Innate Immune Checkpoint Inhibitors: The Next Breakthrough in Medical Oncology?

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ABSTRACT

While immunotherapy has revolutionized the treatment of many types of advanced cancer, most patients still do not derive benefit. The currently available immune checkpoint inhibitors target the adaptive immune system, generating a T-cell antitumor response. However, an antitumor immune response depends on a complex interplay of both innate and adaptive immune cells. The innate immune system is a promising new target, and innate immune checkpoint inhibitors can disrupt inhibitory interactions ("don't eat me" signals) between tumor and both phagocytes and natural killer cells. The checkpoint inhibitor may also provide a stimulatory interaction ("eat me" signal), or this can be achieved through use of combination therapy. This generates antitumor effector functions including phagocytosis, natural cytotoxicity, antibody-dependent effects, and synergistic activation of the adaptive immune system via antigen presentation. This is a rapidly expanding area of drug development, either alone or in combination (with anticancer antibodies or adaptive immune checkpoint inhibitors). Here, we comprehensively review the mechanism of action and up-to-date solid tumor clinical trial data of the drugs targeting phagocytosis checkpoints (SIRPα/CD47, LILRB1/MHC-I, and LILRB2/MHC-I) and natural killer–cell checkpoints (TIGIT/CD112 + CD155, PVRIG/CD112, KIRs/MHC-I, and NKG2A-CD94/HLA-E). Innate immune checkpoint inhibitors could once again revolutionize immune-based cancer therapies.

Introduction

Immune function is governed in part by a complex array of stimulatory and inhibitory cell–surface interactions. A coordinated cancer immunity cascade must occur for immune-based therapies to be effective, including expression/release of cancer antigens, antigen presentation, immune cell activation, immune cell transport and infiltration into tumor, and cancer cell killing by both adaptive and innate mechanisms through interaction of stimulatory and inhibitory processes (1). Inhibitory immune checkpoints exist to maintain physiologic immune responses, that is, to induce tolerance (**Fig. 1**). Immune checkpoint inhibitors have been successful in the treatment of many solid tumors, but the majority of advanced solid tumors do not respond and resistance usually emerges (2, 3). The currently available immune checkpoint inhibitors target CTL antigen-4 (CTLA-4), programmed death-1 (PD-1), and its ligand (PD-L1), all attempting to bolster T-cell activation.

There are many mechanisms of resistance to immunotherapy, including loss of tumor antigen expression, increased expression of tumor inhibitory checkpoint ligands (such as PD-L1) which bind cognate ligands on immune cells, and recruitment of suppressive cell populations by tumor-produced immunosuppressive factors (2, 4, 5). Indeed, infiltration of both innate and adaptive immune cells into tumor has been shown to correlate with improved outcomes in many cancer types (6–9.) Novel treatments targeting the innate immune system are promising candidates in medical oncology.

Innate immune cells are derived from both myeloid and lymphoid lineages. Myeloid cells include monocytes, macrophages, dendritic cells (DC), polymorphonuclear cells, and mast cells. Natural killer (NK) cells are innate lymphoid cells. Innate immune cells activate the adaptive immune system via antigen presentation and execute primary effector functions, such as phagocytosis, natural cytotoxicity, and antibody-dependent cellular cytotoxicity and phagocytosis (ADCC and ADCP, respectively) upon activation of their Fc receptors for antibody (10). In fact, the innate immune system is required to generate antigen-specific T-cell activity (11). Innate immune checkpoint inhibitors, with or without adaptive immune checkpoint inhibitors, may overcome resistance to currently approved immune checkpoint drugs.

Innate immune cell function is regulated by a balance of stimulatory and inhibitory cell–surface interactions (**Fig. 1**). Blocking inhibitory checkpoints ("don't eat me" signals) between tumor and innate immune cells is a novel therapeutic target. The checkpoint inhibitor may also provide a stimulatory interaction ("eat me" signal), or this can be achieved through use of combination therapy. When the balance of stimulatory and inhibitory interactions favors activation of innate immune cells, the result is phagocytosis or natural cytotoxicity of cancer cells. Here, we will review basic science of the checkpoints and the mechanism of action, safety, and efficacy data of all innate immune checkpoint inhibitors currently being studied in solid tumor clinical trials.

Materials and Methods

All clinical data from solid tumor clinical trials on the innate immune checkpoint inhibitors are included. Studies were identified by searching for the checkpoints and drugs using the PubMed database, ClinicalTrials.gov, and abstracts from the following annual meetings: American Association of Cancer Research, American Society of Clinical Oncology, European Society for Medical Oncology, and the Society for Immunotherapy of Cancer. Objective response rate (ORR) is defined as partial response (PR) or complete response (CR),

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Mol Cancer Ther 2021;20:961–74

doi: 10.1158/1535-7163.MCT-21-0041

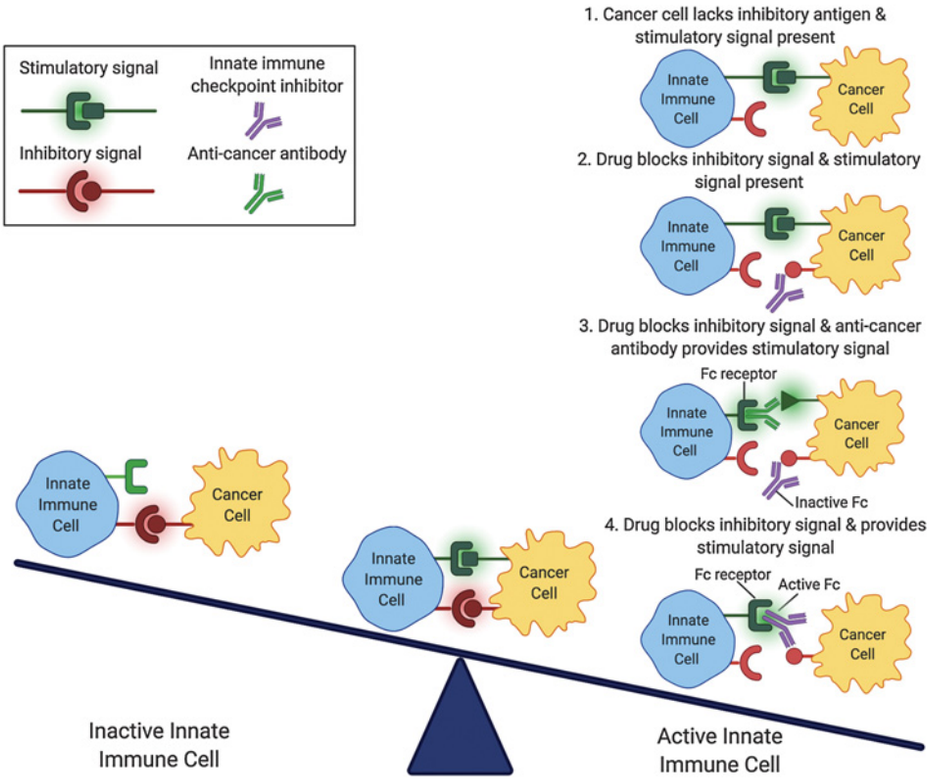

Figure 1.

Innate immune cell–surface regulatory interactions and therapeutic targets. Innate immune cell antitumor function is regulated in part by stimulatory ("eat me") and inhibitory ("don't eat me") cell–surface interactions with the cancer cell, generally involving a receptor on the immune cell and antigen on the cancer cell. Inhibitory interactions ("checkpoints") exist to maintain physiologic immune responses, that is, to induce tolerance. Normal human cells generally avoid immune destruction by expressing more inhibitory than stimulatory antigens. Cancer cells, likewise, can evade the innate immune system by expressing inhibitory antigens and lacking stimulatory antigens (left). Cancer cells may also express stimulatory antigens, activating innate immune cells and resulting in phagocytosis (phagocytes) or natural cytotoxicity (NK cells). Conceptually, there are four scenarios under which the balance of stimulatory and inhibitory signaling favors innate immune cell antitumor effects. First, the cancer cell lacks inhibitory antigens and stimulatory signals are present; radiation/chemotherapy may increase cancer cell expression of stimulatory antigens (right, #1). Second, an innate immune checkpoint inhibitor can block the inhibitory checkpoint while a stimulatory signal is present (right, #2). Third, a combination therapy approach can block the inhibitory checkpoint (with an innate immune checkpoint inhibitor lacking an active Fc region) while a second drug (anticancer antibody) can provide the stimulatory signal upon interacting with innate immune cell Fc receptors (right, #3). Finally, an innate immune checkpoint inhibitor containing an active Fc region can both block the inhibitory checkpoint and provide the stimulatory signal upon interacting with an innate immune cell Fc receptor (right, #4). This final strategy is often limited by hematologic toxicity. Created with BioRender.com.

as assessed in the clinical trial. Disease control rate (DCR) is defined as stable disease (SD), PR, or CR, as assessed in the clinical trial. **Table 1** provides a summary of the available solid tumor clinical safety and efficacy data of the innate immune checkpoint inhibitors. **Tables 2** and **3** list the active clinical trials on ClinicalTrials.gov.

Results

Phagocytosis checkpoint inhibitors

The phagocytes are macrophages, neutrophils, and DCs. Upon activation, phagocytes engulf target cells. Macrophages and neutrophils then directly destroy the target cell (macrophages by lysosomal proteolysis and neutrophils by oxidative burst; ref. 12). DCs, on the other hand, process antigens and load them onto major histocompatibility complex class I (MHC-I) or II (MHC-II) to present to T cells, thereby activating the adaptive immune system (12). Antigen presentation is required for T-cell antitumor activity (10). The amount of tumoral conventional DCs (a subpopulation of DCs even more specialized in antigen presentation) predicts survival across multiple tumor types and predicts response to anti-PD-L1 therapy (9, 13).

Thus, phagocytosis checkpoint inhibitors directly destroy tumor cells and activate the adaptive immune system (via antigen presentation), providing rationale for use with adaptive immune checkpoint inhibitors [targeting CTLA-4 or PD-(L)1], which strengthen T-cell antitumor effects. In addition, while the PD-1–PD-L1 axis is primarily characterized as a T-cell checkpoint, there is evidence of innate immune regulation. Specifically, blockade of the PD-1–PD-L1 axis in mice lacking a functional immune system, other than intact macrophages, generated antitumor response (14). Similarly, PD-L1 knockout tumor cells significantly increased phagocytosis by macrophages (14).

Phagocytosis of cancer cells is regulated by pro- and anti-phagocytic receptor–ligand interactions (**Fig. 1**). The primary prophagocytic ("eat me") signals on cancer cells are calreticulin (interacts with LRP1 on phagocytes), SLAMF7 (interacts with Mac-1 on phagocytes), and tumor antigen bound by antibody (interacts with Fcγ receptor on phagocytes); these antigens are minimally expressed on most normal

Table 1. Results of solid tumor clinical trials of phagocytosis and NK-cell checkpoint inhibitors.

