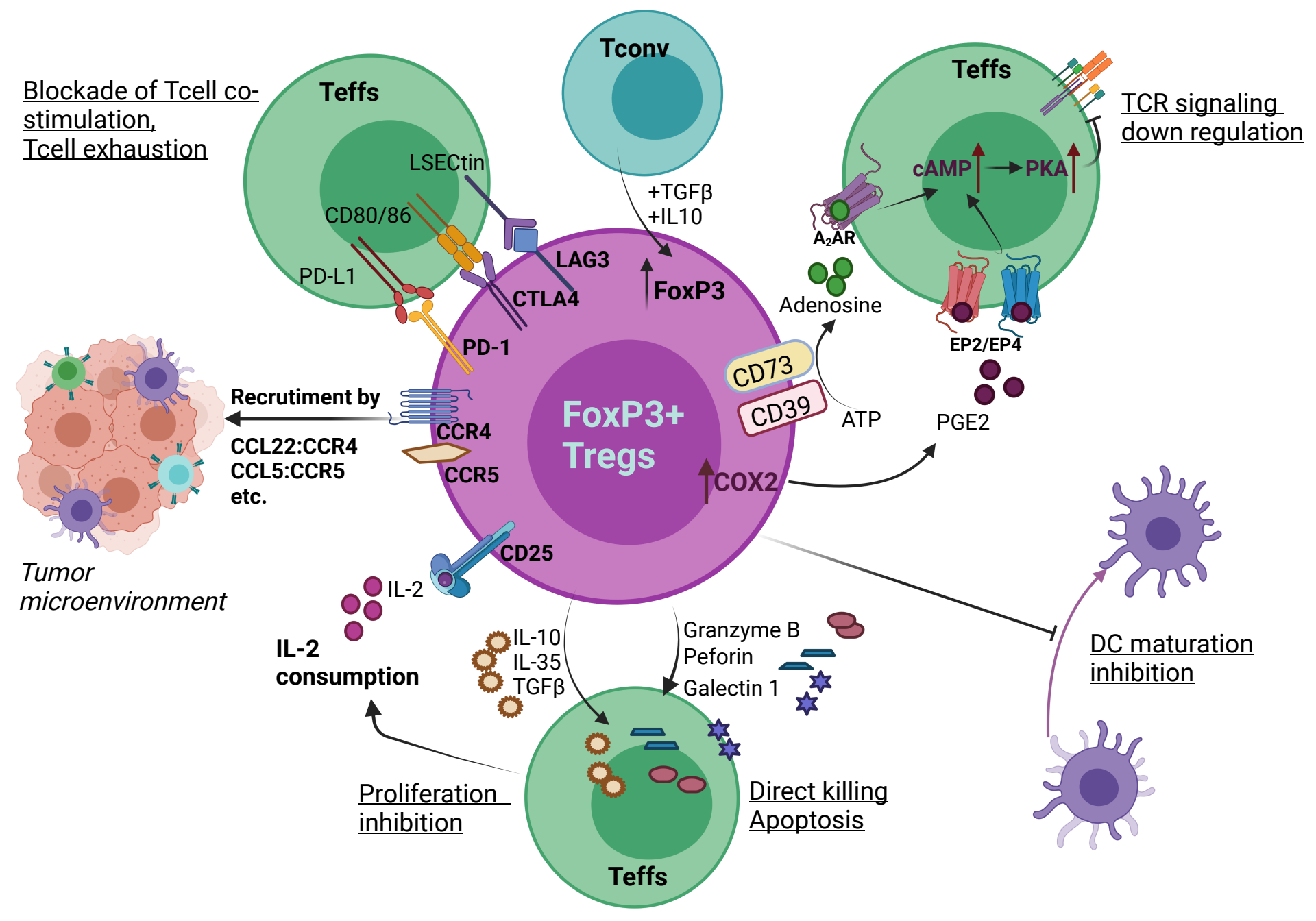
Figure 2. The cAMP-PKA pathway in T cells. As PGE_2 and adenosine converge on the second messenger cAMP downstream of their cognate receptors (EP2/EP4 for PGE_2, A_2aR for adenosine), the cAMP-PKA-Csk pathway serves as a key negative regulator of TCR proximal signaling in T cells. Inhibition of production of PGE_2 and adenosine, receptor antagonists, as well as blockade of ezrin and EBP-50 interaction are current strategies for drug development (indicated by red boxes).

Figure 3. Suppressive mechanisms of regulatory T cells. Tregs are recruited to the TME by chemokines interacting with cognate receptors. The inhibitory function of Tregs on Teffs is exerted through different mechanisms, including proliferation inhibition and direct killing or apoptosis of Teffs; inhibition of DC maturation; TCR signaling down regulation via the adenosine-A_2aR receptor- or PGE_2-EP2/4 receptor-PKA pathway; blockade of co-stimulation and introduction of Teff exhaustion by inhibitory receptors on Tregs. In addition, TGFβ and IL-10 contribute to the conversion of Tconv into peripherally induced Tregs.

Figure 4. Representative immune checkpoint signalling pathways in T cells. Tumor cell/APC ligand interactions with immune checkpoint receptors on T cells initiates inhibitory signaling pathways to prevent proliferation and activation. For example, interaction of PD-L1 with PD-1 deactivates phosphorylation of ZAP70 through SHP2, which suppresses downstream PI3K activation essential for T cell proliferation; CTLA4 competes the interaction of CD80/CD86 with CD28 thus dampening co-stimulation of TCR signaling through SHP2 and PP2A; Interaction of LSEctin with LAG3 inhibits IFN-γ production in T cells; and TIM3 interacts with Galectin 9 to activate Bat3 which subsequently suppresses IFN-γ production.


