Vaccine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Checkpoint blockade in combination with cancer vaccines

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
Anti-PD-1
Anti-PD-L1
Anti-CTLA-4

ABSTRACT

Checkpoint blockade, prevention of inhibitory signaling that limits activation or function of tumor antigen-specific T cells responses, is revolutionizing the treatment of many poor prognosis malignancies. Indeed monoclonal antibodies that modulate signaling through the inhibitory molecules CTLA-4 and PD-1 are now clinically available; however, many tumors, demonstrate minimal response suggesting the need for combinations with other therapeutic strategies. Because an inadequate frequency of activated tumor antigen-specific T cells in the tumor environment, the so-called non-inflamed phenotype, is observed in some malignancies, other rationale partners are modalities that lead to enhanced T cell activation (vaccines, cytokines, toll-like receptor agonists, and other anticancer therapies such as chemo-, radio- or targeted therapies that lead to release of antigen from tumors). This review will focus on preclinical and clinical data supporting the use of cancer vaccines with anti-CTLA-4 and anti-PD-1/PD-L1 antibodies. Preliminary preclinical data demonstrate enhanced antitumor activity although the results in human studies are less clear. Broader combinations of multiple immune modulators are now under study.

1. Rationale for combination of cancer vaccines and checkpoint blockade

Although there has been a longstanding interest in harnessing the immune system to destroy tumors, the discoveries of unique or overexpressed antigens on tumors that could be recognized by T cells and antibodies as targets for immune attack and of antigen-presenting cells such as dendritic cells that are required for stimulation of the tumor antigen-specific T cells dramatically accelerated the development of immunotherapies for malignancies. The ever growing list of tumor antigens and delivery technologies attests to the continued interest in prophylactic and therapeutic cancer vaccines [1,2]. Although there is one FDA approved therapeutic "vaccine" (the cellular therapy Sipuleucel-T) [3] and clinical benefit reported for others in earlier phase studies [4,5], in general, the effectiveness of cancer vaccines has been less robust than initially expected including recent phase III failures [6]. Nonetheless, tumor antigen-specific immune responses correlating with survival have been routinely generated in vaccine studies [7,8], suggesting that strategies to enhance the effectiveness of T cell and antibody responses induced by vaccination may lead to greater anti-tumor activity.

Attempts to enhance tumor killing by immune effectors, previously focused on providing stronger stimuli to increase the magnitude of the response, are now increasingly directed toward improving effector function. While this could be accomplished by strategies to stimulate T cells with greater cytolytic activity ("pushing the gas pedal"), strategies that interfere with immunomodulatory or immunosuppressive mechanisms ("taking the foot off the brake") appear to be equally important. Among these immunomodulatory mechanisms are those employed by suppressive cell types such as regulatory T cells (Treg) and myeloid derived suppressor cells (MDSC) and pathways governed by T cell expressed inhibitory (or "checkpoint") immune receptors (Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4; CD152), and Programmed Death-1 receptor (PD-1; CD279)) and their ligands (B7-1 (CD80) and B7-2 (CD86) and PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; CD273), respectively).

CTLA-4, a type I transmembrane protein of the immunoglobulin superfamily, is expressed by recently activated CD4+ and CD8+ T cells (reviewed in [9]). CTLA-4 crosslinking by antigen presenting cell-expressed CD80 and CD86 following T-cell antigen receptor (TCR)/MHC-peptide engagement inhibits T-cell activation, interleukin (IL)-2 gene transcription, and T-cell proliferation by directly inhibiting TCR signal transduction. Several mechanisms have been proposed for the activity of anti-CTLA-4 antibodies, but blockade of this negative signaling on recently activated tumor antigen-specific T cells is the most straightforward of these mechanisms.