NCT	In combination with	Phase	Cancer type	ORR	DCR	mPFS (months)	Primary toxicity[a]
			SIRPα/CD47				
ALX148 (ALX Oncology, anti-CD47 fusion (CD47 binding domain of SIRPα to inactive human Ig Fc)							
03013218 (22)	Alone, pembrolizumab, trastuzumab, or rituximab	I	Solid and NHL	0% (alone)	16% (alone)	NR	Fatigue, headache, thrombocytopenia
03013218 (23)	Pembrolizumab, trastuzumab	I	Solid	4% (NSCLC) 18% (HNSCC) 19% (gastric)	39% (NSCLC) 41% (HNSCC) 48% (gastric cancer)	NR	Fatigue, AST elevation, anemia
03013218 (24)	Pembrolizumab, trastuzumab, or chemo	I	HNSCC and gastric	50% (ICI-naïve HNSCC) 0% (prior ICI HNSCC) 20% (gastric cancer)	NR	4.6 (ICI-naïve HNSCC) 2 (prior ICI HNSCC) 2.2 (gastric cancer)	Fatigue, AST increase, thrombocytopenia
IBI188 (Innovent Biologics, anti-CD47 humanized IgG4 mAb)							
03763149 (33)	Alone	I	Solid and lymphoma	NR	NR	NR	Nausea, back pain, fatigue
Lemzoparlimab (TJC4, TJ011133) (AbbVie and I-Mab Biopharma, anti-CD47 fully human IgG4 mAb)							
03934814 (32)	Alone	I	Solid	NR	NR	NR	Anemia, fatigue, infusion reaction
Magrolimab (Hu5F9-G4, ONO-7913) (Gilead, anti-CD47 humanized IgG4 mAb)							
02216409 (20)	Alone	I	Solid and heme	NR	NR	NR	Anemia, fatigue, headaches
03558139 (25)	Avelumab	I	Solid	0%	56%	NR	Headache, fatigue, infusion reaction, anemia
02953782 (26)	Cetuximab	I/II	Solid and CRC	7% (KRAS WT CRC) 0% (KRAS MT CRC)	NR (KRAS WT CRC) 45% (KRAS MT CRC)	3.6 (KRAS WT CRC) 1.9 (KRAS MT CRC)	Acneiform rash, dry skin, fatigue
SRF231 (Surface Oncology, anti-CD47 fully human IgG4 mAb)							
03512340 (30)	Alone	I	Solid and heme	0%	NR	NR	Fatigue, headache, pyrexia
			LILRB2/MHC-I				
Etigilimab (OMP-313M32) (Mereo BioPharma, anti-TIGIT IgG1 humanized mAb)							
03119428 (58)	Alone or nivolumab	I	Solid	0%	22%	NR	Rash, fatigue, nausea
MK-4830 (Merck and Agenus, anti-LILRB2 fully human IgG4 mAb)							
03564691 (38)	Alone or pembrolizumab	I	Solid	13%	NR	NR	None reported
Tiragolumab (MTIG7192A, RG6058) (Genentech, anti-TIGIT humanized IgG1/kappa mAb)							
02794571 (52)	Alone or with atezolizumab	I	Solid	0% (alone) 6% (w/atezo)	17% (alone) NR (w/atezo)	NR	Fatigue, anemia
02794571 (52)	Atezolizumab	I	NSCLC (PD-L1+ and ICI-naïve)	50%	79%	NR	Fatigue, anemia
03563716 (54)	Atezolizumab	II	NSCLC[b]	37%	NR	5.6	Rash, infusion reactions
Vibostolimab (MK-7684) (Merck, anti-TIGIT humanized antibody)							
02964013 (55)	Alone or pembrolizumab	I	Solid	3% (alone) 19% (w/pembro)	35% (alone) 47% (w/pembro)	NR	Fatigue, pruritus, anemia
02964013 (56, 57)	Alone or pembrolizumab	I	NSCLC	7% (alone) 5% (w/pembro) 29% (ICI-naïve)	NR	9 (alone) 13 (w/pembro)	Pruritus, fatigue, rash
			PVRIG/CD112				
COM701 (Compugen, anti-PVRIG humanized IgG4 mAb)							
03667716 (62–64)	Alone or nivolumab	I	Solid	NR	57%	NR	Fatigue, nausea, anemia
			KIRs/MHC-I				
Lirilumab (IPH2102, BMS-986015) (Innate Pharma and Bristol-Myers-Squibb, anti-KIR2D fully human IgG4 mAb)							
01750580 (70, 71)	Nivolumab or ipilimumab	I	Solid	24%	52%	NR	Fatigue, pruritus, infusion reaction

(Continued on the following page)

Table 1. Results of solid tumor clinical trials of phagocytosis and NK-cell checkpoint inhibitors. (Cont'd)

NCT	In combination with	Phase	Cancer type	ORR	DCR	mPFS (months)	Primary toxicity[a]
2009–011526–33[c]; (69)	Alone	I	Solid and heme	0%	59%	6.6 (AML) 19.6 (CLL) 5.3 (ovarian)	Asthenia, pruritus, fatigue
NKG2A-CD94/HLA-E							
Monalizumab (IPH2201) (Innate Pharma and AstraZeneca, humanized anti-NKG2A-CD94 IgG4 mAb)							
02671435 (75)	Durvalumab	I	Solid and CRC	8%	38%	NR	Diarrhea
02671435 (76, 77)	Durvalumab, FOLFOX, and (bevacizumab or cetuximab)	I/II	CRC	41% (bevacizumab) 55% (cetuximab)	88%	NR	Fatigue, nausea, peripheral neuropathy
03088059 (78)	Alone	II	HNSCC	0%	22%	1.72	None reported
02643550 (79–82)	Cetuximab	I/II	HNSCC	20%	NR	4.5	Fatigue, pyrexia, headache
02459301 (83)	Alone	I	Gynecologic	0%	41% (dose esc) 18% (dose exp)	NR	Headache, fatigue, vomiting

Abbreviations: AML, acute myeloid leukemia; atezo, atezolizumab; chemo, chemotherapy; CLL, chronic lymphocytic leukemia; CRC, colorectal cancer; DCR, disease control rate; esc, escalation; exp, expansion; heme, hematologic; HNSCC, head/neck squamous cell carcinoma; ICI, anti-PD-(L)1 immune checkpoint inhibitor; mAb, monoclonal antibody; mPFS, median progression-free survival; MT, mutated; NCT, ClinicalTrials.gov identifier; NHL, non-Hodgkin lymphoma; NR, not reported; NSCLC, non-small cell lung cancer; ORR, objective response rate; pembro, pembrolizumab; PFS, progression-free survival; WT, wild-type.

[a]Most common any-grade treatment-related adverse events.

[b]Chemotherapy-naïve, PD-L1+, EGFR and ALK wild-type.

[c]EudraCT (European Union Drug Regulating Authorities Clinical Trials Database) identifier.

cells (4, 15). Radiotherapy and chemotherapy may also increase cancer cell expression of prophagocytic signals, synergizing with phagocytosis checkpoint inhibitors (4, 16). The primary inhibitory checkpoints between phagocytes and tumor cells, respectively, are SIRPα/CD47, LILRB1/MHC-I, and LILRB2/MHC-I (**Fig. 2**). Drugs targeting these checkpoints are typically designed to be given with a prophagocytic signal such as an opsonizing anticancer antibody, radiation, or chemotherapy (**Fig. 1**).

SIRPα/CD47

Signal-regulatory protein-α (SIRPα) is an inhibitory receptor expressed on myeloid phagocytes. SIRPα contains an extracellular Ig domain for ligand binding and intracellular immunoreceptor tyrosine-based inhibition motifs (ITIM; refs. 4, 10). CD47, the ligand for SIRPα, is widely expressed on cancer cells (17). The SIRPα/CD47 interaction results in phosphorylation of the ITIMs and downstream disruption of the cytoskeleton, resulting in inhibition of phagocytosis (17). Blockade of the SIRPα/CD47 axis increases phagocytosis, resulting in cell death and antigen presentation. Tumor killing can be further stimulated if an active Fc domain is supplied, either on the anti-CD47 agent or on a separate anticancer antibody, both of which generate ADCP or ADCC (10). In addition to directly blocking the SIRPα/CD47 interaction, preclinical work has demonstrated an evolving potential drug target. Glutaminyl-peptide cyclotransferase-like protein (QPCTL) is responsible for the formation of pyroglutamate on CD47 at the SIRPα-binding site. Disruption of QPCTL reduces binding of CD47 to SIRPα, enhancing ADCP and ADCC (15).

While CD47 is widely expressed among normal human cells (including hematopoietic cells), SIRPα/CD47 blockade preferentially results in phagocytosis of tumor, as normal human cells typically lack stimulatory "eat me" signals (4, 15). A notable exception is red blood cells (RBC), which are subject to dose-dependent phagocytosis in the setting of SIRPα/CD47 blockade (18). Strategies to minimize anemia include administering a priming dose of the SIRPα-CD47 blocker (resulting in RBC phagocytosis then reticulocytosis, which express higher levels of CD47 and are thus resistant to phagocytosis) or selecting the Fc domain of the anti-CD47 mAb to minimize interactions with the phagocyte Fcγ receptor (either by using an inactive Fc domain or using an Fc domain of IgG2/4, which more weakly interact with Fcγ receptors than do IgG1/3; refs. 19, 20).

ALX148: ALX148 (ALX Oncology) is an engineered fusion protein containing two high-affinity CD47-binding domains of SIRPα linked to an inactive Fc region of human Ig (21). ALX148 is being evaluated in combination with anticancer antibodies and/or with anti-PD-1 agents in both solid [with development focused on non–small cell lung cancer (NSCLC), head/neck squamous cell carcinoma (HNSCC), and gastric/gastroesophageal junction carcinoma] and hematologic malignancies. Phase I efficacy data are encouraging, and treatment appears to be tolerable (including minimal hematologic toxicity).

In a phase I study, 25 patients with advanced refractory solid tumors or non-Hodgkin lymphoma were treated with ALX148 alone (NCT03013218; ref. 22). ORR was 0% and DCR was 16%. The most common treatment-related adverse events (TRAE) were headache (16%), fatigue (12%), dizziness (8%), rash (8%), and thrombocytopenia (8%). Grade ≥3 TRAEs were infection, pancreatitis, thrombocytopenia, and neutropenia (1 each).

Seventy-nine patients with advanced refractory solid tumors were treated with ALX148 in combination with pembrolizumab (NSCLC or HNSCC, $N = 50$) or in combination with trastuzumab ($HER2^+$, primarily gastric/gastroesophageal junction carcinoma, $N = 29$;