Activation of Immune checkpoint pathways
PD-1
CTLA4
LAG3
VISTA
PD-L1
CD80/86
MHCII/LSECtin
VSIG3
Loss of MHC-I antigen presentation
CD8+ T cell
TCR
↓MHC-I
Recruitment and expansion of Tregs
CD4+/CD8+ effector Tcells
Tumor microenvironment
Secretion of tumor-derived soluble inhibitors and immunosuppressive cytokines
Adenosine
IL-10
PGE2
TGFβ
Galectin-1
Metabolic change: IDO overexpression
Induction of Tregs
Tryptophan
IDO
Tcell apoptosis
proliferation


COX2 inhibitor
COX1, COX2
arachidonic acid
PGH2
PGE synthase(mPGES)
PGE2
EP4 antagonist
ATP
CD39
monoclonal antibody
AMP
CD73
monoclonal antibody
Adenosine
A2AR antagonist
PPI blockade
extracellular
intracellular
EP2
EP4
A2AR
CD43
PAG
CD4
CD8
TCR
αs
Adenylyl cyclase
EBP50
Ezrin
Csk
LCK
P
Effector Tcell
cAMP
ATP
R
C
PKA
active PKA
TCR signaling pathway
CREB
CREM
ATF1
IL-2, IL-4,IFNγ, TNFα,


Blockade of Tcell co-stimulation, Tcell exhaustion
Teffs
LSECtin
CD80/86
PD-L1
Tconv
+TGFβ
+IL10
LAG3
CTLA4
FoxP3
PD-1
FoxP3+ Tregs
Teffs
cAMP
PKA
TCR signaling down regulation
A2AR
Adenosine
EP2/EP4
CD73
CD39
ATP
PGE2
COX2
Recrutiment by
CCL22:CCR4
CCL5:CCR5
etc.
CCR4
CCR5
Tumor microenvironment
CD25
IL-2
IL-2 consumption
IL-10
IL-35
TGFβ
Granzyme B
Peforin
Galectin 1
DC maturation inhibition
Proliferation inhibition
Direct killing
Apoptosis
Teffs

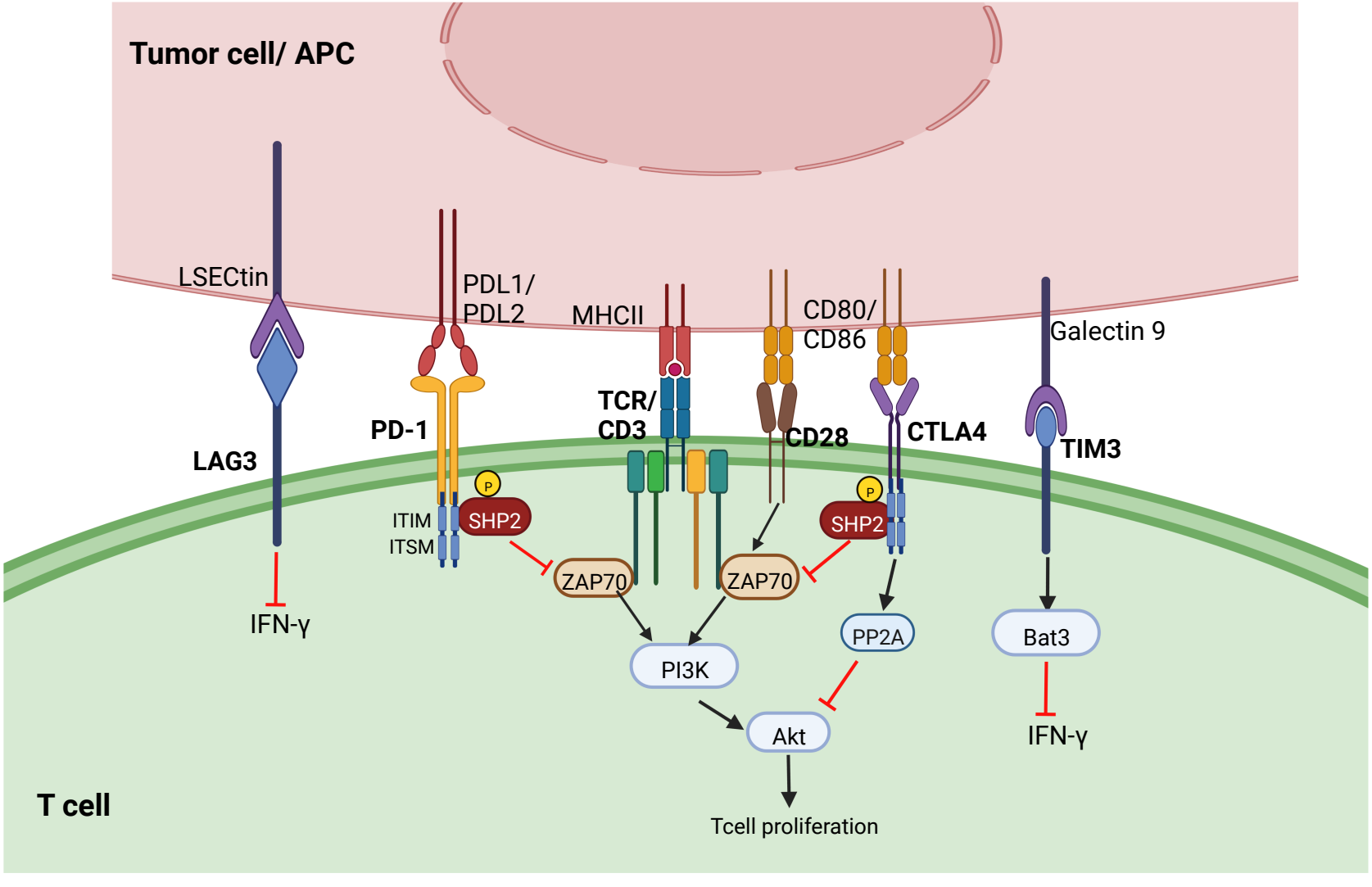

Tumor cell/ APC
LSECtin
PDL1/
PDL2
MHCII
CD80/
CD86
Galectin 9
LAG3
PD-1
TCR/
CD3
CD28
CTLA4
TIM3
P
ITIM
ITSM
SHP2
SHP2
ZAP70
ZAP70
PI3K
PP2A
Bat3
Akt
IFN-γ
IFN-γ
Tcell proliferation
T cell

