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Novel targets for immune-checkpoint inhibition in cancer

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ABSTRACT

Immune-checkpoint inhibitors have revolutionized cancer therapy, yet many patients either do not derive any benefit from treatment or develop a resistance to checkpoint inhibitors. Intrinsic resistance can result from neoantigen depletion, defective antigen presentation, PD-L1 downregulation, immune-checkpoint ligand upregulation, immunosuppression, and tumor cell phenotypic changes. On the other hand, extrinsic resistance involves acquired upregulation of inhibitory immune-checkpoints, leading to T-cell exhaustion. Current data suggest that PD-1, CTLA-4, and LAG-3 upregulation limits the efficacy of single-agent immune-checkpoint inhibitors. Ongoing clinical trials are investigating novel immune-checkpoint targets to avoid or overcome resistance. This review provides an in-depth analysis of the evolving landscape of potentially targetable immune-checkpoints in cancer. We highlight their biology, emphasizing the current understanding of resistance mechanisms and focusing on promising strategies that are under investigation. We also summarize current results and ongoing clinical trials in this crucial field that could once again revolutionize outcomes for cancer patients.

Introduction

Over the last decade, immune-checkpoint inhibitors (ICI) have revolutionized cancer care, offering patients an alternative to chemotherapy or targeted therapies and a chance at long-term remission across many tumor types. The first two immune-checkpoint receptors for which clinically efficient inhibitors were successfully developed were the cytotoxic lymphocyte antigen-4 (CTLA-4) and the programmed death-1 (PD-1) receptor. Most solid tumors and a subset of hematologic malignancies benefit from using one or both drug classes. While ICI were initially evaluated and approved for the treatment of metastatic cancers, their use has now expanded to include early-stage cancer in certain tumor types, such as Triple-Negative Breast Cancer [1] or Non-Small-Cell Lung Cancer (NSCLC) [2]. Despite the significant progress heralded by these checkpoint inhibitors, most patients do not exhibit long-term responses and progress on therapy in the metastatic setting. Both primary and acquired resistance are observed that can stem from intrinsic and extrinsic mechanisms.

Intrinsic resistance can result from a multitude of causes, including neoantigen depletion, faulty antigen-presentation machinery, interferon-gamma defects leading to PD-L1 downregulation and upregulation of immune-checkpoint ligands, immunosuppression and phenotypic changes of tumor cells [3,4].

Extrinsic resistance causes comprise the acquired upregulation of inhibitory immune-checkpoints, which prevent T-cells from exhibiting polyclonal activation. In lung cancer, for example, increased expression of Programmed Cell Death Protein 1 (PD-1), CTLA-4, T-cell Immunoglobulin and Mucin containing protein-3 (TIM-3), Lymphocyte activation gene-3 (LAG-3) and B and T Lymphocyte Attenuator (BTLA) on intratumoral CD8 T-cells appears to be correlated with the natural course of disease progression. Co-expressed inhibitory checkpoint receptors are sequentially upregulated and promote T-cell exhaustion, a state in which T-cells persistently stimulated by antigen exposure gradually lose their effector function and capacity to proliferate [5]. This phenomenon renders restoring T-cell function with PD-1 inhibitors more difficult. Similarly, treatment with PD-1 inhibitors, such as nivolumab, is followed by the upregulation of multiple checkpoint receptors, leading to resistance [6]. Moreover, upregulation of PD-1, CTLA-4 and LAG-3 limit the efficacy of single agent immune-checkpoint inhibitors [7,8] and are a mechanism of acquired resistance.

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Many immune checkpoints have been identified, and there is great progress in understanding their biology. As such, clinical trials are ongoing of novel molecules that may overcome acquired resistance to immune-checkpoint inhibitors and prevent the emergence of extrinsic resistance mechanisms. As will be discussed in this review, some of these studies evaluate the upfront use of these new ICI in association with anti-PD-1/L1 to potentiate anti-PD-1/-L1 efficacy and prevent the emergence of resistance mechanisms. In contrast, others explore their efficacy at progression to overcome tumor immune escape.

In this review, we will discuss the growing landscape of potentially targetable immune-checkpoints in cancer through an in-depth analysis of their biology and of both published and ongoing clinical trials. Fig. 1

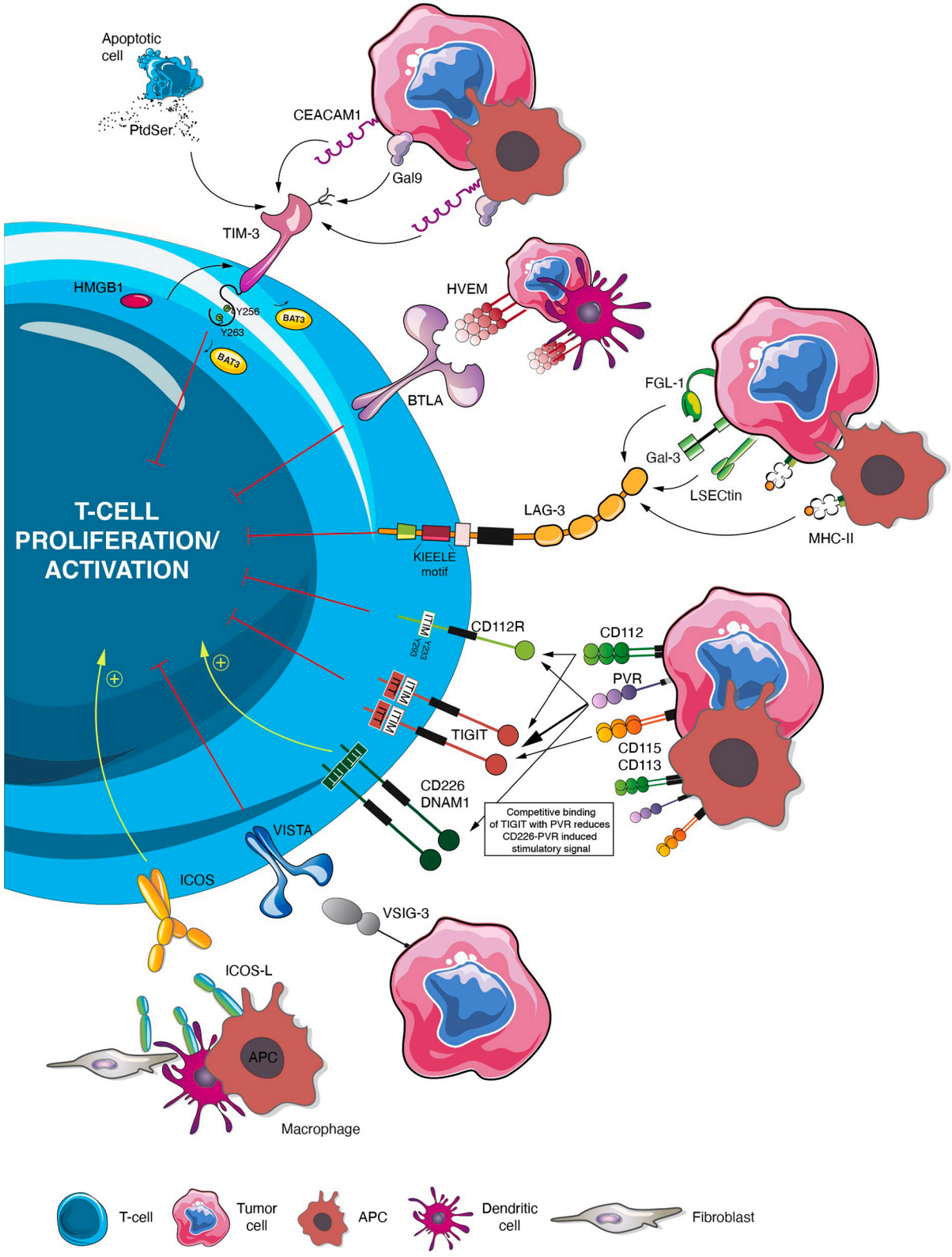

Fig. 1. Current and novel targets of immune-checkpoint inhibition in cancer.

provides an overview of the biological mechanisms of these novel targets.

