Molecular Therapy
Oncology
Review

Promising immunotherapy targets: TIM3, LAG3, and TIGIT joined the party

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Immune checkpoint inhibitors (ICIs) have shown great promise as immunotherapy for restoring T cell function and reactivating anti-tumor immunity. The US Food and Drug Administration (FDA) approved the first immune checkpoint inhibitor, ipilimumab, in 2011 for advanced melanoma patients, leading to significant improvements in survival rates. Subsequently, other immune checkpoint-targeting antibodies were tested. Currently, seven ICIs, namely ipilimumab (anti-cytotoxic T lymphocyte-associated protein 4 [CTLA4]), pembrolizumab, nivolumab (anti-programmed cell death protein 1 [PD-1]), atezolizumab, avelumab, durvalumab, and cemiplimab (anti-PD-L1), have been approved for various cancer types. However, the efficacy of antibodies targeting CTLA4 or PD-1/programmed death-ligand 1 (PD-L1) remains suboptimal. Consequently, ongoing studies are evaluating the next generation of ICIs, such as lymphocyte activation gene-3 (LAG3), T cell immunoglobulin and mucin-domain containing 3 (TIM3), and T cell immunoglobulin and ITIM domain (TIGIT). Our review provides a summary of clinical trials evaluating these novel immune checkpoints in cancer treatment.

INTRODUCTION

Immune checkpoint therapy, which targets regulatory pathways in T cells to enhance anti-tumor immune responses, has led to important clinical advances and provided a new weapon against cancer. The human body has a large repertoire of T cells, each with a unique T cell receptor (TCR) that recognizes antigens as short peptides bound to major histocompatibility complex (MHC) proteins on the surface of antigen-presenting cell (APCs). These antigen/MHC complexes, especially when unique to tumor cells, are the key signal for T cells to attack. The normal immune system could recognize and attack tumor cells. It is now clear that T cell activation requires two signals: (1) MHC-restricted interaction between TCR and MHC-antigen; and (2) CD28 co-stimulation. At the same time, T cell function is held in check by inhibitory receptors and ligands. These immune checkpoint pathways maintain self-tolerance and limit tissue damage during inflammation. However, the tumor cells could hijack these checkpoint pathways by expressing inhibitory molecules such as programmed death-ligand 1 (PD-L1) to evade destruction.[1] Thus, drugs that can destroy the immune checkpoint system, such as cytotoxic T lymphocyte-associated protein 4 (CTLA4), programmed cell death protein 1 (PD-1), and PD-L1 inhibitors, could unleash anti-tumor immunity. Despite the success of anti-CTLA4 and anti-PD-1/PD-L1 antibodies, the field of cancer immunotherapy continues to advance rapidly, with next-generation immune checkpoint inhibitors (ICIs) showing promising results for multiple cancer types. These newer ICIs aim to overcome the limitations of the existing therapies and provide additional treatment options for patients.

T cell immunoglobulin and mucin-domain containing 3

T cell immunoglobulin and mucin-domain containing 3 (TIM3) was first identified in 2002 as a transmembrane protein expressed on CD4+ Th1 cells and CD8+ Tc1 (cytotoxic) cells.[2] Initial studies showed the inhibitory functions of TIM3 and demonstrated that blocking TIM3 could enhance the severity of experimental autoimmune encephalomyelitis (EAE). Subsequent studies showed the expression of TIM3 on regulatory T (Treg) cells, dendritic cells (DCs), natural killer (NK) cells, monocytes, macrophages, and mast cells.[3] TIM3 plays an important role in maintaining immune tolerance and dysregulation of TIM3 leads to development of autoimmune diseases. The negative regulatory functions of TIM3 are dependent on its ligands Galectin-9, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), phosphatidyl serine (PtdSer), and high-mobility group protein B1 (HMGB1). Galectin-9 was the first identified TIM3 ligand and its interactions lead to cell death of Th1 cells,[4] confirming the negative regulatory function of TIM3. On the other hand, CEACAM1 is a heterodimeric TIM3 ligand and its binding facilitates maturation and expression of TIM3, which is a necessity for the inhibitory function of TIM3.[5] The binding of TIM3 with PtdSer results in the phagocytosis of apoptotic cells and cross-presentation (Figure 1). However, the biological relevance of the interaction between TIM3 and PtdSer in T cells remains unknown since T cells have not been shown to clear apoptotic cells.[6] HMGB1 recognizes tumor-derived stress and activates protective immunity. Interestingly, binding of HMGB1 with TIM3 suppresses the sensor function and leads to inhibition of innate immune.[7]

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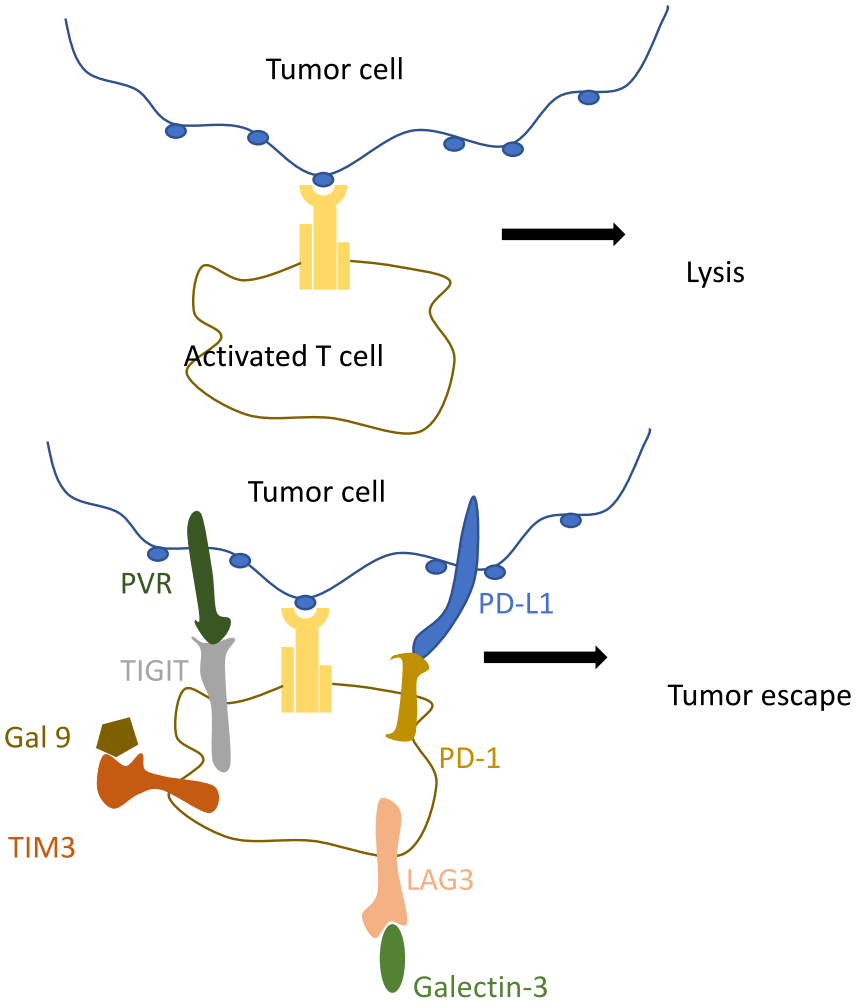

Figure 1. Novel immune checkpoints and their ligands
Ligation of TIM3 with soluble ligand Gal9 and LAG3 with Galectin-3 and TIGIT with tumor-expressed Poliovirus receptor (PVR) leads to the inhibition of T cell function.