Table 1. Targeting the adenosine pathway

Drug	Target	Cancer type	Stage	Clinical trial ID	Treatment detail	Status
TTX-030	anti-CD39 monoclonal antibody	Advanced solid tumor	Phase 1/1b	NCT03884556	in combination with Gemcitabine, paclitaxel, pembrolizumab, docetaxel	Recruiting
TTX-030	anti-CD39 monoclonal antibody	Adult solid tumor	Phase 1/1b	NCT04306900	in combination with immunotherapy or standard chemotherapies	Recruiting
SRF617	anti-CD39 monoclonal antibody	Metastatic Castration Resistant Prostate Cancer	Phase 2	NCT05177770	in combination with etrumadenant and zimberelimab	Recruiting
SRF617	anti-CD39 monoclonal antibody	Advanced solid tumor	Phase 1	NCT04336098	in combination with Gemcitabine, paclitaxel, pembrolizumab	Recruiting
IPH5201	anti-CD39 monoclonal antibody	Advanced solid tumor	Phase 1	NCT04261075	monotherapy or in combination with durvalumab(anti-PD-1) +/- oleclumab(anti-CD73)	Active, not recruiting
CPI-006	anti-CD73 monoclonal antibody	Advanced solid tumor	Phase 1/1b	NCT03454451	alone or in combination with ciforadenant (A2AR antagonist, CPI444) and pembrolizumab (anti-PD1)	Recruiting
NZV930	anti-CD73 monoclonal antibody	Advanced Malignancies	Phase 1	NCT03549000	alone or in combination with PDR001(anti-PD1)/ NIR178(A2AR antagonist)	Recruiting
NZV930	anti-CD73 monoclonal antibody	Advanced solid tumor	early phase 1	NCT04237649	in combination with KAZ954(no information), PDR001 and NIR178.	Recruiting
INCA 0186	anti-CD73 monoclonal antibody	Advanced Solid Tumors	Phase 1	NCT04989387	in combination with INCB106385(dual A2AR/A2BR antagonsit) and/or retifanlimab(anti-PD1)	Recruiting
Oleclumab(MEDI9447)	anti-CD73 monoclonal antibody	Advanced Solid Malignancies	Phase 1	NCT03736473		Completed
Oleclumab(MEDI9447)	anti-CD73 monoclonal antibody	Recurrent, Refractory, or Metastatic Sarcoma	Phase 2	NCT04668300	in combination with durvalumab(anti-PD1)	Recruiting
Oleclumab(MEDI9447)	anti-CD73 monoclonal antibody	Luminal B Breast Cancer	Phase 2	NCT03875573	After radiotherapy, in combination with anti-PD-L1 durvalumab	Recruiting
Oleclumab(MEDI9447)	anti-CD73 monoclonal antibody	Triple Negative Breast Cancer	Phase 2	NCT03616886	combination of chemotherapy (paclitaxel + carboplatin) , durvalumab(anti-PD-L1)	Recruiting
Oleclumab	anti-CD73 monoclonal antibody	Resectable/Borderline Resectable Primary Pancreatic Cancer	Phase 2	NCT04940286	in combination with Gemcitabine, Nab-paclitaxel, durvalumab	Recruiting
Oleclumab	anti-CD73 monoclonal antibody	Pancreatic cancer	Phase1b/2	NCT03611556	MEDI9447(Oleclumab) in combination with pancreatic chemotherapy	Active, not recruiting
Oleclumab	anti-CD73 monoclonal antibody	NSCLC	Phase 1b/2	NCT03381274	together with AZD-4635 (A2AR antagonist) and osimertinib standard treatment	Active, not recruiting
Oleclumab	anti-CD73 monoclonal antibody	Select Advanced Solid Tumors	Phase 1	NCT02503774	MEDI9447 alone ,or in combination with durvalumab (anti-PD1)	Active, not recruiting
BMS-986179	anti-CD73 monoclonal antibody	Malignant Solid Tumor	Phase 1/2	NCT02754141	alone or in combination with nivolumab (anti-PD1)	Active, not recruiting
AB680	anti-CD73 monoclonal antibody	Gastrointestinal Malignancies/Advanced Pancreatic Cancer	Phase 1	NCT04104672	in combination with zimberelimab(anti-PD1), nab-paclitaxel and Gemcitabine	Recruiting
NIR178	A2AR antigonist	Triple Negative Breast Cancer	Phase 1	NCT03742349	in bombination with spartalizumab(anti-PD1),LAG525(anti-LAG3),capmatinib, MCS110, or canakinumat	Recruiting
NIR178	A2AR antigonist	Solid Tumors and Non-Hodgkin Lymphoma	Phase 2	NCT03207867	in combination with PDR001 (anti-PD1)	Recruiting
NIR178 (PBF-509)	A2AR antigonist	non-small cell lung cancer (NSCLC)	Phase 1/2	NCT02403193		Active, not recruiting
AZD-4635	A2AR antagonist	Advanced Solid Malignancies	Phase 1	NCT03980821	Japanese patients	Completed
AZD-4635	A2AR antagonist	Advanced Solid Malignancies	Phase 1	NCT02740985	monotherapy or in combination with durvalumab(anti-PD-1) +/- oleclumab(anti-CD73) and other chem	Active, not recruiting
AZD-4635	A2AR antagonist	metstatic castration-resistant prostate cancer	Phase 2	NCT04495179	in combination with durvalumab(anti-PD1)and cabazitaxel (hormone treatment)	Active, not recruiting
AZD4635	A2AR antagonsit	Prostate cancer	Phase2	NCT04089553	in combination with oleclumab (amti-CD73)+durvalumab(anti-PD1)	Active, not recruiting
CPI444	A2AR antigonist	Advanced solid tumor	Phase 1/1b	NCT02655822	in combination with atezolizumab (anti-PD1)	Completed
CPI444+daratumumab	A2AR antigonist	refractory multiple myeloma	Phase 1b	NCT04280328	In combination with daratumumab (anti-CD38)	Active, not recruiting
EOS100850 (inupadenant)	A2AR antigonist	solid tumor	Phase 1	NCT03873883	single or in combination with Pembrolizumab(anti-PD1) and/or chemotherapy	Recruiting
EOS100850 (inupadenant)	A2AR antigonist	Advanced Solid Tumors	Phase 1/2	NCT05060432	In combined with EOS448 (anti-TIGIT) as a second treatment arm	Recruiting
AB928 (etrumadenant)	Dual adenosine receptor A2AR and A2BR antagonist	metastatic colorectal cancer	Phase 1/2	NCT04660812	in combination with PD-1 treatment and other stantdard chemotherapy	Recruiting
AB928 (etrumadenant)	Dual adenosine receptor A2AR and A2BR antagonist	Metastatic Castrate Resistant Prostate Cancer	Phase 1b/2	NCT04381832	in combination with zimberelimab, AB680(anti-CD73), Enzalutamide and Docetaxel	Recruiting
AB928 (etrumadenant)	Dual adenosine receptor A2AR and A2BR antagonist	GastroEsophageal Cancer/ Colorectal Cancer	Phase 1	NCT03720678	in combination with mFOLFOX(chemotherapy)	Completed
AB928 (etrumadenant)	Dual adenosine receptor A2AR and A2BR antagonist	Triple-Negative Breast Cancer or Ovarian Cancer	Phase 1/1b	NCT03719326	in combination with doxorubicin +/- IPI-549(PI3K inhibitor), or albumin-bound-paclitaxel	Completed
AB928 (etrumadenant)	Dual adenosine receptor A2AR and A2BR antagonist	Advanced Malignancies	Phase 1	NCT03629756	in combination with zimberelimab (AB122,anti-PD-1 antibody)	Completed

