Prospects for TIM3-Targeted Antitumor Immunotherapy

Shin Foong Ngiow1,2, Michele W.L. Teng1,2, and Mark J. Smyth1,2

Abstract

New insights into the control of T-cell activation and proliferation have led to the identification of checkpoint proteins that either up- or downmodulate T-cell reactivity. Monoclonal antibody immunotherapies that are reactive with cytotoxic T lymphocyte antigen 4 or programmed death receptor 1 have shown promising therapeutic outcomes in mice and humans with established cancer, highlighting the fact that cancer immunotherapy using T-cell checkpoint inhibitors is one of the most promising new therapeutic approaches. T-cell immunoglobulin and mucin domain 3 (TIM3) is one of many similar inhibitory molecules that are gaining attention as targets, but it remains relatively poorly studied in oncology. This review discusses our recent probing of the mechanism of action of anti-TIM3 antibody against established spontaneous and experimental tumors in mice, in the context of the exciting possibility of rationally combining agents that promote tumor-specific T-cell activation, proliferation, effector function, and survival. Cancer Res; 71(21); 1–5. ©2011 AACR.

Introduction

Effector T cells can kill cancer cells, and the presence of tumor-infiltrating lymphocytes (TIL) is considered to be an indication of the host immune response to tumor antigens and to reflect the dynamic process of cancer immunoediting (1). However, exceedingly strict biologic limits that are imposed on the immune system to prevent excessive T-cell activation and expansion limit the effectiveness of administered cancer vaccines. Investigators have developed various immunotherapeutic agents to circumvent these biologic restrictions, including (i) vaccine adjuvants, (ii) dendritic cell (DC) activators and growth factors, (iii) T-cell stimulators and growth factors, (iv) immune checkpoint inhibitors, and (v) agents to neutralize or inhibit suppressive cells, cytokines, and enzymes. Alone, each approach will have a limited use in cancer treatment, but in combinations dictated by the biology of the tumor microenvironment, these agents are overwhelmingly likely to have an impact.

The subject of discussion here is the immune checkpoint inhibitors that are now leading the way to the routine translation of immunotherapy in cancer patients. During the past decade, new insights into the mechanisms by which T-cell activation and proliferation are regulated have led to the identification of checkpoint proteins that either up- or downmodulate T-cell reactivity (2, 3). In the presence of active malignancy, pathophysiologic inhibition of T-cell activity may predominate over stimulation. Tumor immunotherapy aims to break effector T-cell anergy and to block suppressive cell types and ligands, allowing effector cells to exert tumor eradication. Much of the recent excitement in the translational field of tumor immunology and immunotherapy has been generated by the recognition that human immune checkpoint proteins can be blocked by specific monoclonal antibodies (mAb) in patients. Promising clinical data showing durable, objective responses and improved survival have already been generated in melanoma with human antibodies directed against cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD-1; refs. 4–6). Excitingly, anti-CTLA-4 (ipilimumab) was recently approved by the U.S. Food and Drug Administration for the treatment of patients with metastatic melanoma. The experience with anti-CTLA-4 therapy and the durable clinical benefit observed provide proof of principle of the effectiveness of antitumor immune modulation and the promise of future clinical immune modulatory antibodies.

Encouragingly, many of the therapeutic effects of these 2 agents and their mechanism of action were somewhat predicted by mouse models of cancer and immunity. Several studies have assessed the mechanism of action of anti-CTLA-4 mAb in mice (7–9) and more recently in humans (10–12). Anti-PD-1 and anti-PD-L1 mAbs have also been studied in some depth in mice and humans (13–15). The mechanism of action of anti-PD-1 and anti-CTLA-4 combinations has also been explored in mice (16). CTLA-4 is ~30% homologous to the costimulatory receptor CD28 and binds with higher avidity to its ligands, B7-1 and B7-2, allowing CTLA-4 to promote termination of immune responses by preventing continued T-cell costimulation and activation. Previous reports have shown that CTLA-4–blocking antibodies promote T-cell activation and render T-effector (Teff) cells resistant to T-regulatory cells (Treg). Both CTLA-4

Authors’ Affiliations: 1Cancer Immunology Program, Trescowthick Laboratories, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia; 2Department of Pathology, University of Melbourne, Parkville, Australia

Note: M.W.L. Teng and M.J. Smyth contributed equally to this work.