TIM3 and cancer immunotherapy

TIM3 is a negative regulator of anti-tumor immunity as its expression is the marker for T cell dysfunction. Mouse model results showed that TIM3+PD-1+ Tumor-infiltrating lymphocytes (TILs) resemble most dysfunctional T cells as they fail to proliferate and produce interleukin (IL)-2, tumor necrosis factor (TNF), and interferon (IFN)-γ.[8] Apart from enforcing exhaustion of CD8 T cells, the mouse colon cancer model revealed that TIM3 is involved in the apoptosis of CD8+ TILs.[9] TIM3 may also enhance the anti-tumor immunity by increasing CD11b+Gr-1+ myeloid suppressor cells (MDSCs). Anti-TIM3 treatment increased CD4+ and CD8+ T cells while decreasing immunosuppressive MDSCs at tumor sites, yielding significantly elevated ratios of CD4+ and CD8+ T cells to Tregs and MDSCs.[10]

Besides the important role TIM3 plays in regulating effector T cells, researchers found that it is highly expressed on Foxp+ Tregs.[11] TIM3+ Tregs are highly effective suppressors of T cell effectors compared with TIM3− Tregs. TIM3+ Tregs secrete more IL-10, CD39, CD73, and transforming growth factor (TGF)-β and express high levels of other checkpoint receptors, including CTLA4, lymphocyte activation gene-3 (LAG3), and PD-1.[12] Although 60% Foxp+ TILs express TIM3 in non-small cell lung cancer (NSCLC) patients, TIM3 is barely expressed on Tregs on peripheral blood, which makes TIM3+ Treg a special kind of tumor tissue resident Treg.[12] Collectively, the expression of TIM3 on tumor Tregs underpins the increased suppressive function.

There is high expression of TIM3 in cancer tissues and TILs. In NSCLC patients, the high expression of TIM3 was correlated with poor clinicopathological parameters such as nodal metastasis and advanced cancer stages.[11] In cervical cancer, 65.1% of cancer tissue were positive for TIM3 staining. The data showed that TIM3-negative patients had significantly higher 5-year survival rate (80%) compared with TIM3-positive patients (46.4%) in cervical cancer.[13] Similarly, TIM3 is highly expressed on tumor cells of gastric cancer, with about 60% of gastric cancer patients reported to be positive for TIM3, which is significantly associated with poor survival.[14] A meta-analysis that investigated the correlation between TIM3 expression and tumor survival showed that TIM3 could serve as prognostic marker for multiple solid tumors, including lung cancer, gastric cancer, colon cancer, hepatocellular carcinoma (HCC), renal cell carcinoma, bladder urothelial carcinoma, and cervical cancer.[15] Taken together, TIM3 could be a promising target for immunotherapy.

Clinical trials on TIM3

Eight anti-TIM3 antibodies or PD-1/TIM3 bispecific antibodies have been under clinical trials since 2016. TSR-022 (Tesaro) was the first anti-TIM3 antibody under clinical trial (Table 1). NCT02817633 is a first-in-human study evaluating the anti-TIM3 antibody TSR-022. The clinical study began in 2016 and is still recruiting patients. However, researchers presented preliminary data on the safety, tolerability, and efficacy of TSR-022 monotherapy (arm 1A) and its combination with the anti-PD-1 antibodies nivolumab (arm 1B) or dostarlimab (arm 1C) at the 2022 American Society of Clinical Oncology (ASCO) annual meeting.[16] A total of 104 patients were included in the study: 46 in arm 1A, seven in arm 1B, and 55 in arm 1C.[16] Treatment-emergent adverse events (TEAEs) of grade 3 or worse severity were observed in 4.3% of patients in arm 1A, 28.6% in arm 1B, and 14.5% in arm 1C. No grade 5 TEAEs were reported. TEAEs led to treatment discontinuation in 2.2% of patients in arm 1A, 28.6% in

Table 1. Clinical trial on TIM3

NCT Number	Year	Drug	Phase	Objective	Company
NCT03744468	2018	BGB-A425	1 and 2	study of BGB-A425 in combination with tislelizumab in advanced solid tumors	BeiGene (Beijing, China)
NCT04370704	2020	INCAGN02390	1 and 2	study of combination therapy with INCMGA00012 (anti-PD-1), INCAGN02385 (anti-LAG3), and INCAGN02390 (anti-TIM3) in participants with select advanced malignancies	Incyte Corporation (Wilmington, DE, USA)
NCT03652077	2018	INCAGN02390	1	a safety and tolerability study of INCAGN02390 in select advanced malignancies	Incyte Corporation (Wilmington, DE, USA)
NCT03099109	2017	LY3321367	1	a study of LY3321367 alone or with LY3300054 in participants with advanced relapsed/refractory solid tumors	Eli Lilly and Company (Indianapolis, IN, USA)
NCT03752177	2018	LY3415244	1	a study of LY3415244 in participants with advanced solid tumors	Eli Lilly and Company (Indianapolis, IN, USA)
NCT03961971	2019	MBG453	1	trial of anti-TIM3 in combination with anti-PD-1 and SRS (Stereotactic radiosurgery) in recurrent GBM	Novartis Pharmaceuticals
NCT02608268	2015	MBG453	1	phase I-Ib/II study of MBG453 as single agent and in combination with PDR001 in patients with advanced malignancies	Novartis Pharmaceuticals
NCT03066648	2017	MBG453	1	study of PDR001 and/or MBG453 in combination with decitabine in patients with AML or high-risk MDS	Novartis Pharmaceuticals
NCT03946670	2019	MBG453	2	A study of MBG453 in combination with hypomethylating agents in subjects with IPSS-R intermediate, high or very-high-risk MDS	Novartis Pharmaceuticals
NCT04266301	2020	MBG453	3	study of efficacy and safety of MBG453 in combination with azacitidine in subjects with intermediate, high or very-high-risk MDS as per IPSS-R, or chronic myelomonocytic leukemia-2	Novartis Pharmaceuticals
NCT04785820	2021	RO7121661	2	A study of RO7121661 and RO7247669 compared with nivolumab in participants with advanced or metastatic squamous cell carcinoma of the esophagus	Roche (Basel, Switzerland)
NCT03869190	2019	RO7121661	1 and 2	study evaluating the efficacy and safety of multiple immunotherapy-based treatments and combinations in patients with urothelial carcinoma (MORPHEUS-UC)	Roche (Basel, Switzerland)
NCT03708328	2018	RO7121661	1	A dose-escalation and expansion study of RO7121661, a PD-1/TIM3 bispecific antibody, in participants with advanced and/or metastatic solid tumors	Roche (Basel, Switzerland)
NCT03489343	2018	Sym023	1	Sym023 (anti-TIM3) in patients with advanced solid tumor malignancies or lymphomas	Symphogen A/S (Copenhagen, Denmark)
NCT03311412	2017	Sym023	1	Sym021 monotherapy, in combination with Sym022 or Sym023, and in combination with both Sym022 and Sym023 in patients with advanced solid tumor malignancies or lymphomas	Symphogen A/S (Copenhagen, Denmark)
NCT04641871	2020	Sym023	1	Sym021 in combination with either Sym022 or Sym023 in patients with advanced solid tumor malignancies	Symphogen A/S (Copenhagen, Denmark)
NCT02817633	2016	TSR-022	1	A study of TSR-022 in participants with advanced solid tumors (AMBER)	Tesaro (Waltham, MA, USA)
NCT03680508	2018	TSR-022	2	TSR-022 (anti-TIM3 antibody) and TSR-042 (anti-PD-1 antibody) in patients with liver cancer	Tesaro (Waltham, MA, USA)
NCT04139902	2019	TSR-022	2	neoadjuvant PD-1 inhibitor dostarlimab (TSR-042) vs. combination of TIM3 inhibitor cobolimab (TSR-022) and PD-1 inhibitor dostarlimab (TSR-042) in melanoma	Tesaro (Waltham, MA, USA)

