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Cancer Immunotherapy: Targeting Checkpoint Blockade

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Abstract

Immune system is developed in such a way that it can efficiently recognize, target and eliminate foreign pathogens effectively, but leave the host self-architecture intact. During the developmental process self-reactive high avidity immune effectors are deleted, and several other mechanisms are put in place to ensure that the self-reactive low avidity immune effectors cannot generate harmful autoimmune reactions. T cells are critical immune effectors of a protective antigen specific adaptive immune response. While engagement of the T cell receptor (TCR) critical for the development of antigen specific T cell response, development of effector function in T cells is fine tuned by positive factors, the co-stimulatory factors, and negative factors, the co-inhibitory receptors. While role of co-stimulation was initially considered critical for the generation of an optimum protective immune response, it is well established that the co-inhibitory molecules play equally essential role in this process. Approaches targeting co-inhibitory receptor mediated immune blockade mechanisms have recently been shown to produce remarkable protective responses in cancer patients. We will here take a brief account of the recent advances towards development of immune checkpoint blockade strategies in cancer immunotherapy.

Keywords: Cancer; Immunotherapy; Immune checkpoint blockade

Abbreviations:

CTLA-4: Cytolytic T Lymphocytes; PD-1: Programmed Death-1 receptor

Key Effectors and Modulators for the Generation of a Productive Antigen Specific Protective Cytolytic T Lymphocyte (CTL) Response

Generation of an antigen specific T cell response is a well-coordinated process involving three key effectors, the antigen presenting cells (APC), CD8 T cells, and CD4 T cells. Antigen presenting cells acquire, process and present the antigen to naive CD8 T cells, that acquire effector function, and go on to target and kill the cells expressing cognate peptide epitopes. CD4 T cells can influence the generation of a protective CD8+ cytolytic T lymphocyte (CTL) response, by facilitating "help" or by "regulating" this process. While the key role of APC towards generation of a productive antigen specific CTL response is processing and presentation of target antigen, denoted as "signal-1", APC do much more to influence the nature of the T cell response generated. It was shown that the antigen presenting cells, besides presenting the antigenic epitopes to T cells also provide accessory signals, the co-stimulatory signals, denoted as "signal-2" that can assist the generation of a productive immune response. However, it is now also well-established now that T cells also receive negative signals, the co-inhibitory signals, from APC and other cells they encounter, and these negative signals play significant role in fine tuning the priming of T cell precursors and development of a potent antigen specific effector response (Figure 1). These co-stimulatory molecules and the co-inhibitory molecules offer opportunities to modulate the generation of a desired antigen specific immune response.

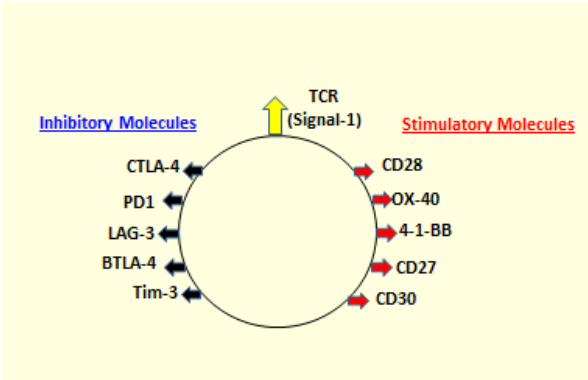

Figure 1: Signal-1 and signal-2 involved in T cell response generation. Generation of an effective antigen specific T cells response involves signal-1 provided by engagement of T cell receptor (TCR) with antigenic epitope presented on the antigen presenting cells (APC). Besides the signal-1, the quality of antigenic response generated is modulated by engagement of several accessory molecules (signal-2) that can impart a co-stimulatory signal or a co-inhibitory signal, to augment or inhibit the T cell response generated.