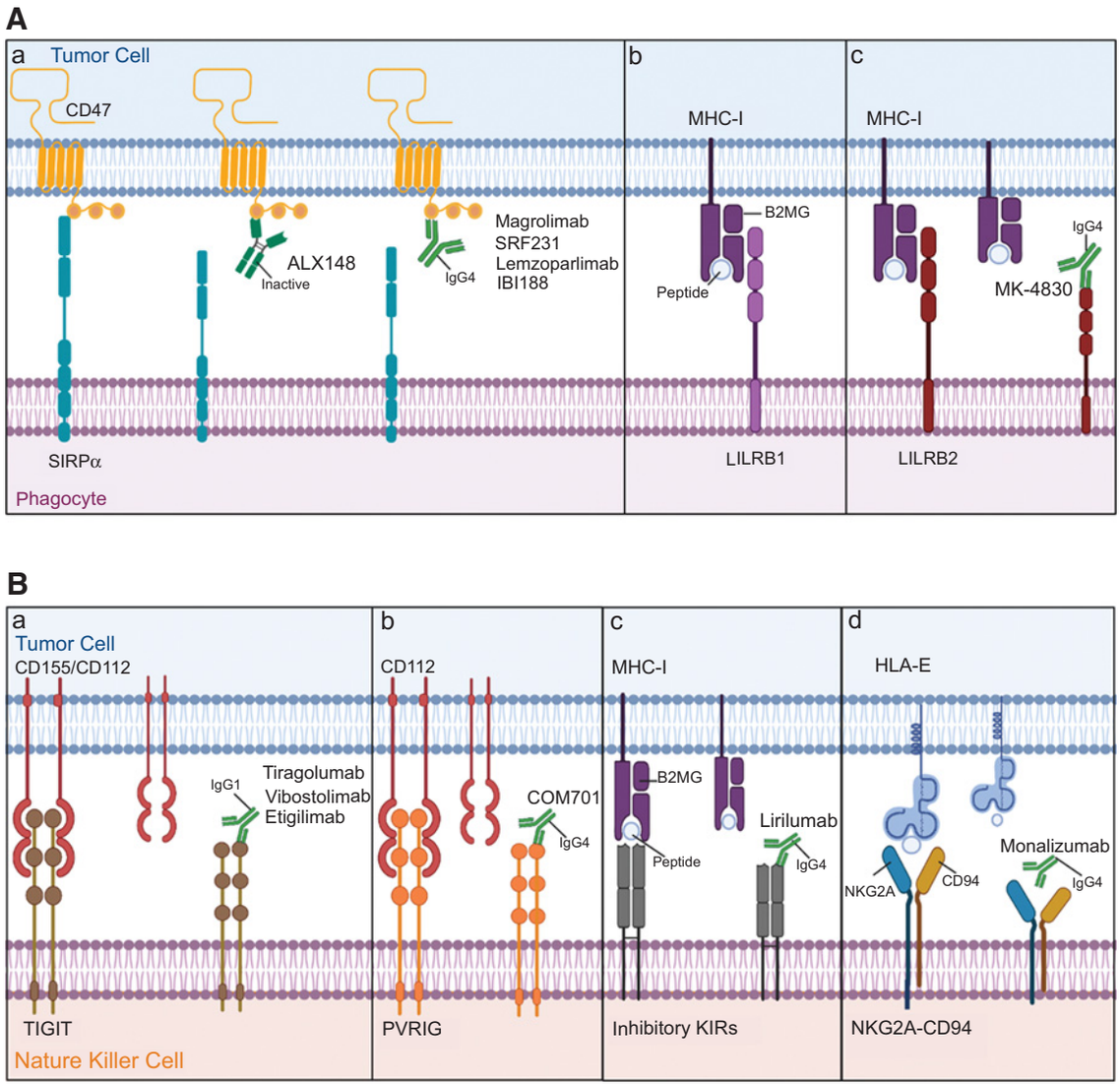

Figure 2.

Innate immune checkpoints and inhibitors. Tumor cells express ligands ("don't eat me" signals) which interact with phagocyte (**A**) and NK (**B**) cell-surface receptors, stimulating signaling pathways which inhibit phagocytosis and natural cytotoxicity, respectively. The phagocytosis checkpoints (**A**) include (a) SIRPα/CD47, (b) LILRB1/MHC-I, and (c) LILRB2/MHC-I. Drugs are being developed to interrupt these signaling pathways, thereby increasing phagocytosis of tumor cells; drugs with presented/published clinical trial data are shown. (a) The anti-CD47 drugs are ALX148 (engineered fusion protein - two high-affinity CD47 binding domains of SIRPα linked to an inactive Fc), magrolimab (anti-CD47 humanized IgG4 mAb), SRF231 (anti-CD47 fully human IgG4 mAb), lemzoparlimab (anti-CD47 humanized IgG4 mAb), and IBI188 (anti-CD47 humanized IgG4 mAb). No compounds blocking the LILRB1/MHC-I axis have available clinical trial results (b). MK-4830 is an anti-LILRB2 fully human IgG4 mAb (c). The NK checkpoints (**B**) include (a) TIGIT/CD155 + CD112, (b) PVRIG/CD112, (c) Inhibitory KIRs/MHC-I, and (d) NKG2A-CD94/HLA-E. Drugs are being developed to interrupt these signaling pathways, thereby increasing natural cytotoxicity of tumor cells; drugs with presented/published clinical trial data are shown. Tiragolumab, vibostolimab, and etigilimab are anti-TIGIT humanized IgG1 mAbs (a). COM701 is an anti-PVRIG humanized IgG4 mAb (b). Lirilumab is an anti-KIR2D fully human IgG4 mAb (c). Monalizumab is an anti-NKG2A-CD94 humanized IgG4 mAb (d). Created with BioRender.com. B2MG, beta-2-microglobulin; Fc, immunoglobulin fragment crystallizable region; HLA, human leukocyte antigen; Ig, immunoglobulin; KIR, killer cell immunoglobulin-like receptor; LILRB1, leukocyte immunoglobulin-like receptor B1; LILRB2, leukocyte immunoglobulin-like receptor B2; mAb, monoclonal antibody; MHC, major histocompatibility complex; NKG2A, NK group 2 member A; PVRIG, poliovirus receptor related immunoglobulin domain containing; SIRPα, signal-regulatory protein-α; TIGIT, T-cell immunoglobulin and immunoreceptor tyrosine-based inhibition motifs domain.

NCT03013218; ref. 23). Patients with NSCLC previously received pembrolizumab or had PD-L1 tumor proportion score <50%, patients with HNSCC had progressed on platinum-based therapy, and HER2$^+$ patients had previously received trastuzumab. ORR and DCR, respectively, were 4% and 39% for NSCLC, 18% and 41% for HNSCC, and 19% and 48% for gastric/gastroesophageal junction carcinoma. Treatment was well tolerated. Any grade TRAEs included fatigue (11%), aspartate aminotransferase (AST) increase (9%), alanine aminotransferase (ALT) increase (8%), anemia (8%), and thrombocytopenia (6%).

An overlapping patient cohort was subsequently presented (NCT03013218; ref. 24). Patients included 52 with HNSCC that had progressed on platinum-based therapy (with or without prior

anti-PD-1 therapy) and were treated with ALX148 and pembrolizumab, one with previously untreated HNSCC treated with ALX148, pembrolizumab, and platinum doublet, and 30 with $HER2^+$ gastric/gastroesophageal junction cancer that had progressed on platinum doublet with trastuzumab and were treated with ALX148 and trastuzumab with or without ramucirumab/paclitaxel. ORR, median progression-free survival (mPFS, months), and median overall survival (mOS, months) were 40%, 4.6, and not reached in the previously treated anti-PD-1 naïve HNSCC arm treated with ALX148/pembrolizumab; 0%, 2.0, and 7.4 in the anti-PD-1 experienced HNSCC arm treated with ALX148/pembrolizumab; and 20%, 2.2, and 8.1 in the gastric/gastroesophageal junction cancer arm treated with ALX148/trastuzumab. TRAEs in the arms treated with ALX148 and either pembrolizumab or trastuzumab were fatigue (18%), AST increase (11%), thrombocytopenia (10%), ALT increase (9%), anemia (9%), and pruritus (9%).

Magrolimab (Hu5F9-G4, ONO-7913): Magrolimab (Gilead, recently acquired from Forty Seven) is an anti-CD47 humanized IgG4 mAb (18). It is being evaluated in solid tumors and hematologic malignancies. Anemia is common but can be mitigated by administration of a priming dose of magrolimab with intrapatient dose escalation (20). Thrombocytopenia and leukopenia are usually not severe. There are many ongoing phase I–III trials evaluating magrolimab in both solid and hematologic cancers (**Table 2**).

In a phase I study, 60 patients with refractory advanced solid tumors (plus 2 patients with diffuse large B-cell lymphoma) were treated with magrolimab (NCT02216409; ref. 20). ORR was 3% (ovarian and fallopian tube carcinoma). The most common hematologic toxicities were anemia (57%, typically with a hemoglobin decrease of 1–2 g/dL after the priming dose with rapid subsequent improvement; 4 patients required transfusions), lymphopenia (18%), and thrombocytopenia (11%). The most common nonhematologic toxicities were fatigue (64%), headache (50%), fever and chills (45% each), and hyperbilirubinemia (34%, usually transient unconjugated hyperbilirubinemia associated with anemia during the priming period). Thus, efficacy of magrolimab monotherapy appears to be limited.

In a subsequent phase Ib study, 34 patients with refractory solid tumors (primarily ovarian cancer) were treated with magrolimab and avelumab (NCT03558139; ref. 25). Among the 18 response-evaluable patients with ovarian cancer, ORR was 0% and DCR was 56%. TRAEs were similar.

Magrolimab was evaluated with cetuximab in patients with solid tumors (phase Ib, $N = 32$) and previously treated colorectal cancer (phase II, $N = 46$; NCT02953782; ref. 26). The most common any-grade TRAEs were aceniform rash (36%), dry skin (33%), fatigue (32%), infusion reaction (31%), headache (30%), diarrhea (23%), nausea (23%), chills (23%), and anemia (22%); 4% of patients discontinued treatment due to an AE. Among KRAS wild-type colorectal cancer ($N = 30$), ORR was 7% (all cetuximab-experienced), mPFS was 3.6 months, and mOS was 10.1 months. Among KRAS-mutant colorectal cancer ($N = 40$), ORR was 0%, DCR was 45%, mPFS was 1.9 months, and mOS was 10.4 months. Tumor biopsies showed increases in tumor-infiltrating macrophages.

SRF231: SRF231 (Surface Oncology) is an anti-CD47 fully human IgG4 mAb (27–29). In preclinical models, SRF231 binds to RBC CD47 but does not cause RBC phagocytosis (27). It is being developed in both solid and hematologic malignancies. Among 37 response-evaluable patients with advanced refractory solid ($N = 45$) and hematologic malignancies ($N = 1$) treated with SRF231, ORR was 0% with SD reported (phase I, NCT03512340; ref. 30). The most common TRAEs were fatigue (43%), headache (35%), and fever (30%).

Lemzoparlimab (TJC4, TJ011133): Lemzoparlimab (AbbVie and I-Mab Biopharma) is an anti-CD47 fully human IgG4 mAb with a novel conformational epitope, resulting in minimal binding to human RBCs and platelets in preclinical models (31). It is being evaluated in both and solid tumors. A phase I trial of lemzoparlimab in patients with solid tumors and lymphoma is ongoing (NCT03934814). Thus far, 20 patients with advanced refractory solid tumors were treated with lemzoparlimab in dose escalation (part 1; ref. 32). The most common any grade TRAEs were anemia (30%, primarily transiently during cycle 1 with average reduction in hemoglobin 1.5 g/dL), fatigue (25%), infusion reaction (20%), and diarrhea (15%); no grade ≥3 TRAEs were reported. Part 2 is planned, which will evaluate lemzoparlimab with pembrolizumab or rituximab.

IBI188: IBI188 (Innovent Biologics) is an anti-CD47 humanized IgG4 mAb that is being evaluated in both solid and hematologic malignancies (10). Among 20 patients with refractory/advanced solid tumors or lymphoma treated with IBI188, the most common any-grade TRAEs were nausea (35%), back pain (35%), fatigue (30%), vomiting (20%), elevated bilirubin (20%), and anemia (15%). Grade ≥3 TRAEs were elevated bilirubin, thrombocytopenia, and anemia (5% each; NCT03763149; ref. 33).

LILRB1/MHC-I

Leukocyte Ig-like receptor B1 (LILRB1) is expressed on both innate (monocytes, macrophages, eosinophils, basophils, DCs, and certain NK cells) and adaptive (certain T and B cells) immune cells (4). MHC-I, expressed on nucleated cells (including cancer cells), is a heterodimer composed of a heavy α-chain and a β2-microglobulin chain (4, 34). Antigen-bound MHC-I on cancer cells is recognized by cytotoxic T cells, and engagement of MHC-I by the T-cell receptor and CD8 results in cytotoxicity (4, 34). However, expression of MHC-I on cancer cells has been correlated with resistance to phagocytosis, likely due to an inhibitory interaction between LILRB1 on phagocytes and the β2-microglobulin subunit of MHC-I on cancer cells (4, 35). While cytotoxic T-cell antitumor activity is dependent upon intact interaction with MHC-I, specifically blocking the LILRB1/β2-microglobulin axis is a possible innate immune drug target. There are currently no active clinical trials targeting this checkpoint, which may be explained by (i) the relatively recent discovery that the LILRB1/MHC-I interaction correlates with resistance to phagocytosis in cancer cells (in 2018; ref. 4) and (ii) drugs cannot interfere with the MHC-I/T-cell receptor interaction).