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Table 1. *Continued*

NCT Number	Year	Drug	Phase	Objective	Company
NCT04823624	2021	MBG453	2	MBG453 in lower-risk MDS	Novartis Pharmaceuticals
NCT04150029	2020	MBG453	2	A study of MBG453 in combination with azacitidine and venetoclax in AML patients unfit for chemotherapy	Novartis Pharmaceuticals
NCT03940352	2019	MBG453	1	HDM201 in combination with MBG453 or venetoclax in patients with AML or high-risk MDS	Novartis Pharmaceuticals
NCT04878432	2021	MBG453	2	STIMULUS MDS-US: sabatolimab added to HMA (Hypomethylating Agents) in higher-risk MDS	Novartis Pharmaceuticals
NCT04283526	2020	MBG453	1	study of select combinations in adults with myelofibrosis	Novartis Pharmaceuticals
NCT04623216	2021	MBG453	1 and 2	sabatolimab as a treatment for patients with AML and presence of measurable residual disease after allogeneic stem cell transplantation	Novartis Pharmaceuticals
NCT04810611	2021	MBG453	1	phase Ib study of select drug combinations in patients with lower-risk MDS	Novartis Pharmaceuticals
NCT04097821	2019	MBG453	1 and 2	platform study of novel ruxolitinib combinations in myelofibrosis patients	Novartis Pharmaceuticals
NCT04812548	2021	MBG453	2	A study of sabatolimab in combination with azacitidine and venetoclax in high- or very-high-risk MDS participants	Novartis Pharmaceuticals

GBM, glioblastoma; MDS, myelodysplastic syndrome.

arm 1B, and 9.0% in arm 1C. Dose-limiting toxicities (DLTs) were observed in 3.0% of patients (one out of 33) in arm 1A, 40.0% of patients (two out of five) in arm 1B and no DLTs were reported in arm 1C.[16] Overall, the combination of TSR-022 and dostarlimab was well tolerated and demonstrated preliminary anti-tumor activity.

The PD-L1/TIM3 bispecific antibody LY3415244 (Eli Lilly) was dropped from the clinical pipeline and phase 1 clinical trial (NCT03752177) terminated during dose escalation because of unfavorable risk/benefit ratio. Another anti-TIM3 antibody LY3321367 (Eli Lilly) is under phase 1 clinical trial alone or in combination with anti-PD-L1 antibody LY300054 (NCT03099109). Initial data show that LY3321367 is well tolerated as a monotherapy and in combination with LY3300054.[17] No DLT or DLT-equivalent toxicities have been reported so far. TEAEs of grade 3 or worse severity occurred in two out of 37 patients (5.4%) (one asymptomatic lipase increases and one dyspnea) who were under the monotherapy. For combination therapy, TEAEs of grade 3 or worse such as diarrhea, hyperglycemia, and renal failure were reported in three out of 91 patients (3.3%).[18]

The anti-TIM3 antibody MBG453 (Novartis) is now under phase1-3 clinical trial with not only anti-PD-1 agent but also chemotherapy drugs. One completed phase 1 clinical trial NCT03066648 shows that a combination of MBG453 with decitabine is safe and well tolerated and demonstrated durable clinical responses in patients with acute myeloid leukemia (AML).[19,20] The most common adverse events (AEs) of the combined therapy were similar to decitabine treated alone, consisting of thrombocytopenia (45.8%), neutropenia (50.0%), anemia (33.3%), and febrile neutropenia (29.2%), respectively.[20] Only three with AML discontinued treatment due to an AE regardless of relationship to treatment. Five out of 48 patients had grade 3 and none had grade 4/5 AEs. The objective response rate (ORR) for the 40 evaluable AML patients was 40.0% and median Duration of Response (mDOR) was 12.6 months. Estimated 12-month progression-free survival (PFS) rate was 27.9% (95% confidence interval [CI], 14.9%–42.5%).[20]

Also, MBG453 combined with spartalizumab (anti-PD-1 antibody) was well tolerated with preliminary signs of anti-tumor activity in multiple solid tumors.[21,22]

In addition, phase 1 clinical trial (NCT03489343) of Sym023, a human anti-TIM3 antibody (Symphogen Biotechnology), showed that the antibody is safe and well tolerated in patients with locally advanced, unresectable, or metastatic solid tumor malignancies or lymphomas. The maximum tolerated dose was not reached and two out of 24 patients (8%) had grade 3 or higher TEAEs.[23] Assessment of pharmacokinetics, immunogenicity, and pharmacodynamic markers is underway. Other anti-TIM3 antibodies, such as Sym023, INCAGN02390, BGB-A425 or PD-1/TIM3 bispecific antibody, RO7121661, are in the phase 1 clinical trial. The safety and efficacy of them as single agents or in combination with other ICIs remain unknown.

