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Beyond Cancer Vaccines: A Reason for Future Optimism with Immunomodulatory Therapy

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Abstract

Despite significant scientific knowledge in the field of cancer immunology, therapeutic strategies using cancer vaccines to generate antitumor immunity have historically resulted in only modest clinical benefit. Disappointing results from prior cancer vaccine trials are likely due to multifactorial causes. Perhaps, most importantly, is the role of inherent tumor induced immune suppression and enhanced immunologic tolerance. Current research directed towards understanding the mechanisms of immunologic tolerance has led to the development of promising therapeutic immune regulatory antibodies that inhibit immunologic checkpoints and subsequently enhance immunologic antitumor activity. This review discusses the prior challenges associated with cancer vaccines and describes how, by breaking immune inhibition and facilitating immune stimulation, immune regulatory antibodies show great promise in the treatment of a variety of tumors.

Introduction

The origin of cancer immunotherapy can be traced back to the work of Dr. William Coley who, in the 1890s, found that when bacterial products (Coley's toxins) were administered to patients with inoperable cancers, dramatic responses were seen.^{1,2} Though considered unconventional and controversial for years, the principle of harnessing the immune system to treat cancer has ultimately retained great promise. Specific active immunotherapy, through therapeutic cancer vaccines, is a unique approach to cancer therapy by enlisting the patient's own immune system, largely through activation of T-cells, to recognize and destroy tumors. It is possible that this approach, based upon the immune system's exquisite selectivity, could mount a durable antitumor immune response with fewer side effects than traditional chemotherapy.

Despite the promise of cancer vaccines, initial trials met numerous challenges which limited their clinical utility. Though the reason for prior disappointing clinical results was likely multifactorial, recent research suggests that important mechanisms involved in immunologic inhibition may be a primary reason previous cancer vaccines failed. Immunotherapeutic modalities, aimed at manipulating these immunologic checkpoints, are now proving indispensable in the field of cancer immunotherapy.

On March 25, 2011, the US Food and Drug Administration (FDA) approved ipilimumab (Yervoy™, Bristol-Myers Squibb, Princeton, NJ) for the treatment of patients with unresectable or metastatic melanoma. Ipilimumab is the first therapy for unresectable or metastatic melanoma to demonstrate a significant improvement in overall survival based on the results of a randomized Phase III trial³ and works by blocking cytotoxic T-lymphocyte associated antigen (CTLA)-4. CTLA-4 inhibition enhances antitumor immunity by preventing inhibitory signals that would otherwise downregulate T-cell activation. The FDA's recent approval of ipilimumab exemplifies the promise immunomodulatory antibodies provide. It also validates the use of interleukin-2 (aldesleukin), which should be rightfully considered as the first effective therapy for melanoma which relies solely on immune modulation and has no direct effect on the tumor. This review will focus on the challenges experienced in the development of therapeutic cancer vaccines and how novel strategies to modulate the immune system, particularly through abrogation of immune inhibitory mechanisms and enhancement of costimulatory pathways, is reason to retain great optimism for the future.

Types of Cancer Vaccine Platforms, Associated Challenges, and Clinical Results

A variety of approaches have been used in vaccine development with the goal of inducing clinically relevant antitumor immune responses, each found to have particular challenges. Perhaps the simplest cancer vaccine strategy consists of a peptide/small epitope vaccine delivered intradermally or subcutaneously with an immunologic adjuvant.⁴ Cloning of a number of shared melanoma-associated antigens and cancer-testis antigens (i.e. MAGE-1,-3) expressed specifically by solid tumors, generated enthusiasm for peptide/small epitope vaccination trials to elicit specific antitumor immunity.⁵ Unfortunately, peptide/small epitope vaccines combined with incomplete Freund's adjuvant were insufficiently immunogenic and did not elicit robust antitumor immunity in the absence of exogenous cytokines.⁶ A prior Phase III trial for patients with resected, high-risk melanoma in the adjuvant setting compared GM2-ganglioside vs. interferon- α 2B. Patients treated with interferon- α 2B, as opposed to the GM2-ganglioside vaccine, had improved relapse-free and overall survival.⁷ One Phase II trial for patients with metastatic melanoma showed a high response rate of 42% (13/31 patients) when the peptide vaccine, gp100 was combined with interleukin-2 (IL-2).⁸ Other Phase II trials, however, combining gp100 with IL-2, did not demonstrate as high of a response rate.⁹ Despite varying results in prior Phase II studies of the gp100 vaccine in combination with IL-2, the benefit of combining gp100 with IL-2 appears promising as suggested by the recent favorable results of a randomized, Phase III trial.¹⁰ In this study, patients with advanced melanoma who received IL-2 in combination with gp100 vs. IL-2 alone had improved clinical response (16% vs. 6%, $p=0.03$) and progression-free survival (2.2 months vs. 1.6 months, $p=0.008$), respectively. Overall survival was also improved for patients who received the gp100 vaccine in combination with IL-2, though the result was not statistically significant (17.8 months vs. 11.1 months, $p=0.06$).