Corresponding Author: Mark Smyth, Cancer Immunology Program, Peter MacCallum Cancer Centre, Locked Bag 1, A Beckett Street, Victoria 8006, Australia. Phone: 61-3-9656-3729; Fax: 61-3-9656-1411; E-mail: mark.smyth@petermac.org

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blockade and cell-intrinsic CTLA-4 deficiency have been shown to decrease the suppressive function of CD4\(^+\) Tregs (17). Therapeutic antitumor activity is believed to be reflected by an increase in the tumor infiltrating CD8\(^+\) T cell memory formation, function, and maintenance (18). By contrast, PD-1 functions via different immune signaling pathways than CTLA-4 and is likely to have a different spectrum of effects when blocked. PD-1 is expressed on T cells following T-cell receptor activation. Binding of this receptor to its cognate ligands, PD-L1 and PD-L2, downregulates signals by the T-cell receptor, promoting T-cell anergy and apoptosis, thus leading to immune suppression (19). PD-1/PD-L1 blockade results in loss of peripheral tolerance and the initiation of autoimmunity. It is also recognized that the PD-1/PD-L1 pathway is an important element contributing to tumor-mediated immune suppression. These observations support the idea that murine preclinical therapeutic experiments may be an important guide to the conduct of trials employing abrogation of immune checkpoint proteins in T cells in patients.

A variety of other molecules that similarly regulate T-cell activation are being assessed as targets of cancer immunotherapy (Fig. 1). One of these inhibitory molecules that has gained considerable attention recently is T-cell immunoglobulin and mucin domain 3 (TIM3), a member of a relatively newly described TIM family (20). TIM3 was first reported as
expressed by IFN-γ-secreting T-helper 1 (Th1) cells and subsequently on DCs, monocytes, CD8+ T cells, and other lymphocyte subsets (21, 22). Binding of TIM3 by its ligand, galectin-9, results in Th1 cell death, suggesting a role for TIM3 in negatively regulating Th1 responses (23). Blockade of TIM3 has been shown to increase IFN-γ-secreting T cells (24), and TIM3 expressed on monocytes and macrophages has also been implicated in phagocytosis of apoptotic cells (25).

**Key Findings**

To date, few studies have extensively assessed the mechanism of action of anti-TIM3 mAb against tumors. Instead, investigators have characterized the comparatively defective cytokine effector function of TIM3+PD-1+CD8+ T-cell populations in experimental tumors in mice (26) or tumor antigen-specific TIM3+PD-1+CD8+ T cells in advanced melanoma in humans (27). Although TIM3 appears to be a potentially promising target for cancer immunotherapy, the mechanism of action of anti-TIM3 mAb and its activity alone and in combination with other immunomodulatory mAbs in experimental and spontaneous mouse models of cancer have not been assessed to any great extent. In 3 different experimental mouse tumor models (CT26, WT3, and MC38), we recently showed that the therapeutic effect of anti-TIM3 required both CD8+ and CD4+ T cells (28). In the MC38 tumor model, we further showed that IFN-γ production from CD8+ T cells is critical for the efficacy of anti-TIM3 mAb therapy. Although anti-TIM3 therapy alone had a modest effect against methylocholanthrene-induced fibrosarcomas, impressively, the combination of anti-TIM3 and anti-PD-1 mAbs significantly suppressed established tumor growth and even resulted in cures in a small proportion of these treated mice (28). The increase in antitumor effect achieved by combining anti-TIM3 with anti-CTLA-4 and/or PD-1 mAbs compared with single therapy alone was also observed in 6 different experimental mouse tumor cell lines (28). Of importance, anti-TIM3 alone or in combination with anti-CTLA-4 and/or anti-PD-1 was well tolerated, and no overt autoimmunity was observed in treated tumor-bearing mice. Overall, our study strongly supports the potential of blocking TIM3 in combination with other immune checkpoint inhibitors for the treatment of cancer.