Co-stimulatory molecules: Co-stimulation, besides TCR signal, is essential for T cells to reach an optimum activation threshold necessary for acquisition of effector function. TCR signals in the absence of co-stimulation can lead to the development of anergy/tolerance in T cells. CD28, a member of immunoglobulin super-family, was the first co-stimulatory signal to be identified to augment T cell proliferation, interleukine-2 (IL-2) synthesis and expression of anti-apoptotic effector molecule Bcl-XL [1]. While all murine T cells

constitutively express CD28, only 80% of human T cells constitutively express CD28 molecules. Among human T cells, all CD4 T cells express CD28, however, only 50% of CD8 T cells express CD28, implying an interesting role for CD28 in the biology of human CD4 and CD8 T cells. Since the identification of CD28, many additional co-stimulatory molecules, belonging to tumor necrosis factor receptor (TNFR) super family, have been identified, for example TNFR family members, OX-40, 4-1-BB, CD27, CD30, HVEM, among which OX-40, 4-1-BB and CD27 have been characterized in relatively greater details [2]. While CD28 is constitutively expressed on T cells, TNFR member family co-stimulatory molecules can either exhibit low to moderate constitutive expression (CD27 and HVEM) or are induced following antigen encounter (OX-40, H-1BB, CD30), thereby suggesting distinct roles for co-stimulatory signals, for example constitutively expressed primary co-stimulatory molecules and the secondary co-stimulatory molecules, induced following activation.

Co-inhibitory molecules: While role of co-stimulatory molecules in modulation of immune response was identified first, it is now also known that several co-inhibitory molecules, for example Cytotoxic T Lymphocyte Antigen-4 (CTLA-4), Program Death-1 (PD-1), Lymphocyte Activation Gene-3 (LAG-3), T Cell Immunoglobulin-3 (Tim-3), B and T Lymphocyte Attenuator (BTLA-4) etc., also play critical role in this process [3]. We here discuss the significant features of two key inhibitory molecules, CTLA-4 and PD-1 that have been characterized in significant details and have advanced to clinical application stages. CTLA-4 was the first in the list of molecules identified that transmits negative signal to the T cells. CTLA-4 is a homologue of CD28 that also belongs to immunoglobulin super family. Like CD28, CTLA-4 has two ligands, B7.1 and B7.2 (CD80 and CD86). Interestingly, initial findings demonstrating a CTLA-4 antibody facilitate T cell proliferation led to belief that CTLA-4 was a co- stimulatory molecule [4]. However, subsequent studies showed that CTLA-4 antibody that was immobilized or cross-linked to a secondary antibody inhibited T cell responses triggered by anti-CD3 and anti-CD28 antibodies [5,6], thereby suggesting that positive effects of CTLA-4 antibody on T cell proliferation in initial study might have resulted due to mitigation of negative signals imparted by CTLA-4. Animal studies showing lymphoproliferative disorders with early lethality in CTLA-4 deficient mice further supported a negative role for CTLA-4 in T cell activation schema [7]. CTLA-4 expression is transiently induced on T cells following activation, blocking an optimum priming of antigen specific T cells. Cytolpasmic region of CTLA-4 has an immunoreceptor tyrosinase inhibitory motif (ITIM) that upon engagement of CTLA-4 recruits Src homology region 2 containing phosphatases (SHP)-1 and 2 reduces T cell activation by increasing threshold for activation, up-regulating indolamine 2, 3-dioxygenase (IDO) and down-regulating IL-2 production.

PD-1 is another co-inhibitory molecule that has been characterized in greater details, and has turned out to be an even more promising target in the field of cancer immunotherapy than CTLA-4. Interestingly, PD-1 was identified from a T-cell hybridoma undergoing programmed cell death [8], however, subsequent studies in animal models and in ex-vivo systems established that PD1 plays a critical role in limiting the effector function of T cells [3,9,10]. PD1 has a wide expression profile, with expression on T cells, regulatory T cells (Tregs), exhausted T cells, B cells, activated monocytes, dendrtitic cells (DC), and natural killer (NK) cells. PD1 expression is induced on T cells in response to persistent chronic infection and correlates with "exhausted" phenotype of these T cells [11]. Expression of tumor infiltrating lymphocytes (TIL) also indicated towards PD1 being a biomarker of exhausted anti-tumor T cells. While CTLA-4 acts at the activation phase of the T cells, PD1 works at a later stage limiting the T cell activity in peripheral tissues in case of inflammatory response to infection and to limit autoimmunity. PD1 has two ligands, B7-H1 (PD-L1) and B7-DC (PD-L2). The intracellular domain of PD1 contains an ITIM motif and immunoreceptor tyrosinase-based switch motif (ITSM). PD-1 engagement by its ligands results in transmission of the negative signal to T cells by phosphorylation of ITIM and ITSM motifs by recruitment of SHP-2 domain containing tyrosinase phosphatases that result in dephosphorylation of downstream TCR signal transmitters and inhibition of T cell function. Interestingly, there are significant differences in PD-1 and CTLA-4 mediated immune suppression mechanisms, such that the PD-1 mediated inhibitory signals target both the phosphoinositide-3-kinase (PI-3K) and Akt activities, while CTLA-4 mediated inhibitory signals target Akt [10]. PD-1 engagement also leads to down-regulation of BCl-XL, thereby suggesting that it has indirect role in cell death rather than directly functioning as a death inducer, as originally postulated.