Plasmid DNA vaccines, similar to peptide/small epitope vaccines, are another relatively simple cancer vaccine to manufacture. Unlike peptide vaccines, plasmid DNA vaccines do not depend on a particular HLA type because the protein product will be processed *in vivo* by host antigen presenting cells (APCs).¹¹ Unfortunately, many DNA vaccines have faced the challenge of limited immunogenicity *in vivo* in humans.¹² In some models, the immunogenicity of a particular DNA vaccine could be enhanced by using xenogeneic DNA, a closely related version of the gene from a different species.¹³ The xenogeneic plasmid DNA vaccine (Oncept, Merial Ltd.) was shown to improve overall survival in dogs with

canine mucosal melanoma and, in fact, became the first therapeutic cancer vaccine licensed in the US (USDA, 2007).¹⁴

To improve immunogenicity that often limits the efficacy of small epitope and DNA vaccines, recombinant viral and bacterial vaccines have been explored as a way to delivery defined tumor antigens using gene transfer.^{15,16} Though the immunologic potential is high, toxicities and neutralizing immunity to the vector backbone remain significant challenges. Prior phase III trials have not demonstrated a survival benefit with this approach.¹⁷

Dendritic cells (DCs), professional APCs and effective primers of T-cells, have been developed as a vaccine platform. Despite their promise, their heterogeneity often complicates clinical development,¹⁸ and they are often defective when obtained from cancer patients.^{19,20} A promising 30% response rate was seen in an early clinical trial of a peptide-pulsed DC vaccination for melanoma,²¹ but a subsequent Phase III trial disappointingly closed early secondary to clear inferiority of this DC vaccine compared to dacarbazine.²²

Cancer vaccines can be derived from whole tumor cells that deliver a diverse panel of tumor antigens. Since tumor cells are often not inherently immunogenic, tumor cell vaccine approaches require genetic modification or modification of tumor cells with costimulatory molecules, of which granulocyte-macrophage colony-stimulating factor (GM-CSF) has been shown to be particularly effective.^{23,24} CanvaxinTM, a tumor cell vaccine, initially displayed clinical promise based on perceived favorable results for patients with Stage IV melanoma after complete surgical resection.²⁵ Unfortunately, the phase III trial was stopped prematurely due to a survival disadvantage compared to observation alone. A second tumor cell vaccine, OncoVax^R, demonstrated benefits in a phase III trial of 254 patients with resected stage II and stage III colon cancer. Patients who received OncoVax^R were shown to have improved recurrence free survival. Despite this benefit, though available in Europe, financial investment into its developmental program has lagged, and it is still not approved by the FDA.^{26,27} Reniale^R is another tumor cell vaccine that has demonstrated successful clinical results. A 558-patient, open-label phase III clinical study of Reniale^R showed decreased risk of disease progression at 5 years for vaccinated patients compared to those observed alone.²⁸ Significant financial challenges, similar to those facing OncoVax^R, have been preventing plans for an international, phase III, double-blind, randomized, placebo-controlled trial to secure regulatory approval. Lastly, ProphageTM (vitespen; formerly HSPPC-96) is an autologous tumor cell derived heat shock protein vaccine that has been tested in Phase III studies in two populations: Stage IV melanoma and renal cell carcinoma in the adjuvant setting.^{29,30} While both of these studies missed their primary endpoints, retrospective analysis suggested that patients with earlier stage disease and patients who received a greater number of vaccinations may have experienced benefit, hypotheses that require prospective validation.

Radiographic Response Rate May Misguide Potential Benefits of Immunotherapy

In 2004, the Surgery Branch of the National Cancer Institute reviewed 440 patients treated with a variety of cancer vaccines and showed a disappointingly low overall objective response rate of only 2.6%.³¹ An update to the 2004 study was performed in 2011 with a review of an additional 936 patients treated with cancer vaccines since 2004.³² An objective response rate (not including stable disease) remained similar at 3.6%. The response rate varied somewhat among the various vaccine platforms: 3.7% with peptide vaccines; 4.2% with dendritic cell vaccines; 0.9% with recombinant viral vaccines; 2% with tumor cell vaccines; and 6.7% with DNA plasmid vaccines. Though disappointingly low, these

response rates are somewhat predictable as early phase oncology clinical trials, when considered together, have response rates on the order of 3.8% to 11%.^{33,34}

Though response rate is important, overall survival is perhaps the most important and least subjective study endpoint in therapeutic oncology trials. Even in the absence of a convincing improvement in disease response rate, some vaccine trials for men with asymptomatic or minimally symptomatic castrate resistant metastatic prostate cancer demonstrated an overall survival benefit.^{35–37} Though methodology questions regarding these trials remain, the presence of a survival benefit without a convincing tumor response suggests the standard oncologic response evaluation criteria in solid tumors (RECIST) may need to be revised when considering immunotherapeutic approaches.