LILRB2/MHC-I

LILRB2 is also expressed on both innate (monocytes, macrophages, basophils, and DCs) and adaptive ($CD4^+$ T cells) immune cells and interacts with MHC-I on nucleated cells (unknown specific ligand; ref. 4). LILRB2 is also referred to as ILT4, LIR2, monocyte/macrophage Ig-like receptor 10, MIR-10, and CD85d. It has been shown preclinically that LILRB2 antagonism promotes macrophage maturation and activation (36).

MK-4830: MK-4830 (Merck and Agenus) is an anti-LILRB2 fully human IgG4 mAb (37). In a phase I study, 84 patients with advanced refractory solid tumors were treated with MK-4830 alone ($N = 50$) or in combination with pembrolizumab ($N = 34$; NCT03564691; ref. 38). Among all patients, ORR was 13%; most (91%) were in the

Table 2. Clinical trials of phagocytosis checkpoint inhibitors registered on ClinicalTrials.gov.

NCT	Status	Phase	Cancer type	In combination with	Enrollment[a]
			SIRPα/CD47		
ALX148 (ALX Oncology, anti-CD47 fusion (CD47 binding domain of SIRPα to inactive human Ig Fc)					
03013218	Recruiting	I	Solid cancers and NHL	Pembrolizumab, chemo, or anticancer antibodies	184
04417517	Recruiting	I/II	High risk MDS	Azacitadine	63
AO-176 (Arch Oncology, anti-CD47 humanized IgG2 mAb)					
03834948	Recruiting	I/II	Solid cancers	Alone or paclitaxel	132
04445701	Recruiting	I/II	Multiple myeloma	Alone, dexamethasone or bortezomib	102
BI 765063 (OSE-172) (OSE Immunotherapeutics and Boehringer Ingelheim, anti-SIRPα humanized IgG4 mAb)					
03990233	Recruiting	I	Solid cancers	Alone or BI-754091	116
CC-90002 (Celgene, anti-CD47 humanized IgG4 mAb)					
02367196	Not yet open	I	Hematologic neoplasms	Alone or rituximab	60
02641002	Terminated	I	AML, MDS	Alone	28
CC-95251 (Celgene, anti-SIRPα humanized mAb)					
03783403	Recruiting	I	Hematologic and solid cancers	Monotherapy, rituximab, or cetuximab	230
DSP107 (Kahr Medical, SIRPα and 4-1BB ligand bi-functional fusion protein)					
04440735	Recruiting	I	Solid cancers	Alone or atezolizumab	100
FSI-189 (Gilead, anti-SIRPα antibody)					
04502706	Not yet open	I	NHL	Alone or rituximab	63
HX009 (Waterstone Pharmaceuticals, anti-PD-1 and -CD47 bispecific antibody fusion protein)					
04097769	Recruiting	I	Solid cancers	Monotherapy	37
IBI322 (Innovent Biologics, recombinant anti-CD47/PD-L1 bispecific antibody)					
04338659	Not yet open	I	Solid cancers	Alone	45
04328831	Recruiting	I	Solid cancers	Alone	218
IBI188 (Innovent Biologics, anti-CD47 humanized IgG4 mAb)					
04511975	Recruiting	I	MDS	Azacitadine	32
04485052	Not yet open	I	AML	Azacitadine	126
04485065	Not yet open	I	MDS	Azacitadine	12
03763149	Not yet open	I	Hematologic and solid cancers	Alone	42
03717103	Recruiting	I	Hematologic and solid cancers	Alone or rituximab	92
IMC-002 (ImmuneOncia Therapeutics, anti-CD47 fully human IgG4 mAb)					
04306224	Recruiting	I	Solid cancers and lymphoma	Alone	24
Lemzoparlimab (TJC4, TJ011133) (AbbVie and I-Mab Biopharma, anti-CD47 fully human IgG4 mAb)					
04202003	Recruiting	I/II	AML and MDS	Alone	42
03934814	Recruiting	I	Solid cancers and lymphoma	Alone, pembrolizumab, or rituximab	88
Magrolimab (Hu5F9-G4, ONO-7913) (Gilead, anti-CD47 humanized IgG4 mAb)					
02216409	Completed	I	Solid cancers	Alone	88
03558139	Not yet open	I	Solid cancers	Avelumab	32
03248479	Recruiting	I	AML, MDS	Alone or azacitadine	257
03922477	Recruiting	I	AML	Atezolizumab	21
02678338	Completed	I	AML, MDS	Alone	20
03527147	Recruiting	I	NHL	Acalabrutinib and Rituximab	88
04403308	Recruiting	I	Solid cancers	Alone	12
02953509	Recruiting	I/II	NHL	Rituximab	422
04435691	Recruiting	I/II	AML	Azacitadine or venetoclax	38
04541017	Not yet open	I/II	Mycosis fungoides, Sezary syndrome	Mogamulizumab	100
02953782	Completed	I/II	Solid cancers	Cetuximab	78
03869190	Recruiting	I/II	Urothelial cancer	Atezolizumab	385
04313881	Recruiting	III	MDS	Azacitadine	180
SHR-1603 (Jiangsu HengRui Medicine, anti-CD47 humanized IgG4 mAb)					
03722186	Not yet open	I	Hematologic and solid cancers	Alone	128
04282070	Recruiting	I	Nasopharyngeal cancer	Alone	40
03710265	Recruiting	I	Solid cancers	Alone	112
SL-172154 (Shattuck Labs, bifunctional fusion protein SIRPα-Fc-CD40 L against CD47 and CD40)					
04406623	Recruiting	I	Ovarian cancer	Alone	40
04502888	Not yet open	I	SCC of the head/neck or skin	Alone (intratumoral)	18
SRF231 (Surface Oncology, anti-CD47 fully human IgG4 mAb)					
03512340	Completed	I	Hematologic and solid cancers	Alone	148
TG-1801 (TG Therapeutics and Novimmune, anti-CD47/CD19 bispecific antibody)					
03804996	Recruiting	I	B-cell lymphoma	Alone or ublituximab	16
TTI-621 [Trillium Therapeutics, anti-CD47 recombinant fusion (CD47 binding domain of human SIRPα fused human IgG1 Fc)]					
02890368	Terminated	I	Solid tumors, mycosis fungoides[b]	Alone, anti-PD-(L)1, PEG-IFN-α2a, T-Vec, or RT	56
02663518	Recruiting	I	Hematologic and solid cancers	Alone, rituximab, or nivolumab	260

(Continued on the following page)

Table 2. Clinical trials of phagocytosis checkpoint inhibitors registered on ClinicalTrials.gov. (Cont'd)

NCT	Status	Phase	Cancer type	In combination with	Enrollment[a]
TTI-622 [Trillium Therapeutics, anti-CD47 recombinant fusion (CD47 binding domain of human SIRPα fused human IgG1 Fc)]					
03530683	Recruiting	I	Lymphoma or multiple myeloma	Alone, rituximab, anti-PD-1, or PI	156
ZL-1201 (Zai Lab, anti-CD47 humanized IgG4 mAb)					
04257617	Recruiting	I	Hematologic and solid cancers	Alone	65
LILRB2/MHC-I					
MK-4830 (Merck and Agenus, anti-LILRB2 fully human IgG4 mAb)					
03564691	Recruiting	I	Solid tumors	Alone, pembrolizumab, chemo, or lenvatinib	290

Abbreviations: AML, acute myeloid leukemia; chemo, chemotherapy; mAb, monoclonal antibody; MDS, myelodysplastic syndrome; NCT, ClinicalTrials.gov identifier; NHL, non-Hodgkin lymphoma; PI, proteasome inhibitor; RT, radiation therapy; SCC, squamous cell carcinoma.
[a]Projected or actual.
[b]Percutaneously accessible solid tumors or mycosis fungoides for intratumoral injection.

combination group, 45% were anti-PD-1 naïve, and some were durable (>1 year). TRAEs occurred in 52% of patients and most were grade 1–2.

NK-cell checkpoint inhibitors

NK cells contain both stimulatory and inhibitory receptors, serving as possible drug targets (**Fig. 2**). NK-cell stimulatory receptors include DNAM-1 (also called CD226), NKG2C, NKG2D, and natural cytotoxicity receptors (NKP30, NKP44, and NKP46); these interact with cell-surface and soluble markers of stressed cells, including tumor cells, such as cell-surface NK ligands (10, 39–41). The result is natural cytotoxicity, via secretion of cytokines (IFNγ, TNFα, and GMCSF), and DC recruitment and activation via production of chemoattractants (such as CCL5, CXL1, and CXL2; refs. 10, 39, 40). Thus, NK-cell inhibitory checkpoint inhibitors, like phagocytosis checkpoint inhibitors, strengthen the adaptive immune antitumor response. NK cell–mediated recruitment and activation of DCs has been demonstrated in preclinical melanoma models and may predict response to anti-PD-1 therapy (13, 42).

Healthy cells express low levels of stimulatory ligands and high levels of inhibitory ligands; NK cells identify cancer cells by the opposite pattern; however, cancer cells can evade detection by altering ligand expression (43). Intratumoral NK-cell activity and infiltration has been correlated with improved outcomes in multiple solid tumor types and stages (44–49). The primary inhibitory checkpoints between NK cells and tumor cells, respectively, are TIGIT/CD112 + CD155, PVRIG/CD112, KIRs/MHC-I, and NKG2A-CD94/HLA-E (**Fig. 2**). NK-cell inhibitory checkpoint inhibitors are frequently being evaluated in combination with anti-PD-(L)1 therapy.

TIGIT/CD112 + CD155

T-cell Ig and ITIM domain (TIGIT) is an ITIM-containing inhibitory receptor expressed on NK cells, and also T-cell subsets ($CD4^+$ T cells, $CD8^+$ T cells, and regulatory T cells; ref. 10). The ligands for TIGIT are CD112 (also called PVRL2 and nectin-2) and CD155 (also called PVR), both of which are expressed on tumor cells (10). TIGIT competes for binding to CD112 and CD155 with DNAM-1, a stimulatory NK-cell receptor (43). In preclinical models, TIGIT blockade has been shown to increase anti-tumor NK-cell activity and $CD8^+$ T-cell cytokine production/cytotoxicity; antitumor T-cell effect was NK-cell dependent (43, 50, 51). Preclinical data suggests synergy between TIGIT blockade and PD-(L)1 blockade, a strategy implemented in clinical trials (43, 50, 52).

Tiragolumab (MTIG7192A, RG6058): Tiragolumab (Genentech) is an anti-TIGIT humanized IgG1 mAb designed to inhibit the interaction of TIGIT with CD155 (52, 53). In a phase I study, 73 patients with refractory advanced solid tumors were treated with tiragolumab alone (phase Ia, $N = 24$) or in combination with atezolizumab (phase Ib, $N = 49$) (NCT02794571; ref. 52). In phase Ia, ORR was 0% and DCR was 17%. In phase Ib, ORR was 6% ($N = 3$, all with $PD\text{-}L1^+$ tumors, 2 with NSCLC and 1 with HNSCC) and DCR was not reported. Given promising efficacy, an expansion cohort was initiated in $PD\text{-}L1^+$, checkpoint inhibitor naïve patients. This included 14 patients with NSCLC treated with tiragolumab and atezolizumab, in whom ORR was 50% and DCR was 79%. TRAEs occurred in 67% of patients in phase Ia (most common: fatigue in 38%) and 59% of patients in phase Ib (most common: anemia in 31%). Grade 3–4 TRAEs were uncommon.