LAG3

LAG3 was first identified in 1990 as a novel transmembrane protein expressed in activated NK cells and T cells.[24] LAG3 is highly

Table 2. Clinical trials on LAG3

NCT Number	Year	Phase	Drugs	Objective	Company
NCT01968109	2013	1 and 2	BMS-986016	an investigational immunotherapy study to assess the safety, tolerability and effectiveness of anti-LAG33 with and without anti-PD-1 in the treatment of solid tumors	BMS (New York, NY, USA)
NCT02061761	2014	1 and 2	BMS-986016	safety study of anti-LAG3 in relapsed or refractory hematologic malignancies	BMS (New York, NY, USA)
NCT02658981	2016	1	BMS-986016	anti-LAG3 alone and in combination with nivolumab treating patients with recurrent GBM (anti-CD137 arm closed 10/16/18)	BMS (New York, NY, USA)
NCT02966548	2016	1	BMS-986016	safety study of BMS-986016 with or without nivolumab in patients with advanced solid tumors	BMS (New York, NY, USA)
NCT03662659	2018	2	BMS-986016	an investigational study of immunotherapy combinations with chemotherapy in patients with gastric or GEJ cancers	BMS (New York, NY, USA)
NCT03743766	2019	2	BMS-986016	nivolumab, BMS-936558 in combination with relatlimab, BMS-986016 in patients with metastatic melanoma naive to prior immunotherapy in the metastatic setting	BMS (New York, NY, USA)
NCT03623854	2019	2	BMS-986016	nivolumab and relatlimab in treating participants with advanced chordoma	BMS (New York, NY, USA)
NCT03642067	2019	2	BMS-986016	study of nivolumab and relatlimab in patients with microsatellite stable advanced CRC	BMS (New York, NY, USA)
NCT04150965	2020	1 and 2	BMS-986016	immuno-oncology drugs elotuzumab, anti-LAG3, and anti-TIGIT	BMS (New York, NY, USA)
NCT04326257	2020	2	BMS-986016	personalized immunotherapy in patients with recurrent/metastatic SCCHN (Head and neck squamous cell carcinoma) that have progressed on prior immunotherapy	BMS (New York, NY, USA)
NCT03459222	2018	1 and 2	BMS-986016	an investigational study of immunotherapy combinations in participants with solid cancers that are advanced or have spread	BMS (New York, NY, USA)
NCT03607890	2018	2	BMS-986016	study of nivolumab and relatlimab in advanced mismatch repair deficient cancers resistant to prior PD-(L)1 inhibitor	BMS (New York, NY, USA)
NCT04080804	2019	2	BMS-986016	study of safety and tolerability of nivolumab treatment alone or in combination with relatlimab or ipilimumab in head and neck cancer	BMS (New York, NY, USA)
NCT03440437	2018	1	FS118	FS118 first-in-human study in patients with advanced malignancies after PD-1/PD-L1-containing therapy	F-star Delta Limited (Cambridge, UK)
NCT00351949	2006	1	IMP321	IMP321 1 trial in metastatic renal cell carcinoma	Immutep (NSW, Australia)
NCT00349934	2006	1	IMP321	IMP321 plus first-line paclitaxel in metastatic breast carcinoma	Immutep (NSW, Australia)
NCT00732082	2008	1	IMP321	LAG3 and gemcitabine for treatment of advanced pancreas cancer	Immutep (NSW, Australia)
NCT02614833	2015	2	IMP321	IMP321 (eftilagimod alpha) as adjunctive to a standard chemotherapy paclitaxel metastatic breast carcinoma	Immutep (NSW, Australia)
NCT02676869	2016	1	IMP321	1 study of IMP321 (Eftilagimod alpha) adjuvant to anti-PD-1 therapy in unresectable or metastatic melanoma	Immutep (NSW, Australia)
NCT03252938	2017	1	IMP321	feasibility and safety of IMP321 for advanced-stage solid tumors	Immutep (NSW, Australia)

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Table 2. *Continued*

NCT Number	Year	Phase	Drugs	Objective	Company
NCT03625323	2019	2	IMP321	combination study with soluble LAG3 fusion protein eftilagimod alpha (IMP321) and pembrolizumab in patients with previously untreated unresectable or metastatic NSCLC, or recurrent PD-X refractory NSCLC or with recurrent or metastatic SCCHN	Immutep (NSW, Australia)
NCT04252768	2020	1	IMP321	a study in hormone receptor-positive metastatic breast carcinoma patients to test a new schedule of Efti (IMP321, eftilagimod alpha) as adjunctive to a weekly treatment regimen of paclitaxel	Immutep (NSW, Australia)
NCT03538028	2018	1	INCAGN02385	a safety and tolerability study of INCAGN02385 in select advanced malignancies	Incyte Corporation (Wilmington, DE, USA)
NCT04370704	2020	1 and 2	INCAGN02385	study of combination therapy With INCMGA00012 (anti-PD-1), INCAGN02385 (anti-LAG3), and INCAGN02390 (anti-TIM3) in participants with select advanced malignancies	Incyte Corporation (Wilmington, DE, USA)
NCT03219268	2017	1	MGD013	a study of MGD013 in patients with unresectable or metastatic neoplasms	MacroGenics (Rockville, MD, USA)
NCT04082364	2019	2 and 3	MGD013	combination margetuximab, INCMGA00012, MGD013, and chemotherapy 2/3 trial in HER2+ gastric/GEJ cancer (MAHOGANY)	MacroGenics (Rockville, MD, USA)
NCT03005782	2016	1	REGN3767	study of REGN3767 (anti-LAG3) with or without REGN2810 (anti-PD-1) in advanced cancers	Regeneron Pharmaceuticals (Tarrytown, NY, USA)
NCT04140500	2019	1	RO7247669	dose-escalation study of a PD-1-LAG3 bispecific antibody in patients with advanced and/or metastatic solid tumors	Roche (Basel, Switzerland)
NCT03311412	2017	1	Sym022	Sym021 monotherapy and in combination with Sym022 or Sym023 in patients with advanced solid tumor malignancies or lymphomas	Symphogen A/S (Copenhagen, Denmark)
NCT03489369	2018	1	Sym022	Sym022 (anti-LAG3) in patients with advanced solid tumor malignancies or lymphomas	Symphogen A/S (Copenhagen, Denmark)
NCT02817633	2016	1	TSR-033	a study of TSR-022, an anti-TIM3 monoclonal antibody, in patients with advanced solid tumors (AMBER)	Tesaro (Waltham, MA, USA)
NCT03250832	2017	1	TSR-033	study of TSR-033 with an anti-PD-1 in patients with advanced solid tumors	Tesaro (Waltham, MA, USA)
NCT03849469	2019	1	XmAb22841	a study of XmAb22841 monotherapy and in combination with pembrolizumab in subjects with selected advanced solid tumors	Xencor (Monrovia, CA, USA)
NCT04112498	2019	1	relatlimab	a bioavailability study of relatlimab in combination with nivolumab	BMS (New York, NY, USA)
NCT03724968	2019	2	relatlimab	nivolumab plus relatlimab or ipilimumab in metastatic melanoma stratified by MHC-II expression	BMS (New York, NY, USA)
NCT03470922	2018	2 and 3	relatlimab	a study of relatlimab plus nivolumab versus nivolumab alone in participants with advanced melanoma	BMS (New York, NY, USA)
NCT04095208	2019	2	relatlimab	combination of nivolumab plus relatlimab in patients with advanced or metastatic soft-tissue sarcoma: a proof-of-concept randomized II study	BMS (New York, NY, USA)
NCT03044613	2017	1	relatlimab	nivolumab +/− relatlimab prior to chemoradiation with II/III gastro/esophageal cancer	BMS (New York, NY, USA)
NCT03978611	2019	1	relatlimab	a study to assess safety and efficacy of relatlimab with ipilimumab in participants with advanced melanoma who progressed on anti-PD-1 treatment	BMS (New York, NY, USA)