To address the issue of varied radiographic responses during ipilimumab therapy, the radiographic responses of 487 patients treated on three multicenter Phase II clinical trials of ipilimumab were analyzed, and four patterns were identified: decreased baseline lesions without new lesions; durable stable disease; initial increase in total tumor burden but eventual response; and response of initial lesions with development of new lesions.³⁸ Interestingly, all patterns of radiographic assessment were associated with favorable survival. Due to these observations, immune-related response criteria (irRC) were proposed to evaluate the benefits of immunotherapy, previously potentially misrepresented by traditional RECIST criteria. The main difference of irRC compared to traditional RECIST criteria is that by irRC, the development of new lesions is considered part of the “total tumor burden” and not immediately as overall progressive disease. IrRC are already being used in place of traditional RECIST criteria in current clinical protocols, and prospective validation of irRC’s correlation with additional clinical endpoints is ongoing.

Challenges with Vaccination Approaches and Promise of Immune Regulatory Antibodies

Immunotherapeutic approaches via cancer vaccination alone, as described above, have been disappointing for a number of different reasons. Perhaps, most importantly, is current awareness of the significant role immunosuppressive checkpoints play in halting the desired T-cell tumor reactivity cancer vaccines attempt to achieve.

The immune system has established an elaborate system of self regulation to prevent against excessively damaging immune responses. Though essential for proper immunologic homeostasis, in the presence of active malignancy, inhibition of immune reactivity may predominate over stimulation. Over the past decade, various immune regulatory circuits that contribute to these immunologic checkpoints have been elucidated. Immune regulatory antibodies that block inhibitory pathways or enhance activating pathways have been developed for clinical use. These regulatory antibodies are revitalizing interest in solid tumor immunotherapy and are resulting in promising clinical outcomes where prior cancer vaccination efforts had traditionally fallen short.

Antibodies that Abrogate Immune Inhibition

Cytotoxic T-Lymphocyte Associated (CTLA)-4 Blocking Antibodies

The most well studied and clinically advanced immune regulatory antibodies block immunologic checkpoints by targeting CTLA-4. CTLA-4 is a member of the CD28:B7 immunoglobulin superfamily and is normally expressed at low levels on the surface of naïve effector T cells (Teffs) and regulatory T-cells (Tregs).³⁹ In response to stimulation of a naïve T-cell through the T-cell receptor (TCR), CTLA-4 localizes to the plasma membrane and competes with CD28 for B7, ultimately turning off TCR signaling.^{40,41} The immune

inhibitory function of CTLA-4 is of vital importance as CTLA-4 $-/-$ mice die within one month of birth due to diffuse lymphoproliferation and fatal autoimmune tissue destruction.⁴² CTLA-4 is additionally involved in Treg induced immune inhibition as conditional knockout mice that lack CTLA-4 exclusively in the CD4⁺Foxp3⁺ Treg cell compartment develop systemic lymphoproliferation.⁴³

Preclinical studies suggested the benefit of CTLA-4 inhibition, particularly in combination with GM-CSF, resulting in regression of tumors in a variety of preclinical models: a SM1 mammary tumor model,⁴⁴ a B16 melanoma model,⁴⁵ and a transgenic murine prostate cancer model.⁴⁶ CTLA-4 inhibition also enhanced other vaccination strategies including those involving a poxvirus vaccine and DNA vaccines such as tyrosine-related protein-2 and prostate-specific membrane antigen.^{47,48} In combination with radiotherapy, CTLA-4 inhibition has also demonstrated promise by inhibiting tumors outside of the radiation field in the TSA breast carcinoma and MCA38 colon cancer mouse models.⁴⁹

Two human, monoclonal antibodies that block CTLA-4 have been developed, tremelimumab (Pfizer, New York, NY) and ipilimumab (Bristol Myers-Squibb, Princeton, NJ). In a phase II trial of 246 patients with metastatic melanoma, Tremelimumab was associated with a 21% clinical benefit rate (overall response + stable disease).⁵⁰ An 18 patient Phase II study of Tremelimumab in patients with advanced esophageal and gastric cancer, however, only had one patient with a partial response, but remarkably, this patient has experienced a durable response and had remained well at 32.7 months.⁵¹ Further development of Tremelimumab for metastatic melanoma was halted in April 2008 after Pfizer discontinued a randomized phase III trial based upon interim review results suggesting that Tremelimumab would not demonstrate superiority over standard chemotherapy. These results may have been complicated, however, by an unintentional crossover from chemotherapy to compassionate use ipilimumab. Further development of tremelimumab is under consideration.

Ipilimumab, the other human antibody inhibiting CTLA-4, is the first agent to demonstrate an overall survival benefit in a phase III trial for patients with unresectable stage III or stage IV melanoma.³ In this trial, patients who had progressed on prior therapy were randomized in a 3:1:1 ratio to receive ipilimumab plus a peptide vaccine (gp100), ipilimumab alone, or the peptide vaccine (gp100) alone as the control arm. Median overall survival in the combination ipilimumab and peptide vaccine arm (10.0 months) was similar to the ipilimumab alone arm (10.1 months) but significantly better than the peptide vaccine alone arm (6.4 months, $p=0.003$). Based upon the results of the phase III trial and expected future promise, ipilimumab was approved by the FDA on March 25, 2011, greatly expanding awareness and access of this promising therapeutic antibody.