In a prospective, randomized, double-blind, placebo-controlled phase II study, 135 patients with chemotherapy-naïve, $PD\text{-}L1^+$, EGFR and ALK wild-type, locally advanced/metastatic NSCLC were randomized 1:1 to atezolizumab with or without tiragolumab (CITYSCAPE, NCT03563716; ref. 54). After a median follow-up of 10.9 months, tiragolumab + atezolizumab significantly improved ORR (37% vs. 21%) and median PFS (5.6 vs. 3.9 months). Patients in the tiragolumab arm experienced similar AEs as the placebo arm. TRAEs, grade ≥3 TRAEs, and AEs leading to treatment withdrawal occurred in 81%, 15%, and 8% of patients treated with tiragolumab + atezolizumab and 72%, 19%, and 10% of patients treated with placebo + atezolizumab. Tiragolumab is currently being studied in multiple phase III clinical trials, focused on esophageal squamous cell carcinoma and NSCLC (**Table 3**).

Vibostolimab (MK-7684): Vibostolimab (Merck) is an anti-TIGIT IgG1 humanized mAb which is being evaluated in early-phase clinical trials in solid tumors, with a focus on NSCLC (55). In a phase I study, 34 patients with refractory advanced solid tumors were treated with vibostolimab alone and 47 patients in combination with pembrolizumab (NCT02964013; ref. 55). In the monotherapy and combination therapy groups, respectively, ORR was 3% and 19% and DCR was 35% and 47%. AEs occurred in 53% (6% grade ≥3) of patients treated with monotherapy and 65% (12% grade ≥3) of patients treated with combination therapy. The most common AEs were fatigue, pruritus, infusion reaction, anemia, nausea, and rash.

A 120-patient NSCLC dose expansion was initiated (NCT02964013; refs. 56, 57). Patients were treated with either vibostolimab alone or in combination with pembrolizumab and analysis was stratified by prior anti-PD-(L)1 therapy. Thus, the cohorts included anti-PD-(L)1 refractory NSCLC treated with vibostolimab alone ($N = 41$) or in combination with pembrolizumab ($N = 38$), and anti-PD-(L)1 naïve refractory NSCLC ($N = 41$). ORR was 7% in anti-PD-(L)1 refractory NSCLC treated with

Table 3. Clinical trials of NK-cell checkpoint inhibitors registered on ClinicalTrials.gov.

NCT	Status	Phase	Cancer type	In combination with	Enrollment[a]
TIGIT/CD112 + CD155					
AB154 (Arcus Biosciences, anti-TIGIT human IgG1 mAb)					
03628677	Recruiting	I	Solid cancers	Alone or zimberelimab	66
04262856	Recruiting	II	Lung Cancer	Zimberelimab or AB928	150
ASP8374 (PTZ-201) (Astellas, anti-TIGIT fully human IgG4 mAb)					
03945253	Completed	I	Solid cancers	Alone	6
03260322	Not yet open	I	Solid cancers	Alone or pembrolizumab	300
BGB-A1217 (BeiGene, anti-TIGIT humanized IgG1-variant mAb)					
04047862	Recruiting	I	Solid cancers	Tislelizumab	39
BMS-986207 (Bristol Meyers Squibb, anti-TIGIT human IgG1 mAb with null Fcγ receptor)					
04570839	Recruiting	I/II	Solid cancers	COM701 and nivolumab	100
02913313	Not yet open	I/II	Solid cancers	Alone or nivolumab	170
04150965	Recruiting	I/II	Multiple myeloma	Alone or pomalidomide and dexamethasone	104
Etigilimab (OMP-313M32) (Mereo BioPharma, anti-TIGIT IgG1 humanized mAb)					
03119428	Terminated	I	Solid cancers	Alone or nivolumab	33
SGN-TGT (SEA-TGT) (Seattle Genetics, anti-TIGIT human mAb)					
04254107	Recruiting	I	Hematologic and solid cancers	Monotherapy or pembrolizumab	111
Tiragolumab (MTIG7192A, RG6058) (Genentech, anti-TIGIT humanized IgG1/kappa mAb)					
04584112	Recruiting	I	Triple-negative breast cancer	Atezolizumab or chemo	60
04045028	Recruiting	I	Multiple myeloma, NHL	Alone, daratumumab, or rituximab	52
02794571	Recruiting	I	Solid cancers	Alone, atezolizumab, or chemo	400
04524871	Recruiting	I/II	HCC	Atezolizumab and bevacizumab	100
03281369	Recruiting	I/II	Gastric or esophageal cancer	Atezolizumab or chemo	410
03869190	Recruiting	I/II	Urothelial cancer	Atezolizumab	385
03193190	Recruiting	I/II	Pancreatic cancer	Atezolizumab or chemo	260
04300647	Recruiting	II	Cervical cancer	Atezolizumab	160
04543617	Recruiting	III	Esophageal SCC	Atezolizumab	750
04540211	Recruiting	III	Esophageal SCC	Atezolizumab or chemo	450
03563716	Not yet open	III	NSCLC	Atezolizumab	135
04294810	Recruiting	III	NSCLC	Atezolizumab	500
04513925	Recruiting	III	NSCLC	Atezolizumab	800
04256421	Recruiting	III	NSCLC	Atezolizumab or chemo	400
Vibostolimab (MK-7684) (Merck, anti-TIGIT humanized antibody)					
02964013	Recruiting	I	Solid cancers	Alone, pembrolizumab, or chemo	432
04305054	Recruiting	I/II	Melanoma	Pembrolizumab	135
04305041	Recruiting	I/II	Melanoma	Pembrolizumab and MK-1308	200
04303169	Recruiting	I/II	Melanoma	Pembrolizumab	65
04165070	Recruiting	II	Non–small cell lung cancer	Pembrolizumab or chemo	90
PVRIG/CD112					
COM701 (Compugen, anti-PVRIG humanized IgG4 mAb)					
03667716	Recruiting	I	Solid cancers	Alone or nivolumab	140
04570839	Recruiting	I/II	Solid cancers	BMS-986207 and nivolumab	100
IPH2101 (1–7F9) (Innate Pharma and Bristol-Myers-Squibb, anti-KIR2D fully human IgG4 mAb)					
01217203	Completed	I	Multiple myeloma	Lenalidomide	15
01256073	Completed	I	AML	Alone	21
00552396	Completed	I	Multiple myeloma	Alone	32
00999830	Completed	II	Multiple myeloma	Alone	27
01222286	Completed	II	Smoldering myeloma	Alone	30
01248455	Terminated	II	Smoldering myeloma	Alone	9
Lirilumab (IPH2102, BMS-986015) (Innate Pharma and Bristol-Myers-Squibb, anti-KIR2D fully human IgG4 mAb)					
01750580	Completed	I	Solid cancers	Ipilimumab	22
02252263	Completed	I	Multiple myeloma	Elotuzumab	44
03203876	Not yet open	I	Solid cancers	Nivolumab and ipilimumab	21
03532451	Not yet open	I	Bladder cancer	Nivolumab	43
03347123	Not yet open	I/II	Hematologic and solid cancers	Epacadostat and nivolumab	11
01714739	Completed	I/II	Solid cancers	Nivolumab and ipilimumab	337
02813135	Recruiting	I/II	Solid cancers	Nivolumab	397
01592370	Not yet open	I/II	Hematologic neoplasms	Nivolumab	375
01687387	Completed	II	Acute myeloid leukemia	Alone	152
02599649	Terminated	II	MDS	Alone, nivolumab, and/or azacitadine	10
02399917	Terminated	II	AML	Azacitadine	36

(Continued on the following page)

Table 3. Clinical trials of NK-cell checkpoint inhibitors registered on ClinicalTrials.gov. (Cont'd)

NCT	Status	Phase	Cancer type	In combination with	Enrollment[a]
02481297	Completed	II	Leukemia	Rituximab	7
03341936	Recruiting	II	Head and neck cancer	Nivolumab	58
			NKG2A-CD94/HLA-E		
Monalizumab (IPH2201) (Innate Pharma and AstraZeneca, humanized anti-NKG2A-CD94 IgG4 mAb)					
02459301	Completed	I	Gynecologic cancers	Alone	59
02921685	Recruiting	I	Hematologic neoplasms	Alone	18
02671435	Not yet open	I/II	Colorectal cancer	Durvalumab and cetuximab	383
02643550	Recruiting	I/II	HNSCC	Alone, cetuximab, or anti-PD(L)1	140
02557516	Terminated	I/II	Chronic lymphocytic leukemia	Alone	22
02331875	Terminated	I/II	HNSCC	Alone	3
03088059	Recruiting	II	HNSCC	Alone, durvalumab	340
04307329	Not yet open	II	Breast cancer	Trastuzumab	38
03822351	Not yet open	II	NSCLC	Durvalumab	189
03794544	Not yet open	II	NSCLC	Durvalumab	80
03833440	Recruiting	II	NSCLC	Durvalumab	120
04590963	Recruiting	III	HNSCC	Cetuximab	600

Abbreviations: AML, acute myeloid leukemia; chemo, chemotherapy; HCC, hepatocellular carcinoma; HNSCC, head/neck squamous cell carcinoma; mAb, monoclonal antibody; NCT, ClinicalTrials.gov identifier; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; SCC, squamous cell carcinoma.
[a]Projected or actual.

vibostolimab monotherapy, 5% in anti-PD-(L)1 refractory NSCLC treated with vibostolimab in combination with pembrolizumab, and 29% in anti-PD-(L)1 naïve NSCLC treated with vibostolimab in combination with pembrolizumab (with a trend toward higher ORR among patients with PD-L1 tumor proportion score ≥ 1%). Many responses were durable. Across all patients, TRAEs occurred in approximately 70% of patients and most frequently were pruritus, fatigue, rash, fever, arthralgia, and anorexia. Grade 3–4 TRAEs occurred in approximately 15% of patients and included lipase elevation, hypertension, and pneumonitis (the latter after receiving combination therapy and resulted in death).

Etigilimab (OMP-313M32): Etigilimab (Mereo BioPharma) is an anti-TIGIT IgG1 humanized mAb (58). Preclinical studies of intratumor immune cells demonstrated increased NK-cell cytotoxicity and T-cell (both $CD4^+$ and $CD8^+$) activation/infiltration (59). However, ORR was 0% and DCR 22% among 18 patients with refractory advanced solid tumors treated with etigilimab in a phase I study reported in 2018 (NCT03119428; ref. 58). This study was terminated August 2020 and no additional clinical trials are currently registered.

PVRIG/CD112

Poliovirus receptor related Ig domain containing (PVRIG, also called CD112 receptor) is an inhibitory receptor expressed on NK cells and $CD8^+$ T cells which recognizes CD112 but not CD155 on tumor cells (41, 51). Like TIGIT, PVRIG also competes with the NK cell–activating receptor DNAM-1 for binding to CD112. In preclinical studies, anti-PVRIG therapy has been shown to increase NK-cell cytotoxicity and $CD8^+$ T-cell cytokine production/cytotoxicity (51, 60).

COM701: COM701 (Compugen) is an anti-PVRIG humanized IgG4 mAb (61, 62). Twenty-eight patients with advanced refractory solid tumors were treated with COM701 with ($N = 16$) or without ($N = 12$) nivolumab in a phase I study (NCT03667716; refs. 62–64). In the entire population DCR was 57%. No patients stopped treatment due to toxicity. The most common treatment-emergent AEs (TEAE) were fatigue (46%), nausea (31%), and anxiety (23%) in patients with COM701 alone; with combination therapy, 88% of TEAEs were grade 1–2 (anemia, edema, rash, and fatigue most commonly).