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Table 2. *Continued*

NCT Number	Year	Phase	Drugs	Objective	Company
NCT03704077	2019	2	relatlimab	an investigational immunotherapy study of relatlimab plus nivolumab compared to various standard-of-care therapies in previously treated participants with recurrent, advanced or metastatic gastric cancer or GEJ adenocarcinoma	BMS (New York, NY, USA)

GEJ, gastroesophageal junction.

structurally homologous to CD4 molecule, and has been shown to bind MHC-II with higher affinity than CD4. Although LAG3 has been shown to affect the function of CD8+ T cells and NK, data on the LAG3 ligand remains elusive. An *in vitro* study speculated that Galectin-3 and liver sinusoidal endothelial cell lectin (LSECtin) might be the potential ligand for LAG3.[25] LAG3 has been shown to negatively regulate the functions of T cells. A previous study showed that inhibition of LAG3 could enhance the proliferation of CD4 T cells and increase the production of pro-inflammatory cytokines.[26] This result was consistent with another study that also showed that blocking LAG3 could increase the proliferation of both CD4+ and CD8+ T cells.[27] The inhibitory function of LAG3 was further demonstrated in the LAG3 knockout mice, which showed that both CD4+ and CD8+ T cells undergo increased cell expansion following infection.[28]

Besides regulating homeostasis of effector T cells, LAG3 inhibits the functions of Tregs. A study showed that LAG3+ Tregs have more suppressive ability compared with LAG3− Tregs.[29] In addition, LAG3-deficient Tregs exhibit enhanced Treg proliferation and function, which leads to the mitigation of autoimmune diseases.[30]

LAG3 and cancer immunotherapy

Elevated expression of LAG3 has been detected on different types of TILs, including CD4+ TILs, CD8+ TILs, and Tregs.[31] It was reported that co-expression of LAG3 with other immune checkpoint molecules indicates the most dysfunctional status of CD8+ TILs.[32] LAG3 has low expression level on inactive CD8+ T cells but is overexpressed on CD8+ T cells that infiltrate various tumor types, including ovarian cancer, HCC, renal cell carcinomas, and other solid tumors.[33] Also, LAG3 signaling could mediate chronic exhaustion of CD4+ T cells during cancer recurrence.[34] During the progression of tumors, CD4+ T cells that infiltrate the tumor exhibit characteristics of chronic exhaustion. This is accompanied by an increase in the expression of the inhibitory receptor LAG3.[35] LAG3 interacts with MHC-II molecules and negatively regulates the expansion of CD4+ T cells, as well as suppresses their cytokine response. Studies have shown that a specific motif called KIEELE in the cytoplasmic tail of LAG3 is crucial for inhibiting effector CD4+ T cells.[36] This motif mediates intracellular signaling and prevents T cells from entering the growth phase of the cell cycle.

Tumor-infiltrating Tregs show elevated levels of Foxp3 and inhibitory molecules such as PD-1, CTLA4, TIM3, and LAG3. The LAG3-expressing Tregs secrete more inhibitory cytokines such as IL-10 and TGF-β.[37] Within the tumor microenvironment (TME), Tregs are known to hinder anti-tumor immune responses by impeding cytokine production and enhancing suppressor activity. LAG3 plays a crucial role in maximizing the suppressive function of Tregs and contributes to their regulatory phenotype.[37] Recent studies have demonstrated that LAG3 promotes the differentiation of Tregs, while blocking LAG3 reduces Treg induction.[37]

Besides the immune-suppressive function, LAG3 is reported to co-express with PD-1 in NSCLC, HCC, and colorectal cancer (CRC), as confirmed by both immunohistochemistry and RNA sequencing data.[38] LAG3+ PD-1+ T cells are the most exhausted T cells, thus biospecific antibody was developed to restore the function of these cells. The FDA has approved relatlimab, a LAG3-blocking antibody, combined with nivolumab for adult and pediatric patients 12 years of age or older with unresectable or metastatic melanoma.[39]

The suppressive function and negative regulatory role on T cells demonstrate that LAG3 is a promising target for cancer immunotherapy.

Clinical trials on LAG3

IMP321, a soluble LAG3 fusion protein developed by Immutep, consists of the four extracellular immunoglobulin (Ig) domains of LAG3 to the Fc portion of human IgG1. The first clinical trial (NCT00351949) on IMP321 was completed in 2009 and showed that seven of the eight metastatic renal cell carcinoma patients had stable disease (Table 2).[40] The tolerance and efficacy of IMP321 combined with chemotherapy agent paclitaxel was then investigated in HER2-negative metastatic breast cancer (NCT00349934). At the 6-month endpoint, 90% of the breast cancer patients had experienced a clinical benefit. The ORR was 50% based on Response Evaluation Criteria In Solid Tumours (RECIST) criteria, which compared favorably with the 25% response rate observed in patients on paclitaxel monotherapy.[41] Another clinical trial (NCT02614833) demonstrated that the combination of paclitaxel and IMP321 yielded 47% partial response rate and a disease control rate (DCR) of 87% in metastatic breast cancer.[42] Elsewhere, NCT00732082 showed that IMP321 combined with gemcitabine, a chemotherapy drug in pancreatic cancer, was safe and no severe AEs were observed. However, there were no significant differences when comparing pre- and post-treatment levels of monocytes, dendritic cells, and T cells, probably due to suboptimal dosing.[43]

