Seminars in Cancer Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

Emerging targets in cancer immunotherapy

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ARTICLE INFO

Keywords:
Immuno-oncology
Checkpoints
Tumor-infiltrating lymphocytes
Macrophages
Natural killer

ABSTRACT

The first generation of immune checkpoint inhibitors (anti-CTLA-4 and anti-PD-1/PD-L1) targeted natural immune homeostasis pathways, co-opted by cancers, to drive anti-tumor immune responses. These agents led to unprecedented results in patients with previously incurable metastatic disease and may become first-line therapies for some advanced cancers. However, these agents are efficacious in only a minority of patients. Newer strategies are becoming available that target additional immunomodulatory mechanisms to activate patients' own anti-tumor immune responses. Herein, we present a succinct summary of emerging immune targets with reported pre-clinical efficacy that have progressed to active investigation in clinical trials. These emerging targets include co-inhibitory and co-stimulatory markers of the innate and adaptive immune system. In this review, we discuss: 1) T lymphocyte markers: Lymphocyte Activation Gene 3 [LAG-3], T-cell Immunoglobulin- and Mucin-domain-containing molecule 3 [TIM-3], V-domain containing Ig Suppressor of T cell Activation [VISTA], T cell ImmunoGlobulin and ITIM domain [TIGIT], B7-H3, Inducible T-cell Co-stimulator [ICOS/ICOS-L], CD27/CD70, and Glucocorticoid-Induced TNF Receptor [GITR]; 2) macrophage markers: CD47/Signal-Regulatory Protein alpha [SIRPα] and Indoleamine-2,3-Dioxygenase [IDO]; and 3) natural killer cell markers: CD94/NKG2A and the Killer Immunoglobulin-like receptor [KIR] family. Finally, we briefly highlight combination strategies and potential biomarkers of response and resistance to these cancer immunotherapies.

1. Introduction

Cancer immunotherapy is now considered a pillar of cancer treatment, alongside surgery, chemotherapy, and radiation. Ipilimumab and nivolumab/pembrolizumab are among the earliest immune checkpoint inhibitors (targeting CTLA-4 and PD-1, respectively) and are now moving from second-line to become first-line therapies of choice in advanced non-small cell lung cancer and melanoma [1,2]. Treatment with these agents can induce resistance through upregulation of additional immune checkpoints, highlighting a need for new antitumor immune activating agents [3]. Emerging drugs target not only lymphocytes associated with adaptive immunity − via blockade of immune-inhibitory checkpoints or as agonists of immunostimulatory pathways − but also innate immune processes mediated by macrophages and natural killer (NK) cells, pathways of broad relevance across many types of solid and hematopoietic cancers (markers summarized in Fig. 1). The following emerging immune targets in cancer immunotherapy were selected based on their advanced stage of development in preclinical/clinical studies and on the limited number of review articles available describing some of these targets.

2. Adaptive immunity

2.1. Inhibitory lymphocyte receptors

2.1.1. LAG-3

Lymphocyte Activation Gene 3 (LAG-3) is a surface receptor expressed on activated T cells, an exhaustion marker with immunosuppressive activity. Major histocompatibility complex class II (MHC-II) is a ligand for LAG-3; additional ligands (*e.g.*, L-selectin and galectin-3) have also been identified [4]. Regulatory T cells (Tregs) expressing LAG-3 have enhanced suppressive activity, whereas cytotoxic CD8+ T cells expressing LAG-3 have reduced proliferation rates and effector cytokine production in cancer and autoimmune diabetes [5–7]. A splice variant of LAG-3 cleaved by metalloproteinases and secreted in the cellular microenvironment has immune-activating properties when bound to MHC-II on antigen presenting cells [8].