Targeting immune checkpoint blockade for an effective cancer immunotherapy

Remarkable success of vaccines against infectious agents fueled the quest to develop similar approaches against cancer, resulting in identification of several cancer associated antigens, characterization of immunogenic tumor antigen epitopes, and development of several vaccine strategies to induce a protective anti-tumor immune response [12]. However, initial enthusiasm was dampened by poor overall results observed with active specific immunization based cancer immunotherapy strategies. Since most human cancers develop due to the transformation of normal cells, and because developmental cascade of immune system ensures that host reactive high avidity T cells are eliminated, it became evident that host immune system was inherently ill-equipped to generate a protective anti-tumor immune response. In addition, tumor microenvironment employs multiple immune suppressive mechanisms to dysfunction tumor reactive immune repertoire (Figure 2). To overcome these limitations, adoptive immunotherapy strategies were developed, whereby host T cells were harvested, programmed to function as killer T cells and administered back to the patients [13-16]. Among these strategies included generation of lymphokine activated non-specific killer T cells, ex-vivo expansion of tumor infiltrating lymphocytes (TIL) generation of antigen specific anti-tumor effectors through transgenic TCR or chimeric antigen receptor mediated approaches [13-18]. Interestingly CAR based approaches targeting CD19 molecule has produced remarkable clinical results [19].

While adoptive immunotherapy based approaches on one hand have produced encouraging results, immune checkpoint blockade based approaches (Figure 2), have also come a long way to produce equally remarkable clinical results. CTLA-4 was the first immune checkpoint molecule pursued as a target for cancer immunotherapy. While CTLA-4 was not tumor specific in its expression profile, preclinical data showing anti-tumor effect of anti-CTLA-4 antibody in case of immunogenic tumors, and along with GM-CSF transduced cellular vaccine in case of weakly immunogenic tumors, provided sufficient rationale for follow up studies to examine its clinical efficacy in cancer patients. Of the two anti-CTLA-4 antibodies, ipilimumab and tremelimumab [20,21], used in initial trials, both the antibodies produced objective responses in ~10% of cancer patients. However, in randomized Phase III clinical trials while tremmelimumab showed no

survival benefit, ipilimumab produced a survival benefit of 3.5 months [21]. With ipilimumab being the first therapy to show survival benefit for metastatic melanoma, this lead to its approval by the FDA for the treatment of advanced melanoma in 2010.