Lymphocyte activation gene-3 (LAG-3)

LAG-3 is a co-inhibitory receptor expressed by immune cells and a type I transmembrane protein of the Ig superfamily [9]. Its extracellular domain harbors four Ig-like domains that share about 20% homology with the CD4 receptor. LAG-3 is expressed on the surface of lymphocytes, such as CD4 T cells, CD8 T cells, natural killer (NK) cells, and regulatory T cells (Treg) [10]. LAG-3 acts as a negative regulator of T-cell proliferation and effector T-cell activation [11,12], although the precise molecular pathways remain to be elucidated [13]. Several ligands of LAG-3 have been described, such as the major histocompatibility complex class II (MHC-II) [14], the fibrinogen-like protein 1 (FGL-1) [15], Galectin-3 (Gal-3) [16], and LSECTin [17]. Since the interaction of LAG-3 with MHC-II is of higher affinity than with other ligands, LAG-3 is thought to interfere with the CD4-MHC-II interaction, thereby dampening T-cell activation [14]. However, the interaction between LAG-3 and MHC-II is probably more complex than just competitive

Table-1
Completed and ongoing phases III trials.

Target	Drug/intervention	Tumor type	Patients	Results	Clinical trial
LAG-3 + PD-1	Relatlimab + Nivolumab Vs Nivolumab	Metastatic melanoma, 1st line	714	HR for PFS 0.75 [95%CI 0.62 to 0.92]	Relativity-047 (NCT03470922)
	Relatlimab + Nivolumab Vs Nivolumab	Stage III or IV resected melanoma, adjuvant	1050	Ongoing	Relativity-098 (NCT05002569)
	Relatlimab + Nivolumab Vs TAS102 or regorafenib	Metastatic colorectal cancer	700	Ongoing	Relativity-123 (NCT05328908)
	Fianlimab + Cemiplimab vs Pembrolizumab	Unresectable locally advanced or metastatic melanoma, 1st line	1590	Ongoing	NCT05352672
	Fianlimab + Cemiplimab vs Pembrolizumab	Stage IIC to IV resected melanoma, adjuvant	1530	Ongoing	NCT05608291
TIM-3	MBG453 + azacytidine vs placebo + azacitidine	Intermediate/high risk myelodysplastic syndrome; Chronic Myelomonocytic Leukemia	530	Ongoing	NCT04266301
TIGIT + PD-1	Ociperlimab + Tislelizumab vs Pembrolizumab	Locally advanced unresectable, or metastatic NSCLC, PD-L1 $\geq$ 50%, 1st line	660	Ongoing	NCT04746924
	Ociperlimab + Tislelizumab + Concurrent Chemoradiotherapy (cCRT) Followed by Ociperlimab + Tislelizumab vs Tislelizumab + cCRT Followed by Tislelizumab vs cCRT Followed by Durvalumab	Locally advanced, unresectable stage III NSCLC	700	Ongoing	NCT04866017
	Atezolizumab + Tiragolumab vs Atezolizumab	Unresectable esophageal squamous cell carcinoma after definitive concurrent chemo-radiotherapy	750	Ongoing	SKYSCRAPER-07 (NCT04543617)
	Atezolizumab + Tiragolumab vs Atezolizumab	Locally advanced unresectable, or metastatic NSCLC PD-L1 $\geq$ 50%, 1st line	660	No improvement in PFS (unpublished results)	SKYSCRAPER-01 (NCT04294810)
	Tiragolumab + Atezolizumab + Platinum-Pemetrexed vs Pembrolizumab + Platinum	Locally advanced unresectable, or metastatic NSCLC	540	Ongoing	SKYSCRAPER-06 (NCT04619797)
	Tiragolumab + Atezolizumab + Carboplatin + Etoposide vs placebo + Atezolizumab + Carboplatin + Etoposide	Extensive-Stage Small Cell Lung Cancer, 1st line	490	HR for OS 1.04 [95% CI 0.79 to 1.36] HR for PFS 1.11 [95% CI 0.89 to 1.38]	SKYSCRAPER-02 (NCT04256421)
	Tiragolumab + Atezolizumab + Carboplatin + Etoposide vs placebo + Atezolizumab + Carboplatin + Etoposide	Extensive-Stage Small Cell Lung Cancer, 1st line	123	Ongoing	SKYSCRAPER-02C (China) (NCT04665856)
	Atezolizumab + Tiragolumab + Paclitaxel + Cisplatin vs Paclitaxel + Cisplatin	Unresectable locally advanced, recurrent, or metastatic esophageal carcinoma, 1st line	461	Ongoing	SKYSCRAPER-08 (NCT04540211)
	Tiragolumab + Atezolizumab vs Durvalumab	Locally advanced, unresectable stage III NSCLC, maintenance	829	Ongoing	SKYSCRAPER-03 (NCT04513925)
	Pembrolizumab + Vibostolimab vs Pembrolizumab	Resected, high-Risk Stage II-IV melanoma, adjuvant	1560	Ongoing	MK-7684A-010/ KEYVIBE-010 (NCT05665595)
	Domvanalimab + Zimberelimab + Chemotherapy vs Nivolumab + Chemotherapy	Locally advanced unresectable or metastatic gastric, gastroesophageal junction, and esophageal adenocarcinoma, 1st line	970	Ongoing	STAR-221 (NCT05568095)
	Domvanalimab + Zimberelimab + platinum-based chemotherapy vs Pembrolizumab platinum-based chemotherapy	Metastatic NSCLC, 1st line	720	Ongoing	STAR-121 (NCT05502237)
	Domvanalimab + Durvalumab vs Durvalumab Stage III	Locally advanced, unresectable stage III NSCLC, maintenance	860	Ongoing	PACIFIC-8 (NCT05211895)
NKG2A	Monalizumab + Cetuximab vs Cetuximab	Metastatic HNSCC relapsing after platinum-based chemotherapy and PD-(L)-1 inhibitor	370	Closure for futility (unpublished)	INTERLINK-1 (NCT04590963)
NKG2A + PD-L1; CD73 + PD-L1	Monalizumab + Durvalumab vs Oleclumab + durvalumab vs Durvalumab + Placebo	Locally advanced, unresectable stage III NSCLC, maintenance	999	Ongoing	PACIFIC-9 (NCT05221840)

binding, and other mechanisms contribute to the inhibitory effect of LAG-3. LAG-3 seems to recognize stable complexes of peptide and MHC-II selectively, and not all MHC-II universally [18]. The binding of LAG-3 to a particular stable peptide-MCH-II complex suppresses the responsiveness to the antigen in the T-cell. Interaction of LAG-3 with Gal-3 or FGL-1 also leads to T-cell inactivation in vitro [15,16]. Interestingly, FGL-1 is upregulated in tumors but not in normal tissues [15]. For example, FGL-1 expression has been described on the surface of tumor cells in NSCLC, which has been shown to be associated with a worse prognosis [15]. FGL-1 levels were furthermore associated with resistance to PD-1/PDL-1 blockade. In mouse models, targeting the FGL-1-LAG-3 interaction achieved anti-tumor activity dependent on CD8 and CD4 T cells. Moreover, the concurrent blockade of LAG-3 and PD-1 led to a higher anti-tumor activity than that of LAG-3 alone in various preclinical models [19,20].