LAG-3+ tumor-infiltrating lymphocytes (TILs) have been reported in melanoma, colon, pancreatic, breast, lung, hematopoietic, and head and neck cancer patients [9–15], in association with aggressive clinical features. Antibody-based LAG-3 blockade in multiple cancer mouse models restores CD8+ effector T cells and diminishes Treg populations,

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http://dx.doi.org/10.1016/j.semcancer.2017.10.001
Received 7 July 2017; Received in revised form 29 September 2017; Accepted 1 October 2017

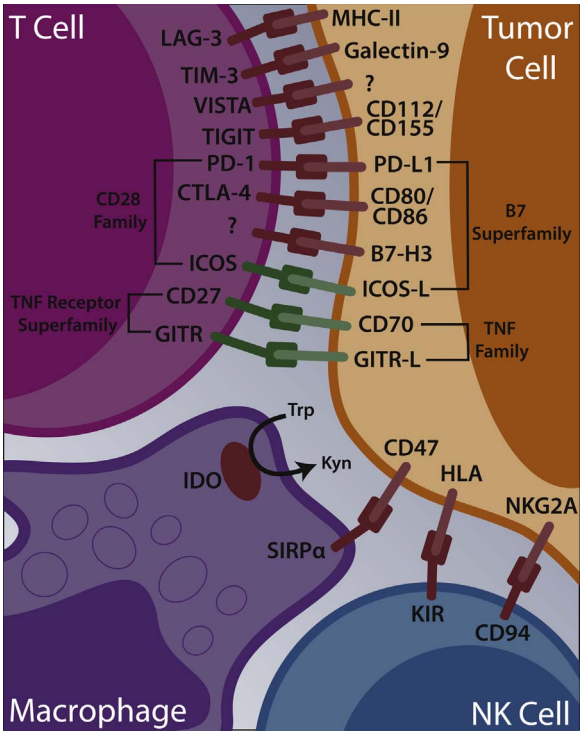

Fig. 1. Overview of emerging targets for cancer immunotherapy. Immune inhibitory interactions are marked in red, and immune co-stimulatory interactions are marked in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

an effect enhanced when combined with anti-PD-1 [16,17]. A recent study in a metastatic ovarian cancer mouse model showed that LAG-3 blockade leads to upregulation of other immune checkpoints (PD-1, CTLA-4, and TIM-3), and combination therapy targeting LAG-3, PD-1, and CTLA-4 increases functional cytotoxic T cell levels while reducing Tregs and myeloid-derived suppressor cells [18].

Multiple early phase clinical trials are testing antagonistic LAG-3 agents in combination with anti-PD-1 and/or anti-CTLA-4 therapy (Table 1). In view of the activating properties of soluble secreted LAG-3, a soluble agonist LAG-3 antibody (IMP321) was tested in advanced solid malignancies as a single agent [19], and demonstrated sufficient tolerability and efficacy to warrant advancement to phase II.

2.1.2. TIM-3

T-cell Immunoglobulin- and Mucin-domain-containing molecule 3 (TIM-3) is an immune-inhibitory molecule first identified on CD4+ Th1 (helper) T-cells and CD8+ Tc1 (cytotoxic) T-cells [20], then later on Th17 T-cells [21], regulatory T-cells [22,23], and innate immune cells [24–26]. TIM-3 is activated primarily by its widely-expressed ligand, galectin-9 [27], leading to effector T-cell death through calcium influx, cellular aggregation, and apoptosis [28]. When TIM-3 signalling is active, interferon-producing T-cells become exhausted, resulting in Th1 suppression and immune tolerance [28–30]. TIM-3 expression is commonly observed during chronic infection, as a characteristic marker of exhausted T cells [31–35].

In cancer, tumor-infiltrating lymphocytes expressing TIM-3 have been observed in melanoma [36,37], non-Hodgkin lymphoma [38], lung, [22], gastric [39,40], and other cancers [41–44]. In these studies, Tim-3 is co-expressed with PD-1 and associated with effector T-cell exhaustion and dysfunction. This phenomenon is also observed in mouse models of solid [45] and hematologic [46] cancers, where Tim3 + PD1 + CD8+ T-cells exhibit an exhausted phenotype characterized by reduced proliferation and defective production of IL-2, TNFα, and IFN-γ. In contrast, TIM-3 positive Treg display increased expression of effector molecules and are more immunosuppressive than their TIM-3 negative counterparts [47,48].