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http://dx.doi.org/10.1016/j.vaccine.2015.10.057
0264-410X/

However, a role for anti-CTLA-4 antibody in modulating regulatory T cells (Treg) has also been demonstrated [10]. Natural Treg (nTregs), key to maintenance of self-tolerance and potent inhibitors of T cell activation, constitutively express CTLA-4 [11,12], suggesting they could be targeted by anti-CTLA-4 antibody, but this has been a complex issue as studies have observed increases, decreases, and variable changes in nTreg after anti-CTLA-4 blockade [13–15]. These contradictory observations could in part arise from the IgG subtype of anti-CTLA-4 antibody studied [16,17]. A more consistent finding has been that the ratio of effector T cells to Treg, particularly in the tumor microenvironment, may increase following CTLA-4 blockade. In this scenario, dose level of anti-CTLA-4 antibody may be important. Kavanagh [13] observed that CTLA-4 blockade expanded Treg at low doses, but expanded effector T cells only at high dose. However, regardless of Treg frequency, these cells do require CTLA-4 expression in order to impair costimulatory molecule expression on DC [18]. In sum, the effects of CTLA-4 blockade would be expected to enhance the activation of T cells by vaccines; however, not only might vaccines be enhanced by anti-CTLA-4 therapy, but vaccines may enhance efficacy of anti-CTLA-4 therapy. For example, anti-CTLA-4 therapy alone has activity in animal models with intrinsically immunogenic tumors [19,20], but this therapy is ineffective alone in non-immunogenic tumors [21] and is only FDA-approved for the treatment of melanoma, a tumor often viewed as intrinsically immunogenic. These observations suggest that greater efficacy of anti-CTLA-4 antibodies may be achieved by optimizing the immunogenicity of in situ tumors or the host immune response to the tumor with vaccines.

PD-1 is also expressed by activated T cells. Binding of PD-1 to tissue (or infiltrating immune cell)-expressed PD-L1 and antigen presenting cell-expressed PD-L2 down-regulates signals by the TCR, promoting T-cell anergy and apoptosis, and reduction in production of TNF-alpha, IFN-γ and IL-2 thus leading to impaired T cell function [22]. In contrast to CTLA-4 which exerts its effects mainly at the site of T cell activation, the major role of PD1 is to limit autoimmunity by T cells infiltrating inflamed peripheral tissues expressing their cognate antigen. Similarly, in the tumor microenvironment, PD-1 expression by previously activated antigen-specific infiltrating T cells, limits their anti-tumor function [23,24]. Indeed, studies have demonstrated absence of PD-1 expression on CD8+ T cells in tumor-draining LNs despite PD-1 expression by tumor-infiltrating antigen-specific CD8+ T cells suggesting PD-1 is induced when T cells re-encounter their cognate antigen [25]. Blockade of PD-1/PD-L1 or PD-L2 interactions enhances and prolongs the activity of tumor specific-effector T cell responses and induces memory responses [26]. This would be expected to enhance vaccine induced T cell responses; however, vaccine induced T cell responses may enhance the activity of PD-1 blockade by promoting the T cell infiltration into tumors upon which PD-1 blockade acts. An important observation is that clinical benefit from PD-1 blockade is more frequent in PD-L1 expressing tumors, but PD-L1 is heterogeneously expressed, mainly at sites of both lymphocytic infiltration and intratumoral IFNγ expression [27] suggesting that IFNγ produced by infiltrating T cells induces PDL1 expression [28], which in turn suppresses the activity of PD1+ T cells (the adaptive resistance hypothesis) [29]. Preliminary evidence from cancer vaccine studies suggests that T cells specific for the vaccinating antigen upregulate PD-1 [30,31] while non-specific T cells do not. These data also suggest that for some tumors, it may be necessary to activate and promote intratumoral accumulation of tumor antigen-specific, IFNγ-producing T cells with vaccines in order to observe significant activity of PD-1 blockade.