Various anti-LAG-3 agents have entered clinical trials. Relatlimab is a human IgG4 monoclonal antibody targeting LAG-3 and blocking interaction with its ligands, including MHC-II. Its anti-tumor activity and safety were demonstrated in phase I/II trials in patients with tumors refractory to anti-PD-1/L1 therapy, [21]. In the phase III RELATIVITY-047 trial, Relatlimab was further tested as first-line treatment in combination with nivolumab for metastatic melanoma [22]. The association showed an improvement in progression-free survival (PFS) over nivolumab alone (hazard ratio for progression or death, 0.75 (95% CI 0.62–0.92); P = 0.006). The combination was deemed safe, with 18.9% of the patients experiencing Grade 3 or 4 treatment-related adverse events (AEs) versus 9.7% of patients in the nivolumab group. Overall survival data are still awaited. Nivolumab and relatlimab demonstrated a more modest clinical activity in melanoma patients after progression on anti-PD-1/-L1 in the phase I/IIa Relativity-020 trial, with an overall response rate (ORR) of 12% and 9.2% after one or more anti-PD1 containing regimen, respectively [23]. The combination of nivolumab and relatlimab also showed promising results in the neoadjuvant setting in stage III or oligometastatic stage IV melanoma [24]. Among 30 patients, 57% had a pathological complete response rate after 2 cycles, and no grade 3–4 immune-related AEs were reported. As a comparison, in the OPACIN-NEO trial, the association of ipilimumab (anti-CTLA-4) and nivolumab (anti-PD1) showed a pathological complete response rate of 47 and 57% for the ipilimumab 3 mg/kg - nivolumab 1 mg/kg and ipilimumab 1 mg/kg – nivolumab 3 mg/kg regimens respectively, but with many more immune-related adverse events (grade 3–4 immune-related AEs of 40% and 20% within 12 weeks for each regimen respectively) [25]. Relatlimab is currently being tested in combination with nivolumab in various tumor types (cf. Table 1).

Favezelimab is another antibody targeting LAG-3, which is currently being evaluated in a phase III trial in microsatellite-stable (MSS) metastatic colorectal cancer (NCT05064059). In a phase I trial, including patients with metastatic colorectal cancer and gastric cancer, favezelimab, in combination with pembrolizumab, had a favorable safety profile but only a modest anti-tumor activity, with an overall response rate of 6% and 11.6 in patients with colorectal cancer [26], and gastric cancer, respectively [27]. Favezelimab is also being tested in lymphomas (NCT03598608) (see Table 2).

Another LAG3 inhibitor, Ieramilimab (LAG525) was tested in multiple tumor types in a phase I/II trial [28]. The combination with the anti-PD1 spartalizumab led to a response in PD-1/L1 naïve patients but showed only modest anti-tumor activity in patients pre-treated with PD-1/-L1 inhibitors (ORR < 10%; 5.3%, 6.3% and 9.1% in patient with RCC, mesothelioma and melanoma respectively, 0% for NSCLC and TNBC) [29]. Other strategies, such as combining the targeting of PD-1 and LAG-3 with a single molecule, are also in development, like RO7247669, an anti-PD1-LAG3 bispecific antibody, or Tebotelimab, a bispecific DART® (Dual-Affinity Re-Targeting) molecule that selectively inhibits PD-1 and LAG-3 (NCT04082364).

An additional LAG3 inhibitor, Fianlimab, was tested in combination with cemiplimab in an advanced melanoma phase I trial, with an

Table-2
Selected completed and ongoing phases I and II trials.

Target	Drug/intervention	Phase	Tumor type Clinical trial
LAG-3 + PD-L1	Nivolumab + relatlimab	II	Multiple solid tumors (NCT01968109)CRC (NCT03642067) Metastatic soft-tissue sarcoma (NCT04095208)HNSCC (NCT04080804; NCT04326257)NSCLC (NCT04205552) (NCT02750514)Renal cell carcinoma (NCT02996110)Gastric cancer (NCT02935634)Metastatic melanoma (NCT04552223, NCT03743766, NCT03724968) Hepatocellular carcinoma (NCT04567615)Metastatic basal cell carcinoma (NCT03521830)Metastatic NSCLC (NCT04623775)Metastatic CRC (NCT03867799)Gastro-esophageal junction adenocarcinoma (NCT03704077 NCT03662659, NCT03610711, NCT04062656) Metastatic ovarian cancer (NCT046111269)Multiple myeloma (NCT04150965)
	REGN3767 + cemiplimab	II	Breast cancer (NCT01042379) Advanced solid tumors (NCT04706715)
	LAG525 + spartalizumab	II	TNBC (NCT03499899) Advanced solid tumors (NCT03365791, NCT02460224) Melanoma (NCT03484923
LAG-3 + PD-1 + TIM-3	INCAGN02385 + INC-MGA00012 + INCAGN02390	II	Advanced solid tumors (NCT04370704)
TIM-3 + PD-1	BMS-986258 + nivolumab	II	Advanced solid tumors (NCT03446040)
	BGB-A425 + tislelizumab	II	Advanced solid tumors (NCT03744468)
	TSR-022 + TSR-042	II	Liver cancer (NCT03680508) Melanoma (NCT04139902)
TIGIT + PD-L1	Tiragolumab + atezolizumab	II	Cervical cancer (NCT04300647)Gastric and esophageal adenocarcinoma (NCT03281369)Urothelial carcinoma (NCT03869190)Pancreatic adenocarcinoma (NCT03193190)NSCLC (NCT03563716, NCT04619797)SCLC (NCT04308785)HNSCC (NCT04665843, NCT03708224)
	Domvanalimab + zimberelimab	II	NSCLC (NCT04262856) Advanced solid tumors (NCT03628677)
	BMS-986207 + nivolumab		Advanced solid tumors (NCT02913313, NCT04570839)
CD112R +/- PD-1	COM701 +/- nivolumab	I	Advanced solid tumors (NCT03667716)
B7-H3	DS-7300a	II	Advanced solid tumors (NCT04145622)
	Enoblituzumab	II	Prostate cancer (NCT02923180)

(continued on next page)

Table-2 (*continued*)

Target	Drug/intervention	Phase	Tumor type Clinical trial
BTLA	JS004	I	Advanced solid tumors (NCT04278859)
	TAB004	I	Advanced solid tumors (NCT04137900)
NKG2A	Monalizumab	II	Metastatic HNSCC (NCT02643550), (NCT03088059)Breast cancer (NCT04307329)Chronic lymphoid leukemia (NCT02557516)
NKG2A + PD-1	Monalizumab + durvalumab	II	CRC (NCT04145193)Advanced solid tumors (NCT02671435)NSCLC (NCT03822351) (NCT038223519) (NCT03833440)
CD200	Samalizumab	II	AML (NCT03013998)Multiple myeloma (NCT00648739)
CD73 + PD-1	Durvalumab + Oleclumab	II	Sarcoma (NCT04668300)
ICOS + PD-1	JTX-4014 + Vopratelimab	I	NSCLC (NCT04549025)
GITR	TRX518-001	I	Melanoma (NCT01239134)
GITR + PD-1	REGN6569 + Cemiplimab	I	Advanced Solid Tumor (NCT04465487)
VISTA	CI 8993	I	Advanced solid tumors (NCT04475523)
	HMBD-002	I	Advanced solid tumors (NCT05082610)
VISTA, PD-L1 & PD-L2	CA-170	I	Advanced solid tumors and lymphomas (NCT02812875)

interesting ORR of 62.5% and an acceptable toxicity profile [30]. This combination is also being tested in NSCLC [31].

Sym002, BI 754111, HLX26, TSR-033 and INCAGN02385 are other anti-LAG-3 antibody in development, alone or in combination with other immune checkpoints inhibitors (NCT03489369, NCT04370704, NCT03005782, NCT05287113, NCT05584137, NCT03250832, NCT03156114, NCT03433898).

