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Immunological Targets for Immunotherapy: Inhibitory T Cell Receptors

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Abstract

Tumor development is characterized by the accumulation of mutational and epigenetic changes that transform normal cells and survival pathways into self-sustaining cells capable of untrammelled growth. Although multiple modalities including surgery, radiation, and chemotherapy are available for the treatment of cancer, the benefits conferred are often limited. The immune system is capable of specific, durable, and adaptable responses. However, cancers hijack immune mechanisms such as negative regulatory checkpoints that have evolved to limit inflammatory and immune responses to thwart effective antitumor immunity. The development of monoclonal antibodies against inhibitory receptors expressed by immune cells has produced durable responses in a broad array of advanced malignancies and heralded a new dawn in the cancer armamentarium. However, these remarkable responses are limited to a minority of patients and indications, highlighting the need for more effective and novel approaches. Preclinical and clinical studies with immune checkpoint blockade are exploring the therapeutic potential antibody-based therapy targeting multiple inhibitory receptors. In this chapter, we discuss the current understanding of the structure, ligand specificities, function, and signaling activities of various inhibitory receptors. Additionally, we discuss the current development status of various immune checkpoint inhibitors targeting these negative immune receptors and highlight conceptual gaps in knowledge.

Keywords

Immunotherapy; Inhibitory receptors; PD-1; CTLA-4; TIM-3; TIGIT; LAG-3; BTLA; VISTA

1 Introduction

Cancer cells produce tumor antigens (TA) that are recognized by T cells and can induce tumor rejection [1]. The presence of CD8 tumor-infiltrating T lymphocytes (TIL) is usually a marker of good clinical outcome in multiple primary solid tumors [2-5]. However,

spontaneous and vaccine-induced TA-specific T cells often fail to impede the growth of tumors in patients with advanced cancer [6, 7].

Multiple negative immunoregulatory pathways impede T cell-mediated tumor destruction in the tumor microenvironment (TME), contributing to the paradoxical coexistence of TA-specific CD8⁺ T cells and tumor progression in cancer patients. Among them, inhibitory receptors (IR) like PD-1 and CTLA-4 play a critical role in dampening T cell functions. Immunotherapies with immune checkpoint inhibitors directed against these immunoregulatory pathways provide long-term clinical benefits to patients with a growing range of solid tumors [8].

The development of monoclonal antibodies (mAb) targeting immune checkpoint receptors cytotoxic T lymphocyte associated antigen-4 (anti-CTLA-4) and programmed death 1 (PD-1) are proof of this therapeutic strategy. In this review, we discuss the preclinical and early clinical data supporting the rationale for current and future combinatorial therapeutic strategies targeting inhibitory immune checkpoints.

1.1 Inhibitory T Cell Receptors

1.1.1 Inhibitory T Cell Receptors: CTLA-4

CTLA-4: Structure and Ligands: Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4, CD152) is an activation-induced glycoprotein that belongs to the immunoglobulin (Ig) superfamily. CTLA-4 is homologous to the T cell costimulatory protein CD28; but where CD28 provides the costimulatory signal required for antigen-specific T cell activation and expansion after the initial interaction between T cell receptor (TCR) and antigen presenting cells (APCs), CTLA-4 downregulates T cell responses [9-12]. CTLA-4 contains an extracellular V domain, a transmembrane domain, and a cytoplasmic tail. CTLA-4 cytoplasmic tail is structurally and functionally similar to CD28: it has no intrinsic catalytic activity but contains *both* a YVKM motif that can bind phosphatidylinositol 3-kinase (PI3K), protein phosphatase 2 A (PP2A) and SHP-2 *and* a separate proline-rich motif able to bind SH3 containing proteins [13].

CTLA-4 is constitutively expressed on regulatory T cells (Tregs), while expression on CD8⁺ T cells primarily occurs after initial activation. T regs primarily store CTLA-4 intracellularly within endosomes—providing a large intracellular pool that can be rapidly cycled to the cell surface upon activation. CTLA-4 has two natural ligands found on APCs: CD80 (B7.1) or CD86 (B7.2) [14-16].

CTLA-4: Signaling and Function: Unlike CD28 and PD-1 which are robustly expressed on cell surfaces, CTLA-4 is primarily distributed intracellularly where it is constitutively present as a homodimer [17, 18]. Although CTLA-4 signaling has been shown to be linked to phosphorylation of CD3ζ [19], disruption of ZAP-70 microclusters [20], and interaction with PI3K [21] or SHP-2 [22] or serine/threonine phosphatase PP2A [23], multiple other studies have shown that CTLA-4 inhibitory signaling was unrelated to each of these interactions [24-28]. Molecular imaging experiments have shown that both T regs and CD8⁺ T cells compete for the same ligands at the immune synapse in a cell-intrinsic fashion [29].

This suggests that upon antigen exposure, CTLA-4 binds CD80 and CD86 with greater affinity and avidity compared to CD28, enabling it to outcompete CD28 for ligand binding [30, 31] and argues that some measure of the inhibitory activity of CTLA-4 is due to ligand-dependent signaling. However, CTLA-4 inhibitory activity also results in ligand downregulation on APC via a transendocytic mechanism [32]. This mechanism is stimulated by TCR engagement, is cell-extrinsic, and has been observed in both T regs and CD8+ T cells [32]. Overall, these findings suggest that the primary inhibitory effect of CTLA-4 is to control access of CD28 to CD80/CD86 ligands and argues that the effects of CTLA-4 signaling are complex, contradictory and context-dependent.

Separately, other data suggest that some measure of CTLA-4's inhibitory effects on the T reg compartment is mediated by either intratumoral Treg depletion or reduced Treg suppressive activity [33-36]. CTLA-4 therapy is associated with an increase in the CD8 T cell-Treg ratio within tumors [37-43]. The effect of CTLA-4 blockade on the Treg compartment appears to be Fc-gamma receptor (Fc- γ R) dependent and is associated with the presence of Fc- γ R expressing macrophages [44, 45]. This effect is isotype dependent and antibodies with improved Fc effector function are associated with improved activity preclinically [46].

CTLA-4: Preclinical and Clinical Data: The discovery of the inhibitory function of CTLA-4 led to a series of experiments testing CTLA-4 inhibition in various murine tumor models. In 1996, Leach and colleagues demonstrated that antibody-mediated CTLA-4 blockade led to tumor rejection of transplantable mouse colon cancer and fibrosarcoma [47]. CTLA-4 blockade resulted in immunologic memory as previously challenged mice subsequently rejected implanted tumors without additional CTLA-4 blockade. CTLA-4 blockade was ineffective as a single-agent in B16 melanoma and SM1 mammary carcinoma [48, 49], although combining CTLA-4 blockade with GM-CSF-secreting vaccines resulted in tumor eradication [48, 49].

These results spurred the development of two anti-CTLA-4 mAb: ipilimumab (MDX-010; Medarex and Bristol-Myers Squibb) and tremelimumab (CP-675,206 or ticilimumab; Pfizer and Medimmune). Although both ipilimumab and tremelimumab are fully humanized mAb, ipilimumab belongs to the IgG1 κ class and has a half-life of 12–14 days, while tremelimumab is a IgG2 mAb with a longer half-life of 22 days. The first clinical data came from a dose-escalation study in patients with advanced melanoma where authors reported two partial responses in a cohort of 17 patients treated with a single-dose of ipilimumab 3 mg/kg [50]. Subsequent studies tested a variety of doses and schedules in various diseases including melanoma [51] and lymphoma [52]. These early studies revealed three hallmark features: a clear *dose-response relationship* with greater responses at higher doses (albeit with a higher incidence of toxicity), a unique spectrum of “immune related adverse events” (irAE) that reflected tissue specific inflammation, and a small fraction of *durable* responders of approximately 20% [53].

Ipilimumab was subsequently evaluated in two phase III studies in melanoma: ipilimumab compared to gp100 vaccine in previously treated HLA-A*0201-positive melanoma (MDX010-020) [54]; and ipilimumab/dacarbazine combination compared to dacarbazine/

placebo in treatment naïve melanoma (MDX010-024/CA184-024) [55]. In both studies, the previously seen hallmarks were observed proving that immunotherapy provided durable disease control in a subset of patients and led to regulatory approval for this indication. Pooled clinical trial data in advanced melanoma patients with long-term follow-up suggests that survival curves plateau at 3 years in 20–26% of treated patients [53].

Early clinical trials of tremelimumab suggested comparable efficacy to ipilimumab, although both agents have not been directly compared. Tremelimumab exposure resulted in durable responses in a minority of patients and a similar profile of irAEs including colitis and rash. The dose and schedule chosen for further study was 15 mg/kg every 3 months, reflecting in part the longer half-life of tremelimumab compared to ipilimumab. In a phase II study in advanced melanoma, response rate was 7%, although 21% had disease control and most of these were durable [56]. A subsequent phase III trial that compared tremelimumab to chemotherapy was terminated after an interim-analysis showed inferior survival for the investigational arm [57].