Table 2. Targeting the PGE2 pathway

Drug	Target	Cancer type	Stage	Clinical trial ID	Treatment detail	Status
Aspirin	COX2 inhibitor	Colorectal cancer liver meta	Phase 2/3	NCT03326791		Recruiting
Aspirin	COX2 inhibitor	Colorectal Cancer	Phase 3	NCT02647099	patients with PIK3	Recruiting
Aspirin	COX2 inhibitor	Colorectal Cancer	Phase 3	NCT02607072	Asian patients, Ace	Recruiting
Aspirin	COX2 inhibitor	Gastric Cancer	Phase 3	NCT04214990	Early Gastric Cance	Recruiting
Aspirin	COX2 inhibitor	Ovarian cancer	Early phase 1	NCT05080946	with neoadjuvant c	Recruiting
Aspirin	COX2 inhibitor	Colon cancer	Phase 3	NCT02467582		Active, not recruiting
Aspirin	COX2 inhibitor	Stage III colorectal cancer	Phase 3	JCOG1503C		
Aspirin	COX2 inhibitor	Dukes C and High Risk Dukes	Phase 3	NCT00565708		Active, not recruiting
Aspirin	COX2 inhibitor	Colon Cancer	Phase 3	NCT02301286		Recruiting
Aspirin	COX2 inhibitor	Colon Cancer	Phase 3	NCT03464305		Recruiting
Aspirin	COX2 inhibitor	Non Metastatic Solid Tumou	Phase 3	NCT02804815	breast, colorectal, g	Recruiting
Aspirin	COX2 inhibitor	Prostate cancer	Phase 2/3	NCT03103152	with or without Vit	Completed
Celecoxib	COX2 inhibitor	Breast Cancer	Phase 3	NCT02429427		Completed
Celecoxib	COX2 inhibitor	Pancreatic cancer	Phase 2	NCT03498326	in combination witl	Recruiting
Celecoxib	COX2 inhibitor	NSCLC	Phase 1/2	NCT00046839	In addition to field	Completed
Celecoxib	COX2 inhibitor	Hereditary Non-Polyposis Co	Phase 1	NCT00001693		Completed
Aspirin	COX2 inhibitor	Colorectal Cancer	Phase 2	NCT03638297	change to celebrex	Recruiting
Celecoxib	COX2 inhibitor	colon carcinoma	Phase 2	NCT03026140	in combination witl	Recruiting
Grapiprant	EP4 antagonist	Microsatellite Stable Colorec	Phase 1	NCT03658772	alone or incombina	Recruiting
Grapiprant	EP4 antagonist	Metastatic Inflammatory Bre	Phase 1/2	NCT05041101	in combination witl	Recruiting
AN0025	EP4 antagonist	advanced Solid Tumors	Phase 1	NCT04975958	in combination witl	Recruiting
AN0025	EP4 antagonist	advanced solid tumors	Phase 1b	NCT04432857	incombination with	Recruiting
TPST-1495	EP2+EP4 antagonist	solid tumors	phase 1a/1b	NCT04344795		Recruiting
ONO-4578	EP4 antagonist	advanced or metastatic solid	phase 1	NCT03155061		Active, not recruiting
INV1120	EP4 antagonist	advanced solid tumors	Phase 1	NCT04443088		Recruiting
BMS-986310	EP4 antagonist	Advanced Solid Tumors	Phase 1/2	NCT03661632	alone or in combin	Completed

Table 3. Targeting soluble factors.