In a recently completed trial for previously untreated unresectable or metastatic melanoma, ipilimumab (10mg/kg) in combination with dacarbazine was recently shown to demonstrate superior overall survival (11.2 months) to dacarbazine alone (9.1 months). Survival at 1-year, 2-years, and 3-years was higher for the ipilimumab and dacarbazine combination group compared to dacarbazine alone for a hazard ratio for death with combination ipilimumab and dacarbazine of 0.72 ($p<0.001$).⁵² An additional phase III trial of ipilimumab in the adjuvant setting for resected high risk melanoma is ongoing (NCT00636168).

Though ipilimumab has been most thoroughly studied in melanoma, ipilimumab has shown promising efficacy in a variety of additional malignancies. A phase II study in patients with metastatic renal cell carcinoma demonstrated that five out of 40 patients met criteria for a partial response by RECIST criteria.⁵³ Patients with castrate resistant, metastatic prostate cancer have been shown to have a decrease in PSA and response in visceral metastases in

response to ipilimumab in combination with GM-CSF.⁵⁴ Unlike prior cancer vaccine approaches that attempted to elicit T-cell immunoreactivity against a specific tumor antigen, CTLA-4 inhibition causes generalized immunoreactivity. It is expected, therefore, that this may remain an encouraging therapeutic strategy in a variety of additional malignancies as monotherapy, or in combination with relevant, tumor specific vaccination strategies.

Programmed Death (PD)-1 Blocking Antibodies

Although CTLA-4 inhibition remains the most thoroughly investigated and clinically developed strategy for abrogating peripheral tolerance, programmed death (PD)-1 is another inhibitory receptor and promising therapeutic target. PD-1 is expressed on T cells, B cells, and myeloid cells after activation,⁵⁵ and its ligands, PD-L1 and PD-L2, are expressed on APCs, tumor cells, and other cells found in the inflammatory microenvironment.⁵⁶ Interaction between PD-L1 and PD-1 appears to contribute to tumor induced immune suppression through multiple immunosuppressive pathways including induction of T-cell death and enhancement of resistance of tumor cells to T-cell mediated apoptosis.^{57,58} High levels of PD-L1 correlate with poor clinical outcomes in a variety of tumors including: pancreatic, ovarian, renal, urothelial, head and neck, and melanoma.^{59–65}

Two PD-1 inhibitory antibodies have been developed and tested in clinical trials: CT-011 (Cure Tech, Yavne, Israel) and BMS-936558 (previously MDX-1106, Bristol-Myers Squibb, Princeton, NJ, USA).^{66–68} Results from these trials suggest these antibodies are well tolerated and can result in disease response in patients with solid tumors. A Phase II trial of 21 patients with treatment refractory metastatic non-small cell lung cancer, RCC, melanoma, or prostate cancer shows treatment with MDX-1106 demonstrated activity in patients with RCC and melanoma without serious toxicity.⁶⁷

In addition to immune regulatory antibodies directly targeting PD-1, an alternative strategy has involved targeting the PD-1 ligand, PD-L1. Research involving a preclinical murine model of leukemia has shown that an anti-PD-L1 antibody resulted in decreased tumor burden and prolonged survival.⁶⁹ Clinical trials using MDX-1105, an anti-PD-L1 antibody, are ongoing.

Associated Side Effects of Immune Checkpoint Inhibiting Antibodies

As expected, immune regulatory antibodies that block inhibitory checkpoints, such as anti-CTLA-4 and anti-PD-1, have been associated with a spectrum of immune related side effects, traditionally termed, immune related adverse events (irAEs). The most commonly reported adverse events after anti-CTLA-4 inhibition include: diarrhea/colitis, dermatitis/rash, vomiting, nausea, and fatigue.⁷⁰ Enterocolitis is the most threatening inflammatory condition associated with CTLA-4 inhibition and was seen in 15% of patients with melanoma treated with ipilimumab at 10mg/kg.⁷¹ Following the recent approval of ipilimumab and its expected widespread use, early recognition and treatment of potentially life-threatening enterocolitis will be essential. Fortunately, corticosteroids can be extremely effective in managing this unique toxicity, and studies have not shown high-dose steroids compromise responses to ipilimumab.⁷² In cases of steroid refractory enterocolitis, tumor necrosis factor- α blockade with infliximab has been effective.⁷³ Autoimmune hypophysitis is another less frequent irAE occurring in 5% of 163 patients treated with ipilimumab in one study.⁷⁴ Some patients in this study required steroid and/or hormonal therapy, and in some cases the condition was irreversible.