Further development in the anti-CTLA-4 arena has focused on means of enhancing the therapeutic index through several means including: linkage to a proprietary masking peptide that requires cleavage by tumor associated proteases within TME *and* structural modifications to enhance antibody dependent cellular cytotoxicity (ADCC) and/or Fc- γ R activity. BMS-986218 is a nonfucosylated form of ipilimumab with the same amino acid sequence and ligand blocking properties as ipilimumab that is made in cells deficient for alpha-(1,6)-fucosyltransferase (Fut8). This nonfucosylated (NF) moiety has increased affinity for Fc- γ R and increased ADCC [44, 46, 58]. The increased Fc- γ R affinity and ADCC activity of CTLA-4 NF may improve the T reg depletion and CD8 activity enhancement seen with ipilimumab. BMS-986218 is currently in dose escalation in a phase I study of all solid tumors (NCT03110107). BMS-986249 is a CTLA-4 probody with an identical amino acid sequence and ligand blocking properties to ipilimumab that contains a proprietary masking peptide at the antigen-binding site that is covered by a protease-cleavable linker. This linker is cleaved by tumor associated proteases found in the TME and theoretically limits drug activation to tumor sites. BMS-986249 is being studied a phase I study of all solid tumors (NCT03369223).

1.2 Inhibitory T Cell Receptors: PD-1

1.2.1 PD-1: Structure and Ligands—The PD-1 receptor consists of a single N-terminal IgV-like domain, a transmembrane domain, and a cytoplasmic tail. The cytoplasmic tail contains immunoreceptor tyrosine based inhibitory motifs (ITIM) and immunoreceptor tyrosine-based switch motifs (ITSM). Upon engagement, ITIM and ITSM are phosphorylated and act as docking sites for SH2 domain-containing protein tyrosine phosphatase (Shp)-1 and Shp-2 [59, 60]. While Shp-1 dampens various signaling cascades, Shp-2 positively regulates growth factor or hormone receptor signaling; oncogenic Shp-2 signaling has been implicated in multiple malignancies primarily through activation of the RAS-ERK signaling pathway [60–63]. PD-1 receptor binds to two known ligands: programmed death ligand 1 (PD-L1 or B7-H1) [64] and programmed death ligand 2 (PD-L2 or B7-DC) [65, 66]. PD-1 is upregulated by TA-specific CD8⁺ T cells and tumor infiltrating

lymphocytes (TIL) in cancer patients and negatively regulates T cell function through the engagement of PD-L1, which is upregulated by human tumors including melanoma [67-70].

1.2.2 PD-1: Signaling and Function—The PD-1 and PD-L1-PD-L2 interaction plays an important role in maintaining peripheral tolerance and immune homeostasis but is hijacked by tumors to escape immune surveillance. In cancer, PD-L1 ligation has at least three dominant effects on TME- intrinsic T cells: *inhibition of cell cycle progression*, *generation of induced regulatory T cells (iTreg)*, and *T cell metabolic reprogramming*. T cells naturally lack cyclin expression and typically reside in G0 phase. CDK inhibitor p27^{kip1} is ubiquitously expressed within T cells where it interacts with Cdk2 until ubiquitin-dependent degradation of p27^{kip1} by SCFSkp2 ubiquitin ligase initiates cell cycle progression. However, PD-L1 ligation suppresses *SKP2* transcription resulting in accumulation of p27^{kip1} and inhibition of cell cycle progression [71, 72]. TGF- β is a pleiotropic cytokine produced in large amounts within TME that directly inhibits T cell proliferation, activation and effector function in a *Smad3*-dependent, *Smad2*-independent fashion [73]. Synergizing with TGF- β , PD-L1 promotes iTreg cell conversion and enhances and sustains Foxp3 expression to increase the suppressive function of iTreg cells [74]. While naïve T cells are dependent on oxidative phosphorylation, following activation T cells utilize glycolysis as their primary means of energy generation. Upon PD-L1 ligation, T cells are unable to engage in glycolysis or amino acid metabolism; but have an increased rate of fatty acid β -oxidation (FAO) due to increased expression of carnitine palmitoyltransferase I (CPT1) [75]. These exhausted T cells are unable to lyse tumor cells and/or produce cytokines upon tumor recognition.

Separately, PD-1 activation appears to suppress TCR signaling [76, 77], ICOS costimulatory signaling [77], and CD28 co-stimulatory signaling both directly [78] and indirectly via dephosphorylation of CD28 by PD-1–recruited Shp2 phosphatase [79].

1.2.3 PD-1: Preclinical and Clinical Data—CTLA-4 blockade and PD-1 blockade affect T cell-dependent antitumor immunity through distinct and nonredundant mechanisms. PD-1 primarily inhibits T cell activity within the TME through a cell-intrinsic mechanism at the effector phase which primarily relies upon the presence of chronically activated TA-specific T cells within TME [80, 81]. CTLA-4 attenuates T cell activating in the priming phase via cell-intrinsic and cell-extrinsic mechanisms by inducing expansion of both CD8⁺ T cells and ICOS⁺ Th1-like CD4 effector T cells [82]. The combination of dual anti-PD-1 blockade and anti-CTLA-4 checkpoint blockade result in distinct and nonoverlapping immunological changes driven by distinct mechanisms [83, 84].

The development of mAb targeting PD-1/PD-L1 has fundamentally changed the treatment of advanced cancer by providing clinical benefits in multiple solid tumors. These mAb are primarily humanized IgG4 targeting PD-1 receptor (nivolumab, pembrolizumab, and cemiplimab) or IgG1 lambda targeting PD-L1 (atezolizumab, durvalumab, and avelumab). Anti-PD-1 or anti-PD-L1 mAb have shown impressive activity in multiple cancer types including melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), renal cell carcinoma, relapsed Hodgkin's lymphoma, head and neck squamous cell carcinoma (HNSCC), urothelial carcinoma, microsatellite unstable or mismatch repair-

deficient tumors, Merkel cell carcinoma, and cutaneous squamous cell carcinoma (cSCC). These data are summarized in Table 1.

While the durable responses observed with anti-PD-1/anti-PD-L1 mAb underscore a new reality in the management of patients with advanced cancers, it is clear that a distinct group of patients either do not respond or do not achieve durable responses. To describe and classify resistance mechanisms to immunotherapy, investigators have introduced the terms: “primary resistance” (lack of response to immunotherapy), “acquired resistance” (initial response to immunotherapy, followed by nonresponse) and “adaptive resistance” (tumor cell intrinsic adaptation to immune recognition that manifests as primary resistance, mixed response or acquired resistance) [85]. Primary and adaptive resistance may be due to cell-intrinsic processes including increased oncogenic signaling through MAPK [86] or Wnt/ β -catenin [87] pathways or via PTEN loss [88]; loss of interferon-gamma (IFN γ) signaling [89-91]; and impaired T cell responses because of low neoantigen load or impaired antigen presentation [92]. Cell-extrinsic processes that could result in primary and/or adaptive resistance arise from factors in TME other than tumor cells including T regs, myeloid derived suppressor cells (MDSCs), tumor associated macrophages (TAM) and T cell inhibitory receptor expression [93-95]. These factors variably combine to inhibit the development of effective antitumor immune responses. Therapeutic strategies designed to target one or more of these mechanisms are the focus of clinical trials going forward.

Conversely, two distinct factors have emerged as candidate predictive biomarkers of response to PD-1 blockade. First, IFN γ gene expression is strongly associated with the T cell-inflamed phenotype required for clinical response to anti-PD-1/anti-PD-L1 mAb [96]. Secondly, response to PD-1 blockade appears to be reliant on the presence of CD8+ PD-1+ T cells [97]. In murine models, it appears that the majority of CD8+ PD-1+ T cells that proliferate upon PD-1 blockade belong to a CD8+ CXCR5+ T cell subset with a stem-like phenotype maintained by TCF-1 mediated transcriptional program [98].

1.3 Inhibitory T Cell Receptors: TIM-3

1.3.1 TIM-3: Structure and Ligands—T-cell immunoglobulin and mucin domain containing protein-3 (TIM-3), also known as Hepatitis A virus cellular receptor 2 (HAVCR2), is an important negative regulator of innate and adaptive immunity [99]. Of the eight TIM genes in the mouse, only three are conserved in humans: *Havcr1* (TIM-1), *Havcr2* (TIM-3), and *Timd4* (TIM-4). The TIM-3 locus in humans located at 5q33.2 contains the IL-4 gene cluster. Polymorphisms in the TIM family of genes (specifically TIM-1) are associated with airway hyperreactivity [100], suggesting that the TIM-3 family of molecules are broadly involved in Th1/2 differentiation and allergic diseases. Human TIM-3 consists of a distal variable immunoglobulin (IgV)-like domain bound to a proximal mucin domain both of which are extracellular and connected to an intracellular cytoplasmic tail that is involved in phosphotyrosine-dependent signaling [101-105]. The IgV-like domain of TIM-3 binds multiple ligands including carcinoembryonic antigen cell adhesion molecule 1 (CEACAM1) [106], high mobility group protein B1 (HMGB1) [107], galectin-9 [101, 102, 108] and phosphatidylserine (PS) [109, 110].