T-cell immunoglobulin and mucin domain-3 (TIM-3)

TIM-3 is a surface glycoprotein encoded by the hepatitis A virus cellular receptor 2 (HAVCR2) gene and a member of the TIM family of immunoregulatory proteins [32,33]. TIM-3 is expressed by various types of immune cells: T Helper-1, CD8 T cells, regulatory T cells (T-reg), myeloid cells, natural killer cells and mast cells [34]. TIM-3 comprises an extracellular N-terminal immunoglobulin variable domain (v-domain), a mucin stalk, a transmembrane domain and an intracytoplasmic domain. When TIM-3 is not bound to its ligand, the HLA-B-associated transcript 3 (BAT3) protein is attached to its intracellular v-domain. The binding of BAT3 to the v-domain leads to the recruitment of the activated LCK kinase. This mechanism is thought to allow T-cell activation [34]. Four ligands have been described for TIM-3: galectin-9 (Gal-9), phosphatidylserine (PtdSer), high-mobility group protein B1 (HMGB1), and carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) [34]. Gal-9 is found at the surface of tumor cells, or in a soluble form secreted by tumor cells or antigen-presenting cells (APCs) [34]. CEACAM1 is found on the surface of some tumor cells [35] and macrophages, monocytes and dendritic cells. PtdSer is a phospholipid released from apoptotic cells. HMGB1 is a non-histone protein with multiple functions [36]. It can be bound to DNA released from dying cells or secreted by tumor cells.

Gal-9 and CEACAM1 bind to two different regions of the extracellular v-domain, but both induce the same intracellular downstream effect: phosphorylation of the two tyrosine residues Tyr256 and Tyr263, upon which the BAT3 protein unbinds from the intra-cytoplasmic domain of TIM-3 [37]. This allows the binding of the tyrosine kinase FYN. FYN is supposed to have a competitive role to BAT3, as it binds to TIM-3 at the same location as BAT3, but leads to T-cell inactivation via an interaction with the phosphoprotein associated with glycolipid-enriched membranes (PAG) protein and CSK tyrosine kinase [38]. Consequently, the binding of TIM-3 to its ligand in T-cells ultimately leads to a decrease in T-cell receptor signalling, T-cell inhibition and to cell death [37,39]. TIM-3 is indeed found on exhausted tumor-infiltrating CD8 lymphocytes in various cancers [40,41]. T-reg cells in the tumor microenvironment also frequently express TIM-3, which has been shown to be associated with a worse prognosis [42]. TIM-3 upregulation has been shown after a first response to anti-PD-1 therapy, in mouse models and in patients who developed acquired resistance to anti-PD-1 [43]. Moreover, in mouse models, the inhibition of TIM-3 led to a restoration of anti-PD-1 activity, and prolonged survival after anti-PD-1 failure [43]. Other pre-clinical models showed the superiority of dual blockade of PDL-1 and TIM-3 over PD-1 blockade alone in tumor control [41,44]. The targeting TIM-3 could also affect tumor growth by modulating T-reg activity. In pre-clinical models of head and neck cancer, dual TIM-3 and PD-1 blockage induced a depletion of tumor infiltrating TIM-3 + T-reg cells, associated with better tumor growth control [45], underlining the highly complex immunoregulatory mechanisms of TIM-3.

Based on these results, molecules targeting TIM-3 have entered clinical trials, for which some preliminary results are available. Sabatolimab (MBG453), a monoclonal antibody targeting TIM-3, was tested in phase I/II trials either alone (133 patients) or in combination (86 patients) with spartalizumab, in patients with advanced solid tumors[44]. The combination was relatively well tolerated, with grade 3–4 AEs suspected to be treatment-related reported in only 9% of patients. Efficacy, however, was somewhat disappointing, as no patient had an objective response with sabatomimab monotherapy, and only 5 patients presented a partial response (6%, lasting 12–27 months) in the combination group.

LY3321367, another TIM-3-targeting antibody, was evaluated alone or in combination with LY300054, an anti-PD-L1 antibody, in a phase Ia/Ib trial of patients with solid tumors [46]. No dose-limiting toxicity occurred in the dose escalation cohorts, neither with monotherapy nor combination therapy. A monotherapy expansion cohort of non-small cell lung cancer patients demonstrated a poor overall response rate, with only 1 partial response. However, more stable disease and longer median PFS (7.3 versus 1.9 months) were observed in patients with prior response to standard-of-care anti-PD-1/L1 therapy versus anti-PD-1/L1 refractory patients. Nevertheless, the interpretation of these findings is limited due to the relatively small sample size. A combination expansion cohort revealed an overall response rate of 4%, a disease control rate of 42% and a median PFS ranging from 1.5 to 3.7 months in patients with various tumor types. Interestingly, many patients developed anti-drug antibodies, although with no apparent repercussions on exposure and target engagement, as shown by pharmacokinetic and pharmacodynamics studies.

The LY3321367 and LY300054 combination was also evaluated in 82 patients with microsatellite instability-high/mismatch repair-deficient (MSI-H/dMMR) tumors [47]. Patients with no prior anti-PD-1/L1 therapy received the anti-PD1 LY300054 in monotherapy (40 patients) or combination with LY3321367 (20 patients). Grade 3 or 4 treatment-related AEs occurred in 3 patients (7.1%) receiving the combination. An objective response rate of 45% was observed for patients treated with the combination therapy that were anti-PD1/L1 naïve patients (versus 32.5% for the anti-PD1 LY300054 monotherapy). A more limited treatment efficacy (ORR 4.5) was observed in patients with anti-PD-1/L1 resistant MSI-H/dMMR tumors.

Cobolimab (TSR-022/GSK4069889) was tested in a phase I trial in patients with advanced solid tumors either as a monotherapy or combined with anti-PD1 antibodies (dostarlimab and nivolumab). The

combination was safe, with less than 20% of grade ≥3 treatment-related AEs, and an ORR of 42.9% in melanoma patients [48].

A phase I trial investigated the safety and efficacy of LY3415244, a bispecific antibody against TIM-3 and PD-L1, in 12 patients with advanced solid tumors [49]. The study was terminated early, however, as two patients presented treatment-related anaphylactic reactions, and all patients developed anti-drug antibodies. Multiple other anti-TIM-3 antibodies are currently being tested in phase I/II trials, alone or in combination with anti-PD1/L1 in liver cancer (NCT03680508), melanoma (NCT04139902, NCT04370704), non-small cell lung cancer (NCT04931654), glioblastoma (NCT03961971), esophageal squamous cell carcinoma (NCT04785820), or in trials including patients with various advanced tumor types (NCT03311412, NCT03744468, NCT04641871, NCT03708328 and NCT02817633).

T cell immunoglobulin and ITIM domain (TIGIT)

TIGIT is another member of the immunoglobulin superfamily, first described in 2009 [50]. It is composed of an extracellular variable domain, a transmembrane stalk, and an intracellular tail. The intracellular domain contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) domain and an Ig tail-tyrosine (ITT)-like motif that are implicated in the regulatory signal transduction within the immune cell [51]. TIGIT is expressed in CD8 + T cells, memory and regulatory CD4 + T cells, T-reg and NK cells [52]. Various ligands for TIGIT have been described, like PVR (CD155), CD112 (nectin-2), and CD113 (nectin-3) [50]. These ligands have been described on tumor cells and antigen-presenting cells. PVR expression, in particular, was described and associated with worse prognosis in many tumor types, including colon cancer, NSCLC, melanoma and pancreatic cancer. [53–56].

TIGIT binding with PVR leads to decreased TCR expression and p-ERK mediated signalling in CD8 + T-cells [51,57], thus reducing T-cell activation and proliferation. In NK cells, the interaction of TIGIT with its ligand decreases cytokine release and cytotoxic function by recruiting the SHP-1 phosphatase by the TIGIT ITIM and ITT-like intracellular domains, leading to a blockade of the MAPK signalling [58]. Other mechanisms are involved in the regulation of T-cell activity by TIGIT. CD226 (DNAM-1) and CD96 are other receptors at the T-cell surface that share identical ligands with TIGIT, and their competitive binding interaction also plays a role in regulating T-cell activation. CD226 is a co-stimulatory receptor which activates T-cells upon binding to its ligand PVR. TIGIT, having a higher affinity for PVR than CD226 [50], interferes with the CD226-PVR interaction, thereby decreasing CD226-mediated T-cell activation [59]. Moreover, TIGIT directly interferes with CD226 homo-dimerization, leading to a decrease in CD226 signaling [60].