Patients who experience irAEs appear to derive greater benefit from anti-CTLA-4 therapy. In a phase I study, all three responding patients (1 complete response and 2 partial responses) had grade III/IV toxicities. Only three of 11 nonresponders had similar toxicities.⁷⁵ Further

support for the association between irAEs and clinical benefit has been seen in larger studies.^{72,76} Monitoring for predisposition to the development of these irAEs and attempting to separate the therapeutic benefits of anti-CTLA-4 therapy from irAEs are areas of ongoing investigation.

Though PD-1 inhibitory antibody therapy can be additionally associated with immune mediated side effects, its profile of adverse effects is somewhat unique from those associated with CTLA-4 inhibition. Preclinical work has shown that PD-1 genetic knockout mice develop organ-specific autoimmunity, such as dilated cardiomyopathy, nephritis, and arthritic changes.^{77,78} In a phase I trial of the PD-1 inhibiting antibody, CT-011, no maximum tolerated dose (MTD) was reached at doses up to 6mg/kg.⁶⁶ This was similar to results for another PD-1 antibody, MDX-1106, as no MTD was reached at doses up to 10mg/kg.⁶⁸ Only 1 patient developed arthritis and 2 patients developed asymptomatic increase of thyroid stimulating hormone in a subsequent phase II trial of 21 patients who received an antibody directed against PD-1.⁶⁷ Pneumonitis may be another rare side effect which is relatively unique to PD-1 blockade.

Antibodies that Enhance Immune Stimulation

CD40 Agonist Antibodies

In addition to immune regulatory antibodies that break peripheral tolerance, immune regulatory antibodies that enhance stimulatory pathways are an additional area of great promise. CD40, a member of the TNF receptor super family, is expressed on APCs, endothelial cells, and platelets. Activation by its ligand, CD40L, located on activated T-cells, enhances effective tumor immunity by “licensing” the APC for T-cell activation; substituting for T-cell “help”;⁷⁹ and possibly preventing tumor induced apoptosis of DCs.⁸⁰ Given the promise of CD40 agonist therapy in improving tumor immunity, several CD40 agonist, immune stimulating antibodies have been developed.

CP-870,893 (Pfizer, New York, NY) is a fully human IgG2 antibody that has been tested in Phase I clinical trials in patients with solid tumors. Compared to ipilimumab and anti-PD-1 antibodies, a much lower dose was tolerated with the most common side effect being a cytokine release syndrome associated with chills, fever, and rigors. Although one trial with CP-870,893 alone in repetitive doses showed only stable disease with no responses,⁸¹ another with a single administration of CP-870,893 reported that 27% of the melanoma patients experienced a partial response at day 43.⁸² CP-870,893 was tested in 21 patients with surgically unresectable pancreatic ductal carcinoma.⁸³ Results indicated that 4/21 patients had a partial response and 11/21 had stable disease in this traditionally difficult to treat malignancy. The mechanism of CP-870,893’s therapeutic effects is believed to involve the activation of tumor-associated macrophages, illustrating the important role of immunologic manipulation of the tumor microenvironment. A second CD-40 agonist antibody, dacetuzumab, has demonstrated safety and efficacy in a Phase I study of patients with recurrent or refractory non-Hodgkin’s lymphoma.⁸⁴

4-1BB

4-1BB is another member of the TNF receptor superfamily and a marker of T-cell activation. Preclinical work suggests that 4-1BB functions to enhance antigen-specific T-cell activity, and 4-1BB agonist antibodies can facilitate regression of established murine tumors.⁸⁵ A phase I dose escalation trial involving patients with refractory cancer has explored the use of a 4-1BB agonist antibody; out of 47 patients with melanoma, 3 patients achieved a partial response, and 6 patients experienced stable disease.⁸⁶ Recent development, however, has slowed due to two cases of fatal hepatic toxicity.

Glucocorticoid-Induced Tumor Necrosis Factor Receptor Family-Related Gene (GITR)

A third member of the TNF receptor superfamily, glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR), is a type I transmembrane protein expressed on inactive T-cells and up-regulated following T-cell activation.⁸⁷ Ligation of GITR enhances immunity by providing a costimulatory signal that enhances T-cell proliferation and effector functions as well as inhibits the immune suppressive effects of Tregs.⁸⁸ Research in preclinical models has demonstrated ligation of GITR by the monoclonal antibody, DTA-1, at the time of inoculation with a poorly immunogenic tumor led to the rejection of a secondary challenge with the same tumor.⁸⁹ Enhanced primary and recall CD8+ T-cell responses, with associated increases in tumor immunity, has also been observed when vaccination with tumor antigens was combined with GITR ligation.⁹⁰ Treatment as monotherapy with an antibody agonist directed at GITR resulted in regression of small, established B16 melanoma tumors.⁹¹ Current phase I trials of GITR ligation for patients with melanoma are underway.