Table 3. Clinical trials on TIGIT

NCT Number	Year	Phase	Drugs	Objective	Company
NCT03628677	2018	1	AB154	a study to evaluate the safety and tolerability of AB154 in participants with advanced malignancies	Arcus Biosciences (San Francisco, CA, USA)
NCT04262856	2020	2	AB154	study to evaluate monotherapy and combination immunotherapies in participants with PD-L1-positive non-small cell lung cancer	Arcus Biosciences (San Francisco, CA, USA)
NCT04656535	2020	0 and 1	AB154	AB154 combined with AB122 for recurrent glioblastoma	Arcus Biosciences (San Francisco, CA, USA)
NCT04736173	2021	3	AB154	study to evaluate monotherapy compared to combination immunotherapies in participants with PD-L1 positive non-small cell lung cancer	Arcus Biosciences (San Francisco, CA, USA)
NCT04791839	2021	2	AB154	safety and efficacy of zimberelimab (AB122) in combination with domvanalimab (AB154) and etrumadenant (AB928) in patients with previously treated non-small cell lung cancer	Arcus Biosciences (San Francisco, CA, USA)
NCT04047862	2019	1	BGB-A1217	study of BGB-A1217 in combination with tislelizumab in advanced solid tumors	BeiGene
NCT04693234	2021	2	BGB-A1217	AdvanTIG-202: anti-PD-1 monoclonal antibody tislelizumab (BGB-A317) combined with or without anti-TIGIT monoclonal antibody ociperlimab (BGB-A1217) in participants with previously treated recurrent or metastatic cervical cancer	BeiGene
NCT04866017	2021	3	BGB-A1217	tislelizumab plus BGB-A1217 versus durvalumab when co-administered with concurrent chemoradiotherapy (cCRT) in lung cancer	BeiGene
NCT04732494	2021	2	BGB-A1217	AdvanTIG-203: anti-PD-1 monoclonal antibody tislelizumab (BGB-A317) combined with or without anti-TIGIT monoclonal antibody ociperlimab (BGB-A1217) in participants with recurrent or metastatic esophageal squamous cell carcinoma	BeiGene
NCT04746924	2021	3	BGB-A1217	a study of ociperlimab with tislelizumab compared to pembrolizumab in participants with untreated lung cancer	BeiGene
NCT04150965	2019	1 and 2	BMS-986207	immuno-oncology drugs elotuzumab, anti-LAG3 and anti-TIGIT	BMS (New York, NY, USA)
NCT04570839	2020	1	BMS-986207	COM701 in combination with BMS-986207 and nivolumab in subjects with advanced solid tumors	BMS (New York, NY, USA)
NCT02913313	2016	1 and 2	BMS-986207	an investigational immunotherapy study of BMS-986207 given alone and in combination with nivolumab or with nivolumab and ipilimumab in solid cancers that are advanced or have spread	BMS (New York, NY, USA)
NCT04354246	2020	1	COM902	COM902 (a TIGIT inhibitor) in subjects with advanced malignancies	Compugen (Tel Aviv-Yafo, Israel)
NCT04353830	2020	1	IBI939	a study evaluating the safety, tolerability, and initial efficacy of recombinant human anti-T cell immunoreceptor with Ig and ITIM domains (TIGIT) monoclonal antibody injection (IBI939) in subjects with advanced malignant tumors	Innovent Biologics (Suzhou, China)
NCT04672356	2020	1	IBI939	a study to evaluate the safety, tolerability and efficacy of IBI939 in combination with sintilimab in patients with advanced lung cancer	Innovent Biologics (Suzhou, China)
NCT04672369	2020	1	IBI939	a study to evaluate the efficacy of IBI939 in combination with sintilimab in patients with advanced NSCLC	Innovent Biologics (Suzhou, China)

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Table 3. *Continued*

NCT Number	Year	Phase	Drugs	Objective	Company
NCT04305054	2020	1 and 2	MK-7684	safety and efficacy of pembrolizumab in combination with investigational agents or pembrolizumab alone in participants with first-line (1L) advanced melanoma (MK-3475-02B)	MSD (Kenilworth, NJ, USA)
NCT04305041	2020	1 and 2	MK-7684	safety and efficacy of pembrolizumab in combination with investigational agents in participants with PD-1-refractory melanoma	MSD (Kenilworth, NJ, USA)
NCT04303169	2020	1 and 2	MK-7684	safety and efficacy of pembrolizumab in combination with investigational agents or pembrolizumab alone in participants with stage III melanoma who are candidates for neoadjuvant therapy	MSD (Kenilworth, NJ, USA)
NCT04738487	2021	3	MK-7684	coformulation of pembrolizumab/vibostolimab (MK-7684A) versus pembrolizumab (MK-3475) monotherapy for PD-L1-positive metastatic NSCLC (MK-7684A-003)	MSD (Kenilworth, NJ, USA)
NCT04725188	2021	2	MK-7684	pembrolizumab/vibostolimab coformulation (MK-7684A) or pembrolizumab/vibostolimab coformulation plus docetaxel versus docetaxel for metastatic NSCLC with progressive disease after platinum doublet chemotherapy and immunotherapy (MK-7684A-002)	MSD (Kenilworth, NJ, USA)
NCT02964013	2016	1	MK-7684	study of vibostolimab alone and in combination with pembrolizumab in advanced solid tumors (MK-7684-001)	MSD (Kenilworth, NJ, USA)
NCT04165070	2019	2	MK-7684	KEYMAKER-U01 substudy 1: efficacy and safety study of pembrolizumab (MK-3475) plus chemotherapy when used with investigational agents in treatment-naive participants with advanced NSCLC (MK-3475-01A/KEYMAKER-U01A)	MSD (Kenilworth, NJ, USA)
NCT02861573	2016	1 and 2	MK-7684	study of pembrolizumab (MK-3475) combination therapies in metastatic castration-resistant prostate cancer (MK-3475-365/KEYNOTE-365)	MSD (Kenilworth, NJ, USA)
NCT03563716	2018	2	MTIG7192A	a study of MTIG7192A in combination with atezolizumab in chemotherapy-naive patients with locally advanced or metastatic NSCLC	Genentech (San Francisco, CA, USA)
NCT04665856	2020	3	MTIG7192A	study of atezolizumab plus carboplatin and etoposide with or without tiragolumab in participants with untreated extensive-stage small cell lung cancer	Genentech (San Francisco, CA, USA)
NCT04619797	2020	2	MTIG7192A	a study of tiragolumab in combination with atezolizumab plus pemetrexed and carboplatin/cisplatin versus pembrolizumab plus pemetrexed and carboplatin/cisplatin in participants with previously untreated advanced non-squamous non-small cell lung cancer	Genentech (San Francisco, CA, USA)
NCT04832854	2021	2	MTIG7192A	a study evaluating the safety and efficacy of neoadjuvant and adjuvant tiragolumab plus atezolizumab, with or without platinum-based chemotherapy, in participants with previously untreated locally advanced resectable stage II, IIIA, or select IIIB NSCLC	Genentech (San Francisco, CA, USA)
NCT04540211	2020	3	MTIG7192A	a study of atezolizumab plus tiragolumab in combination with paclitaxel and cisplatin compared with paclitaxel and cisplatin as first-line treatment in participants with unresectable locally advanced, unresectable recurrent, or metastatic esophageal carcinoma	Genentech (San Francisco, CA, USA)