TIGIT expression has been reported in several tumors, such as NSCLC and SCLC, melanoma, and colorectal cancers, and is a marker of poor prognosis [61]. TIGIT is frequently co-expressed with PD-1 in CD8 + T cells of the tumor microenvironment, constituting a potential mechanism of immune evasion and ICI resistance [62].

Many drugs targeting TIGIT have entered clinical development. Tiragolumab, the most advanced anti-TIGIT antibody in its clinical development, has been studied in several tumor types. In the phase II CITYSCAPE trial, Tiragolumab combined with the anti-PD-L1 atezolizumab was evaluated in NSCLC [63]. Among 135 randomized patients, the combination demonstrated a significant improvement in ORR (37% vs 21%) and PFS (5.6 vs 3.9 months) compared to Atezolizumab. Notably, the ORR increased to 66% in patients with tumors with a PD-L1 ≥ 50%. The treatment was described as safe, with comparable rates of treatment-related AEs between both groups (~20%). The predictive effect of TIGIT expression was also assessed in the trial, with 49 out of 105 patients being defined as TIGIT-high (≥5% on IC). TIGIT expression did not affect PFS (HR 0.62, 95% CI 0.30–1.32). The phase III SKYSCRAPER-01 trial sought to follow up on the promising results of CITYSCAPE, evaluating the same regimen for the first-line treatment of metastatic NSCLC with PD-L1 ≥ 50%. However, the combination treatment did not improve PFS and the OS results are still awaited [64]. In the phase III SKYSCRAPER-06 study (NCT04619797), the combination of tiragolumab and atezolizumab in association with chemotherapy is being tested in metastatic NSCLC regardless of PD-L1 expression versus pembrolizumab plus pemetrexed and platinum-based chemotherapy,. While in the phase III SKYSCRAPER-03 trial (NCT04513925), atezolizumab and tiragolumab are being compared with durvalumab in treating unresectable stage III NSCLC.

In SCLC, the phase III trial SKYSCRAPER-02 evaluated tiragolumab or placebo, in combination with the carboplatin, etoposide and atezolizumab, in the first-line metastatic setting [65]. At an interim analysis, the trial failed to meet its co-primary endpoint of PFS improvement, with a PFS of 5.4 versus 5.6 months ($p = 0.35$).

Tiragolumab is being evaluated in several other indications, such as unresectable locally advanced esophageal squamous cell carcinoma associated with atezolizumab in the SKYSCRAPER-07 phase III trial (NCT04543617) or in hematological malignancies (NCT05315713, NCT04045028).

Domvanalimab, an IgG1 monoclonal antibody targeting TIGIT and reducing immunosuppression of T/NK cells, has been evaluated in NSCLC in the ARC-7 randomized phase II trial [66]. In combination with the anti-PD1 agent zimberelimab, domvanalimab showed superior ORR (41% vs 27%) and PFS (12 vs 5.4 months) versus zimberelimab in metastatic NSCLC with PD-L1 ≥ 50%. A third arm of the trial evaluated the triple combination of zimberelimab, domvanalimab and the adenosine receptor antagonist A2a and A2b etrumadenant, with results comparable to zimberelimab + domvanalimab. An ongoing phase III in NSCLC evaluates the combination of zimberelimab and domvanalimab with chemotherapy, compared to the standard of care consisting of pembrolizumab and chemotherapy (NCT05502237). Furthermore, domvanalimab is assessed in unresectable stade III NSCLC in association with durvalumab (NCT05211895), and zimberelimab. Furthermore, domvanalimab is also being tested in other cancer types such as upper gastrointestinal tract tumors (NCT05568095, NCT05329766) and melanoma (NCT05130177).

Ociperlimab, a third anti-TIGIT antibody, was evaluated in association with the anti-PD-1 tislelizumab and platinum-based chemotherapy in patients with metastatic squamous and non-squamous NSCLC [67]. The ORR was 45.9% in squamous NSCLC, and 25.6% in non-squamous NSCLC (95% CI: 0.1, 0.4), with 57% of patients developing grade ≥3 AEs.

Various other anti-TIGIT are in development, such as vibostolimab [68], M6223 [69], IBI939 [70], etigilimab [71] or BAT6021 (NCT05073484).

V-domain Ig suppressor of T-cell activation (VISTA)

VISTA, encoded by the *Vsir* gene located on chromosome 10 (10q22.1), is a transmembrane protein with an extracellular IgV domain of the B7 protein family. It is also known as PD-1 homolog (PD-1H), B7-H5, V-set immunoregulatory receptor (VSIR), stress-induced secreted protein 1 (SISPQ), and differentiation of embryonic stem cells 1 (Dies1) [72]. It shares a 22% homology with PD-L1. VISTA is expressed on various immune cells, such as monocytes, dendritic cells, macrophages, circulating granulocytes, T cells, T-reg, and TILs, where it acts as an immune regulatory checkpoint [73,74]. VISTA is found predominantly in tissues presenting high levels of infiltrating leukocytes in humans [74]. Little is known about the ligands, co-receptors and signalling pathways through which VISTA regulates T-cells activity. The V-Set and Immunoglobulin domain containing 3 (VSIG3) [75], which is upregulated in gastric, colorectal and liver cancer [76], and the P-selectin glycoprotein-1 (PSGL1) are binding partners, the latter at an acidic pH [77]. The VISTA-VSIG3 interaction inhibited T-cell proliferation and cytokine production in vitro [75]. As mentioned above, evidence from in vitro studies support the role of VISTA as an immune regulatory

checkpoint [73,74]. In a mouse model of autoimmune encephalomyelitis, blocking VISTA led to an increase in T-cell auto-immunity [78]. In another murine model of acute hepatitis, a VISTA-specific monoclonal antibody agonist directly inhibited T-cell activation and suppressed T-cell mediated acute inflammation [79]. VISTA expression on both T-cell and APC seems to contribute to the inhibition of T-cell activation, as in vitro analyses showed that VISTA deletion on both APC and T-cells led to a further increase in T-cell proliferation when compared to VISTA deletion in a single cell type [79]. Unlike other immune checkpoint, VISTA is also expressed on naïve T cells, where it maintains quiescence and promotes peripheral immune tolerance [73].

In tumors, VISTA expression has been identified in pancreatic cancer [80,81], melanoma, [80] mesothelioma [82], NSCLC, [83] breast cancer [84], colorectal cancer [85], renal clear cell carcinoma [86], endometrial and ovarian cancer [87]. Based on data from The Cancer Genome Atlas, mesothelioma showed the most significant VISTA expression among all cancer types [88]. Interestingly, VISTA expression on biopsies collected from patients upon progression after an initial response to immune checkpoint inhibitors was increased compared to pre-treatment biopsies, suggesting VISTA's potential role in resistance anti-PD1/-L1 therapies in melanoma [62]. Blando and colleagues compared the microenvironments of an immune-checkpoint inhibitor-sensitive (melanoma) and a resistant tumor (pancreatic cancer). They observed a significantly higher number of VISTA-positive cells in the pancreatic stromal area compared with melanoma, despite lower infiltration of CD3, CD4, and CD8 T cells, supporting a role for VISTA as an immune regulatory checkpoint in pancreatic cancer.

Drugs targeting VISTA, alone or in combination with the PD-L1 blockade, have been developed and are currently the object of early clinical trials (cf. Table 1), with the first results expected soon.