Drug	Target	Cancer type	Stage	Trial ID	Treatment detail	Status
Galunisertib	TGFβ receptor inhibitor	advanced, or recurrent NSCLC, HCC	Phase1/2	NCT02423343	Galunisertib(LY2157299), in combination with Nivolumab(anti-PD1)	Completed
Galunisertib	TGFβ receptor inhibitor	metastatic castration-resistant prostate cancer	Phase 2	NCT02452008	in combination with enzalutamide	Recruiting
Galunisertib	TGFβ receptor inhibitor	Hepatocellular Carcinoma (HCC)	Phase 1	NCT02906397	in combination with Stereotactic Body Radiotherapy (SBRT)	Completed
Galunisertib	TGFβ receptor inhibitor	Metastatic Unresectable Pancreatic Cancer	Phase1/2	NCT01373164	in combination with Gemcitabine	Completed
Galunisertib	TGFβ receptor inhibitor	Rectal Adenocarcinoma	Phase 2	NCT02688712	in combination with chemotherapy and radiation therapy	Recruiting
Galunisertib	TGFβ receptor inhibitor	metastatic pancreatic cancer	Phase 1	NCT02734160	in combination with (durvalumab anti-PD-L1)	Completed
Fresolimumab	anti-TGFβ monoclonal antibody	metastatic breast cancer	Phase 2	NCT01401062	in combination with radiation therapy	Completed
Fresolimumab	anti-TGFβ monoclonal antibody	Relapsed Malignant Pleural Mesothelioma	Phase 2	NCT01112293		Completed
Fresolimumab	anti-TGFβ monoclonal antibody	early stage NSCLC	Phase 1/2	NCT02581787	in combination with Stereotactic Ablative Radiotherapy	Active, not recruiting
GC1008	anti-TGFβ monoclonal antibody	Relapsed Malignant Pleural Mesothelioma	Phase 2	NCT01112293		Completed
GC1008	anti-TGFβ monoclonal antibody	Renal cell carcinoma, malignant melanoma	Phase 1	NCT00356460		Completed
SAR-439459	anti-TGFβ monoclonal antibody	Advanced Malignant Solid Neoplasm	Phase 1	NCT04729725	in combination with cemiplimab(PD-L1 inhibitor)	Recruiting
TASO-001	TGF-β2 targeting anti-sense oligonucleotide	advanced or metastatic solid tumor	Phase 1	NCT04862767	in combination With Recombinant Interleukin-2(Aldesleukin)	Recruiting
AVID200	TGFβ1/TGFβ3 protein trap	Malignant Solid Tumor	Phase 1	NCT03834662		Active, not recruiting
GM-CT-01	Galectin1 inhibitor	solid tumor	Phase1	NCT00054977	with or without 5-Fluorouracil (5-FU)	Completed
OTX008	Galectin1 inhibitor	solid tumor	Phase 1	NCT01724320	Oncoethix GmbH	Unknown
GR-MD-02	Galectin3 inhibitor	metastatic melanoma	Phase1	NCT02117362	in combination with ipilimumab (anti-CTLA4)	Completed
GR-MD-02	Galectin3 inhibitor	melanoma, NSCLC, HNSCC	Phase1	NCT02575404	in combination with pembrolizumab (anti-PD1)	Active, not recruiting
LYT-200	anti-Galectin9 monoclonal antibody	Cholangiocarcinoma,Colorectal Cancer, Pancreatic Cancer	Phase 1/2	NCT04666688	in combination with chemotherapy(Gemcitabine/nab-paclitaxel) or anti-PD1	Recruiting
Pegilodecakin	recombinat rIL10	metastatic pancreatic cancer	Phase 3	NCT02923921	Pegilodecakin(LY3500518), in combination with FOLFOX versus FOLFOX alone	Completed
Pegilodecakin	recombinat rIL10	advanced solid tumor	Phase 1b	NCT02009449	monotherapy or in combination with chemotherapy or immunotherapy	Active, not recruiting
SHR1701	bifunctional anti-PD-L1/TGF-βRII	solid tumor	Phase 1	NCT03774979		Recruiting
SHR1701	bifunctional anti-PD-L1/TGF-βRII	Squamous Cell Carcinoma of Head and Neck	Phase 2	NCT04650633		Recruiting
TST005	bi-specific antibody of PD-L1 and TGF-βRII	Locally Advanced or Metastatic Cancers	Phase 1	NCT04958434		Recruiting