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Table 3. *Continued*

NCT Number	Year	Phase	Drugs	Objective	Company
NCT04543617	2020	3	MTIG7192A	a study of atezolizumab with or without tiragolumab in participants with unresectable esophageal squamous cell carcinoma whose cancers have not progressed following definitive concurrent chemoradiotherapy	Genentech (San Francisco, CA, USA)
NCT04513925	2020	3	MTIG7192A	a study of atezolizumab and tiragolumab compared with durvalumab in participants with locally advanced, unresectable stage III NSCLC	Genentech (San Francisco, CA, USA)
NCT02794571	2016	1	MTIG7192A	safety and pharmacokinetics (PK) of escalating doses of tiragolumab as a single agent and in combination with atezolizumab and/or other anti-cancer therapies in locally advanced or metastatic tumors	Genentech (San Francisco, CA, USA)
NCT03119428	2017	1	OMP-313M32	a study of OMP-313M32 in subjects with locally advanced or metastatic solid tumors	OncoMed Pharmaceuticals (Redwood City, CA, USA)
NCT04256421	2020	3	tiragolumab	a study of atezolizumab plus carboplatin and etoposide with or without tiragolumab in patients with untreated extensive-stage small cell lung cancer	Roche (Basel, Switzerland)
NCT04294810	2020	3	tiragolumab	a study of tiragolumab in combination with atezolizumab compared with placebo in combination with atezolizumab in patients with previously untreated locally advanced unresectable or metastatic PD-L1-selected NSCLC	Roche (Basel, Switzerland)
NCT03281369	2017	1 and 2	tiragolumab	a study of multiple immunotherapy-based treatment combinations in patients with locally advanced unresectable or metastatic gastric or GEJ or esophageal cancer (morpheus-gastric and esophageal cancer)	Roche (Basel, Switzerland)
NCT04300647	2020	2	tiragolumab	a study of tiragolumab plus atezolizumab and atezolizumab monotherapy in participants with metastatic and/or recurrent PD-L1-positive cervical cancer	Roche (Basel, Switzerland)
NCT04665843	2021	2	tiragolumab	a study of atezolizumab plus tiragolumab and atezolizumab plus placebo as first-line treatment in participants with recurrent/metastatic PD-L1 positive squamous cell carcinoma of the head and neck	Roche (Basel, Switzerland)
NCT04308785	2021	2	tiragolumab	a study of atezolizumab with or without tiragolumab consolidation in limited stage small cell lung cancer	Roche (Basel, Switzerland)
NCT04584112	2020	1	tiragolumab	a study of the safety, efficacy, and pharmacokinetics of tiragolumab in combination with atezolizumab and chemotherapy in participants with triple-negative breast cancer	Roche (Basel, Switzerland)
NCT04933227	2021	2	tiragolumab	a study to explore the efficacy and safety of atezolizumab plus tiragolumab and chemotherapy in first-line HER2-negative unresectable, recurrent or metastatic gastric cancer or adenocarcinoma of GEJ	Roche (Basel, Switzerland)
NCT04045028	2019	1	tiragolumab	a study to evaluate the safety, tolerability, PK, pharmacodynamics, and preliminary activity of tiragolumab in participants with relapsed or refractory MM or with relapsed or refractory B cell non-Hodgkin lymphoma	Roche (Basel, Switzerland)
NCT03708224	2019	2	tiragolumab	preoperative immunotherapy in patients with squamous cell carcinoma of the head and neck	Roche (Basel, Switzerland)

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Table 3. *Continued*

NCT Number	Year	Phase	Drugs	Objective	Company
NCT03869190	2019	1 and 2	tiragolumab	study evaluating the efficacy and safety of multiple immunotherapy-based treatments and combinations in patients with urothelial carcinoma (MORPHEUS-UC)	Roche (Basel, Switzerland)
NCT04524871	2020	1 and 2	tiragolumab	a study evaluating the efficacy and safety of multiple immunotherapy-based treatment combinations in patients with advanced liver cancers (Morpheus-Liver)	Roche (Basel, Switzerland)
NCT03193190	2017	1 and 2	tiragolumab	a study of multiple immunotherapy-based treatment combinations in participants with metastatic pancreatic ductal adenocarcinoma (Morpheus-Pancreatic Cancer)	Roche (Basel, Switzerland)

The combination of IMP321 with another checkpoint inhibitor, pembrolizumab (anti-PD-1 antibody) (NCT02676869), in melanoma was completed in 2019. The data showed that the combination was well tolerated with no grade 3 or greater TEAEs. Tumor reduction was observed in eight out of 16 patients (50%).[44] Besides, the combination of IMP321 and pembrolizumab was also investigated in metastatic NSCLC and head and neck carcinoma patients (NCT03625323) and the preliminary data demonstrated an ORR of 47% and a DCR of 82%.[45] One ongoing clinical trial investigates the feasibility and safety of different routes of drug delivery (NCT03252938). Preliminary results from this study indicated that IMP321 could be safely administered up to 30 mg through both intratumoral and intraperitoneal routes. Five out of 12 patients had stable disease, five had progressive disease, while two experienced clinical progression.[46]

BMS-986016 or relatlimab, an anti-LAG3 (BMS), has undergone 20 clinical trials since 2013. Although none of the clinical trial is completed, some preliminary data have been reported. The first clinical trial assesses the safety, tolerability, and effectiveness of the anti-LAG3 antibody with and without anti-PD-1 in the treatment of solid tumors (NCT01968109). Initial results demonstrated tolerability with occurrence of grade 3/4 TEAEs in 9% of patients. Preliminary clinical activity showed 16% ORR and 45% DCR in patients with melanoma who were previously treated with anti-PD-1/PD-L1 therapy.[47] MGD013 is a bispecific PD-1 and LAG3 dual-affinity protein, and initial data presented at the 2020 ASCO annual meeting demonstrated good tolerability in metastatic solid tumors. There were also reports of TEAEs including rash (13.2%), hypothyroidism (11.3%), immune-mediated hepatitis (3.8%), pancreatitis (1.9%), colitis (1.9%), adrenal insufficiency (1.9%), and hyperthyroidism (1.9%). Preliminary evidence of anti-tumor activity of MGC018 has been observed, with ORR ranges from 9% to 21% for different cancers.[48]

Other anti-LAG3 antibodies such as LAG525, TSR-033, INCAGN02385, REGN3767, Sym022, and XmAb22841 or bispecific antibodies such as RO7247669 and FS118 are under various clinical trial stages, and data on safety and efficacy are limited.

T cell Ig and ITIM domain

T cell Ig and ITIM domain (TIGIT) was first identified in 2009 as a surface protein expressed on regulatory, memory, and activated T cells that exerts immunosuppressive effects by binding to poliovirus receptor and modulating cytokine production by dendritic cells.[49] TIGIT is also referred to as Vsig9, Vstm3, and WUCAM[50] and has two ligands: CD155 (PVR or Necl-5) and CD112 (nectin-2, also known as PRR2 or PVRL2). TIGIT contains an Ig variable domain, a transmembrane domain, and an immunoreceptor tyrosine-based inhibitory motif, and it can bind CD155 and inhibit the cytotoxicity function of NK cells.[51] Besides, TIGIT can suppress cytokine production (IFN-γ) via the NF-κB pathway. The function of CD112 and TIGIT interaction needs to be clarified. TIGIT gains its immunosuppressive function by competing with CD266 (DNAM-1) or CD96. CD226 and CD96 bind to the same ligands, and together with TIGIT form a pathway in which CD226 delivers a positive co-stimulatory signal, while CD96 and TIGIT deliver inhibitory signals. This pathway is comparable with the CTLA4/B7/CD28 pathway.[52] Besides, the expression of TIGIT on Tregs mediates suppressive function by inhibiting proinflammatory cell Th1 and Th17 response.

TIGIT and cancer immunotherapy

TIGIT was reported to be upregulated in various malignancies, including melanoma, breast cancer, NSCLC, colon adenocarcinoma (COAD), gastric cancer, AML, and multiple myeloma (MM).[53] The expression of TIGIT on TILs was associated with poor survival in melanoma patients.[54] These data reveal the essential function of TIGIT in cancer immunology, validating it as a promising target for immunotherapy.