CD112R

CD112R, also known as PVRIG (Poliovirus Receptor (PVR)-related Ig Domain) is a transmembrane receptor belonging to the PVR family and a member of the DNAM1/TIGIT/PVR axis [89]. It comprises an extracellular IgV domain, a transmembrane segment and an intracellular domain harboring an ITIM-like region with two tyrosine residues: Y233 and Y293. The Y233 and its phosphorylation are implicated in the intracellular CD112R-mediated signal transduction [89]. CD112R is expressed predominantly on NK and T-cells, mainly effectors and memory CD8 + cells, and is found in various tumors, such as kidney, ovarian, lung and prostate cancer and acute myeloid leukemia [90]. CD112R binds to CD112 (also called PVRL2 or Nectin-2), a transmembrane protein of the nectin family, found in various cells and tissues, which is also a ligand for TIGIT [91]. CD112 is also expressed in tumor cells, dendritic cells, tumor-associated macrophages, and non-immune cells such as fibroblasts or endothelial cells [89,92]. CD112 expression levels in tumor cells often correlate with CD112R expression in TILs. Co-expression of both proteins has been described in the same tumor sample, supporting a role for the CD112R-CD112 interaction in regulating the tumoral immune microenvironment [92]. CD112R inhibits TCR-signaling in T-cells and T-cell activation in vitro [89]. In preclinical models of various tumor types, blocking the CD112R-CD112 interaction promoted T-cell and NK-cell activation and was associated with a slower tumor progression [93]. Moreover, dual blockade of CD112R with TIGIT or PD-L1 further enhanced T-cells activation and tumor control [92–95].

CD112R blockade is currently being tested in clinical trials, with preliminary results reported of a phase I clinical trial evaluating COM701, a CD112R-targeting antibody that prevents its binding to CD112 [96]. Sixteen patients received COM701 alone, and 12 received COM701 plus nivolumab. While one patient in each group had a partial response, stable disease was observed for several, with a clinical benefit rate of 69% and 75% with monotherapy or combination therapy, respectively. COM701 was generally well tolerated and is currently being tested in triple combination with anti-TIGIT and anti-PD-L1. Preliminary results from 13 patients treated in the phase I dose escalation study revealed a favorable safety profile with no dose-limiting toxicities and 23% of stable disease [97]. A phase II testing of this triple combination is ongoing.

Inducible Co-stimulatory receptor (ICOS)

ICOS (or CD278) is a co-stimulatory receptor expressed on T-cells, structurally a homodimeric protein, and a CD28/CTLA-4 family member [98]. ICOS is constitutively expressed on T-regs, whereas on CD4 + and CD8 + T-cells, its expression is induced by TCR activation. In TILs, ICOS expression varies according to subtype, with the most significant expression being observed in T-reg, followed by CD4 + and CD8 + T-cells [99]. The interaction of ICOS with its ligand ICOS-L (CD275) induces the production of pro-inflammatory cytokines such as IFNγ and TNFα in T-effectors cells, whereas in T-regs, it leads to the production of anti-inflammatory cytokines such as IL-10. ICOS-L is expressed on antigen-presenting cells, B-cells, macrophages and dendritic cells, and other cells from the tumor micro-environment, such as fibroblasts and endothelial cells, where TNF promotes its expression-α [100]. The ICOS-ICOS-L interaction can thus play a dual role in tumor evolution: a pro-tumor effect by maintaining T-regs activity and an anti-tumor effect by promoting CD4 + and CD8 + T-cells [101]. ICOS expression in T-regs cells has indeed been shown to be associated with a worse outcome in gastric cancer [102], while higher ICOS expression on Th1-CD4 + cells is associated with better survival in colorectal cancer [103]. Interestingly, higher ICOS expression has also been demonstrated after anti-CTLA-4 antibody exposure in pre-clinical models, suggesting a potential role for ICOS in anti-CTLA-4 mediated anti-tumor response [104]. Moreover, the same authors demonstrated that concurrent anti-CTLA-4 and ICOS stimulation led to a better anti-tumor effect than anti-CTLA-4 alone [105].

Currently, different agents targeting ICOS are under clinical development. These agents aim to achieve an anti-tumor activity through two distinct mechanisms: (1) an antagonist activity against ICOS + T-reg cells, to deplete them in the tumor micro-environment, and (2) an agonist activity for ICOS + effector T cells.

KY1044, an anti-ICOS monoclonal antibody, was shown to induce antibody-dependent cell-mediated cytotoxicity of ICOS + T-regs cells and increase T-effector cells to T-regs ratio [106]. It has been studied alone or in combination with atezolizumab in a phase I study, with a favorable safety profile but a low ORR of 5% [107]. A phase II study is ongoing (NCT03829501).

Vopratelimab, on the other hand, is an antibody with a dual mechanism of action, depleting ICOS + T-reg and exerting an agonist effect on T-effector cells [108]. The depleting effect on T-reg might be due to the higher expression of ICOS on T-reg, compared to CD4 and then CD8 + T-cells, which could result in antibody-dependent cell-mediated cytotoxicity with greater predominance in the T-reg compartment of the microenvironment. Vopratelimab showed anti-tumor activity in various in vitro tumor models, further enhanced when associated with anti-PD-1. Nevertheless, vopratelimab displayed limited anti-tumor activity in the ICONIC first-in-human phase I/II trial, where it was evaluated alone or in combination with nivolumab in patients with advanced solid tumors [109] (ORR: 1.4% for vopratelimab alone, 2.3% for the combination).

The development of Feladilimab (GSK3359609), another ICOS agonist, was recently stopped by GlaxoSmithKline [110]. Finally, MEDI-570, a pure antagonistic anti-ICOS antibody, which depletes ICOS-expressing cells, has demonstrated an interesting ORR of 33% in a phase I trial in relapse/refractory angioimmunoblastic T-Cell Lymphoma, with a favorable safety profile [111].

B & T Lymphocyte Attenuator (BTLA)

BTLA is a member of the CD28 family of receptors, which shares similarities with CTLA-4 and PD-1, regarding its structure and function [112]. BTLA is found on the surface of T-cells, B-cells and macrophages. The only BTLA ligand described so far is Herpesvirus entry mediator (HVEM), a member of the TNF-receptor family found on B cells, DCs, and T cells [113]. HVEM binding of BTLA leads to a decrease in T-cell activation and proliferation through the recruitment of SHP-1 and SHP-2 upon phosphorylation of the BTLA's intracellular ITIM and immunoreceptor tyrosine-based switch motifs (ITSM) [114]. Whereas BTLA is widely expressed on naïve T-cells, its expression decreases typically with T-cell maturation and activation. Conversely, BTLA expression was shown to be maintained on tumor-antigen-specific CD8 + TILs throughout differentiation, with a potential role in T-cell exhaustion [115]. Pre-clinical studies have demonstrated that simultaneous targeting of PD-1 and BTLA can enhance T-cell response, thus providing a rationale for simultaneous targeting [115,116].

BTLA targeting is currently being evaluated in clinical trials with icatolimab, a monoclonal anti-BTLA antibody (NCT05427396, NCT05000684, NCT04137900). Very early results showed a favorable safety profile, however, with limited signal for clinical activity at this stage [117,118].

Glucocorticoids-induced TNF receptor family-related protein (GITR)

GITR (also called TNFRSF18/CD357/AITR) is a co-stimulatory receptor and a member of the TNFR gene superfamily, including 4–1BB and OX40. GITR is constitutively expressed on T-regs at high levels and on naïve and memory T-cells at lower levels [119]. GITR expression increases upon activation of T-cells. GITRL (TNFSF18), identified as the ligand of GITR, is predominantly found on activated APCs. Upon binding to its ligand, GITR, like other members of the TNFR family, can activate the transcription nuclear factor-κB (NF-κB) pathways, resulting in reduced T-cell apoptosis and T-cell expansion. In T-effector cells, activation of GITR leads to T-cell expansion and activation [120]. In T-regs, the effect of GITR activation is less straightforward as it seems to decrease the T-reg suppressive function of T-effector cells [121,122]. GITR targeting by monoclonal agonist antibody led to tumor regression in pre-clinical models [123].

In early clinical trials, GITR targeting agents such as TRX518 or BMS986156 have shown disappointing results, with low clinical activity so far [124–126].