Table 4. Targeting Tregs

Drug	Target	Cancer type	Stage	Trial ID	Treatment detail	Status
Daclizumab	anti-CD25 monoclonal antibody	Adult T-cell leukemia	Phase 1/2	NCT00001941	Zenapax(Trademark)	Completed
Daclizumab	anti-CD25 monoclonal antibody	Leukemia, lymphoma	Phase 1/2	NCT00002681	plus IL-2 aldesleukin	Completed
Daclizumab	anti-CD25 monoclonal antibody	Stage IV melanoma	Phase 1/2	NCT00847106	Dendritic cell-based vaccines	Completed
LMB-2	anti-CD25+immunotoxicn (Anti-Tac(Fv)-PE38)	Chronic lymphocytic leukemia	Phase 2	NCT00077922		Completed
LMB-2	anti-CD25+immunotoxicn (Anti-Tac(Fv)-PE38)	Cutaneous T-Cell Lymphomas	Phase 2	NCT00080535		Completed
LMB-2	anti-CD25+immunotoxicn (Anti-Tac(Fv)-PE38)	Adult T-Cell Leukemia	Phase1/2	NCT00924170	in combination with Fludarabine and Cyclophosphamide	Completed
90 Y-HAT	anti-CD25 monoclonal antibody	Non-Hodgkin's Lymphoma and Lymphoid Leukemia	Phase1/2	NCT00001575	yttrium 90-labeled humanized anti-Tac	Completed
RO7296682 (RG6292)	anti-CD25 monoclonal antibody	Solid tumor	Phase 1	NCT04158583		Active, not recruiting
RO7296682 (RG6292)	anti-CD25 monoclonal antibody	Solid tumor	Phase 1	NCT04642365	in combination with atezolizumab (anti-PD-L1)	Recruiting
AZD-8701	antisense oligonucleotide (ASO) targeting FOXP3 mRNA	Advanced solid tumor	Phase 1	NCT04504669	alone or in combination with Durvalumab(anti-PD1]	
Mogamulizumab	anti-CCR4 monoclonal antibody	Relapsed or refractory Cutaneous T-Cell Lymphoma	Phase 2	NCT04745234		Recruiting
Mogamulizumab	anti-CCR4 monoclonal antibody	Locally advanced or metastatic solid tumors	Phase1/2	NCT02705105	in combination with Nivolumab(anti-PD1)	Recruiting
Mogamulizumab	anti-CCR4 monoclonal antibody	Relapsed or Refractory Diffuse Large B Cell Lymphoma	Phase1/2	NCT03309878	in combination with Pembrolizumab(anti-PD1)	Completed
Mogamulizumab	anti-CCR4 monoclonal antibody	Advanced and/or Metastatic Solid Tumors	Phase 1/2	NCT02281409		Active, not recruiting
KW-0761 (Mogamulizumab)	anti-CCR4 monoclonal antibody	Adult T-cell Leukemia-lymphoma	Phase 2	NCT00920790		Completed
KW-0761 (Mogamulizumab)	anti-CCR4 monoclonal antibody	Rreviously Treated Peripheral T-cell Lymphoma (PTCL)	Phase 2	NCT01611142		Completed
KW-0761 (Mogamulizumab)	anti-CCR4 monoclonal antibody	Peripheral T/NK-cell Lymphoma	Phase 2	NCT01192984		Completed
KW-0761 (Mogamulizumab)	anti-CCR4 monoclonal antibody	Adult T-Cell Leukemia-Lymphoma (ATL) and Peripheral T-Cell lymphoma (PT	Phase 1	NCT00355472		Completed
FLX475	anti-CCR4 monoclonal antibody	Advanced or Metastatic Gastric Cancer	Phase 2	NCT04768686	in combination with pembrolizumb(anti-PD1)	Completed
FLX475	anti-CCR4 monoclonal antibody	Advanced Melanoma	Phase 2	NCT04894994	in combination with Ipilimumab(anti-CTLA4)	Recruiting
FLX475	anti-CCR4 monoclonal antibody	Advanced Cancer	Phase1/2	NCT03674567	monotherapy, or in combination with pembrolizumb(anti-PD1)	Recruiting
BMS-813160	dual CCR2 and CCR5 antagonist	Non-small Cell Lung Cancer (NSCLC) or Hepatocellular Carcinoma (HCC)	Phase 2	NCT04123379	in combination with nivolumab(anti-PD1) and BMS-986253 (anti-IL-8)	Recruiting
GS-1811(ITX-1811)	anti-CCR8 monoclonal antibody	Advanced Solid Tumors	Phase 1	NCT05007782	monotherapy and in combination with pembrolizumab(anti-PD1)	Recruiting
BL-8040	CXCR4 antagonist	Metastatic Pancreatic Cancer	Phase 2	NCT02907099	in combination with pembrolizumab (anti-PD1)	Recruiting
BKT140	CXCR4 antagonist	Multiple Myeloma	Phase 1/2	NCT01010880		Active, not recruiting
BMS-936564	anti-CXCR4	Acute Myelogenous Leukemia and Selected B-cell Cancers	Phase 1	NCT01120457		Completed
Plerixafor	CXCR4 antagonist	Metastatic Pancreatic Cancer	Phase 2	NCT04177810	in combination with cemiplimab(anti-PD1)	Completed
CXCR4 antagonist	CXCR4 antagonist	metastatic renal cell carcinoma		NCT03891485	new CXCR4 antagonists (PCT/IB2011/000120/ EP2528936B1/ US2013/0079292A1), also treated with nivolumab (anti-PD	Recruiting
ASP1948	anti-Nrp1 monoclonal antibody	Locally advanced or metastatic solid tumors	phase 1	NCT04094506		Recruiting
ASP1948	anti-Nrp1 monoclonal antibody	advanced solid tumor	phase 1	NCT03565445	as single treatment or in combination with anti-PD1 Nivolumab or Pembrolizumab	Active, not recruiting