PD1 is the second immune checkpoint target molecule that has produced remarkable clinical responses in cancer patients. As mentioned before, PD1 has two ligands, PDL-1 and PDL-2. PD-1 engagement of T cells transmits inhibitory signal, resulting in dysfunction of T cell function. Tumor infiltrating lymphocytes (TIL) from many different types of cancers have been found to express PD-1, and many type of tumors cells have been shown to express PD-1 ligands. For example, PD-L1 expression has been shown to be expressed in solid tumor models, and PD-L2 expression has been shown to be significantly up-regulated in B cell cancer systems. Tumor specific expression profiles for these molecules together with preclinical observation provided the rationale for clinical investigations targeting the PD1-PDL1/PDL2 checkpoint blockade. Interestingly, while approaches targeting PD-1 can block both PDL-1 and PDL-2 triggered responses, approaches targeting PDL-1 or PDL-2 just block cascades triggered with these individual molecules. Therefore, it was reasoned that the side effects produced by targeting the PD1 ligand blockade would be significantly lower than the PD1 blockade approach. Clinical trials were carried out with antibodies targeting PD1 as well as with antibodies targeting PD-L1, and results have been remarkable [22-24]. Two antibodies targeting PD1, nivolumab and lambrolizumab, produced 30-50% objective responses in melanoma patients [25]. The protective effect observed was not just limited to melanoma in case of non-small cell lung cancer patients 33% response rate was observed in case of Squamous Cell Cancer (SCC) and 12% in case of non-SCC, with nivolumab antibody. PDL1 targeting antibody, BMS-936559, produced 8% ORR in SCC and 12% in non-SCC, with an overall survival at 1 and 2 years of 44% for SCC and 41% for non-SCC [23,25]. Promising results were also observed in case of renal cell cancer (RCC), in phase I trial of MPDL3280A, with an overall survival rate (ORR) of 13% [25]. Interestingly ORR was 20% in PDL-1 positive cohorts and 10% in PDL-1 negative cohorts. Interestingly, Nivolumab produced an overall survival (OS) rate of 72% at 1 year, and 52% at 3 year OS. Interestingly the safety profiles of PD1/PDL-1/2 targeting have been significantly better than the CTLA-4, makes PD1/PDL-1/2 blockade more appealing. These remarkable results have led to FDA approval of two PD-1 checkpoint blocking antibodies, Merk`s keytruda and Bristol Meyer`s Squibb`s nivolumab, for the treatment of melanoma. Given that CTLA-4 and PD-1 checkpoint blockade works at different stages of the response cycle, combinatorial approaches simultaneously targeting both these molecules have been envisioned, and initial results from such combinatorial approaches are quite encouraging. Approaches are also underway to combine active specific immunization as well as adoptive immunotherapy with immune checkpoint blockade, and they could further improve the clinical success rate observed so far with immune checkpoint strategies.

Conclusions

The field of cancer immunotherapy has come out of the times of doubts, frustration and failures, and has ushered into the times of hope and promise. While CAR based approaches have generated enthusiasm among proponents of adoptive immunotherapy, success of CTLA-4 and PD-1 based approaches have also brought cheers to the proponents of checkpoint blockade strategies. While initial success has been encouraging, clinical data also points towards additional work required to improve the success rates. While initial immune intervention strategies targeting PD-1 and the CTLA-4 have been to the most part were used as standalone approaches, combinatorial approaches are under development that could further improve the success rate of these approaches. In addition, recent success is also going to encourage detailed characterization of additional immune checkpoint molecules, for example, B7-H3, B7-H7, BTLA, LAG-3, Tim-3, and reveal their therapeutic potential in different disease settings including cancer. While lot remains to be done, it is now amply clear that with the right tools and strategies, it is indeed possible to educate the host immune system to effectively target cancer.

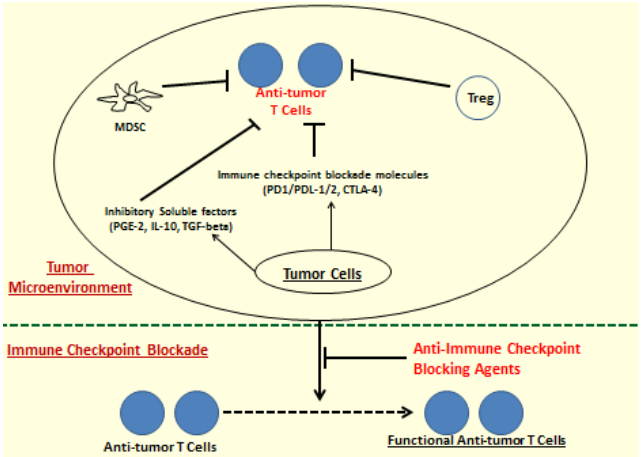

Figure 2: Tumor microenvironment and the immune checkpoint blockade strategy.A growing tumor employs multiple immune-inhibitory mechanisms to counter the anti-tumor immune mechanisms. Among these includes secretion of immunosuppressive soluble factors, such as IL-10, TGF-beta, VEGF, PGE-2 etc.; recruitment of Treg, MDSC; as well as induction of immune checkpoint blockade molecules, such as PD1/PD-L1/2, that causes immune dysfunction of anti-tumor T cells. Agents blocking engagement of immune checkpoint molecules, such as anti-PD1 and anti-PD-L1 antibodies, prevent transmission of negative signals to T cells thereby rescuing the anti-tumor immune effectors from tumor microenvironment mediated immune dysfunction and generating protective anti-tumor immune response. IL-10, interleukine-10; PGE-2, prostaglandin E2; Treg, regulatory T cells; MDSC, myeloid derived suppressor cells.

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