KIRs/MHC-I

Killer cell Ig-like receptors (KIR) are expressed on most NK cells and a minority of T cells (primarily $CD8^+$ memory T cells) and recognize MHC-I on tumor cells (10, 65). There are numerous individual KIRs, with nomenclature determined by the number of extracellular Ig-like domains ("2D" or "3D" following "KIR") and the size of the cytoplasmic tail (long/"L" or short/"S"; refs. 65, 66). KIRs can either be activating or inhibitory, and inhibitory KIRs typically have a long cytoplasmic tail. The inhibitor KIRs include KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, and KIR3DL3 (11, 39, 65, 66). Individual KIRs bind HLA class I subgroups.

In fact, all NK cells express at least one inhibitory receptor (either a KIR or NKG2A) and normal tissues express low levels of NK cell–activating ligands, allowing for self-tolerance (67). Loss or mutation of tumor MHC-I generates an antitumor NK-cell response by responding to missing self (11, 65). On the contrary, MHC-I molecule upregulation allows cancer cells to evade immune destruction. In 1999, it was shown that KIR-HLA mismatches between donor and recipient during hematopoietic stem cell transplant for acute myeloid leukemia generated a T cell–independent graft-versus-leukemia effect, by preventing the inhibitory interaction between KIRs on donor NK cells and MHC-I on recipient cancer cells (68). Hence, the development of many anti-KIR drugs has focused on hematologic malignancies.

Combination approaches include blocking KIRs (or NKG2A-CD94, see below) with the addition of an anti-PD-(L)1 agent, anti CTLA-4 agent, or anticancer antibody (the latter increasing NK-cell ADCC; ref. 66). Blockade of CTLA-4 or PD-(L)1 can stimulate cytokine secretion from T cells (such as IL2), enhancing NK-cell function. Conversely, blockade of KIRs can stimulate cytokine secretion from NK cells (such as IFNγ), enhancing myeloid and T-cell antitumor function (40).

Lirilumab (IPH2102, BMS-986015, BMS-986015-01): Lirilumab (Innate Pharma and Bristol-Myers-Squibb), is a fully human IgG4 mAb directed against a common epitope shared by KIR2D (i.e., anti-KIR2DL1–3 effect; ref. 69). This is identical to the structure of IPH2101

(previously developed in malignant hematology) except for one mutation introduced into the constant region of the heavy chain to increase yield and prevent half-antibody formation that can occur with human IgG4 (69). Lirilumab is being evaluated in both solid and hematologic malignancies (leukemia, lymphoma, and multiple myeloma).

In a phase I study, 136 patients with refractory advanced solid tumors were treated with lirilumab in combination with nivolumab ($N = 136$, NCT01714739) or ipilimumab ($N = 22$, NCT01750580; refs. 70, 71). TRAEs occurred in approximately 70% of patients in each cohort and most commonly were fatigue, pruritus, infusion reaction, nausea/vomiting, rash, and diarrhea. Grade 3–4 TRAEs occurred in 13% of the nivolumab cohort and 9% of the ipilimumab cohort, including grade 4 thrombocytopenia ($N = 1$), grade 3 pancreatitis ($N = 1$), and grade 3 radiation skin injury ($N = 1$). Overall toxicity in the nivolumab cohort was similar to nivolumab monotherapy, with the exception of manageable infusion reactions. Among 29 response-evaluable patients with platinum-refractory HNSCC, ORR was 24% and DCR was 52%.

A separate phase I trial included patients with both hematologic ($N = 22$) and solid malignancies (breast $N = 6$, ovarian $N = 7$, pancreatic $N = 1$, and endometrial $N = 1$) treated with lirilumab (EudraCT 2009-011526-33; ref. 69). Among all patients (including those with hematologic malignancy), any grade TRAEs were primarily mild and occurred in 68% of patients. Most commonly these were pruritus (19%), asthenia (16%), fatigue (14%), infusion reaction (14%), and headache (11%). Grade 3–4 TRAEs occurred in 19% of patients and included elevated lipase, lymphopenia, presyncope, elevated bilirubin, abnormal liver function tests, urticaria, and angioedema. Among only solid tumor patients, ORR was 60% (4 breast cancer, 4 ovarian cancer, and 1 pancreas cancer). mPFS was not reached for breast cancer and 5.3 months for ovarian cancer. Additional phase I–II trials are currently recruiting patients with solid tumors (**Table 3**).

NKG2A-CD94/HLA-E

NK group 2 member A (NKG2A) and CD94 form a heterodimeric ITIM-containing (on NKG2A) inhibitory receptor, which is expressed on cytotoxic lymphocytes (NK cells and a subset of $CD8^+$ T cells; refs. 10, 72–74). NKG2A is the first inhibitory receptor expressed during NK-cell development (66). After differentiation, NK cells may coexpress NKG2A with KIRs or NKG2A may be lost (66). The NKG2A-CD94 heterodimer recognizes the nonclassical MHC-I HLA-E, which is upregulated on both hematologic and solid tumors (10, 39, 43, 72). In contrast, classical MHC-I molecules are often downregulated on tumor, allowing immune evasion (73). Increased tumor expression of HLA-E has been associated with decreased NK-cell cytotoxicity and worse prognosis in both hematologic and solid cancers (39, 43). Synergistic antitumor effect with PD-(L)1 blockade has been noted in preclinical models, an effect which is dependent on both NK and $CD8^+$ T cells (72).

Monalizumab (IPH2201): Monalizumab (Innate Pharma and AstraZeneca) is a humanized IgG4 mAb which binds to the NKG2A-CD94 heterodimeric receptor, blocking its interaction with HLA-E and promoting effector NK- and $CD8^+$ T-cell functions (39, 73). In preclinical studies, monalizumab alone promoted NK-cell cytotoxicity, monolizumab with durvalumab promoted NK- and $CD8^+$ T-cell effector functions, and monalizumab with cetuximab enhanced NK cell–mediated ADCC (72, 73). Monalizumab is being evaluated in solid tumors (primarily colorectal cancer, HNSCC, NSCLC, and gynecologic cancer), often in combination with anticancer antibodies and/or anti-PD-(L)1 agents.

Initially, 55 patients with refractory advanced solid tumors [including 40 patients with microsatellite stable (MSS) metastatic colorectal cancer (mCRC) in dose expansion] were treated with monalizumab and durvalumab (NCT02671435; ref. 75). In the colorectal cancer expansion cohort, among 37 response-evaluable patients, ORR was 8% and DCR was 38%. In the entire population, 48% had any grade TRAEs (diarrhea most common) and grade ≥3 TRAEs were rare.

Development in colorectal cancer was continued. In a phase I/II study of treatment-naïve MSS mCRC, 18 patients were treated with monalizumab + durvalumab + FOLFOX + bevacizumab and 17 patients were treated with monalizumab + durvalumab + FOLFOX + cetuximab (RAS/BRAF wild-type and left-sided tumor only; NCT02671435; refs. 76, 77). ORR and DCR, respectively, were 41% and 88% in the bevacizumab cohort and 53% and 88% in the cetuximab cohort. Any TEAE occurred in nearly all patients. In the bevacizumab cohort, the most common TEAEs were fatigue, nausea, and peripheral neuropathy (any TEAE grade ≥3 78%). In the cetuximab cohort, the most common TEAEs were peripheral neuropathy, rash, and dermatitis acneiform (any TEAE grade ≥3 71%).

Monalizumab monotherapy was also evaluated in a phase II trial in refractory advanced HNSCC, where 27 patients [100% prior platinum, 59% prior anti-PD-(L)1 agent] (UPSTREAM, NCT03088059; ref. 78). The site of primary tumor included oral cavity, oropharynx, hypopharynx, and larynx. ORR was 0%, DCR was 22%, median PFS was 7.4 weeks, and median OS was 28 weeks. Grade 3 or higher TEAEs occurred in 59% of patients; however, none were attributed to treatment. Prespecified ORR was not met and the study was closed at interim analysis for futility.

Development in HNSCC was continued, but in combination with cetuximab. In a multicenter phase I/II trial, 40 patients with recurrent or metastatic HNSCC [100% prior platinum, 45% prior anti-PD-(L)1 agent, 13% prior cetuximab, and no more than two prior lines of therapy for advanced disease] were treated with monalizumab and cetuximab (NCT02643550; refs. 79–81). ORR was 28% [36% in anti-PD-(L)1-naïve patients and 17% in anti-PD-(L)1-experienced patients]. Among all patients, median duration of response was 5.6 months, median PFS was 4.5 months, and median OS was 8.3 months. Treatment was well tolerated. The most common monalizumab-related AEs were fatigue (17%), pyrexia (13%), and headache (10%). Six percent of TRAEs were grade 3–4. In a separate expansion cohort, 40 patients with recurrent or metastatic HNSCC previously treated with both platinum and an anti-PD-(L)1 agent were treated with monalizumab and cetuximab, and early results show ORR 20% (all PR) (82). A randomized phase III trial of monalizumab and cetuximab is recruiting in this population (**Table 3**).

In addition, 58 patients with refractory advanced gynecologic malignancies (platinum-sensitive ovarian, platinum-resistant ovarian, squamous cervical, and epithelial endometrial carcinoma) were treated with monalizumab monotherapy (NCT02459301; ref. 83). Antitumor efficacy was poor. ORR and DCR, respectively, in the dose-ranging cohort were 0% and 41% and in the dose-expansion cohort were 0% and 18%. Overall treatment was well tolerated and none of the AEs were felt to be immune mediated. The most common TRAEs were headache, fatigue, nausea/vomiting, and abdominal pain (>15% each). Grade ≥3 TRAEs included lymphopenia (19%), anemia (16%), vomiting (5%), fatigue (3%), and neutropenia (2%).

Discussion

While immune checkpoint inhibitors targeting the PD-(L)1 and CTLA-4 axes have revolutionized cancer treatment, novel

immune-based therapies are desperately needed. Innate immune checkpoint inhibitors alone or in combination (with anticancer antibodies or adaptive immune checkpoint inhibitors) are a promising novel anticancer strategy. These drugs can unleash the innate immune system against tumor, generating phagocytosis and natural cytotoxicity (both of which can be enhanced with the addition of an anticancer antibody), and synergistically activate the adaptive immune system via antigen presentation (often combining well with adaptive immune checkpoint inhibitors).

As repeatedly noted, there is considerable overlap in checkpoint expression and effects of checkpoint modulation between the innate and adaptive immune systems. TIM-3 and LAG-3 are inhibitory receptors expressed on both innate and adaptive immune cells (10, 11, 84, 85). While they serve primary as adaptive immune checkpoints (with blockade resulting in T-cell stimulation), there is evidence of innate immune effects (86–89). There are many areas of ongoing and future research, and studies may elucidate a larger role of these checkpoints in antitumor efficacy.

Phagocytosis and NK-cell checkpoint inhibitors are currently being evaluated in clinical trials with solid tumor safety and efficacy data summarized here. In specific solid tumor types, several agents have demonstrated promising early efficacy signals (ALX148 in HNSCC and gastric cancer, tiragolumab and vibostolimab in NSCLC, and monalizumab in colorectal cancer and HNSCC). Currently, phase III trials are underway for tiragolumab (esophageal SCC and NSCLC) and monalizumab (HNSCC). While clinical development is still early and ongoing monitoring is needed, innate immune checkpoint inhibitors (as monotherapy) do not appear to frequently cause immune-related AEs, as inhibitors of PD-(L)1 and CTLA-4 do, likely due to low expression of prophagocytic signals on normal tissues (90, 91). In addition, strategies have been developed to mitigate on-target toxicities, such as cytopenias seen with anti-CD47 agents.

The development of innate immune checkpoint inhibitors continues at a rapid pace. **Tables 2** and **3** list the active clinical trials on ClinicalTrials.gov and there are many additional compounds in preclinical development. The use of novel genetic screening techniques may identify additional cell-surface regulators of innate immune cell function, serving as new therapeutic targets (92). Innate immune checkpoint inhibitors are an exciting new therapeutic class in both solid and hematologic malignancies that have the potential to expand the use of immunotherapy in many cancer types, significantly improving patient outcomes.