Inhibition of TIM-3 alone tends to have little effect on tumor growth in pre-clinical mouse models, despite some evidence supporting a reversal of immune cell exhaustion [36,45,49–51]. However, combined targeting of PD-1 and TIM-3 leads to a substantial reduction in tumor growth − better than either pathway alone − in numerous preclinical *in vivo* models [36,45,46,51], supporting the concept that malignant cells become resistant to PD-1 checkpoint blockade by activating another immune checkpoint. Indeed, mouse models partially responsive to PD-L1 inhibition upregulated TIM-3 expression in resistant tumors [43,52], and addition of TIM-3 blockade was successful in overcoming that resistance. Upregulation of TIM-3 has also been observed in patients receiving PD-L1 monotherapy, suggesting it may represent a form of adaptive resistance to this therapy [52]. Four early phase clinical trials are underway that attempt to combine anti PD-L1 therapy with agents targeting TIM-3 (Table 1).

2.1.3. TIGIT

TIGIT (T cell Immunoglobulin and ITIM domain) is a transmembrane protein receptor that acts as an immune checkpoint on T and NK cells by way of two immunoreceptor tyrosine-based inhibitory motifs (ITIM) in its cytoplasmic tail [53]. There are two prominent TIGIT ligands (CD155 and CD112), mostly expressed on antigen presenting cells, and one recently-discovered ligand called nectin-2 [54]. TIGIT immunosuppressive actions appear to mimic CTLA-4 interactions with the B7 cell surface receptor. Binding of CD155 to CD226 (a receptor on T and NK cells) leads to activation of effector functions which are inhibited when CD155 binds to TIGIT instead [53].

Mice with TIGIT deficiency are sensitive to autoimmune arthritis [55]. In cancer, TIGIT blockade leads to tumor regression, increased survival, and resistance to tumor re-challenge in melanoma and colon cancer mouse models [56,57]. High expression of *TIGIT* mRNA and increased levels of TIGIT+ lymphocytes by flow cytometry have been reported in human renal cell carcinoma, melanoma, lung, breast, and esophageal cancers. [10,56,58–62]

In melanoma, NY-*ESO*-1-specific TIGIT+ CD8+ T cells co-express other immune checkpoint markers, such as PD-1 and TIM-3 [59]. Blockade of TIGIT and PD-1 *in vitro* increased IFN-γ and TNF-α production from tumor-specific CD8+ T cells. A population of early effector TILs that express TIGIT and other inhibitory receptors (LAG-3, TIM-3, and PD-1) but retain their functional phenotype has been reported in lung cancer patients [10]. *TIGIT* gene expression is demonstrable among a subset of basal-like breast cancers where, like other biomarkers of immune recognition, it is associated with improved survival in what is otherwise an aggressive disease [62]. TIGIT inhibitors are still in early phase development, but at least two agents (MTIG7192A, OM*P*-313M32) are being investigated in human trials (Table 1).

2.1.4. B7-H3

B7-H3 (CD276) is a member of the B7 superfamily of immune modulatory ligands, closely related to B7-H1 (PD-L1), B7-DC (PD-L2), B7-H2 (ICOS-L), and CTLA-4 ligands B7-1/B7-2 (CD80/CD86) [63]. The role of B7-H3 in immune regulation is controversial [64], as early studies described it as immune co-stimulator [63,65–72], but subsequent studies have shown a co-inhibitory role [73–81].

B7-H3 is highly expressed in normal tissues, [63]. and has been shown to be overexpressed in melanoma [82] and numerous carcinomas [83–88]; in most cases, expression is associated with worse outcomes. Enoblituzumab (MGA271), a monoclonal antibody targeting B7-H3, inhibits tumor growth in renal and bladder carcinoma xenografts [89] and is currently being investigated in at least four phase 1 clinical trials, including in combinations with pembrolizumab or ipilimumab. Preliminary single agent results (NCT01391143) report good tolerability and tumor shrinkage (2–69% at 12 weeks) across several