1.3.2 TIM-3: Signaling and Function—Unlike PD-1 (ITIM/ITSM), neither TIM-3 nor LAG-3 has classical signaling motifs in their cytoplasmic tails. Rather, the cytoplasmic tails of TIM-3 in both humans and mice contain five conserved tyrosine residues, of which two are located in the proximal region, while three are located in the distal region of the cytoplasmic tail. Of these, Y256 and Y263 can be phosphorylated by either ITK [101] or Src kinases [104]. Upon galectin-9 mediated signaling, phosphorylation of Y256 and Y263 residues results in release of Bat3 (HLA-B-associated transcript 3) from the C-terminal tail of TIM-3 [104, 111]. Under steady state conditions, bound Bat3 recruits tyrosine protein kinases Fyn/Lck and p85 regulatory subunit of PI3K forming an intracellular complex that blocks SH2 domain binding sites, thereby promoting TIM-3 inhibitory function while promoting T cell expansion [111]. However, while this mechanism accounts for the downstream signaling pathway of the galectin-9–TIM-3 interaction on T cells, it is unclear if this mechanism is redundant across other TIM-3 ligands (CEACAM1, HMGB1, and PS) and in other cells besides T cells.

High levels of TIM-3 expression are associated with T cell dysfunction—a process first identified in the setting of chronic HIV infection [112]. In cancer, TIM-3 is upregulated by TILs in both human and murine models [113, 114]. It should be noted that in both chronic viral infection and cancer, while TIM-3 expression is often associated with PD-1 expression on CD8⁺ antigen-specific T cells, TIM-3 is also expressed by some exhausted T cells lacking PD-1 expression [112]. Further, within CD8⁺ PD-1⁺ T cells, TIM-3 expression represents a more deeply exhausted T cell population compared to either PD-1⁺ single-positive CD8⁺ T cells [113]. Besides CD8 TIL, TIM-3 is expressed on Tregs and multiple innate immune cell types NK cells and APCs. On Tim-3⁺Fox-3⁺Tregs, represent a subset of highly activated/suppressive Tregs, which also express high-level PD-1, CTLA-4, and LAG-3 [115–118].

1.3.3 TIM-3: Preclinical and Clinical Data—In both models of cancer and chronic viral infection, exhausted CD8⁺ and CD4⁺ T cells coexpress multiple inhibitory receptors which cooperate to diminish the function of these TA-specific T cells. Dual PD-1/TIM-3 blockade is markedly more effective in restoring T cell effector function than either TIM-3 blockade or PD-1 blockade alone in models of both chronic viral infection [67, 119, 120] and multiple cancers including melanoma [121], non-small cell lung cancer [122], and follicular lymphoma [123]. In the context of cancer, preclinical data from human and mice models demonstrates the potency of dual PD-1/TIM-3 blockade in augmenting TA-specific cells responses in vitro with evidence of reduced tumor growth in vivo [113, 114]. Also, TIM-3 appears to be an adaptive mechanism of resistance to PD-1 blockade in mouse lung tumor models and patients with lung cancer [95]. Overall, these data suggest that the PD-1 and TIM-3 pathways promote T cell dysfunction through synergistic and nonredundant mechanisms; and that dual blockade of PD-1 and TIM-3 reinvigorate effector T cell responses. Separately, in a murine model of breast cancer, TIM-3 expression appeared to regulate the function of CD103⁺ dendritic cells (DCs) through CXCL9 expression. TIM-3 blockade increased influx of CXCR3⁺ CD8 T cells synergized with chemotherapy in a CD8⁺ T cell-dependent fashion [124]. Whether this applies in human cancer models where CD103⁺ DCs are functionally homologous to CD141⁺ plasmacytoid DCs is unknown.

TIM-3 development programs have been disclosed by Novartis (MBG453), Tesaro (recently acquired by GlaxoSmithKline) (TSR-022), Bristol-Myers Squibb (BMS-986258), and Incyte (INCAGN02390). The publically available data regarding structure, TIM-3 target, and ligand specificity along with current development phase and response rates of these agents are summarized in Table 2. Of these, the development of Tesaro's TSR-022 appears to be furthest along with publically reported phase I dose-escalation data.

TSR-022 is an IgG4k isotype humanized mAb that binds with high affinity to TIM-3. The ligand specificity of TSR-022 has not been disclosed. TSR-022 is being studied as monotherapy and in combination with PD-1 inhibitor TSR-042 and LAG-3 inhibitor TSR-033 in the ongoing phase I/II AMBER study ([NCT02817633](#)) in PD-1 refractory melanoma and NSCLC patients. In the part 1A dose-escalation portion of the AMBER study, 46 patients with a variety of solid tumors were enrolled to receive escalating doses of TSR-022 [125]. TSR-022 was administered at doses ranging from 100 mg every 3 weeks to 10 mg/kg every 3 weeks with no dose-limiting toxicities (DLT) observed. Subsequently, 54 patients were enrolled to receive TSR-022 at either 100 mg, 300 mg or 900 mg every 3 weeks in combination with PD-1 inhibitor TSR-042 500 mg every 3 weeks in the part 1C combination dose-escalation. While 57% of patients had at least one treatment emergent adverse event (AE), no DLT were observed, and there was no correlation between dose and AEs across three dose levels. Early signs of clinical activity were observed at both TSR-022 100 mg and TSR-022 300 mg dose levels. Most recently, data from the part 2 combination dose-escalation that evaluated TSR-022 100 mg and TSR-022 300 mg in combination with TSR-042 500 mg in PD-1 refractory melanoma and NSCLC patients was presented. A clear dose–response relationship was observed with greater responses at the TSR-022 300 mg dose compared to TSR-022 100 mg dose in both melanoma and NSCLC [126]. The dose–response relation observed in this study may be related to the greater proportion of patients who achieved steady state TSR-022 pharmacokinetics above threshold at higher doses (900 mg/300 mg every 3 weeks) suggesting that dose optimization is important with this agent [126]. Among 12 NSCLC patients with high PD-L1 (≥ 1), 4 had a confirmed response, while 6 had stable disease, suggesting that enriching for PD-L1 expression could serve as a biomarker enrichment strategy [126].

1.4 Inhibitory T Cell Receptors: TIGIT

1.4.1 TIGIT: Structure and Ligands—TIGIT (also called T cell immunoreceptor with Ig and ITIM domains) is a 26-kDa type I immune receptor of the Ig superfamily that is present on activated T cells, Tregs, follicular T helper cells (Tfh), and natural killer cells (NK) [127–131]. Structure of TIGIT comprises an extracellular IgV domain, type I transmembrane region, a cytoplasmic tail containing ITIM and an immunoglobulin tail tyrosine (ITT)-like motif. In humans, TIGIT is constitutively expressed on a subset of CD4⁺ and CD8⁺ T cells, although the majority of Tregs and NK cells express TIGIT. However, upon chronic antigen stimulation such as with chronic viral infections and cancer, TIGIT is strongly upregulated on CD8⁺ T cells where it is associated with other markers of T cell exhaustion including PD-1, CTLA-4, LAG-3 and TIM-3 [132, 133]. In human cancers, TIGIT is highly expressed on TIL and tumor-infiltrating Tregs and less so on CD4⁺ T effector cells [116, 132, 133].

TIGIT binds to CD155 (PVR) on DCs and macrophages with high affinity and CD112 (PVRL2) with lower affinity [127-131]. Recent work suggests that both CD112R and CD226 (but not TIGIT) on DC compete for CD112 binding on tumor cells [134]. Together with negative receptors CD96 and CD112R along with costimulatory receptor CD226, the TIGIT/CD96/CD112R/CD226 pathway is one in which shared ligands and differential ligand-receptor binding affinities tweak immune response analogous to the CD28/CTLA-4 pathway [129-131, 135]. This complex interplay may actually mediate some element of the negative effects of the TIGIT/CD226 pathway as illustrated by recent work demonstrating that a high TIGIT–CD226 ratio (rather than either singly) determined the suppressive function and stability of T regs in melanoma [136].

Separately, the complexity of the TIGIT system raises the question as to whether this axis through means other than TIGIT blockade including CD155 blockade, CD96 blockade, CD112 receptor (CD112R) blockade and augmenting preferential signaling through CD226 through either CD226 agonism or dominant/negative approach. Preclinically, CD96 blockade appears to be inactive in CD226-deficient mice underscoring the critical need for CD226 in tumor-NK cell and/or APC-NK cell interactions [137]. Other work has shown that combinatory blockade of CD112R and TIGIT enhances TA-specific effector T cell function greater than each singly [134]. Further evaluation studying TIGIT, CD96, and CD112R blockade singly and in combination is ongoing.