Authors' Disclosures

R.W. Lentz reports grants from NIH during the research and preparation of the manuscript. S.S. Mitra reports nonfinancial support from 47Inc during the research and preparation of the manuscript; in addition, S.S. Mitra has a patent for Treatment of Pediatric Brain Tumors with Targeting of CD47 Pathway pending to Siddhartha Mitra. W.A. Messersmith reports other from ALX Therapeutics during the research and preparation of the manuscript. No disclosures were reported by the other authors.

Acknowledgments

This work was supported by NIH NRSA T32CA236734 (to R.W. Lentz).

Received January 15, 2021; revised March 1, 2021; accepted March 29, 2021; published first April 13, 2021.

References

1. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity 2013;39:1–10.
2. Puccini A, Battaglin F, Iaia ML, Lenz HJ, Salem ME. Overcoming resistance to anti-PD1 and anti-PD-L1 treatment in gastrointestinal malignancies. J Immunother Cancer 2020;8:e000404.
3. Haslam A, Prasad V. Estimation of the percentage of US patients with cancer who are eligible for and respond to checkpoint inhibitor immunotherapy drugs. JAMA Netw Open 2019;2:e192535.
4. Feng M, Jiang W, Kim BYS, Zhang CC, Fu Y-X, Weissman IL. Phagocytosis checkpoints as new targets for cancer immunotherapy. Nat Rev Cancer 2019;19:568–86.
5. Jenkins RW, Barbie DA, Flaherty KT. Mechanisms of resistance to immune checkpoint inhibitors. Br J Cancer 2018;118:9–16.
6. Pages F, Galon J, Dieu-Nosjean MC, Tartour E, Sautes-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene 2010;29:1093–102.
7. Barnes TA, Amir E. HYPE or HOPE: the prognostic value of infiltrating immune cells in cancer. Br J Cancer 2017;117:451–60.
8. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou F-S, Bifulco C, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet 2018;391:2128–39.
9. Broz ML, Binnewies M, Boldajipour B, Nelson AE, Pollack JL, Erle DJ, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. Cancer Cell 2014;26:638–52.
10. Demaria O, Cornen S, Daeron M, Morel Y, Medzhitov R, Vivier E. Harnessing innate immunity in cancer therapy. Nature 2019;574:45–56.
11. Rothlin CV, Ghosh S. Lifting the innate immune barriers to antitumor immunity. J Immunother Cancer 2020;8:e000695.
12. Savina A, Amigorena S. Phagocytosis and antigen presentation in dendritic cells. Immunol Rev 2007;219:143–56.
13. Barry KC, Hsu J, Broz ML, Cueto FJ, Binnewies M, Combes AJ, et al. A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments. Nat Med 2018;24:1178–91.
14. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature 2017;545:495–9.
15. Logtenberg MEW, Jansen JHM, Raaben M, Toebes M, Franke K, Brandsma AM, et al. Glutaminyl cyclase is an enzymatic modifier of the CD47-SIRPalpha axis and a target for cancer immunotherapy. Nat Med 2019;25:612–9.
16. Chao MP, Jaiswal S, Weissman-Tsukamoto R, Alizadeh AA, Gentles AJ, Volkmer J, et al. Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47. Sci Transl Med 2010;2:63ra94.
17. Willingham SB, Volkmer J-P, Gentles AJ, Sahoo D, Dalerba P, Mitra SS, et al. The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. Proc Natl Acad Sci U S A 2012;109:6662–7.
18. Advani R, Flinn I, Popplewell L, Forero A, Bartlett NL, Ghosh N, et al. CD47 blockade by Hu5F9-G4 and rituximab in non-hodgkin's lymphoma. N Engl J Med 2018;379:1711–21.
19. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol 2014;5:520.
20. Sikic BI, Lakhani N, Patnaik A, Shah SA, Chandana SR, Rasco D, et al. First-in-human, first-in-class phase I trial of the anti-CD47 antibody Hu5F9-G4 in patients with advanced cancers. J Clin Oncol 2019;37:946–53.
21. Kauder SE, Kuo TC, Harrabi O, Chen A, Sangalang E, Doyle L, et al. ALX148 blocks CD47 and enhances innate and adaptive antitumor immunity with a favorable safety profile. PLoS One 2018;13:e0201832.
22. Lakhani NJ, LoRusso P, Hafez N, Krishnamurthy A, O'Rourke TJ, Kamdar MK, et al. A phase 1 study of ALX148, a CD47 blocker, alone and in combination with established anticancer antibodies in patients with advanced malignancy and non-Hodgkin lymphoma. J Clin Oncol 36:15s, 2018(suppl; abstr 3068).
23. Chow LQM, Gainor JF, Lakhani NJ, Chung HC, Lee K-W, Lee J, et al. A phase I study of ALX148, a CD47 blocker, in combination with established anticancer antibodies in patients with advanced malignancy. J Clin Oncol 37:15s, 2019 (suppl; abstr 2514).

24. Chow LQM, Gainor JF, Lakhani NJ, Lee KW, Chung HC, Lee J, et al. A phase I study of ALX148, a CD47 blocker, in combination with standard anticancer antibodies and chemotherapy regimens in patients with advanced malignancy. J Clin Oncol 38:15s, 2020 (suppl; abstr 3056).
25. Lakhani NJ, Patnaik A, Liao JB, Moroney JW, Miller DS, Fleming GF, et al. A phase Ib study of the anti-CD47 antibody magrolimab with the PD-L1 inhibitor avelumab (A) in solid tumor (ST) and ovarian cancer (OC) patients. J Clin Oncol 38:5s, 2020 (suppl; abstr 18).
26. Fisher GA, Lakhani NJ, Eng C, Hecht JR, Bendell JC, Philip PA, et al. A phase Ib/II study of the anti-CD47 antibody magrolimab with cetuximab in solid tumor and colorectal cancer patients. J Clin Oncol 38:4s, 2020 (suppl; abstr 114).
27. Peluso MO, Adam A, Armet CM, Zhang Li, O'Connor RW, Lee BH, et al. The Fully human anti-CD47 antibody SRF231 exerts dual-mechanism antitumor activity via engagement of the activating receptor CD32a. J Immunother Cancer 2020;8:e000413.
28. Holland PM, Normant E, Adam A, Armet CM, O'Connor RW, Lake AC, et al. CD47 monoclonal antibody SRF231 is a potent inducer of macrophage-mediated tumor cell phagocytosis and reduces tumor burden in murine models of hematologic malignancies. Blood 2016;128:1843.
29. Valentin R, Peluso MO, Lehmberg TZ, Adam A, Zhang Li, Armet CM, et al. The fully human anti-CD47 antibody SRF231 has dual-mechanism antitumor activity against chronic lymphocytic leukemia (CLL) Cells and increases the activity of both rituximab and venetoclax. Blood 2018;132:4393.
30. Patnaik A, Spreafico A, Paterson AM, Peluso M, Chung J-Ku, Bowers B, et al. Results of a first-in-human phase I study of SRF231, a fully human, high-affinity anti-CD47 antibody. J Clin Oncol 38:15s, 2020 (suppl; abstr 3064).
31. Meng Z, Wang Z, Guo B, Cao W, Shen H. TJC4, a differentiated Anti-CD47 antibody with novel epitope and RBC sparing properties. Blood 2019;134:4063.
32. Berlin J, Harb W, Adjei A, Xing Y, Swiecicki P, Seetharam M, et al. 385 A first-in-human study of lemzoparlimab, a differentiated anti-CD47 antibody, in subjects with relapsed/refractory malignancy: initial monotherapy results. J Immunother Cancer 2020;8:A410.
33. Lakhani N, Orloff M, Fu S, Liu Y, Wang Y, Zhou H, et al. 295 First-in-human phase I trial of IBI188, an anti-CD47 targeting monoclonal antibody, in patients with advanced solid tumors and lymphomas. J Immunother Cancer 2020; 8:A322.
34. Maher J, Davies ET. Targeting cytotoxic T lymphocytes for cancer immunotherapy. Br J Cancer 2004;91:817–21.
35. Barkal AA, Weiskopf K, Kao KS, Gordon SR, Rosental B, Yiu YY, et al. Engagement of MHC class I by the inhibitory receptor LILRB1 suppresses macrophages and is a target of cancer immunotherapy. Nat Immunol 2018;19: 76–84.
36. Chen H-M, van der Touw W, Wang YS, Kang K, Mai S, Zhang J, et al. Blocking immunoinhibitory receptor LILRB2 reprograms tumor-associated myeloid cells and promotes antitumor immunity. J Clin Invest 2018;128:5647–62.
37. Zuniga LA, Joyce-Shaikh B, Wilson DC, Cherwinski H, Chen Y, Jeff G, et al. Preclinical characterization of a first-in-class ILT4 antagonist, MK-4830. J Immunother Cancer 2018;6(Suppl 1):115.
38. Siu LL, Wang D, Hilton J, Geva R, Rasco D, Abraham AK, et al. 524O Initial results of a phase I study of MK-4830, a first-in-class anti-immunoglobulin-like transcript 4 (ILT4) myeloid-specific antibody in patients (pts) with advanced solid tumours. Ann Oncol 2020;31:S462.
39. Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. Nat Rev Immunol 2018;18:671–88.
40. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. Nat Rev Cancer 2016;16:7–19.
41. Sanchez-Correa B, Valhondo I, Hassouneh F, Lopez-Sejas N, Pera A, Bergua JM, et al. DNAM-1 and the TIGIT/PVRIG/TACTILE axis: novel immune checkpoints for natural killer cell-based cancer immunotherapy. Cancers 2019;11:877.
42. Böttcher JP, Bonavita E, Chakravarty P, Blees H, Cabeza-Cabrerizo M, Sammicheli S, et al. NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. Cell 2018;172:1022–37.
43. Hodgins JJ, Khan ST, Park MM, Auer RC, Ardolino M. Killers 2.0: NK cell therapies at the forefront of cancer control. J Clin Invest 2019;129:3499–510.
44. Tartter PI, Steinberg B, Barron DM, Martinelli G. The prognostic significance of natural killer cytotoxicity in patients with colorectal cancer. Arch Surg 1987;122: 1264–8.
45. Schantz SP, Campbell BH, Guillamondegui OM. Pharyngeal carcinoma and natural killer cell activity. Am J Surg 1986;152:467–74.
46. Schantz SP, Savage HE, Racz T, Taylor DL, Sacks PG. Natural killer cells and metastases from pharyngeal carcinoma. Am J Surg 1989;158:361–6.
47. Schantz SP, Ordonez NG. Quantitation of natural killer cell function and risk of metastatic poorly differentiated head and neck cancer. Nat Immun Cell Growth Regul 1991;10:278–88.
48. Remark R, Alifano M, Cremer I, Lupo A, Dieu-Nosjean M-C, Riquet M, et al. Characteristics and clinical impacts of the immune environments in colorectal and renal cell carcinoma lung metastases: influence of tumor origin. Clin Cancer Res 2013;19:4079–91.
49. Delahaye NF, Rusakiewicz S, Martins I, Ménard C, Roux S, Lyonnet L, et al. Alternatively spliced NKp30 isoforms affect the prognosis of gastrointestinal stromal tumors. Nat Med 2011;17:700–7.
50. Zhang Q, Bi J, Zheng X, Chen Y, Wang H, Wu W, et al. Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. Nat Immunol 2018;19:723–32.
51. Whelan S, Ophir E, Kotturi MF, Levy O, Ganguly S, Leung L, et al. PVRIG and PVRL2 are induced in cancer and inhibit CD8(+) T-cell function. Cancer Immunol Res 2019;7:257–68.
52. Bendell JC, Bedard P, Bang Y-J, LoRusso P, Hodi S, Gordon M, et al. Phase Ia/Ib dose-escalation study of the anti-TIGIT antibody tiragolumab as a single agent and in combination with atezolizumab in patients with advanced solid tumors [abstract]. In: Proceedings of the Annual Meeting of the American Association for Cancer Research 2020; 2020 Apr 27–28 and Jun 22–24. Philadelphia (PA): AACR; Cancer Res 2020;80(16 Suppl):Abstract nr CT302.
53. Caruso C. Tiragolumab impresses in multiple trials. Cancer Discov 2020;10: 1086–7.
54. Rodriguez-Abreu D, Johnson ML, Hussein MA, Cobo M, Patel AJ, Secen NM, et al. Primary analysis of a randomized, double-blind, phase II study of the anti-TIGIT antibody tiragolumab (tira) plus atezolizumab (atezo) versus placebo plus atezo as first-line (1L) treatment in patients with PD-L1-selected NSCLC (CITYSCAPE). J Clin Oncol 38:15s, 2020 (suppl; abstr 9503).
55. Golan T, Bauer TM, Jimeno A, Perets R, Niu J, Lee J, et al. TIGIT antibody MK-7684 as monotherapy and in combination with pembrolizumab in patients with advanced solid tumors. J Immunother Cancer 2018;6 (Suppl 1):115.
56. Ahn M-J, Niu J, Kim D-W, Rasco D, Mileham KF, Chung HC, et al. 1400P Vibostolimab, an anti-TIGIT antibody, as monotherapy and in combination with pembrolizumab in anti-PD-1/PD-L1-refractory NSCLC. Ann Oncol 2020; 31:S887.
57. Niu J, Nagrial A, Voskoboynik M, Chung HC, Lee DH, Ahn M-J, et al. 1410P Safety and efficacy of vibostolimab, an anti-TIGIT antibody, plus pembrolizumab in patients with anti-PD-1/PD-L1-naive NSCLC. Ann Oncol 2020;31: S891–2.
58. Sharma S, Moore K, Mettu N, Garrido-Laguna I, Ulahannan S, Khemka V, et al. Initial results from a phase 1a/b study of etigilimab (OMP-313M32), an anti-T cell immunoreceptor with Ig and ITIM domains (TIGIT) antibody, in advanced solid tumors. J Immunother Cancer 2018;6(Suppl 1):114.
59. Argast GM, Cancillia B, Cattaruzza F, Yeung P, le Scolan E, Harris R, et al. Anti-TIGIT biomarker study: Inhibition of TIGIT induces loss of Tregs from tumors and requires effector function for tumor growth inhibition [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2018; 2018 Apr 14–18; Chicago, IL. Philadelphia (PA): AACR; Cancer Res 2018;78(13 Suppl):Abstract nr 5627.
60. Xu F, Sunderland A, Zhou Y, Schulick RD, Edil BH, Zhu Y. Blockade of CD112R and TIGIT signaling sensitizes human natural killer cell functions. Cancer Immunol Immunother 2017;66:1367–75.
61. Vaena D, Patnaik A, Hamilton E, Olweny J, Hunter J, Adewoye H, et al. Phase I study of COM701 (a novel checkpoint inhibitor of PVRIG) in patients with advanced solid tumors [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2019; 2019 Mar 29–Apr 3; Atlanta, GA. Philadelphia (PA): AACR; Cancer Res 2019;79(13 Suppl):Abstract nr CT168.
62. Sullivan R, Rasco D, Lim E, Sharma M, Shepard D, Patnaik A, et al. COM701 demonstrates preliminary antitumor activity as monotherapy and in combination with nivolumab in patients with advanced solid tumors [abstract]. In: Proceedings of the Annual Meeting of the American Association for Cancer Research 2020; 2020 Apr 27–28 and Jun 22–24. Philadelphia (PA): AACR; Cancer Res 2020;80(16 Suppl):Abstract nr CT031.
63. Dumbrava EI, Fleming GF, Hamilton E, Sullivan R, Patnaik M, Papadopoulos K, et al. Phase 1 study of the safety, tolerability and preliminary anti-tumor activity of COM701 monotherapy in patients with advanced solid tumors. J Immunother Cancer 2019;7(Suppl 1):282.