Natural killer group 2A (NKG2A)

NKG2A is an inhibitory co-receptor expressed at the surface of NK-cells and to a lesser extent on a subset of CD8 + T-cells [127]. NKG2A forms a heterodimeric protein with CD94, with a cytoplasmic ITIM domain. HLA-E class I molecule, which is ubiquitously expressed, represents the ligand of CD94/NKG2A. When bound to its ligand, CD94/NKG2A inhibits the T-cell effector function by recruiting the SHP-1 and SHP-2 phosphates by its cytoplasmic ITIM domain [128]. Thus, when activated, the CD94/NKG2A dimer dampens NK or CD8 + cell-mediated cytotoxicity. Interestingly, the strength of the interaction of CD94/NKG2A with its ligand depends on the peptide exposed by HLA-E [129]. NKG2A has been shown to be expressed on tumor infiltrating NK cells from patients with liver cancer, where it is associated with an exhausted phenotype [130], and also on bladder cancer cells [131]. In vitro, combined NKG2A and PD-1 blockade enhances NK and CD8 + cytotoxicity and synergically reduces tumor growth [132].

Monalizumab, a monoclonal antibody targeting NKG2A, is currently being evaluated in clinical trials. In a randomized phase II study in patients with stage III non-resectable NSCLC with no signs of progression after chemo-radiotherapy, the combination of monalizumab with the anti-PD-L1 Durvalumab resulted in a greater ORR (35.5% vs 19.9%) and longer PFS (0.42; 95% CI, 0.24–0.72) than durvalumab alone. AEs rate was consistent across treatment arms [133]. The combination of monalizumab and durvalumab is currently evaluated in this setting in the phase III Pacific-9 trial (NCT05221840). The same combination of monalizumab and durvalumab was evaluated in the neoadjuvant setting in NSCLC, where preliminary data showed an encouraging rate of major pathological response rate (30%), which was numerically higher than for durvalumab alone (11.1%) [134]. Monalizumab has also been evaluated in HNSCC. In the INTERLINK-1 phase III trial, the combination of monalizumab with cetuximab was compared to cetuximab alone in patients with HNSCC relapsing after platinum-based chemotherapy and a PD-(L)-1 inhibitor. Unfortunately, after the review from the independent data monitoring committee, Astrazeneca announced the premature closure of the trial for futility [135].

B7 homolog 3 protein (B7-H3)

B7-H3 (CD276) is a cell surface protein of the B7 family. B7-H3 is found on APCs, on the surface of cancer cells and tumor-infiltrating vessels of various solid tumors, such as NSCLC, prostate cancer, HNSCC, pancreatic cancer, breast cancer, and colorectal cancer [136]. On the contrary, B7-H3 is rarely found in normal tissues, making it an interesting target for different strategies of anticancer therapy, such as antibody-drug conjugates or even immunotherapy with CAR-T cels therapies [137]. While B7-H3 ligands are currently unknown, B7-H3 seems to play various roles in cancer. For instance, B7-H3 has been shown to play an immune inhibitory role by reducing T-cell activation and cytokine production [138]. As such, when expressed on tumor cells, B7-H3 is thought to contribute to tumor immune evasion, with B7-H3 expression in tumor cells being associated with poor outcomes in NSCLC and HNSCC. Moreover, B7-H3 expression in NSCLC seems to be associated with poorer response to anti-PD1 therapy.

In animal models, combining anti-PD1 and anti-B7-H3 synergistically increases anti-tumor immune response. Yet, B7-H3 was shown to contribute to some extent to the acquisition of some hallmarks of cancer, such as tumor invasion and metastasis.

Enoblituzumab (MGA271) is a humanized monoclonal antibody targeting B7-H3, with a Fc region specifically designed to boost Fc-mediated activities, including antibody-dependent cell-mediated cytotoxicity. In a phase I study in 179 patients with various tumor types, enoblituzumab proved to be safe without dose-limiting toxicities and with some responses being observed [139]. Enoblituzumab was also evaluated in a phase II in the neoadjuvant prostate cancer setting, where it increased CD8 T-cell density in prostatectomy samples. Unfortunately, a phase II evaluating Enoblituzumab, in combination with the anti-PD-1 Retifanlimab and DART® Tebotelimab in HNSCC was prematurely closed due to safety concerns (NCT04634825).

CD73

The CD73 protein (or 5′-nucleotidase) is a nucleotidase localized at the cell's surface. It is an enzyme that converts adenosine monophosphate (AMP) to adenosine. CD73 is frequently found at the surface of immune cells and various types of tumor cells, where it is generally associated with poor prognosis [140–142]. In the tumor microenvironment, the conversion of AMP to adenosine increases its extracellular levels exerting a potent immunosuppressive effect. Extracellular adenosine binds to, among others, the A2A adenosine receptor (A2AR), found at most immune cells' surface [143]. In T-cells, adenosine-A2AR interaction, inhibits T-cell activation and promotes apoptosis.

Furthermore, CD73 has been shown to promote tumor growth, migration and invasion [144]. In pre-clinical models, CD73 inhibition led to the modification of immune cell infiltration of the tumor microenvironment, with an increase in activated macrophages and CD8 + T-cells and an inhibition of tumor growth [145]. Moreover, the adjunction

of CD73-inhibition to anti-PD1/-L1 was shown to have a synergic anti-tumor effect.

Oleclumab (MEDI9447) is a human IgG1 monoclonal antibody targeting CD73, with an inhibitory effect. In a phase I study, oleclumab alone or combined with durvalumab was safe in patients with pancreatic, colorectal and epidermal growth factor receptor (EGFR)–mutated NSCLC [146]. In the phase II COAST study, oleclumab was evaluated with durvalumab as a consolidation therapy in patients with unresectable stage III NSCLC and no progression after concurrent chemoradiotherapy [133]. Confirmed ORR and PFS were numerically higher when oleclumab was added to durvalumab, compared to durvalumab alone (ORR 30.0 vs 17.9%; HR for PFS 0.44, 95% CI, 0.26–0.75), yet, the trial design was non-comparative. The phase III Pacific-9 trial in this setting is ongoing (NCT05221840). Oleclumab has also been tested in triple-negative breast cancer, in association with durvalumab and carboplatin/paclitaxel chemotherapy, where it did not lead to a better disease control rate at 24 weeks of treatment [147]. In an early-phase trial in metastatic prostate cancer, the clinical efficacy of oleclumab in association with durvalumab and AZD4635, an adenosine A2A receptor antagonist, was also disappointing [148].

Another anti-CD73, uliledlimab was evaluated in combination with toripalimab (an anti-PD-1) in a dose-escalation and dose-expansion phase I/II study [149]. Among 48 patients with NSCLC, the ORR was 12.5% (95%CI: 4.7%-25.2%). In another phase I study evaluating uliledlimab in combination with atezolizumab (an anti-PD-L1), the ORR was 23% among 13 evaluable patients who received $\geq$10 mg/kg of the study drug [150] Other anti-CD73 antibodies are under clinical development in association with various anti-PD1 (NCT05119998, NCT04572152, NCT03454451, NCT04672434). Another interesting approach is also in development, combining anti-CD73 and anti-TGFβ activities through the bispecific antibody AGEN1423. The targeting of both CD73 and TGFβ reduces the adenosine concentration in the tumor micro-environment and inhibits the immunosuppressive effect of TGFβ [151].

Indoleamine 2,3-dioxygenase 1 (IDO-1)

Among the multiple mechanisms of resistance to anti-PD1 therapy, tryptophan catabolism has been shown to play a role. Tryptophan is an amino acid mainly degraded to kynurenine and kynurenic acid by the enzymes IDO-1 and tryptophan 2,3-dioxygenase [152]. An increase in IDO-1 activity decreases the levels of tryptophan and increases the concentration of kynurenine and other metabolites. This mechanism exerts an immunosuppressive effect in the tumor microenvironment through different mechanisms, including activating the aryl hydrocarbon receptor (AhR) by the tryptophan metabolites [153]. AhR activation drives an immunosuppressive response by promoting an increase of tumor-associated macrophages, regulatory T cells, and myelocyte-derived suppressor cells [154]. AhR activation also induces the production of the pro-inflammatory and pro-TME tumorigenic cytokine IL6.

Clinically, a decrease in tryptophan levels and an increase in its metabolites are associated with a worse outcome in various cancers [155–157], similar to the upregulation of IDO-1 [158].