1.4.2 TIGIT: Signaling and Function—In murine models, TIGIT signaling appears to be variably reliant on either ITIM motif (Y233) or ITT-like motif (Y227) with phosphorylation of tyrosine residues in *either* being sufficient for TIGIT's inhibitory signaling, and TIGIT signaling is disrupted only when *both* motifs are mutated [129]. However, in humans, various groups have attributed TIGIT's inhibitory signaling to be dependent on either Y233 ITIM motif [129] or Y227 ITT-like motif [138, 139]—and this remains an unsettled issue. In NK cells, upon CD155 engagement, ITT-like motif is phosphorylated and recruits SHIP1 (SH2 domain-containing inositol-5-phosphatase 1) through cytosolic adaptor Grb2 (growth factor receptor-bound protein 2) or β -arrestin 2. The Grb2–SHIP1 interaction terminates PI3K and MAPK signaling [138], while β -arrestin 2–SHIP1 interaction suppresses IFN- γ production via NF- κ B pathway [139]. In DCs, CD155-TIGIT binding induces IL-10 and inhibits IL-12 production rendering them tolerogenic [130]. Although TIGIT's inhibitory effects on T cell function were initially thought indirect, it was subsequently shown that TIGIT exerted cell-intrinsic inhibitory activity on T cells [131, 140]. This activity was mediated by direct downregulation of T cell receptor (TCR) complex (TCR α , CD3 ϵ) and indirectly via downregulation of TCR signal mediators (such as PLC γ) [140].

Analogous to what is observed with CTLA-4 blockade, effective TIGIT blockade appears to be dependent on Fc- γ R engagement [45]. In tumor bearing mice, TIGIT mAb with attenuated Fc- γ R binding did not demonstrate antitumor activity. In ex vivo experiments, Fc-silent TIGIT mAb variant elicited inferior antigen-specific T cell responses compared to IgG1 TIGIT mAb, an effect that was Fc- γ RIII dependent [45].

1.4.3 TIGIT: Preclinical and Clinical Data—Based on these data, TIGIT blockade has been evaluated in human cancers, singly and in combination with PD-1 blockade. TIGIT development programs have been disclosed by Bristol-Myers Squibb (BMS-986207), Merck (MK-7684), Arcus Biosciences (AB154), Compugen (CGEN-15137/COM902 and CGEN-15207/COM701), Astellas (SP 8374/PTZ-201), and Genentech (MTIG7192A). Table 3 summarizes publically available data regarding structure, TIGIT target and ligand specificity along with current development phase and response rates of these agents. Of these, Merck has publically reported on phase I data of MK-7684 singly and in combination with pembrolizumab.

MK-7684 is an IgG1 isotype humanized mAb that binds with high affinity to TIGIT and blocks the interaction between TIGIT and CD112/CD155. In the phase I dose-escalation study ([NCT02964013](#)), MK-7684 was studied singly ($N=34$) and in combination with pembrolizumab ($N=34$) in patients with advanced solid tumors who had failed standard treatments. Of note, patients did not need to have progressed past PD-1 therapy to be considered for enrollment. No DLTs were observed across any of the dose-levels tested. Treatment emergent AEs were observed in 56% (monotherapy arm) and 60% (combination arm), of which Grade 3/4 AEs accounted for 6% and 11% respectively. No treatment discontinuation occurred as a result of AEs. Response was observed in 1 of 34 (3%) in the monotherapy arm and 8 of 34 (19%) in the combination arm. Dose escalation is ongoing. Dose-escalation data of Genentech's MTIG7192A is not available, although a front-line placebo-controlled study testing atezolizumab/MTIG7192A combination has been launched ([NCT03563716](#)).

While BMS-986207 is an IgG1 mAb with an inert Fc, and AB154 has an IgG1 mAb with ADCC capabilities, the isotypes of CGEN-15137/COM902, CGEN-15207/COM701, SP 8374/PTZ-201, and MTIG7192A are not publically disclosed. These studies are in various phases of early testing with no publically available data.

1.5 Inhibitory T Cell Receptors: LAG-3

1.5.1 LAG-3: Structure and Ligands—Lymphocyte-activation gene 3 (LAG-3 or CD223) is a 498-amino acid type I transmembrane protein that is found on cell surfaces and is part of the immunoglobulin (Ig) superfamily. LAG-3 comprises four extra-cellular Ig-like domains with structural homology to CD4 [141, 142] and an intracellular portion containing a unique motif (KIEELE) [143]. LAG-3's membrane-distal D1 domain contains an “extra loop” that is required for LAG-3 to exert any negative effects [144] and against which most antibodies are targeted. LAG-3 is expressed on activated T cells [145], NK cells [141], plasmacytoid dendritic cells [146], and possibly activated B cells [147], although the latter is contentious.

Similar to CD4, LAG-3 binds to MHC class II molecules; however, unlike CD4, which interacts with MHC class II through multiple residues and with lower affinity, LAG-3 interacts with MHC class II through a small set of amino acids in the D1 domain and with much greater affinity [148-151]. The central role of LAG-3 in mediating effects on NK cells and T cells which lack MHC class II, suggest that LAG-3 has other as yet undiscovered ligands. Recent work suggests that fibrinogen-like protein 1 (FGL1) is a functional LAG-3

ligand [152]. FGL1 belongs to the fibrinogen family of proteins which includes fibrinogen, FGL-1, FGL-2 and clotting factors V, VIII and XIII. FGL-1 is predominantly expressed by the liver, and by human cancers including gastric cancer [153] where it is associated with poor prognosis. FGL-1's fibrinogen-like domain interacts with LAG-3 either at membrane-distal D1 or D2 domains, although the specific means by which the FGL-1-LAG-3 interaction inhibits antigen-specific T cell activation remain unclear. FGL-1 blockade confers similar antitumor immunity to LAG-3 blockade in murine models; suggesting that this may be a potential therapeutic target in human cancer [152].

LAG-3 expression on T effector cells and T regs is constitutively regulated by cleavage of transmembrane peptide connecting extra-cellular Ig-like domains and intracellular KIEELE motif by ADAM10 and ADAM17 metalloproteinases. ADAM10/ADAM17 activity is mediated by distinct TCR signaling-dependent mechanisms and results in release of soluble LAG-3 (sLAG-3), the biological function of which is unknown at this time [154].

1.5.2 LAG-3: Signaling and Function—LAG-3 primarily exerts inhibitory effects on T effector cells and T regs. The inhibitory effects of LAG-3 in CD8 T cells is mediated by cross-linking of LAG-3 and CD3/TCR complex which inhibits TCR-induced T cell proliferation, cytokine production and calcium influx [155]. The exact mechanism by which LAG-3 signaling results in cross-linkage of LAG-3's cytoplasmic domain and CD3/TCR complex is unclear at this time but is dependent upon LAG-3's intracellular KIEELE motif which is conserved across species [156]. It remains unclear whether this mechanism accounts for the effects of LAG-3 in T regs.

LAG-3 also inhibits APC activity in a bidirectional signaling fashion. It has been shown that MHC class II binding to LAG-3 positive T regs inhibits DC activation through a mechanism mediated by an ITAM inhibitory signaling pathway involving FcγRγ and SHP-1 [157]. This inhibition is clearly dependent upon the extracellular domain of LAG-3 as loss of the cytoplasmic domain is insufficient to abrogate suppression of DC function [157]. This inhibitory reverse signaling has also been observed in an ex vivo melanoma model system (Liang B, J Immunol 2008). In the presence of sLAG-3 and LAG-3 transfected cells, MHC class II-positive melanoma (but not MHC class II-negative melanoma) was resistant to Fas-mediated and drug-induced apoptosis by activation of the MAPK/Erk and PI3K/Akt pathways in these cells [157].

1.5.3 LAG-3: Preclinical and Clinical Data—At this time, LAG-3 development programs have been disclosed by Bristol-Myers Squibb (BMS-986016, relatlimab), Merck (MK-4280), Novartis (LAG525), OncoMed (OMP-313M32, etigilimab), iTeos (EOS8844488), i-Mab Biopharma (TJT6), Arcus Bioscience/Stanton Bioscience (AB154), Tesaro (TSR-033), and Prima BioMed/Immutep (IMP321). Aside from IMP321 which was designed as an APC activator, most of the other agents are fully humanized IgG4 mAbs designed to block LAG-3 on CD8 T cells and T regs, while OMP-313M32 (etigilimab) is an IgG1 mAb that has ADCC activity as well. Table 4 summarizes publically available data regarding structure, LAG-3 target and ligand specificity along with current development phase and response rates of these agents.