64. Dumbrava EI, Fleming GF, Hamilton E, Sullivan R, Patnaik M, Papadopoulos K, et al. Phase 1 study of the safety, tolerability and preliminary anti-tumor activity of COM701 monotherapy in patients with advanced solid tumors. J Immunother Cancer 2019;7(Suppl 1):282.
65. Parham P. Immunogenetics of killer cell immunoglobulin-like receptors. Mol Immunol 2005;42:459–62.
66. Pende D, Falco M, Vitale M, Cantoni C, Vitale C, Munari E, et al. Killer Ig-like receptors (KIRs): their role in NK cell modulation and developments leading to their clinical exploitation. Front Immunol 2019;10:1179.
67. French AR, Yokoyama WM. Natural killer cells and autoimmunity. Arthritis Res Ther 2004;6:8–14.
68. Ruggeri L, Capanni M, Casucci M, Volpi I, Tosti A, Perruccio K, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood 1999;94:333–9.
69. Vey N, Karlin L, Sadot-Lebouvier S, Broussais F, Berton-Rigaud D, Rey J, et al. A phase 1 study of lirilumab (antibody against killer immunoglobulin-like receptor antibody KIR2D; IPH2102) in patients with solid tumors and hematologic malignancies. Oncotarget 2018;9:17675–88.
70. Segal NH, Infante JR, Sanborn RE, Gibney GT, Lawrence DP, Rizvi N, et al. 1086P - Safety of the natural killer (NK) cell-targeted anti-KIR antibody, lirilumab (liri), in combination with nivolumab (nivo) or ipilimumab (ipi) in two phase 1 studies in advanced refractory solid tumors. Ann Oncol 2016; 27:vi372.
71. Leidner R, Kang H, Haddad R, Segal NH, Wirth LJ, Ferris RL, et al. Preliminary efficacy from a phase I/II study of the natural killer cell–targeted antibody lirilumab in combination with nivolumab in squamous cell carcinoma of the head and neck. J Immunother Cancer 2016;4:91.
72. van Hall T, André P, Horowitz A, Ruan DFu, Borst L, Zerbib R, et al. Monalizumab: inhibiting the novel immune checkpoint NKG2A. J Immunother Cancer 2019;7:263.
73. André P, Denis C, Soulas C, Bourbon-Caillet C, Lopez J, Arnoux T, et al. Anti-NKG2A mAb is a checkpoint inhibitor that promotes anti-tumor immunity by unleashing both T and NK cells. Cell 2018;175:1731–43.
74. Creelan BC, Antonia SJ. The NKG2A immune checkpoint - a new direction in cancer immunotherapy. Nat Rev Clin Oncol 2019;16:277–8.
75. Segal NH, Naidoo J, Curigliano G, Patel S, Sahebjam S, Papadopoulos KP, et al. First-in-human dose escalation of monalizumab plus durvalumab, with expansion in patients with metastatic microsatellite-stable colorectal cancer. J Clin Oncol 36:15s, 2018 (suppl; abstr 3540).
76. Cho M, Bendell JC, Han S, Naidoo J, Lieu C, Carneiro BA, et al. Durvalumab + monalizumab, mFOLOFX6, and bevacizumab in patients (pts) with metastatic microsatellite-stable colorectal cancer (MSS-CRC). Ann Oncol 2019; 30:v475–532.
77. Wainberg ZA, Diamond JR, Curigliano G, Deva S, Bendell JC, Han S-W, et al. First-line durvalumab + monalizumab, mFOLFOX6, and bevacizumab or cetuximab for metastatic microsatellite-stable colorectal cancer (MSS-CRC). J Clin Oncol 38:4s, 2020 (suppl; abstr 128).
78. Galot R, Le Tourneau C, Saada-Bouzid E, Daste A, Even C, Debruyne PR, et al. A phase II study of monalizumab in patients with recurrent/metastatic (RM) squamous cell carcinoma of the head and neck (SCCHN): results of the I1 cohort of the EORTC-HNCG-1559 trial (UPSTREAM). Ann Oncol 2019;30:v449–50.
79. Cohen R, Fayette J, Posner M, Lefebvre G, Bauman J, Salas S, et al. Phase II study of monalizumab, a first-in-class NKG2A monoclonal antibody, in combination with cetuximab in previously treated recurrent or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN): preliminary assessment of safety and efficacy [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2018; 2018 Apr 14–18; Chicago, IL. Philadelphia (PA): AACR; Cancer Res 2018;78(13 Suppl):Abstract nr CT158.
80. Fayette J, Lefebvre G, Posner MR, Bauman J, Salas S, Even C, et al. Results of a phase II study evaluating monalizumab in combination with cetuximab in previously treated recurrent or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN). Ann Oncol 2018;29:viii374.
81. Cohen RB, Lefebvre G, Posner MR, Bauman JR, Salas S, Even C, et al. Monalizumab in combination with cetuximab in patients (pts) with recurrent or metastatic (R/M) head and neck cancer (SCCHN) previously treated or not with PD-(L)1 inhibitors (IO): 1-year survival data. Ann Oncol 2019;30: v460.
82. Cohen RB, Bauman JR, Salas S, Colevas AD, Even C, Cupissol D, et al. Combination of monalizumab and cetuximab in recurrent or metastatic head and neck cancer patients previously treated with platinum-based chemotherapy and PD-(L)1 inhibitors. J Clin Oncol 38:15s, 2020 (suppl; abstr 6516).
83. Tinker AV, Hirte HW, Provencher D, Butler M, Ritter H, Tu D, et al. Dose-ranging and cohort-expansion study of monalizumab (IPH2201) in patients with advanced gynecologic malignancies: a trial of the Canadian Cancer Trials Group (CCTG): IND221. Clin Cancer Res 2019;25:6052–60.
84. Qin S, Xu L, Yi M, Yu S, Wu K, Luo S. Novel immune checkpoint targets: moving beyond PD-1 and CTLA-4. Mol Cancer 2019;18:155.
85. Andrews LP, Marciscano AE, Drake CG, Vignali DA. LAG3 (CD223) as a cancer immunotherapy target. Immunol Rev 2017;276:80–96.
86. Xu L, Huang Y, Tan L, Yu W, Chen D, Lu C, et al. Increased Tim-3 expression in peripheral NK cells predicts a poorer prognosis and Tim-3 blockade improves NK cell-mediated cytotoxicity in human lung adenocarcinoma. Int Immunopharmacol 2015;29:635–41.
87. Chiba S, Baghdadi M, Akiba H, Yoshiyama H, Kinoshita I, Dosaka-Akita H, et al. Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. Nat Immunol 2012;13:832–42.
88. Andrews LP, Yano H, Vignali DAA. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. Nat Immunol 2019;20: 1425–34.
89. da Silva IP, Gallois A, Jimenez-Baranda S, Khan S, Anderson AC, Kuchroo VK, et al. Reversal of NK-cell exhaustion in advanced melanoma by Tim-3 blockade. Cancer Immunol Res 2014;2:410–22.
90. Ramos-Casals M, Brahmer JR, Callahan MK, Flores-Chávez A, Keegan N, Khamashta MA, et al. Immune-related adverse events of checkpoint inhibitors. Nat Rev Dis Primers 2020;6:38.
91. King GT, Sharma P, Davis SL, Jimeno A. Immune and autoimmune-related adverse events associated with immune checkpoint inhibitors in cancer therapy. Drugs Today 2018;54:103–22.
92. Haney MS, Bohlen CJ, Morgens DW, Ousey JA, Barkal AA, Tsui CK, et al. Identification of phagocytosis regulators using magnetic genome-wide CRISPR screens. Nat Genet 2018;50:1716–27.