In the clinic, despite promising early phase results of IDO-1 inhibitors in combination with anti-PD-1 [159], the ECHO-301/KEYNOTE-252 phase III trial of the IDO-1 inhibitor epacadostat in association with the anti-PD-1 pembrolizumab showed no benefit over pembrolizumab alone in patients with metastatic melanoma [160]. The enthusiasm for IDO-1 inhibitors has since decreased, and multiple trials evaluating epacadostat or other IDO-1 inhibitors such as BMS-986205 were halted [161]. Whether IDO-1 inhibition still has a place in some specific clinical settings or in combination remains under evaluation (NCT03459222, NCT02658890).

CD200

CD200 is a cell-surface type I glycoprotein that comprises two Ig-like domains. CD200 is found in some normal tissues, such as the retina or neurons, and also on the surface of various activated immune cells, such as T-cells, B-cells or dendritic cells [162,163]. The ligand of CD200, CD200R1, is an inhibitory receptor expressed on immune cells, like natural killers, T-cells, B-cells and myeloid cells [164]. Upon activation through CD200 binding, CD200R1 can exert a direct inhibitory effect on NK cell activity [165]. The effect of CD200R1 activation on T-cells activity is less clear. In some pre-clinical models of breast cancer, CD200 blockade had an anti-tumoral effect [166], while other pre-clinical models of melanoma showed no impact on tumor growth control [167].

In the clinic, anti-CD200 molecules are being tested in hematological malignancies, with Samalizumab, an anti-CD200 antibody, being currently evaluated in a chronic lymphocytic leukemia phase I/II study [168].

Biomarkers for novel ICI

The development of these novel immune checkpoint inhibitors has the potential to offer significant benefits to cancer patients. However, as novel ICI continue to evolve, there will be a growing need for new and reliable biomarkers to enhance patient selection and treatment response prediction, optimize treatment outcomes and minimize unnecessary toxicities and treatment costs. At present, these new targets lack validated biomarkers. For example, the role of tumor expression of immune checkpoints has not been clearly established.

For anti-LAG-3 drugs, which are already used in clinical practice, the role of LAG-3 tumor expression was assessed in the Relativity-047 trial, using an immunostaining technique[22]. LAG-3 expression was quantified as the proportion of immune cells with positive staining within the tumor region (comprising the tumor, stroma and invasive margin) in relation to all nucleated cells present in the tumor region in samples containing a minimum of 100 viable tumor cells. LAG-3 expression was prognostic, with better outcomes for patients with LAG-3 $\geq$ 1% in both treatment groups. However, no predictive signal was found for LAG-3 expression, as relatlimab-nivolumab performed better than nivolumab both in LAG-3 positive and LAG-3 negative subgroups. On the other hand, patients with PD-L1 negative tumors seem to benefit more from dual therapy than patients with PD-L1 positive tumors. This subgroup analysis led to the approval of relatlimab-nivolumab only for PD-L1 negative melanoma by the European Medicines Agency. For anti-LAG-3, other approaches evaluating the use of peripheral blood CD8 T-cell signatures or plasma cytokine concentrations as biomarkers seem interesting, but are not clinically validated [169].

Anti-TIM-3 agents also lack any reliable biomarkers. Regarding the response to sabatolimab, several potential predictive biomarkers were assessed in pre-treatment biopsies: expression of CD8, PD-L1, CD163, LAG-3 and TIM-3 by immunohistochemistry, as well as RNA sequencing and gene signatures associated with TIM-3 pathways, or T-cell or interferon-gamma signatures[44]. However, no relationship between these immune biomarkers and response to treatment was observed. Of note, three responding patients had >10% TIM-3–positive staining in biopsy samples. Similarly, no relation was found between baseline TIM-3, PD-L1, and CD8 expression by immunohistochemistry and response to LY3321367. The small sample size of these trials and the low numbers of patients responding to therapy make it difficult to assess these potential biomarkers robustly.

Regarding TIGIT, the link between TIGIT expression and response to TIGIT inhibition has not been adequately studied. Moreover, TIGIT expression is variable not only between different cell types but also according to cellular localization, highlighting that simple assessment of TIGIT expression in a tumor sample may be inefficient in determining its relevance in the immune tumor microenvironment [170].

A deeper evaluation of the complex structure of tumor immune

microenvironment, rather than simple immune checkpoint expression evaluation will probably be necessary to better guide treatment decisions. Comprehensive analyses of genomics, transcriptomics and proteomics may offer a more holistic view of the tumor-immune interactions and may be the way forward in precision immunotherapy.

New trial designs for more efficient evaluation of novel strategies

These novel developments in immunotherapy are moving simultaneously with a shift towards precision medicine in oncology. Identifying multiple potential targets for ICI and emerging new biomarkers will inevitably lead to the stratification of patients into multiple small subgroups according to individual tumor and patient specificities. Answering clinical questions efficiently and timely with traditional large 2-groups randomized trials, which often address one question at a time, will become increasingly difficult. In response to these challenges, innovative approaches have emerged to optimize clinical research time and resources and reduce time from bench to bedside [171]. One of the solutions stands in the use of master protocols that allow for the evaluation of several investigational drugs for one tumor type ("Umbrella studies") or of one investigational agent for several tumor types ("Basket trials"), within the same trial structure [172,173]. The use of a master protocol alleviates the organizational complexity of conducting multiple clinical trials concurrently, and thus may help to speed up the process of drug development [174]. A step further is the development of adaptive platform trials, which allow evaluating multiple interventions for a disease and adapting an ongoing trial according to data of included patients and based on a decision algorithm [175]. These trials often use statistical Bayesian inference models that are well-suited for adaptations based on observations. Bayesian models are increasingly used for dose finding in phase I/II trials [176]. Moreover, since they can take into account information from previously enrolled patients, they were adopted in new multi-arm adaptive trials to routinely change the number of arms and patient randomization distribution amongst them. By prioritizing the treatment arms with the highest chance of success, patient allocation is optimized to make the most of each enrolled patient [176,177].

As such, master protocols allow for an efficient use of control arms and the rapid introduction and evaluation of new promising strategies.

Conclusion

In recent years, there has been significant progress in understanding how cells bypass the immune system. These discoveries have opened the door to potential new treatment modalities. Although promising in preclinical models, many of these strategies did not translate into concrete clinical benefits, such as KIR-L and the antibody lirilumab. Even among the new ICIs that have demonstrated clinical activity, cautious optimism is warranted, given the poor correlation between early positive results and actual clinical benefit. Currently, none of the new ICIs seems to be as promising in terms of tumor control as the inhibition of the PD-1/PD-L1 axis, at least in monotherapy. Therefore, many of these new compounds are tested in combination with an anti-PD-1 antibody, aiming to tackle the resistance to PD-1/PD-L1 blockade and provide synergistic effects to enhance antitumoral immunity.

The future of immunotherapy probably lies in these combination strategies, as well as bispecific antibodies and antibody-drug conjugates and their combination with ICI. It is, however, improbable that a "one size fits all" strategy will work, even within the same tumor type, as the mechanisms of immune-escape and immunomodulation vary. Moreover, targeting immune checkpoints will probably prove insufficient in many situations, as these checkpoints are only one piece of the much larger puzzle that the immune system and tumor microenvironment are. Nevertheless, combinations of ICIs with other immunotherapy strategies, chemotherapy, radiotherapy, or targeted therapies, may offer a solution in certain situations.

Finally, the development of biomarkers to determine the cause of immune escape, and the monitoring of the resistance mechanisms, may provide the key to tailoring ICI strategies and an important step in precision oncology [178] Also, identifying predictive biomarkers of response is as important as defining the ones that predict a lack of benefit, such as oncogene-addicted non-small cell lung cancer [179].

The development of refined diagnostic tools to improve patient selection should be part of the development of every new treatment strategy to improve response rates to ICIs and minimize the number of patients unnecessarily exposed to potential immune-related AEs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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