Table 5. ICI combinations

Drug	Target	Cancer type	Stage	Trial ID	Treatment detail	Status
Nivolumab +Ipilimumab	anti-PD1 +CTLA4	Advanced melanoma	Phase 3	NCT02599402		Completed
Nivolumab +Ipilimumab	anti-PD1 +CTLA4	Advanced Renal cell carcinoma	Phase 2	NCT03117309		Recruiting
Balstilimab +Zalifrelimab	anti-PD1 +CTLA4	Second-Line Cervical Cancer	Phase 2	NCT03894215		Recruiting
Nivolumab+Ipilimumab/Relatlimab	anti-PD-1+ CTLA4/LAG3	Recurrent /Metastatic Squamous Cell Carcinoma of the Head and Neck	Phase 2	NCT04326257	Personalized theray based gene expression of LAG3 and CTLA4	Recruiting
Nivolumab+Ipilimumab/Relatlimab	anti-PD-1+ CTLA4/LAG3	Head and Neck Squamous Cell Carcinoma (HNSCC)	Phase 2	NCT04080804	Nivolumab treatment alone or in combination with relatlimab or ipilimumab	Recruiting
Nivolumab + Relatlimab	anti-PD1 + LAG3	melanoma	Phase 1/1a	NCT01968109	Relatlimab (BMS-986016)	Active, not recruiting
Nivolumab + Relatlimab	anti-PD1 + LAG3	metastatic/unresectable melanoma	Phase 2/3	NCT03470922		Active, not recruiting
Nivolumab + Relatlimab	anti-PD1 + LAG3	Advanced Colorectal Cancer	Phase 2	NCT03642067		Recruiting
Nivolumab + Relatlimab	anti-PD1 + LAG3	Non-small Cell Lung Cancer	Phase 2	NCT04205552		Recruiting
LAGS25(Ieramilimab)+PDR001	anti-LAG3+PD1	Advanced Solid and Hematologic Malignancies	Phase 2	NCT03365791		Completed
LAGS25(Ieramilimab)+PDR001	anti-LAG3+PD1	Advanced Solid Tumors	Phase 1/2	NCT02460224		Completed
XmAb®22841 +Pembrolizumab	Bi-specific antibody targeting CTLA-4 and LAG-3+ PD-1	Advanced solid tumor	Phase 1	NCT03849469	XmAb®22841(Pavunalimab) monotherapy, or in combination with Pembrolizumab	Recruiting
Cobolimab + Nivolumab	anti-TIM3+PD-1	Neoplasmas	Phase 1	NCT02817633		Recruiting
Cobolimab + Dostarlimab	anti-TIM3+PD-1	Liver cancer	Phase 2	NCT03680508	TSR022(Cobolimab),Dostarlimab (TSR-042)	Recruiting
Cobolimab + Dostarlimab	anti-TIM3+PD-1	Melanoma	Phase 2	NCT04139902	TSR022(Cobolimab),Dostarlimab (TSR-042)	Recruiting
MBG453 + spartalizumab	anti-TIM3+PD-1	Recurrent glioblastoma multiforme	Phase 1	NCT03961971		Recruiting
MBG453 + PDR001	anti-TIM3+PD-1	AML/ high risk MDS	Phase 1	NCT03066648		Active, not recruiting
Enoblituzumab + Ipilimumab	anti-B7-H3 + CTLA4	melanoma, SCCHN, NSCLC	Phase 1	NCT02381314	Enoblituzumab (MGA271)	Completed
Enoblituzumab + Pembrolizumab	anti-B7-H3 + PD-1	melanoma, SCCHN, NSCLC, urothelia cancer	Phase 1	NCT02475213	Enoblituzumab (MGA271)	Completed
CI-8993	anti-VISTA	solid tumor	Phase 1	NCT04475523		Recruiting
W0180 +Pembrolizumab	anti-VISTA +PD-1	Locally Advanced or Metastatic Solid Tumors	Phase 1	NCT04564417	W0180 as single agent and in combination with Pembrolizumab	Recruiting
HMBD-002 +Pembrolizumab	anti-VISTA +PD-1	Solid tumor	Phase 1/2	NCT05082610	HMBD-002 as a monotherapy, or in combination with Pembrolizumab	Not yet recruiting
CA170	anti-PD-L1/PD-L2+VISTA antagonist	advanced solid tumors, lymphoma	Phase 1	NCT02812875		Completed
Ociperlimab +Tislelizumab	anti-TIGIT+ PD-1	recurrent or metastatic cervical cancer	Phase 2	NCT04693234	Tislelizumab (BGB-A317),Ociperlimab (BGB-A1217)	Recruiting
Ociperlimab +Tislelizumab	anti-TIGIT+ PD-1	recurrent or metastatic Esophageal squamous cell carcinoma	Phase 2	NCT04732494	Tislelizumab (BGB-A317),Ociperlimab (BGB-A1217)	Recruiting
Ociperlimab +Tislelizumab	anti-TIGIT+ PD-1	Non-small Cell Lung Cancer	Phase 3	NCT04746924	ociperlimab + tislelizumab,or pembrolizumab +placebo	Recruiting
AZD2936	anti-TIGIT/anti-PD-1 bispecific antibody	NSCLC	Phase 1/2	NCT04995523		Recruiting
INCAGN01876	GITR agonist	Advanced Solid Tumors	Phase 1/2	NCT02697591		Completed
INCAGN01876+INCMGA00012	GITR agonist+ anti-PD1	Glioblastoma	Phase 2	NCT04225039	GITR: costimulus for Tcell activation and proliferation, combined with stereotactic radiosurgery	Recruiting
INCAGN01876+INCMGA00012	GITR agonist+ anti-PD1	Cancer of the Head and Neck	Phase 1	NCT04470024	in combination with autophagosome vaccine (DPV-001)	Recruiting
GWN323+ PDR001	GITR agonist+ anti-PD1	Advanced Malignancies and Lymphomas	Phase 1/1b	NCT02740270		Completed
MEDI1873	GITR agonist	Advanced Solid Tumors	Phase 1	NCT02583165		Completed
TRX518	anti-GITR	Stage III or Stage IV Malignant Melanoma or Other Solid Tumor Malignan	Phase 1	NCT01239134		Completed
TRX518	anti-GITR	Advanced Solid Tumors	Phase 1/2	NCT03861403	in combination with cyclophosphamide + Avelumab(anti-PD-L1)	Terminated
TRX518	anti-GITR	Solid Tumors	Phase 1	NCT02628574	in combination with gemcitabine, pembrolizumab, or nivolumab	Completed
KHK2455	IDO1 inhibitor	Advanced or Metastatic Solid Tumors	Phase 1	NCT02867007	in combination with mogamulizumab(anti-CCR4)	Completed