**Implications**

Previous studies (26, 27) implied that the target of anti-TIM3 therapy might be the TIM3+PD-1+ T-cell populations found in established tumors. Of interest, however, in our studies, anti-TIM3 mAb therapy appeared to be effective when administered sometime before the appearance and accumulation of significant TIM3+PD-1+ T-cell populations in CT26 tumor-bearing mice (28). Early (day 7–11) CT26 subcutaneous tumors contained CD8+TIM3+PD-1+ cells but very few detectable TIM3+PD-1+ or TIM3+PD-1 T cells, and yet the tumors clearly responded to anti-TIM3 therapy. The apparent lack of TIM3+ T cells early in tumor progression may be misleading, because the expression of TIM3 may itself be very transient. However, the target and mechanism of anti-TIM3 may also be model dependent, because anti-TIM3 was also effective against established MC38 tumors where TIM3+PD-1+ T cells were the predominant T-cell population among TILs at the commencement of treatment. In this model, maintenance of the CD8/CD4 ratio of MC38 TILs over time correlated with the response to anti-TIM3 mAb (28). However, at this time, it is not possible to strictly discount TIM3+PD-1− or TIM3+PD-1+ T cells as a possible target of anti-TIM3. A very recent study supported the exhausted phenotype of TIM3+PD-1+ T cells in acute myeloid leukemia and showed that combined blockade of PD-1/PD-L1 and TIM3/galectin-9 rescued mice from acute myeloid leukemia lethality (29).

Our studies did not reveal a significant effect of targeting TIM3 on CD11c+ DCs and, instead, showed that the importance of these cells was modest and model dependent (28). However, in addition to binding galectin-9, TIM3 has been reported to be a phagocytic receptor for apoptotic cells by a subset of macrophages, monocytes, and CD8+ splenic DCs. Thus, the effect of anti-TIM3 mAb on tumor cell death and cross presentation of tumor antigens by DC may be worthy of further exploration (25). In a tumor setting, TIM3 can induce the expansion of CD11b+Ly6G+ cells in the spleens of mice implanted with the T-cell lymphoma, EL-4. Treatment of these mice with anti-TIM3 mAb resulted in delayed tumor progression and modulation of these cells (30). Although TIM3 ligation induces cell death in CD4+ Th1 cells (23) and expands CD11b+Ly6G+ cells, paradoxically, similar ligation on DCs can result in the production of proinflammatory cytokines (22, 25, 31), promotion of DC maturation, and development of antimicrobial immunity (22, 32). Collectively, these findings suggest that TIM3 may play different biologic roles in different leukocyte subsets, and unraveling the effects of anti-TIM3 mAb on these subsets will be important for the development of this approach for cancer immunotherapy.