Clinical trials on TIGIT

There are a total of 47 ongoing clinical trials evaluating 10 different anti-TIGIT antibodies, including AB154 (Arcus Biosciences), BGB-A1217 (BeiGene), BMS-986207 (BMS), COM902 (Compugen), IBI939 (Innovent Biologics), MK-7684 (MSD), MTIG7192A (Genentech), and tiragolumab (Roche) (Table 3).

AB154 is designed to promote sustained immune activation and tumor clearance in combination with proven immunotherapeutic strategies. AB154 blocks TIGIT at sub-nanomolar concentrations.[55] Data presented at the 2019 The Society for Immunotherapy of Cancer (SITC) annual meeting showed that AB154 had complete receptor coverage on all TIGIT-expressing peripheral leukocytes (NCT03628677) (Table 3). Besides, AB154 exhibits a favorable safety profile and is undergoing phase 2 evaluation for the treatment of first-line metastatic NSCLC, in combination with zimberelimab (anti-PD-1) and AB928 (a dual A2a/A2b adenosine receptor antagonist) (NCT04262856).[56] Data presented on the 2022 ASCO Monthly Plenary Series showed that AB154 combined with zimberelimab had higher ORR compared with zimberelimab alone (41% vs. 27%) and longer median PFS (12.0 vs. 5.4).[56]

Preliminary data presented at the 33rd SITC annual meeting showed that MK-7684 monotherapy or in combination with KEYTRUDA was generally well tolerated. Grade 3 or greater severity TEAEs occurred in 38% or 43% following the monotherapy or combination therapy, respectively.[57] Details of safety and efficacy of MK-7684 as monotherapy or in combination with KEYTRUDA were published recently.[58] In the study, it was found that 56% of patients receiving monotherapy and 62% receiving combination therapy experienced TEAEs. Grade 3–4 TEAEs occurred in 9% of patients receiving monotherapy and 17% of patients receiving combination therapy. Among the reported TEAEs, fatigue (15%) and pruritus (15%) were the most common with monotherapy, while pruritus (17%) and rash (14%) were the most common with combination therapy.[58] Regarding the treatment effectiveness, the confirmed ORR was 0% with monotherapy and 7% with combination therapy.[58]

OMP-313M32 or etigilimab has been shown to be well tolerated at doses up to 20 mg/kg every 2 weeks.[59] Evidence of immune activation was shown in multiple subjects with immune-related AEs. Early signs of potential efficacy have been observed in subjects with prolonged stable disease.

Tiragolumab and atezolizumab showed clinically meaningful improvements in ORR and PFS in the intention-to-treat patient population compared with placebo plus atezolizumab.[60]BGB-A1217, BMS-986207, COM902, and IBI939 are now in phase 1 clinical trial and there is need for further studies to evaluate their safety and efficacy.

Conclusions and perspectives

The use of ICIs has brought about significant advancements in cancer treatment over the past decade. These therapies, such as ipilimumab, pembrolizumab, and nivolumab, have shown remarkable success in restoring T cell function and reactivating anti-tumor immunity. The approval of ipilimumab in 2011 by the FDA marked a significant milestone in the field of immunotherapy. Subsequently, several other antibodies targeting immune checkpoints, including CTLA4 and PD-1/PD-L1, have been approved for the treatment of various types of cancer.

Despite the notable achievements, the efficacy of antibodies targeting CTLA4 or PD-1/PD-L1 has not been satisfactory in all cases. This realization has prompted researchers to explore novel immune checkpoints as potential targets for ICIs. Three promising candidates that are currently being evaluated in clinical trials are LAG3, TIM3, and TIGIT. By targeting these additional checkpoints, it is hoped that a more comprehensive and effective immune response against tumors can be achieved. These novel ICIs have shown promising results in preclinical studies, prompting the initiation of clinical trials to evaluate their safety and efficacy in cancer patients.

Clinical trials evaluating LAG3 inhibitors, TIM3 inhibitors, and TIGIT inhibitors have commenced, and early results are encouraging. These studies aim to determine the potential of these next-generation ICIs in improving patient outcomes, including overall survival, PFS, and response rates. Additionally, researchers are also investigating the synergy between these novel ICIs and existing therapies, such as anti-PD-1/PD-L1 antibodies, to enhance their effectiveness.

It is important to note that the field of immunotherapy is evolving rapidly, and ongoing research is continuously expanding our understanding of immune checkpoints and their role in cancer. The development of next-generation ICIs holds great promise for improving outcomes in cancer patients, particularly those who do not respond well to current therapies. However, further research is needed to optimize dosing, identify biomarkers for patient selection, and explore potential combination strategies to maximize the benefits of these novel treatments.

Indeed, while ICIs have shown promising results in treating cancer, it is important to recognize that the TME can be highly complex and involve various suppressive mechanisms. These include the presence of suppressive immune cells such as regulatory T cells, MDSCs, Th2 CD4+ T cells, and M2 macrophages, as well as the secretion of immunosuppressive cytokines and metabolites. The presence of these suppressive factors can hinder the effectiveness of ICIs by creating a hostile TME that dampens the function of effector T cells. Therefore, it is crucial to investigate strategies that not only target immune checkpoints but also address these alternative mechanisms of immune suppression. Furthermore, the modulation of cytokines and metabolites in the TME is another avenue of investigation. Strategies that block immunosuppressive cytokines or alter the metabolic balance within the tumor have shown potential in preclinical models. These approaches aim to create a more favorable immune environment that supports the activation and function of effector T cells.

While ICIs have shown significant benefits in some patients, not all individuals respond favorably to these therapies. Therefore, there is a need to explore new strategies to enhance the anti-tumor efficacy of ICIs. One approach that has shown promise is the combination of ICIs with antibody-drug conjugates (ADCs). ADCs are a class of targeted therapies that deliver a cytotoxic drug directly to cancer cells, thereby increasing the specificity and potency of treatment. Researchers have found that combining an ADC with an anti-PD-L1

antibody resulted in potent anti-tumor efficacy compared to using the ADC alone. However, the mechanism underlying the enhanced efficacy of the ADC-ICI combination and its potential side effects are not yet fully understood. Further investigation is necessary to elucidate the precise mechanisms by which the combination therapy exerts its effects and to assess any potential AEs.

In conclusion, while ICIs targeting CTLA4 and PD-1/PD-L1 have revolutionized cancer treatment, there is still room for improvement. The evaluation of novel immune checkpoints, such as LAG3, TIM3, and TIGIT, in clinical trials represents an exciting frontier in immunotherapy. The results of ongoing studies will provide valuable insights into the safety and efficacy of these next-generation ICIs and their potential to further enhance anti-tumor immune responses.

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AUTHOR CONTRIBUTIONS

C.L. conceived the idea for the review article and designed the study framework. C.L. and Y.T. conducted a comprehensive literature search, screened relevant articles, and synthesized the findings. C.L. drafted the initial manuscript and coordinated the revisions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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