KHK2455	IDO1 inhibitor	Advanced bladder cancer	Phase 1	NCT03915405	in combination with avelumab (anti-PD-L1)	Recruiting
Epacadostat(INCB024360)	IDO1 inhibitor	Metastatic Non-Small Cell Lung Cancer	Phase 2	NCT03322540	in combination with pembrolizumab(anti-PD1)	Completed
Epacadostat(INCB024360)	IDO1 inhibitor	Advanced Solid Tumors	Phase 1/2	NCT02318277	in combination with avelumab (anti-PD-L1)	Completed
Epacadostat(INCB024360)	IDO1 inhibitor	Advanced Solid Tumors	Phase 1/2	NCT03347123	in combination with nivolumab(anti-PD1) and ipilimumab(anti-CTLA4) or lirilumab(Anti-KIR2D)	Completed
MK-7162	IDO1 inhibitor	Solid Neoplasms	Phase 1	NCT03364049	In combination with pembrolizumab (MK-3475,anti-PD1)	Completed
NLG802	IDO1 inhibitor	Solid Tumor	Phase 1	NCT03164603		Completed
GDC-0919	IDO1 inhibitor	Solid Tumor	Phase 1	NCT02048709		Completed
GDC-0919	IDO1 inhibitor	Solid Tumor	Phase 1	NCT02471846	in combination with atezolizumab (anti-PD-L1)	Completed
BMS-986205	IDO1 inhibitor	Metastatic Hepatocellular Carcinoma	Phase 1/2	NCT03695250	in combination with nivolumab(anti-PD1)	Active, not recruiting
BMS-986205	IDO1 inhibitor	Melanoma	Phase 3	NCT03329846	in combination with nivolumab(anti-PD1)	Completed
BMS-986205	IDO1 inhibitor	Glioblastoma	Phase 1	NCT04047706	in combination with nivolumab(anti-PD1)and standard radiation therapy with or without temozolomid	Recruiting
BMS-986205	IDO1 inhibitor	Endometrial Carcinosarcoma	Phase 2	NCT04106414	in combination with nivolumab(anti-PD1)	Active, not recruiting
Indoximod	IDO pathway inhibitor	metastatic melanoma	Phase 1/2	NCT02073123	in combination with Ipilimumab/Nivolumab(anti-CTLA4)+ Pembrolizumab(anti-PD1)	Completed*
Indoximod	IDO pathway inhibitor	Metastatic Pancreatic Cancer	Phase 1/2	NCT02077881	in combination with Gemcitabine and Nab-Paclitaxel	Completed
HTI-1090 (SHR9146)	IDO1/TDO dual inhibitor	Advanced Solid Tumor	Phase 1	NCT03208959		Completed
Epacadostat +Durvalumab	IDO inhibitor+ anti PD-1	Head and Neck cancer, lung cancer, urothelial cancer	Phase 1/2	NCT02318277		
Nivolumab + BMS-986205	anti-PD-1+ IDO inhibitor	Endometrial Carcinosarcoma	Phase 2	NCT04106414		Active, not recruiting
Nivolumab + PD-L1+ IDO petide vaccine	anti-PD1+PD-L1/IDO inhibitor	metastatic melanoma	Phase 1/2	NCT03047928		Recruiting
Atezolizumab+Tocilizumab+Etrumadenant	anti-PD-L1+ anti-IL6 receptor+ A2AR/A2BR antagonist	Prostate Adenocarcinoma	Phase 2	NCT03821246		Recruiting
DFF332+ RAD001+PDR001+NIR178	anti-HIF2α+mTOR inhibitor+ anti-PD1+ A2AR antagonist	Advanced/Relapsed Renal Cancer	Phase 1	NCT04895748	Malignaces with HIF stablizing mutations	Recruiting
Zimberelimab + Domvanalimab+ Etrumadenant	anti-PD-1 + TIGIT + A2AR/A2BR antagonists	non-small cell lung cancer	Phase 2	NCT04791839	Previously treated patients	Recruiting
Zimberelimab+Domvanalimab+Etrumadenant	anti-PD1+ anti-TIGIT+A2AR/A2BR antagonist	non-small cell lung cancer	Phase2	NCT04262856	PD-L1 postitive patients	Recruiting
Lpilimumab+ Pembrolizumab + asprin	anit-CTLA4+PD-1+COX inhibitor	Cutaneous melanoma	Phase 2	NCT03396952		
Relatlimab + Nivolumab +BMS-986205 / nivolumab or Ipilimumab	anti-LAG3+ PD-1+IDO inhibitor or anti-CTLA4	advanced cancers	Phase 1/2	NCT03459222		Recruiting
SAR439459 + Cemiplimab	TGFβ inhibitor+ PD-L1	Advanced/Metastatic malignant solid neoplasm	Phase 1b	NCT04729725		
Galunisertib +Nivolumab	TGFβ inhibitor+ PD-1	Recurrent or refractory NSCLC, Hepatocellular carcinoma	Phase 1b/2	NCT02423343		
Evofosfamide(TH-302)	hypoxia-activated bio-reductive prodrug	Glioblastoma	Phase 2	NCT02342379	in combination with bevacizumab	Completed
Evofosfamide(TH-302)	hypoxia-activated bio-reductive prodrug	solid tumor	Phase 1	NCT03098160	in combination with Ipilimumab(anti-CTLA4)	Unknown
DFF332+ RAD001+PDR001+NIR178	anti-HIF2α+mTOR inhibitor+ anti-PD1+ A2AR antagonist	Advanced/Relapsed Renal Cancer	Phase 1	NCT04895748	Malignaces with HIF stablizing mutations	Recruiting
Avelumab+ Axitinib Versus Sunitinib	anti PD-L1+ VEGF2/VEGFR	Advanced Renal Cell Cancer	Phase 3	NCT02684006	Combination of anti-PD-L1 and anti-VEGF2, versus anti-VEGFR monotreatment	Active, not recruiting
Pembrolizumab (MK-3475) + Axitinib Versus Sunitinib	anti PD1+ VEGF2/VEGFR	Renal Cell Carcinoma	Phase 3	NCT02853331	Combination of anti-PD1 and anti-VEGF2, versus anti-VEGFR monotreatment	Active, not recruiting
Atezolizumab+ Bevacizumab Versus Sorafenib	anti PD-L1+ VEGFA/VEGFR	Metastatic Hepatocellular Carcinoma	Phase 3	NCT03434379	Combination of anti-PD-L1 and anti-VEGFA, versus anti-VEGFR monotreatment	Active, not recruiting

* ZakhariafiY,et al. J Immunother Cancer 2021;9