The effects of anti-TIM3 in combination with anti-PD-1 and/or anti-CTLA-4 mAbs against established B16F10, MC38, and CT26 tumors were broad and encouraging (28), and these approaches are worthy of further preclinical development in these and other models to optimize the regimen schedule and further understand the mechanism of action of each combination. Anti-CTLA-4 therapy is more effective against mouse tumors when administered at the time of tumor inoculation, and it appears comparatively more effective in humans, so our experiments may underestimate the potential therapeutic benefit of these combinations. Established B16F10 tumors grow rapidly and are extremely difficult to treat with any single therapy. The anti-B16F10 tumor effect achieved with the anti-TIM3/anti-CTLA-4/anti-PD-1 combination was approximately what we achieved with complete Treg cell depletion (33), an intervention that provokes both innate and adaptive arms of antitumor immunity. These data indicate that targeting CTLA-4, PD-1, and TIM3 can at least be additive, and that each of these pathways has a unique mechanism for preventing an effective antitumor immune response. Just as important, with improved tumor suppression, these combination therapies did not provoke any overt autoimmunity in mice (e.g., vitiligo). Whether this will be the case in humans...
remains to be determined and is of critical importance given the impact of completely blocking CTLA-4 in humans, particularly because the mouse models have not been validated to predict autoimmune side effects. Encouragingly, our preliminary data regarding combined anti-PD-1 and anti-TIM3 treatment of established fibrosarcomas induced de novo by methylcholanthrene suggest that this combination might be effective against at least a fraction of tumors (28). A larger study with pretreatment biopsy correlating target expression among TILs and the genetics of tumors with therapeutic activity from these immunotherapies in this and other spontaneous tumor models would be extremely informative. With the clinical development of anti-PD-1 and anti-PD-L1 treatments in humans that is currently underway, the prospect of combining these or anti-CTLA-4 approaches with anti-TIM3 mAb in diseases such as melanoma, prostate cancer, renal cell carcinoma, and sarcoma is very appealing.

Future Directions

To date, mono-immunotherapy using anti-CTLA-4 or anti-PD-1 mAb in humans has shown promising therapeutic outcomes, proving that immunotherapy with T-cell checkpoint inhibitors is one of the most promising new therapeutic approaches. With these new therapies, long-term stabilization of disease and overall survival of patients may be more valuable endpoints than classical Response Evaluation Criteria in Solid Tumors. In parallel, the identification and characterization of multiple T-cell checkpoints through research will drive the further clinical development of other checkpoint inhibitors, such as TIM3 and lymphocyte-activation-gene 3 (LAG-3). The role of TIM3 expressed on immune cells other than DCs and T cells and the potential toxic effects of targeting TIM3 require further exploration if antihuman TIM3 antibodies are to proceed to clinical development. LAG-3 is located in the CD4 locus (34), and when expressed on activated CD4+ and CD8+ T cells, it negatively regulates T-cell expansion by inhibiting T-cell receptor–induced calcium fluxes, thus controlling the size of the memory T-cell pool (34). LAG-3 signaling is important for CD4+ regulatory T-cell suppression of autoimmune responses, and LAG-3 maintains tolerance to self and tumor antigens via direct effects on CD8+ T cells. A recent study showed that blockade of both PD-1 and LAG-3 could provoke immune cell activation in a mouse model of autoimmunity (35). Taken together, these data show a direct role for LAG-3 on CD8+ T cells and may be another important potential target for checkpoint blockade.

Another attractive alternative is to combine checkpoint inhibitors with antibodies or agonists that activate immune cells. The reversal of anergic/exhausted T cells by checkpoint blockade may allow these cells to be more potently activated and to develop full antitumor effector function. Possible approaches for activating immune cells include the use of antibodies that target costimulatory receptors (e.g., CD137, OX40, CD40, and glucocorticoid-induced TNF receptor–related gene); chemotherapeutics or radiotherapy to trigger immunogenic cell death (36); and vaccines/adjuvants to promote adaptive immunity. Antibodies that target stimulatory molecules, such as CD40 and CD137, have been tested in early-phase clinical trials, and they all have their own spectrum of side effects to be considered. It will now be critical to extensively test the antitumor efficacy and safety of combining immunomodulatory therapies (e.g., T-cell checkpoint inhibitors and T-cell activation agonists) preclinically using emerging mouse models of cancer that are more clinically relevant or in which small-molecule therapeutics have shown promising activity (e.g., mouse models of c-kit mutant gastrointestinal stromal tumor and B-raf mutant melanoma).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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