Cancer Immunotherapy Arsenal: Current and Promising New Approaches

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Submission: May 10, 2017; **Published:** June 08, 2017

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Abstract

The remarkable specificity of the immune cells to antigens has long drawn the attention of immunologists and oncologists to develop immunotherapies to fight cancer. Recent developments in detailed understanding of the biology of tumor/host immune responses have considerably increased our attention in exploring immunotherapeutic treatment approaches. Two major strategies that are getting clinical validation are, targeting immunosuppression pathways in the tumor microenvironment, and raising the overall tumor antigen specific cytotoxic T cell populations to tilt the balance towards immune specific tumor control.

Relieving the negative signaling pathways (immunosuppression) of T cell activation using antibodies to immune checkpoints such as cytotoxic T lymphocyte antigen (CTLA-4) and programmed death-1 (PD-1)/ programmed death ligand-1 (PDL-1) have emerged as an attractive strategy and are now approved by FDA for treating patients suffering from various cancers. Recently, adoptive T cell therapy using chimeric antigen receptor (CAR) T cells has shown a great promise in clinical trials and is being actively pursued to treat cancers by in vitro rising of T cells. In the context of these advances, active immunotherapy either alone or in combination with other tumor targeted approaches are considered as promising ways to unleash a long-lasting and durable responses to cancer.

Keywords: Cancer Immunotherapy; CTLA-4; PD-1; PDL-1; Immunosupression; CAR T cell therapy; Adaptive Immune Therapy; Ipilimumab; Nivolumab

Abbreviations: CTLA-4: Cytotoxic T Lymphocyte Antigen-4; PD-1: Programmed Death; APC: Antigen Presenting Cells; NSCLC: Non-Small Cell Lung Cancer; IgG1: Immunoglobulin G; ALL: Acute Lymphoblastic Leukemia

Introduction

Cancer immunotherapy is an approach of treating cancer by harnessing the body's own immune system potential to fight cancer and differs from chemotherapeutic strategies that target tumor itself. Cancer immunotherapy has gained momentum as multiple evidences suggest the role of immune system in fighting the cancer. Some of the evidences include, presence of tumor specific T lymphocytes in the tumor microenvironment and tumor draining lymph nodes, existence of tumor infiltrating immune cells including lymphocytes and monocytes, increasing cases of cancer in immunosuppressed patients, and evidence of cancer remission with immune modulators.

Increasing availability of tools to understand the biology of tumors/host immune system interactions is providing immunotherapy with more ways to utilize immune system to treat cancers. Although much complicated, the development of endogenous immune responses to cancer will follow some key steps which are explored in immunotherapy [1]. Briefly, tumor specific antigens (for example mutated proteins expressed in cancers, non-mutated proteins preferentially expressed in cancers, differential antigens associated with cancer tissue of origin, and antigens against which thymic immune tolerance is not achieved) released either spontaneously or from dead or necrotic cancer cells during the process of oncogenesis will be taken by dendritic cells leading to their activation/maturation.

The activated dendritic cells present the antigens on the surface of their membrane bound MHC receptors and travel to lymph nodes where they prime the T cells leading to differentiation into tumor antigen specific effector T cells with cytotoxic potential. The priming step involves a primary interaction of T cell receptors with antigen expressed on MHC, and a co-stimulatory interactions of CD28 of T cells and B7 class

of molecules (B7.1 and B7.2) on dendritic cells [2]. Although, T cells can interact with tumor antigens directly, it may not activate T cells because of down regulation of B7 class of molecules on tumor cells limiting the co-stimulation required for T cell activation.

The differentiated T cells travel through blood vessels, infiltrate the tumor microenvironment, and specifically kill tumor cells [3-5]. Tumors are shown to counter host immune responses either by skewing dendritic cell maturation or initiating immune suppressive mechanisms or initiating wrong immune responses or local accumulation of regulatory T cells that would oppose the effector T cell responses. Careful decoding of these biology events of tumor/host immune responses lead to immunotherapy approaches with a focus on blocking the negative regulatory pathways of T cell activity (check point blocking) [6], and engineering T cells and dendritic cells with tumor specific antigens to enhance anti-tumor responses.

Blocking the Negative Regulation of T cell Activity

Researchers have identified and targeted two major immune checkpoint mechanisms of T cell activity in the tumor microenvironment. These include cytotoxic T lymphocyte antigen-4 (CTLA-4) receptor based inactivation of T cells and programmed death (PD-1) pathway of T cell inhibition. Strategies that target both these mechanisms have received clinical validation and emerged as major advancements in cancer immunotherapy [6-8].