OX-40

Similar to CD-40, 4-1BB, and GITR, OX-40 is another costimulatory molecule belonging to the TNF receptor superfamily. Binding of OX-40 to its ligand augments T-cell function and inhibits the suppressive function of T regulatory cells.^{92–95} Preclinical data has shown that mice who undergo surgical resection or irradiation of primary sarcomas or lung cancers, respectively, have improved recurrence rate and overall survival if additionally treated with adjuvant OX-40 agonist antibody.⁹⁶ In patients with resected colon cancers, high levels of OX-40 positive lymphocytes in the tumor were correlated with improved survival.⁹⁷ A murine IgG1 monoclonal antibody against OX-40 was prepared and tested in a phase I trial of 20 patients with low overall toxicity.⁹⁸ Five patients had evidence of tumor regression not meeting criteria for partial response, and there was a dose dependent proliferation of CD4+ T-helper cells, CD8+ T cells, and natural killer (NK) cells. Since the OX-40 antibody in this study was a murine antibody, antimurine antibodies developed in the treated patients which likely limited clinical benefit.

Summary and Future Directions of Therapeutic Cancer Immunotherapy

Although years of challenges with cancer vaccines have produced less than optimal clinical results, there is reason for future optimism. It appears that stimulating host immunity, through vaccination alone, is insufficient to engender an effective antitumor immune response. Though prior failures are likely due to many reasons, inherent and tumor induced immunosuppressive mechanisms likely prevented prior attempts at therapeutic cancer vaccination. Currently available immunomodulatory antibodies that break these inhibitory circuits, such as those directed against CTLA-4 and PD-1, have shown great promise and are indispensable when considering vaccination strategies. Costimulatory antibodies directed at CD40, 4-1BB, GITR, and OX-40 additionally may help shift the balance in favor of enhancing immune mediated antitumor effects. Future immunotherapy clinical trials are needed to evaluate novel combinations of immunomodulatory antibodies and combinations of immunomodulatory antibodies with other systemic therapies including chemotherapy and targeted therapy.

References

1. Nauts HC, Fowler GA, Bogatko FH. A review of the influence of bacterial infection and of bacterial products (Coley's toxins) on malignant tumors in man; a critical analysis of 30 inoperable cases treated by Coley's mixed toxins, in which diagnosis was confirmed by microscopic examination selected for special study. *Acta Med Scand Suppl.* 1953; 276:1–103. [PubMed: 13039964]

2. Coley WB. The Treatment of Inoperable Sarcoma by Bacterial Toxins (the Mixed Toxins of the *Streptococcus erysipelas* and the *Bacillus prodigiosus*). *Proc R Soc Med*. 1910; 3:1–48. [PubMed: 19974799]
3. Hodi FS, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010; 363:711–723. [PubMed: 20525992]
4. Purcell AW, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. *Nat Rev Drug Discov*. 2007; 6:404–414. [PubMed: 17473845]
5. Ding M, Beck RJ, Keller CJ, Fenton RG. Cloning and analysis of MAGE-1-related genes. *Biochem Biophys Res Commun*. 1994; 202:549–555. [PubMed: 8037761]
6. Smith C, Cerundolo V. Immunotherapy of melanoma. *Immunology*. 2001; 104:1–7. [PubMed: 11576213]
7. Kirkwood JM, et al. High-dose interferon alfa-2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIB-III melanoma: results of intergroup trial E1694/S9512/C509801. *J Clin Oncol*. 2001; 19:2370–2380. [PubMed: 11331315]
8. Rosenberg SA, et al. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nat Med*. 1998; 4:321–327. [PubMed: 9500606]
9. Sosman JA, et al. Three phase II cytokine working group trials of gp100 (210M) peptide plus high-dose interleukin-2 in patients with HLA-A2-positive advanced melanoma. *J Clin Oncol*. 2008; 26:2292–2298. [PubMed: 18467720]
10. Schwartzentruber DJ, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. *N Engl J Med*. 2011; 364:2119–2127. [PubMed: 21631324]
11. Greenland JR, Letvin NL. Chemical adjuvants for plasmid DNA vaccines. *Vaccine*. 2007; 25:3731–3741. [PubMed: 17350735]
12. Wolchok JD, et al. Safety and immunogenicity of tyrosinase DNA vaccines in patients with melanoma. *Mol Ther*. 2007; 15:2044–2050. [PubMed: 17726460]
13. Weber LW, et al. Tumor immunity and autoimmunity induced by immunization with homologous DNA. *The Journal of clinical investigation*. 1998; 102:1258–1264. [PubMed: 9739060]
14. Bergman PJ, et al. Long-term survival of dogs with advanced malignant melanoma after DNA vaccination with xenogeneic human tyrosinase: a phase I trial. *Clin Cancer Res*. 2003; 9:1284–1290. [PubMed: 12684396]
15. Wu AJ, et al. Radiotherapy after surgical resection for head and neck mucosal melanoma. *Am J Clin Oncol*. 2010; 33:281–285. [PubMed: 19823070]
16. Rosenberg SA, et al. Immunizing patients with metastatic melanoma using recombinant adenoviruses encoding MART-1 or gp100 melanoma antigens. *J Natl Cancer Inst*. 1998; 90:1894–1900. [PubMed: 9862627]
17. Amato RJ, et al. Vaccination of metastatic renal cancer patients with MVA-5T4: a randomized, double-blind, placebo-controlled phase III study. *Clin Cancer Res*. 2010; 16:5539–5547. [PubMed: 20881001]
18. Liu YJ, Kanzler H, Soumelis V, Gillet M. Dendritic cell lineage, plasticity and cross-regulation. *Nat Immunol*. 2001; 2:585–589. [PubMed: 11429541]
19. Brown RD, et al. Dendritic cells from patients with myeloma are numerically normal but functionally defective as they fail to up-regulate CD80 (B7-1) expression after huCD40LT stimulation because of inhibition by transforming growth factor-beta1 and interleukin-10. *Blood*. 2001; 98:2992–2998. [PubMed: 11698282]
20. Satthaporn S, et al. Dendritic cells are dysfunctional in patients with operable breast cancer. *Cancer Immunol Immunother*. 2004; 53:510–518. [PubMed: 14740176]
21. Nestle FO, et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med*. 1998; 4:328–332. [PubMed: 9500607]
22. Schadendorf D, et al. Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic melanoma: a randomized phase III trial of the DC study group of the DeCOG. *Ann Oncol*. 2006; 17:563–570. [PubMed: 16418308]