BMS-986016 (relatlimab) is an IgG4 mAb that binds with high affinity to LAG-3 on CD8 T cells and T regs, although it remains unclear as to which target is primarily responsible for the effects of the drug. Following single-agent dose-escalation in which no adverse safety signals were seen, BMS-986016 has been explored in combination with nivolumab in PD-1/PD-L1 refractory melanoma (CA224-020/[NCT01968109](#)). In the phase II portion of the study comprising 55 advanced melanoma patients, overall response rates were 12.5% in 48 evaluable patients. However, patients with LAG-3 expression ≥1% on tumor associated immune cells had higher response than LAG-3 expression <1% (20% vs. 7%), suggesting that LAG-3 expression on tumor-associated immune cells was a potential predictive biomarker [158]. Several studies evaluating the BMS-986016/nivolumab combination are ongoing in other indications including PD-1 naïve melanoma ([NCT03743766](#)), and advanced chordoma ([NCT03623854](#)).

Similar to BMS-986016 (relatlimab), MK-4280 is an IgG4 mAb that binds to LAG-3 on CD8 T cells and T regs with high affinity. MK-4280 is being studied both singly and in combination with pembrolizumab in a phase I dose-escalation study in solid tumors ([NCT02720068](#)). In the dose-escalation portion of the study which included 33 patients, 18 patients received MK-4280 monotherapy (7–700 mg every 3 weeks), while 15 were treated with MK-4280/pembrolizumab combination. No adverse safety signals were seen in either monotherapy or combination therapy arms (grade 3/4 adverse events: MK-4280 6%; MK-4280/pembrolizumab 20%). Responses were seen in both monotherapy and combination therapy arms (ORR: MK-4280 6%; MK-4280/pembrolizumab 27%) [159].

LAG525 was also studied singly and in combination with PD-1 inhibitor spartalizumab in a dose-escalation study ([NCT03365791](#)). LAG525 was dosed at 15 dose-levels (0.3 mg/kg up to 1000 mg) either every 2 weeks or either every 4 weeks in combination with spartalizumab (1 mg/kg every 2 weeks or 400 mg every 4 weeks) in the phase I dose-escalation trial. No adverse safety signals were seen with either LAG525 or LAG525/spartalizumab combination (grade 3/4 adverse events: LAG525 8%; MK- LAG525/spartalizumab 8%). Durable responses were seen with LAG525/spartalizumab combination (10%) including 11 partial and 1 complete response [160].

OMP-313M32 (etigilimab) is an investigational LAG-3 inhibitor that is currently being evaluated in advanced cancer patients. Eighteen patients with advanced solid organ cancer were treated in the dose-escalation study with single-agent OMP-313M32 (etigilimab) at doses ranging from 0.3 to 20 mg/kg every 2 weeks ([NCT03119428](#)). As no dose-limiting toxicities were observed, 20 mg/kg was picked as the recommended phase 2 dose. Adverse events were typical for the class. No responses were seen, although four patients (22%) had prolonged stable disease lasting >200 days [161].

Phase I studies of EOS8844488, TJT6, AB154 ([NCT03628677](#)), TSR-033 ([NCT03250832](#)) and IMP321 ([NCT03252938](#)) are planned or ongoing.

1.6 Other Inhibitory T Cell Receptors: BTLA and VISTA

1.6.1 BTLA and VISTA: Structure and Ligands—

B- and T-lymphocyte attenuator (BTLA) is a member of the immunoglobulin superfamily of receptors which includes

coinhibitory (CTLA-4, PD-1, and BTLA) and costimulatory [CD28 and inducible T cell costimulator (ICOS)] molecules. BTLA has an intermediate type Ig fold in ectodomain and an ITIM inhibitory signaling domain in the cytosolic portion of the receptor. Unlike the other members of this family which interact with ligands of the B7 family, BTLA interacts with a member of the TNF receptor superfamily (TNFRSF) termed herpes virus entry mediator (HVEM, also known as TNFRSF14) [162]. However, besides binding to BTLA, HVEM binds for other ligands including two members of TNFRSF including LIGHT (TNFSF14) and lympho-toxin α (LT α), herpes simplex virus glycoprotein D (HSV-1 gD), and CD160. The BTLA binding site on HVEM overlaps but is distinct from the LIGHT binding site, although glycoprotein D inhibits the binding of both ligands [163]. The BTLA binding site on HVEM overlaps with the binding site for HSV-1 gD but is distinct from where LIGHT binds [163].

V-domain Ig suppressor of T cell activation (VISTA) is a type I transmembrane protein that contains a signal peptide, extracellular Ig-V domain, stalk, transmembrane domain linked to a cytoplasmic tail [164]. VISTA's cytoplasmic domain does not contain ITAM, ITIM, or ITSM motifs [165]. VISTA has structural homology with PD-L1 and like PD-L1, potently suppresses T cell activation [164]. In healthy patients, VISTA is abundantly expressed in placental tissue; however, in the context of cancer VISTA is predominately expressed on T regs and MDSCs, which are upregulated in the TME [166, 167]. The receptor that VISTA binds to on human immune cells is unknown at this time.

1.6.2 BTLA and VISTA: Signaling and Function—BTLA is expressed on Th1 but not Th2 cells, B cells and to a lesser extent on macrophages, DCs and natural killer cells [168, 169]. In humans, BTLA expression on T cells is associated with poor prognosis in multiple cancers including diffuse large B-cell lymphoma (DLBCL) [170] and hepatocellular carcinoma (HCC) [171]. Similar to PD-1, BTLA signaling has been shown to exert an inhibitory effect upon antigen-specific T cells; blockade of which enhances the expansion, proliferation, and cytokine production of TA-specific CD8⁺ T cells [172]. However, other data suggests that BTLA signaling plays crucial roles in sustaining activated T cells in multiple models including murine models of graft versus host disease (GVHD) [173], CD4⁺ CD45RB^{high} T cell transfer induced colitis in recombination activating gene (Rag) knockout mice [174] and bacterial infection [175]. Concordantly, in advanced melanoma patients treated with adoptive cell therapy, BTLA expression on TIL correlated with response to therapy [176, 177]. The ubiquitous expression of HVEM and BTLA, coupled with broad ligand interaction of HVEM and the ability of HVEM/BTLA to interact in *cis* or *trans* configurations suggest that the BTLA-HVEM pathway is capable of bidirectional signaling with context dependent outcomes.

In syngeneic murine models, VISTA blockade enhanced infiltration, proliferation, and effector function of CD8 T cells in TME [167]. Combination VISTA/PD-L1 blockade produced greater antitumor effects than either agent alone, suggesting that VISTA and PD-1/PD-L1 signaling have overlapping but nonredundant inhibitory effects on CD8 T cells [178]. The effects of VISTA blockade appears to be mediated by IL-23/IL-17 axis, although how IL-17 regulates TA-specific adaptive immunity remains to be clarified [179].

1.6.3 BTLA and VISTA: Preclinical and Clinical Data—At this time, no BTLA development programs have been disclosed.

VISTA development programs have been disclosed by Curis (CA-170), and Janssen (onvatilimab, JNJ-61610588). CA-170 is an oral small molecule inhibitor of PD-L1, PD-L2, and VISTA. Target specificity was inferred through functional studies. Preclinically, CA-170 administration demonstrated antitumor activity in syngeneic models of MC38 colorectal carcinoma, CT26 colon carcinoma, and B16 melanoma at a level comparable to PD-1 blockade. A dose-escalation trial of CA-170 in advanced solid tumors and lymphoma patients is ongoing ([NCT02812875](#)). Fifty nine patients have been treated across 9 dose levels (50–800 mg daily and 600–1200 mg twice daily). Interestingly, neither dose-limiting toxicities nor immune related adverse events were noted. In 51 evaluable patients, no responses were observed, although 25 patients had stable disease, several of whom had prolonged stable disease [180].

Although a phase I trial of JNJ-61610588 was launched in solid tumors ([NCT02671955](#)), this study was subsequently terminated and no results are available at this time.

2 Conclusions

The use of mAb targeting PD-1/PD-L1 and CTLA-4 has produced durable responses in a multitude of advanced malignancies hitherto considered terminal. The CTLA-4 inhibitor ipilimumab received regulatory approval for the treatment of metastatic melanoma in 2011. Following this, 6 PD-1/PD-L1 inhibitors (cemiplimab, nivolumab, pembrolizumab, atezolizumab, avelumab, and durvalumab) and the PD-1/CTLA-4 inhibitor combination (ipilimumab/nivolumab) have been approved to treat advanced cancers in 13 indications, including the first-ever tissue agnostic approval to treat a broad class of cancers defined by a biomarker (pembrolizumab and ipilimumab/nivolumab for DNA mismatch repair-deficient/microsatellite instability—high (MSI-H) tumors). The use of PD-1/PD-L1 and CTLA-4 inhibitors is characterized by several features including: unusual patterns of response requiring novel radiographic response criteria [immune related response criteria (irRC)] and unique spectrum of toxicities [immune related adverse events (irAE)]. However, adaptive immune resistance is an issue of increasing concern.