Targeting CTLA-4 Receptor

CTLA-4 is a negative regulator of immune activation and is expressed on activated T cells. CTLA-4 on T cells interacts with B7 family of accessory molecules expressed on antigen presenting cells (APC), and prevents interaction of B7of APC and CD28 of T cells [9-11]. Unlike B7 and CD28 interaction, the interaction of CTLA-4 with B7 is inhibitory in nature and blocks the further activation and expansion of T cells, and thus effectively control the over activation of T cells from causing chronic autoimmunity [11-14]. James Allison group in 1996 and 1999 reported that modification of endogenous T cell responses would result in potential anti-tumor effects in murine models. They used fully human IgG1 anti-CTLA-4 monoclonal antibodies to block CTLA-4 and observed reduction in tumor growth in multiple murine tumor models [15,16]. This discovery has opened up the idea of targeting the negative regulatory pathways of T cells as a means to treat cancer.

In 1999, a biotechnology firm Medearex (later acquired in 2010 by Bristol-Mayer Squibb) has initiated clinical trials to test an anti-CTLA-4 antibody (Ipilimumab) in patients suffering with metastatic melanoma. In 2009 and 2010 they showed phase II clinical data with improved clinical activity with encouraging long term survival of previously treated advanced metastatic melanoma [17,18]. Phase III clinical trials with Ipilimumab reported a median survival rate of 10.1 months in

unresectable stage III and IV melanoma patients treated with anti-CTLA-4 monoclonal antibody and glycoprotein 100 (gp100) vaccines relative to 6.1 month survival of patients receiving gp100 alone [19]. Also, patients treated with Ipilimumab after the standard decarbazine therapy showed an overall survival rate of 11.2 months compared to 9.1 months when received standard decarbazine therapy alone. Also, among the patients that received Ipilimumab and standard therapy, ~21% were still alive after three years while it was 12% for patients that received standard therapy [20].

These are some of the first examples where a successful phase III data with improved survival rates is produced for the late stage metastatic melanoma. Ipilimumab is subsequently approved by FDA to treat metastatic melanoma patients for whom the first line of therapies have failed [21]. The improved clinical activity was found to be associated with increased tumor antigen specific T cells, increased pretreatment levels of tumor infiltrating lymphocytes and increased mutation load [22-27]. Infusion of CTLA-4 antibodies was also found to lead to some side effects such as colitis, dermatitis, demonstrating its role in peripheral tolerance. Many clinical trials are in progress to test if the anti CTLA-4 antibody therapy holds promise for other types of cancers such as prostate and renal cancers. Also, researchers are testing the hypothesis of further extending the survival rates by combining the immune modulating anti-CTLA-4 therapy with treatments that directly target tumor growth, angiogenesis (targeted therapies) [28].

Several other companies attempted clinical trials with IgG2 isotype of CTLA4 antibody, tremelimumab, for metastatic melanoma. Although phase I and II showed some response, phase III trials did not show any improved clinical anti-tumor activity and the trials were terminated [29]. However, investigations are currently underway using Tremelimumab either as monotherapy against metastatic mesothelioma or in combination with other immunotherapeutic drugs for multiple cancers, including non-small cell lung cancer, squamous cell carcinoma of the head and neck, bladder, pancreatic, gastric and liver cancers. Recent reports suggested that tremelimumab monotherapy did not demonstrate survival benefit for metastatic mesothelioma patients in phase IIb trials. However, the same group published a positive clinical outcome for tremelimumab in combination with durvalumab (PD-1 antibody) for non-small cell lung cancer in a phase Ib study [30].

Targeting PD-1 pathway

Programmed Death-1 (PD-1) is another inhibitory receptor expressed on the surface of T cells that are chronically active and exhausted, such as T cells in the tumor micro environment. PD-1 interacts with ligands PDL-1 and occasionally with PDL-2 that are expressed on surface of antigen presenting cells or other immune cells or tumor itself. The PD-1/PDL-1 (or PDL-2) Interactions render T cell inactive and prevents further proliferation and cytokine production [31]. Unlike CTLA-4, PD-1

does not interfere with co-stimulation of T cells, but interferes with signaling mediated by T cell antigen receptor [32]. It was found that PDL-1 is expressed on tumor after exposure to cytokines released from activated T cells indicating that PD-1/PDL-1 pathway acts to protect the tumor cells from T cell attack [31]. So targeting these interactions using antibodies against PD-1 and PD L-1 to block this negative regulation pathway has gained momentum [33-39]. Two antibodies, nivolumab and pembrolizumab are approved recently in 2015 by FDA for the treatment of various cancers based on the clinical data that improved the overall survival [40,41].

Pembrolizumab is IgG4 monoclonal anti-PD-1 antibody and was first approved anti-PD-1 antibody by FDA for the treatment of unresectable or metastatic melanomas after treatment with ipilimumab [41]. Pembrolizumab is also under clinical trials for the treatment of non-small cell lung cancer (NSCLC). Nivolumab is a fully human IgG4 monoclonal anti-PD-1 antibody, manufactured by Bristol-Myers Squibb and is found to be effective against advanced melanoma (72.9% Survival rate after 1 year and 22% after 3 years in Phase III trial [42], renal carcinoma and non-small cell lung cancer. Among several forms of immunotherapy, nivolumab appears to have high response rate. This may be because of wide distribution of PD-1 in various tissues (lymphoid, myeloid, dendritic, micro vascular endothelial cells) and organs (heart, lung, pancreas, muscle and placenta) suggesting that interactions of PD-1 with its ligand may be important in regulating T cells in peripheral tissues and inhibition of these with nivolumab may trigger a broader activation of T cells responses.