The foregoing supports the hypothesis that vaccines combined with either CTLA-4 or PD-1 blockade should have greater anti-tumor activity on theoretical grounds. Before considering the data generated by testing these hypotheses, it is important to consider that there should be different effects of combinations of vaccines with the different checkpoint blockade molecules. As described above, CTLA-4 and PD-1 are thought to have their predominant effect at different phases of the immune cycle with some overlap, but they also act on T cell populations of different avidities. Strong TCR signals result in the greatest CTLA-4 induction and transport to the immunological synapse [32] and therefore, CTLA-4 inhibits T cells with high avidity receptors to a greater degree [33]. Therefore, CTLA-4 blockade could theoretically be more relevant for very potent vaccines or when booster doses of vaccine are given. PD-1 is induced in, and inhibits to a greater degree, T cells that have received a weak TCR signal [34] and therefore would be expected to inhibit T cells with low avidity receptors. Because most identified tumor antigens are "self" antigens to which high affinity TCR have been deleted in the thymus, PD-1 blockade could theoretically be more relevant for weaker vaccines [35].

2. Combinations of anti-CTLA-4 antibody plus vaccines

Early studies demonstrated a consistently greater anti-tumor effect for combinations of anti-CTLA-4 antibody and vaccines compared with either alone in murine models [21,36–38]. For example, in the rapidly growing B16-BL6 melanoma model, although administration of anti-CTLA-4 antibody (hamster IgG mAb clone 9H10) alone had no effect, and a vaccine based on irradiated GM-CSF-producing B16-BL6 cells only delayed growth, the combination of the vaccine and anti-CTLA-4 Ab blockade caused rejection of all tumors when started the same day or within 4 days of tumor implantation [36]. Other studies also supported the concept that anti-CTLA-4 therapy enhances and maintains the vaccine-induced stimulation of CD8+ effectors and their trafficking to tumors. Dos Santos [39] reported a greater percentage of tumor-infiltrating T cells were CD8+ when a vaccine based on *T. cruzi* encoding NY-ESO-1 was administered with anti-CTLA-4 Ab (Mouse IgG2b clone 9D9), than when the antibody was administered alone.

More recently, studies have suggested that an important function of anti-CTLA-4 antibody is to favorably alter the intratumoral balance of effector T cells (CD4+ and CD8+) and Treg [40] rather than to deplete or inhibit Treg. In fact, greater absolute numbers of intratumoral or tumor draining Treg may occur after anti-CTLA-4 therapy in conjunction with vaccines but there is a greater expansion in the absolute number of effector T cells. Alterations in this balance have been associated with increased tumor rejection and survival. These results do require use of a vaccine that can by itself activate effector T cells capable of tumor infiltration as has been observed with the GVAX (GM-CSF expressing tumor cell) vaccines. Similarly, in a B16 melanoma model, anti-CTLA-4 antibody (9D9) administered concurrently with an alphaviral vector VRP-TRP2 increased intratumoral CD4 and CD8+ T cells and decreased intratumoral CD4+ FOXP3+ Treg, which was associated with prolonged survival compared with vaccine or anti-CTLA-4 alone [41]. This study also provided evidence for potential immunosuppressive mechanisms that would not be addressed purely by anti-CTLA-4 therapy. Increased CD4+ intratumoral T cells occurred with anti-CTLA-4 antibody regardless of whether the immunogen was a tumor-specific antigen containing vaccine or a control vaccine. There was an increase in PD-1 expression by the intratumoral CD4+Foxp3− population, but not among the CD4+Foxp3− splenocytes or CD8+ TILs. These cells were not cytolytic, but rather it was thought they could be immunosuppressive. Indeed, others have observed that PD-1 ligation could convert Th1 cells into Treg [42,43]. In contrast, others have suggested that CD4+ T cells may be necessary for the effectiveness of combined anti-CTLA-4 Ab and vaccine [44]. Studies have reported a requirement for other cell types not directly activated by

Please cite this article in press as: Morse MA, Lyerly HK. Checkpoint blockade in combination with cancer vaccines. Vaccine (2015), http://dx.doi.org/10.1016/j.vaccine.2015.10.057