LAG-3, TIM-3, and TIGIT comprise the next generation of inhibitory receptor targets that are being explored in clinical trials. Our current understanding indicates that these receptors provide an overlapping and nonredundant mechanism of regulating immune responses with PD-1; suggesting that coblockade with anti-PD-1 mAbs will improve antitumor immune responses over PD-1 therapy alone. This hypothesis is borne out by early data in dose-escalation trials hinting at responses to LAG-3, TIM-3, and TIGIT blockade at least in small subset of patients. However, the rational evaluation of these agents requires an increased understanding of both the *specialized functions* of these receptors and their ligands and their effects on *TME components* besides effector T cells including Tregs, TAMs and MDSC. This will permit the rational design of combinatorial clinical trials particularly in combination with existing therapies; and the determination of biomarkers of response to these novel immune checkpoints.

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Response and survival data of PD-1 inhibitors or PD-1/CTLA-4 inhibitor combinations across multiple tumor types

Table 1

Study	Indication	Dose	Overall response rate	Landmark survival
<i>Nivolumab with ipilimumab</i>				
CheckMate-067 (Larkin J, NEJM 2015; Wolchok JD, NEJM 2017)	Previously untreated metastatic melanoma (BRAF V600E mutant or wild type)	Ipilimumab 3 mg/kg every 3 weeks with nivolumab 1 mg/kg every 3 weeks for 4 doses; then nivolumab 3 mg/kg every 2 weeks for 2 years Comparators: nivolumab 3 mg/kg every 2 weeks for 2 years vs. ipilimumab 3 mg/kg every 3 weeks for 4 doses	58% (ipi/nivo) vs. 44% (nivo) vs. 19% (ipi)	Median PFS: 11.5 months (ipi/nivo) vs. 6.9 months (nivo) vs. 2.9 months (ipi) [HR 0.43 for ipi/nivo vs. ipi] 3-year PFS rate: 39% (ipi/nivo) vs. 32% (nivo) vs. 10% (ipi) Median OS: unreached (ipi/nivo) vs. 37.6 months (nivo) vs. 19.9 months (ipi) [HR 0.55 for ipi/nivo vs. ipi] 3-year OS rates: 58% (ipi/nivo) vs. 52% (nivo) vs. 34% (ipi)
CheckMate-214 (Motzer RJ, NEJM 2018)	Previously untreated metastatic intermediate-/poor-risk RCC	Ipilimumab 3 mg/kg every 3 weeks with nivolumab 1 mg/kg every 3 weeks; then nivolumab 3 mg/kg every 2 weeks for 2 years Comparator: sunitinib 50 mg daily	42% (ipi/nivo) vs. 27% (sunitinib)	Median PFS: 11.6 months (ipi/nivo) vs. 8.4 months (sunitinib) [HR 0.82] 2-year PFS rate: 72% (ipi/nivo) vs. 63% (sunitinib) Median OS: unreached (ipi/nivo) vs. 26.0 months (sunitinib) [HR 0.63] 1-year OS rate: 80% (ipi/nivo) vs. 72% (sunitinib)
CheckMate-142 (Overman MJ, J Clin Oncol 2018; Overman MJ, Lancet Oncol 2017)	Metastatic MSI-H/dMMR colorectal cancer after failure of first-line chemotherapy	Ipilimumab 1 mg/kg every 3 weeks with nivolumab 3 mg/kg every 3 weeks; then 2 weeks for 2 years	55%	Median PFS: not reached 1-year PFS rate: 71% Median OS: not reached 1-year OS rate: 85%
<i>Nivolumab</i>				
CheckMate-238 (Weber J, NEJM 2017)	Resected high-risk (stage IIIB/C/D and IV) melanoma (BRAF V600E mutant or wild type)	Nivolumab 3 mg/kg every 2 weeks for 1 year Comparator: ipilimumab 10 mg/kg every 3 weeks for 4 doses, then every 12 weeks for 1 year	N/A	Median PFS: not reached 1-year RFS rate: 71% (nivo) vs. 61% (ipi) Median OS: not reached 1-year OS rate: not reported
CheckMate-066 (Robert C, NEJM 2015)	Previously untreated metastatic melanoma (BRAF V600E mutant or wild type)	Nivolumab 3 mg/kg every 2 weeks for 1 year Comparator: Dacarbazine 1000 mg/m ² every 3 weeks	40% vs. 14% (dacarbazine)	Median PFS: 5.1 months (nivo) vs. 2.2 months (dacarbazine) 1-year PFS rate: not reported Median OS: not reached (nivo) vs. 10.8 months (dacarbazine) 1-year OS rate: 73% (nivo) vs. 42% (dacarbazine)
CheckMate-026 (Carbone DP, NEJM 2017)	Previously untreated metastatic PD-L1 positive (≥ 5%) NSCLC Note: study enrolled patients with PD-L1 1% but primary efficacy analyses were conducted in patients with PD-L1 ≥ 5%	Nivolumab 3 mg/kg every 2 weeks Comparator: Platinum-based chemotherapy every 3 weeks for up to six cycles	26% (nivo) vs. 33% (chemotherapy)	Median PFS: 4.2 months (nivo) vs. 5.9 months (chemotherapy) 1-year PFS rate: 24% (nivo) vs. 23% (chemotherapy) Median OS: 14.4 months (nivo) vs. 13.2 months (chemotherapy) 1-year OS rate: 56% (nivo) vs. 54% (chemotherapy)
CheckMate-017 (Brahmer J, NEJM 2015; NSCLC Hom L, J Clin Oncol 2017)	Previously treated metastatic squamous NSCLC	Nivolumab 3 mg/kg every 2 weeks Comparator: Docetaxel 75 mg/m ² every 3 weeks	20% (nivo) vs. 9% (docetaxel)	Median PFS: 3.5 months (nivo) vs. 2.8 months (docetaxel) 1-year PFS rate: 21% (nivo) vs. 6% (docetaxel) Median OS: 9.2 months (nivo) vs. 6.0 months (docetaxel) 1-year OS rate: 42% (nivo) vs. 24% (docetaxel)
CheckMate-057 (Borghaei H, NEJM 2015)	Previously treated metastatic non-squamous NSCLC	Nivolumab 3 mg/kg every 2 weeks Comparator: Docetaxel 75 mg/m ² every 3 weeks	19% (nivo) vs. 12% (docetaxel)	Median PFS: 2.3 months (nivo) vs. 4.2 months (docetaxel) 1-year PFS rate: 19% (nivo) vs. 8% (docetaxel) Median OS: 12.2 months (nivo) vs. 9.4 months (docetaxel) 1-year OS rate: 51% (nivo) vs. 39% (docetaxel)