CAR T- cell based adaptive Therapy

Rosenberg et al. have developed chimeric antigen receptor (CAR) T cell based adaptive immunotherapy technology which involves genetic engineering of patient T cells to target tumor cell. CARs are developed in such a way that they will provide T cell with exquisite tumor antigen specific recognition, T cell activation and proliferation. The classic CARs have antigen recognition domain, a hinge domain, a transmembrane domain and an intracellular domain [43]. The antigen recognition domain is a single chain variable region of tumor antigen specific antibody and imparts specificity to recognize tumor cells [44]. The hinge region gives flexibility and transduces signals [45]. Transmembrane domain can be derived from variety of transmembrane receptors including CD4, CD10, CD28, and OX40 are found to influence the function of CAR T cells [46-48]. The intracellular domain is a signaling domain and is responsible for transmitting activation and co-stimulatory signals to T cells. The presence of a variety of co-stimulatory signaling molecules CD4, CD27, CD28, CD134, CD138, in signaling domain provide CAR T cells with functions such as T cell proliferation, cytokine production, and tumor lytic activities [49,50]. A variety of viral and non-viral platforms were developed to infuse CAR T cells into patients.

The major advantage of CAR T cell therapy is that it is not affected by tumor evading mechanisms to mount an immune response. It is independent of MHC dependent antigen presentation which generally limits endogenous T cell response when tumors down regulate MHC expression on their surface [51]. CARs can detect a variety of antigens expressed on tumor cell surface including proteins, lipids, carbohydrates for which antibodies can be generated [52]. CAR therapy can overcome the limitations of poor endogenous T cell immune response that are the result of cancer cell induced down regulation of the co-stimulatory molecules [53].

Although CAR T cell therapy is relatively a new immunotherapy approach, initial clinical results are eye-catching. Shannon et al. in 2014 reported that 27 (90%) out of 30 acute lymphoblastic leukemia (ALL) patients that have undergone CAR T cell therapy targeting CD19 (CTL019) responded with a complete remission [54]. A high response is achieved even for patients with previously failed stem-cell transplantation (15 out of 30). Sustained remission was achieved even after 6 months with event free remission in and survival rate of 67% and overall survival rate of 78%. CTL019 was also found to be persistent in the body at 68% even after 6 months indicating that these cells also show high in vivo proliferation.

This unprecedented early success of CAR T cell therapy raised enormous interest and now became the focus of many clinical trials. In addition to hematological malignancies such as acute lymphoblastic leukemia, clinicians also started attempting to test this approach in solid tumors. It is assumed that treating solid tumors with this approach is much more challenging than treating hematological malignancies given the local strong immune suppressive environment of tumors, shortage of specific antigens, and possible toxicity to normal tissues. Exploration of new targets, safe delivery mechanisms, optimized design of CAR vectors, and combination therapies are underway to treat solid tumors with CAR T cell therapy. Authors are redirected elsewhere for a more detailed description of challenges and strategies to treat solid tumors using CAR T cell therapy [55].

Conclusion

Until now chemotherapy, surgery, and radiation are the major treatment strategies for cancer. Now a fourth weapon namely cancer immunotherapy has been deployed. Recent advances in immunotherapy approaches such as checkpoint blockade and CAR T cell therapies are bringing new hope for patients with advanced disease. The attributes of target specificity, low toxicity and the ability to activate immune system holds a great promise for the immunotherapy. Blocking the CTLA-4, PD-1/PDL-1 check points using their respective antibodies bring about a drastic shift in the way cancer therapy is operated because of the following reasons. First, these antibodies target the events involved in T cell activation rather than tumor itself. Second, the therapy is not used to activate immune cells to target tumors,

but to relieve negative regulation path ways that block immune response.

In addition, to blocking the CTLA-4, PD-1/PDL-1, some other negative regulators LAG-3 or TIM-3 are also being actively pursued in clinical trials. Given CTLA-4 and PD-1/PDL-1 are two distinct negative regulations, combination therapies targeting both pathways for possible synergistic effects are being explored in a large way for various cancers. CAR T cell immunotherapy also showed a great success in treating B cell malignancies and efforts are in full swing to apply the therapy to solid tumors as well. Apart from these approaches, many researchers are also attempting to identify patient specific biomarkers to develop effective patient specific and potent immunotherapeutic strategies. Despite these advances, immunotherapy still treats only a fraction of cancer patients and investigators are still searching for answers on why many can't benefit from immunotherapy. Nonetheless, a new concept to treat cancer patients with a great promise for future is brought into light.

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