23. Pandha H, Eaton J, Greenhalgh R, Soars D, Dalglish A. Immunotherapy of murine prostate cancer using whole tumor cells killed ex vivo by herpes simplex viral thymidine kinase/ganciclovir suicide gene therapy. *Cancer Gene Ther.* 2005; 12:572–578. [PubMed: 15803141]
24. Dranoff G, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci U S A.* 1993; 90:3539–3543. [PubMed: 8097319]
25. Hsueh EC, et al. Prolonged survival after complete resection of disseminated melanoma and active immunotherapy with a therapeutic cancer vaccine. *J Clin Oncol.* 2002; 20:4549–4554. [PubMed: 12454111]
26. Vermorken JB, et al. Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. *Lancet.* 1999; 353:345–350. [PubMed: 9950438]
27. Johnson RS, Walker AI, Ward SJ. Cancer vaccines: will we ever learn? *Expert Rev Anticancer Ther.* 2009; 9:67–74. [PubMed: 19105708]
28. Jocham D, et al. Adjuvant autologous renal tumour cell vaccine and risk of tumour progression in patients with renal-cell carcinoma after radical nephrectomy: phase III, randomised controlled trial. *Lancet.* 2004; 363:594–599. [PubMed: 14987883]
29. Testori A, et al. Phase III comparison of vitespen, an autologous tumor-derived heat shock protein gp96 peptide complex vaccine, with physician's choice of treatment for stage IV melanoma: the C-100-21 Study Group. *J Clin Oncol.* 2008; 26:955–962. [PubMed: 18281670]
30. Wood C. A vaccine for renal cancer. *Lancet.* 2008; 372:1460–1461. [PubMed: 18970972]
31. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med.* 2004; 10:909–915. [PubMed: 15340416]
32. Klebanoff CA, Acquavella N, Yu Z, Restifo NP. Therapeutic cancer vaccines: are we there yet? *Immunol Rev.* 2011; 239:27–44. [PubMed: 21198663]
33. Horstmann E, et al. Risks and benefits of phase 1 oncology trials, 1991 through 2002. *N Engl J Med.* 2005; 352:895–904. [PubMed: 15745980]
34. Roberts TG Jr, et al. Trends in the risks and benefits to patients with cancer participating in phase 1 clinical trials. *Jama.* 2004; 292:2130–2140. [PubMed: 15523074]
35. Kantoff PW, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med.* 2010; 363:411–422. [PubMed: 20818862]
36. Small EJ, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. *J Clin Oncol.* 2006; 24:3089–3094. [PubMed: 16809734]
37. Kantoff PW, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J Clin Oncol.* 2010; 28:1099–1105. [PubMed: 20100959]
38. Wolchok JD, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res.* 2009; 15:7412–7420. [PubMed: 19934295]
39. Alegre ML, et al. Regulation of surface and intracellular expression of CTLA4 on mouse T cells. *J Immunol.* 1996; 157:4762–4770. [PubMed: 8943377]
40. van der Merwe PA, Bodian DL, Daenke S, Linsley P, Davis SJ. CD80 (B7-1) binds both CD28 and CTLA-4 with a low affinity and very fast kinetics. *J Exp Med.* 1997; 185:393–403. [PubMed: 9053440]
41. Linsley PS, et al. Intracellular trafficking of CTLA-4 and focal localization towards sites of TCR engagement. *Immunity.* 1996; 4:535–543. [PubMed: 8673700]
42. Tivol EA, et al. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity.* 1995; 3:541–547. [PubMed: 7584144]
43. Wing K, et al. CTLA-4 control over Foxp3+ regulatory T cell function. *Science.* 2008; 322:271–275. [PubMed: 18845758]
44. Hurwitz AA, Yu TF, Leach DR, Allison JP. CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma. *Proc Natl Acad Sci U S A.* 1998; 95:10067–10071. [PubMed: 9707601]