Study	Indication	Dose	Overall response rate	Landmark survival
CheckMate-032 (Antonia SJ, Lancet Oncol 2016)	Limited/extensive stage platinum-refractory SCLC	Nivolumab 3 mg/kg every 2 weeks Comparator: nivolumab plus ipilimumab (1 mg/kg plus 1 mg/kg, 1 mg/kg plus 3 mg/kg, or 3 mg/kg plus 1 mg/kg, intravenously) every 3 weeks for four cycles, followed by nivolumab 3 mg/kg every 2 weeks	10% (nivo) vs. 21% (ipi/nivo—all arms)	Median PFS: 1.4 months (nivo) vs. 1.4–2.6 months (ipi/nivo—all arms) 1-year PFS rate: not reported Median OS: 4.4 months (nivo) vs. 6.0–7.7 months (ipi/nivo—all arms) 1-year OS rate: 33% (nivo) vs. 35–43% (ipi/nivo—all arms)
CheckMate-025 (Morzer RJ, NEJM 2015)	Previously TKI-refractory metastatic RCC	Nivolumab 3 mg/kg every 2 weeks Comparator: everolimus 10 mg daily	25% (nivo) vs. 5% (everolimus)	Median PFS: 4.6 months (nivo) vs. 4.4 months (everolimus) 1-year PFS rate: not reported Median OS: 25.0 months (nivo) vs. 19.6 months (everolimus) 1-year OS rate: not reported
CheckMate-205 (Younes A, Lancet Oncol 2016; Armand P, J Clin Oncol 2018)	Relapsed Hodgkin's lymphoma (after failure of autoSCT and brentuximab)	Nivolumab 3 mg/kg every 2 weeks Comparator: none	69%	Median PFS: 14.7 months 1-year PFS rate: not reported Median OS: not reached 1-year OS rate: 92%
CheckMate-141 (Ferris RL, NEJM 2016)	Recurrent/metastatic chemotherapy-refractory HNSCC	Nivolumab 3 mg/kg every 2 weeks Comparator: single-agent chemotherapy (methotrexate, docetaxel, or cetuximab)	13% (nivo) vs. 6% (chemotherapy)	Median PFS: 2.0 months (nivo) vs. 2.3 months (chemotherapy) 6-month PFS rate: 20% (nivo) vs. 10% (chemotherapy) Median OS: 7.5 months (nivo) vs. 5.1 months (chemotherapy) 1-year OS rate: 36% (nivo) vs. 17% (chemotherapy)
CheckMate-275 (Sharma P, Lancet Oncol 2017)	Metastatic urothelial carcinoma (progressed during/ following platinum-containing chemotherapy; within 12 months of adjuvant/ neoadjuvant platinum-containing chemotherapy)	Nivolumab 3 mg/kg every 2 weeks Comparator: none	20% [16% (PD-L1 < 1%), 24% (PD-L1 1%), 28% (PD-L1 5%)]	Median PFS: 2.0 months 1-year PFS rate: not reported Median OS: 8.7 months 1-year OS rate: not reported
CheckMate-142 (Overman MJ, Lancet Oncol 2017)	MSI-H/dMMR tumors	Nivolumab 3 mg/kg every 2 weeks Comparator: none	31% Tumor PD-L1 expression: 1% 31%, <1% 69% Immune cell PD-L1 expression: rare 35%, intermediate 31%, numerous 34%	Median PFS: not reached 1-year PFS rate: 50% Median OS: not reached 1-year OS rate: 73%
CheckMate-040 (El-Khoueiry AB, Lancet 2017)	Advanced HCC (following sorafenib or intolerant to sorafenib)	Nivolumab in escalating doses (0.1–10 mg/kg every 2 weeks) in virus negative, HCV-infected and HBV-infected cohorts Comparator: none	15% (dose-escalation phase) and 20% (nivolumab 3 mg/kg in dose-expansion)	Median PFS: 9.9 months 6-month PFS rate: 37% Median OS: not reached 6-month OS rate: 83% 9-month OS rate: 74%
<i>Pembrolizumab</i>				
KEYNOTE-054 (Eggermont AMM, NEJM 2018)	Resected high-risk (stage IIIA/B/C/D) melanoma (BRAF V600E mutant or wild type)	Pembrolizumab 300 mg every 3 weeks for 1 year Comparator: placebo	N/A	Median PFS: not reached 1-year RFS rate: 75% vs. 61% (placebo) 18 month RFS rate: 71% vs. 61% (placebo) Median OS: not reached 1-year OS rate: not reported
KEYNOTE-006 (Robert C, NEJM 2015; Schachter J, Lancet 2017)	Previously untreated metastatic melanoma (BRAF V600E mutant or wild type)	Pembrolizumab (10 mg/kg every 2 weeks for 2 years; 10 mg/kg every 3 weeks for 2 years) Comparator: ipilimumab 3 mg/kg for 4 doses	37% (pembro q2) vs. 36% (pembro q3) vs. 13% (ipi)	Median PFS: 5.6 months (pembro q2) vs. 4.1 months (pembro q3) vs. 2.8 months (ipi) 2-year PFS rate: 31% (pembro q2) vs. 28% (pembro q3) vs. 14% (ipi) Median OS: not reached (pembro q2 or pembro q3) vs. 16.0 months (ipi)

Study	Indication	Dose	Overall response rate	Landmark survival
KEYNOTE-189 (Gandhi L, NEJM 2018)	Previously untreated metastatic non-squamous NSCLC in combination with carboplatin and pemetrexed	Cisplatin 75 mg/m ² or carboplatin AUC 5 AND pemetrexed 500 mg every 3 weeks AND pembrolizumab 200 mg every 3 weeks Comparator: Cisplatin 75 mg/m ² or carboplatin AUC 5 AND pemetrexed 500 mg every 3 weeks AND placebo	48% (Carbo/Pem/Pem) vs. 19% (Carbo/Pem/placebo)	2-year OS rate: 55% (pembro q2) vs. 55% (pembro q3) vs. 43% (ipi) Median PFS: 8.8 months (Carbo/Pem/Pem) vs. 4.9 months (Carbo/Pem/placebo) 1-year PFS rate: 34% (Carbo/Pem/Pem) vs. 17% (Carbo/Pem/placebo) Median OS: not reached (Carbo/Pem/Pem) vs. 11.3 months (Carbo/Pem/placebo) 1-year OS rate: Carbo/Pem/Pem vs. Carbo/Pem/placebo <1% TPS: 62% vs. 52% 1–49% TPS: 72% vs. 51% 50% TPS: 73% vs. 48%
KEYNOTE-407 (Paz-Ares L, NEJM 2018)	Previously untreated metastatic squamous NSCLC in combination with carboplatin and paclitaxel/nab-paclitaxel	Carboplatin AUC 6 AND paclitaxel 200 mg/m ² or nab-paclitaxel 100 mg/m ² (D1/8/15) AND pembrolizumab 200 mg every 3 weeks Comparator: Carboplatin AUC 6 AND paclitaxel 200 mg/m ² or nab-paclitaxel 100 mg/m ² (D1/8/15) AND placebo	58% (pem-chemo) vs. 38% (chemo)	Median PFS: 6.4 months (pem-chemo) vs. 4.8 months (chemo) <1% TPS: 6.3 months vs. 5.3 months 1–49% TPS: 7.2 months vs. 5.2 months 50% TPS: 8.0 months vs. 4.2 months 1-year PFS rate: not reported Median OS: 15.9 months (pem-chemo) vs. 11.3 months (chemo) <1% TPS: 15.9 months vs. 10.2 months 1–49% TPS: 14.0 months vs. 11.6 months 50% TPS: not reached vs. not reached 1-year OS rate: 65.2% (pem-chemo) vs. 48.3% (chemo) <1% TPS: 64% vs. 43% 1–49% TPS: 66% vs. 50% 50% TPS: 63% vs. 51%
KEYNOTE-024 (Reck M, NEJM 2016)	Previously untreated metastatic squamous PD-L1 positive NSCLC (≥ 50% TPS)	Pembrolizumab 200 mg every 3 weeks Comparator: chemotherapy	45% (pembro) vs. 28% (chemo)	Median PFS: 10.3 months (pembro) vs. 6.0 months (chemo) 6-month PFS rate: 62.1% (pembro) vs. 50.3% (chemo) Median OS: not reached vs. not reached 6-month OS rate: 80.2% (pembro) vs. 72.4% (chemo)
KEYNOTE-040 (Cohen EEW, Lancet 2019)	Recurrent/metastatic chemotherapy-refractory HNSCC	Pembrolizumab 200 mg every 3 weeks Comparator: single-agent chemotherapy (methotrexate, docetaxel, or cetuximab)	15% (pembro) vs. 10% (chemotherapy)	Median PFS: 2.1 months (pembro) vs. 2.3 months (chemotherapy) 6-month PFS rate: not reported Median OS: 8.4 months (pembro) vs. 6.9 months (chemotherapy) 1-year OS rate: 37% (pembro) vs. 27% (chemotherapy)
KEYNOTE-087 (Chen R, J Clin Oncol 2017)	Relapsed/refractory Hodgkin's lymphoma (after failure of autoSCT and brentuximab)	Pembrolizumab 200 mg every 3 weeks Comparator: none	69%	Median PFS: not reached 6-month PFS rate: 72.4% Median OS: not reached 6-month OS rate: 99.5%
KEYNOTE-013 (Zinzani PL, Blood 2017)	Relapsed/refractory primary mediastinal B-cell lymphoma	Pembrolizumab 200 mg every 3 weeks for 2 years Comparator: none	41%	Median PFS: not reached 1-year PFS rate: not reported Median OS: not reached 1-year OS rate: not reported
KEYNOTE-052 (Balar AV, Lancet Oncol 2017)	Cisplatin ineligible bladder cancer	Pembrolizumab 200 mg every 3 weeks Comparator: none	24%	Median PFS: 2.0 months 6-month PFS rate: 30% Median OS: not reached 6-month OS rate: not reported
KEYNOTE-164 (Le DT, NEJM 2015; Le DT, J Clin Oncol 2018)	Metastatic MSI-H/dMMR colorectal cancer after failure of ≥ 2 lines of chemotherapy	Pembrolizumab 200 mg every 3 weeks Comparator: none	32%	Median PFS: 4.1 months 12-month PFS rate: 41% Median OS: not reached 12-month OS rate: 76%
KEYNOTE-061 (Shitara K, Lancet 2018)	Advanced chemotherapy refractory gastric cancer with PD-L1 positive (PD-L1 positive by CPS ≥ 1%) tumors	Pembrolizumab 200 mg every 3 weeks for up to 2 years Comparator: paclitaxel	16% (pembro) vs. 14% (paclitaxel)	Median PFS: 1.5 months (pembro) vs. 4.5 months (paclitaxel) 1-year PFS rate: 14% (pembro) vs. 9% (paclitaxel) Median OS: 9.1 months (pembro) vs. 8.3 months (paclitaxel) 1-year OS rate: 40% (pembro) vs. 27% (paclitaxel) 18-month OS rate: 26% (pembro) vs. 15% (paclitaxel)
KEYNOTE-158 (Chung HC, J Clin Oncol 2018)	Advanced chemotherapy refractory cervical cancer with PD-L1 positive (PD-L1 positive by CPS ≥ 1%) tumors	Pembrolizumab 200 mg every 3 weeks Comparator: none	13%	Median PFS: 2.1 months 1-year PFS rate: not reported Median OS: 9.4 months 1-year OS rate: not reported

Study	Indication	Dose	Overall response rate	Landmark survival
KEYNOTE-244 (Zhu AX, Lancet Oncol 2018)	Advanced HCC (following sorafenib or intolerant to sorafenib)	Pembrolizumab 200 mg every 3 weeks Comparator: none	17%	Median PFS: 4.9 months 1-year PFS rate: 28% Median OS: 12.9 months 1-year OS rate: 54%
KEYNOTE-017/CITN-09 (Nghiem PT, NEJM 2016)	Previously untreated metastatic Merkel cell carcinoma	Pembrolizumab 200 mg every 3 weeks Comparator: none	56%	Median PFS: 6.8 months 6-month PFS rate: 67% Median OS: not reported 1-year OS rate: not reported
<i>Atezolizumab</i>				
IMvigor211 (Powles T, Lancet 2018)	Metastatic urothelial carcinoma (progressed during/ following platinum-containing chemotherapy; within 12 months of adjuvant/ neoadjuvant platinum-containing chemotherapy)	Atezolizumab 1200 mg every 3 weeks Comparator: chemotherapy (vinflunine 320 mg/m ² , paclitaxel 175 mg/m ² , or docetaxel 75 mg/m ² every 3 weeks)	All patients: 13.4% (atezo) vs. 13.4% (chemo) In PD-L1 5% (IC2/3) patients: 23.0% (atezo) vs. 21.6% (chemo)	Median PFS: 2.1 months (atezo) vs. 4.0 months (chemo) In PD-L1 5% (IC2/3) patients: 2.4 months (atezo) vs. 4.2 months (chemo) 1-year PFS rate: not reported Median OS: 11.1 months (atezo) vs. 10.6 months (chemo) 1-year OS rate: 39.2% (atezo) vs. 32.4% (chemo)
OAK (Rittmeyer A, Lancet 2017)	Previously treated metastatic squamous and non-squamous NSCLC	Atezolizumab 1200 mg every 3 weeks Comparator: docetaxel 75 mg/m ² every 3 weeks	All patients: 14% (atezo) vs. 13% (docetaxel) In PD-L1 5% (IC2/3) patients: 22.5% (atezo) vs. 12.5% (docetaxel)	Median PFS: 2.8 months (atezo) vs. 4.0 months (docetaxel) In PD-L1 5% (IC2/3) patients: 4.1 months (atezo) vs. 3.6 months (docetaxel) 1-year PFS rate: not reported Median OS: 13.8 months (atezo) vs. 9.6 months (docetaxel) 1-year OS rate: 55% (atezo) vs. 51% (docetaxel) In PD-L1 5% (IC2/3) patients: 61% (atezo) vs. 45% (docetaxel) 18-month OS rate: 40% (atezo) vs. 27% (docetaxel) In PD-L1 5% (IC2/3) patients: 46% (atezo) vs. 29% (docetaxel)
<i>Avelumab</i>				
JAVELIN Solid Tumor (Patel MR, Lancet Oncol 2017)	Metastatic urothelial carcinoma (progressed during/ following platinum-containing chemotherapy; within 12 months of adjuvant/ neoadjuvant platinum-containing chemotherapy)	10 mg/kg every 2 weeks	17%	Median PFS: 6.3 weeks 6-month PFS rate: 23% Median OS: 6.5 months 6-month OS rate: 53%
JAVELIN Merkel 200 (Kaufman HL, Lancet Oncol 2016; Kaufman HL, J Immunother Cancer 2018)	Metastatic Merkel cell carcinoma	10 mg/kg every 2 weeks	33%	Median PFS: 2.7 months PFS rates: 29% (1-year) and 26% (2-year) Median OS: 12.6 months OS rates: 50% (1-year) and 36% (2-year)
<i>Durvalumab</i>				
Study 1108 (Powles T, JAMA Oncol 2017)	Metastatic urothelial carcinoma (progressed during/ following platinum-containing chemotherapy; within 12 months of adjuvant/ neoadjuvant platinum-containing chemotherapy)	10 mg/kg every 2 weeks	17.8%	Median PFS: 1.5 months PFS rate: not reported Median OS: 18.2 months OS rates: 55% (1-year)
PACIFIC (Antonia SJ, NEJM 2017)	Unresectable stage III NSCLC following concurrent platinum-based chemotherapy and radiation therapy	10 mg/kg every 2 weeks for 1 year Comparator: placebo	28.4%	Median PFS: 16.8 months (durvalumab) vs. 5.6 months (placebo) 1-year PFS rate: 55.9% (durvalumab) vs. 35.3% (placebo) Median OS: 23.2 months (durvalumab) vs. 14.6 months (a)

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Study	Indication	Dose	Overall response rate	Landmark survival
<i>Cemiplimab</i> EMPOWER (Migden MR, NEJM 2018)	Metastatic/locally advanced cutaneous SCC (not candidates for surgery or RT)	350 mg every 3 weeks	47% (phase II study), 50% (phase I study)	Not reported

Table 2

Development progress of TIM-3 inhibitors

Product	Company and partner (if applicable)	Structure	TIM-3 target and ligand specificity	Phase of development	ORR
MBG453	Novartis	Fully humanized, undisclosed	Undisclosed	Phase I dose-escalation (NCT02608268)	Unreported
TSR-022	Tesaro and GlaxoSmithKline	Fully humanized, IgG4 _k	Undisclosed	Phase I dose-escalation and dose-expansion (select malignancies) (NCT02817633)	All tumors: Melanoma: TSR-022 100 mg: 0/11 TSR-022 300 mg: 3/20 NSCLC: TSR-022 100 mg: 1/11 TSR-022 300 mg: 4/28 (4/12 with PD-L1+)
BMS-986258 (ONO7807)	Bristol Myers Squibb (five prime therapeutics)	Fully humanized, IgG1 _k	Undisclosed	Phase I dose-escalation (NCT03446040)	Unreported
INCAGN02390	Incyte (Agenus)	Fully humanized, IgG1 _k	CC'-FG binding cleft and phosphatidylserine	Phase I dose-escalation (NCT03652077)	Unreported

Table 3

Development progress of TIGIT inhibitors

Product	Company and partner (if applicable)	Structure	TIGIT target	Phase of development	ORR
MK-7684	Merck	Fully humanized, IgG1	CD155/CD112	Phase I dose-escalation and dose-expansion in PD-1 naïve and refractory tumors (select malignancies) (NCT02964013)	Monotherapy: 1/34 (3%) Combination with pembrolizumab: 8/34 (19%)
BMS-986207	Bristol Myers Squibb	Fully humanized, IgG1 with inert Fc	CD155/CD112	Phase I dose-escalation and dose-expansion in PD-1 refractory tumors (select malignancies) (NCT02913313)	Unreported
CGEN-15137/COM902	Compugen	Unknown	TIGIT	Unreported	Unreported
CGEN-15207/COM701	Compugen	Unknown	PVR	Unreported	Unreported
MTIG7192A	Genentech	Unknown	TIGIT	Phase I dose-escalation and dose-expansion in PD-1 refractory tumors (select malignancies) (NCT02794571)	Unreported
		Unknown	TIGIT	Randomized phase II study of MTIG7192A/atezolizumab vs. placebo/atezolizumab in PD-1/chemotherapy naïve metastatic NSCLC (NCT03563716)	Unreported
SP 8374/PTZ-201	Astellas	Unknown	TIGIT	Unreported	Unreported

Table 4

Development progress of LAG-3 inhibitors

Product	Company and partner (if applicable)	Structure	LAG-3 target and ligand specificity	Phase of development	ORR
BMS-986016 (relatlimab)	Bristol Myers Squibb	Fully humanized, IgG4	Undisclosed	Phase I dose-escalation (NCT01968109)	12.5%
MK-4280	Merck	Fully humanized, IgG4	Undisclosed	Phase I dose-escalation (NCT02720068)	6% (MK-4280); 20% (MK-4280/pembrolizumab)
LAG525	Novartis	Fully humanized, IgG4	Undisclosed	Phase I dose-escalation (NCT03365791)	Unknown (LAG525); 10% (LAG525/spartalizumab)
OMP-313M32 (etigilimab)	OncoMed	Fully humanized, IgG4	Undisclosed	Phase I dose-escalation (NCT03446040)	No responses reported, 22% with prolonged stable disease
TSR-033	Tesaro and GlaxoSmithKline	Fully humanized, IgG4	Undisclosed	Phase I dose-escalation (NCT03250832)	Unreported
ABI54	i-Mab Biopharma	Undisclosed	Undisclosed	Phase I dose-escalation (NCT03628677)	Unreported
IMP321	Prima BioMed/Immutep	Undisclosed	Undisclosed	Phase I dose-escalation (NCT03252938)	Unreported