45. van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. *J Exp Med*. 1999; 190:355–366. [PubMed: 10430624]
46. Hurwitz AA, et al. Combination immunotherapy of primary prostate cancer in a transgenic mouse model using CTLA-4 blockade. *Cancer Res*. 2000; 60:2444–2448. [PubMed: 10811122]
47. Espenschied J, et al. CTLA-4 blockade enhances the therapeutic effect of an attenuated poxvirus vaccine targeting p53 in an established murine tumor model. *J Immunol*. 2003; 170:3401–3407. [PubMed: 12626601]
48. Gregor PD, et al. CTLA-4 blockade in combination with xenogeneic DNA vaccines enhances T-cell responses, tumor immunity and autoimmunity to self antigens in animal and cellular model systems. *Vaccine*. 2004; 22:1700–1708. [PubMed: 15068853]
49. Dewan MZ, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res*. 2009; 15:5379–5388. [PubMed: 19706802]
50. Kirkwood JM, et al. Phase II trial of tremelimumab (CP-675,206) in patients with advanced refractory or relapsed melanoma. *Clin Cancer Res*. 2010; 16:1042–1048. [PubMed: 20086001]
51. Ralph C, et al. Modulation of lymphocyte regulation for cancer therapy: a phase II trial of tremelimumab in advanced gastric and esophageal adenocarcinoma. *Clin Cancer Res*. 2010; 16:1662–1672. [PubMed: 20179239]
52. Robert C, et al. Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. *N Engl J Med*. 2011
53. Yang JC, et al. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. *J Immunother*. 2007; 30:825–830. [PubMed: 18049334]
54. Fong L, et al. Potentiating endogenous antitumor immunity to prostate cancer through combination immunotherapy with CTLA4 blockade and GM-CSF. *Cancer Res*. 2009; 69:609–615. [PubMed: 19147575]
55. Freeman GJ, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med*. 2000; 192:1027–1034. [PubMed: 11015443]
56. Hirano F, et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. *Cancer Res*. 2005; 65:1089–1096. [PubMed: 15705911]
57. Dong H, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med*. 2002; 8:793–800. [PubMed: 12091876]
58. Azuma T, et al. B7-H1 is a ubiquitous antiapoptotic receptor on cancer cells. *Blood*. 2008; 111:3635–3643. [PubMed: 18223165]
59. Hino R, et al. Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. *Cancer*. 2010; 116:1757–1766. [PubMed: 20143437]
60. Nomi T, et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. *Clin Cancer Res*. 2007; 13:2151–2157. [PubMed: 17404099]
61. Hamanishi J, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. *Proc Natl Acad Sci U S A*. 2007; 104:3360–3365. [PubMed: 17360651]
62. Thompson RH, et al. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. *Cancer Res*. 2006; 66:3381–3385. [PubMed: 16585157]
63. Nakanishi J, et al. Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. *Cancer Immunol Immunother*. 2007; 56:1173–1182. [PubMed: 17186290]
64. Wu C, et al. Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. *Acta Histochem*. 2006; 108:19–24. [PubMed: 16530813]

65. Hsu MC, et al. Increase of programmed death-1-expressing intratumoral CD8 T cells predicts a poor prognosis for nasopharyngeal carcinoma. *Mod Pathol*. 2010; 23:1393–1403. [PubMed: 20657553]
66. Berger R, et al. Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. *Clin Cancer Res*. 2008; 14:3044–3051. [PubMed: 18483370]
67. Brahmer JRTS, Powderly J, et al. Phase II experience with MDX-1106 (ONO-4538), an anti-PD-1 monoclonal antibody, in patients with selected refractory or relapsed malignancies. *J Clin Oncol*. 2008; 27(suppl):abstract 3015.
68. Brahmer JRTS, Wollner I. Safety and activity of MDX-1106 (ONO-4538), an anti-PD-1 monoclonal antibody, in patients with selected refractory or relapsed malignancies. *J Clin Oncol*. 2008; 26(suppl):abstract 3006.
69. Zhang L, Gajewski TF, Kline J. PD-1/PD-L1 interactions inhibit antitumor immune responses in a murine acute myeloid leukemia model. *Blood*. 2009; 114:1545–1552. [PubMed: 19417208]
70. Weber J. Ipilimumab: controversies in its development, utility and autoimmune adverse events. *Cancer Immunol Immunother*. 2009; 58:823–830. [PubMed: 19198837]
71. Wolchok JD, et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. *Lancet Oncol*. 2010; 11:155–164. [PubMed: 20004617]
72. Downey SG, et al. Prognostic factors related to clinical response in patients with metastatic melanoma treated by CTL-associated antigen-4 blockade. *Clin Cancer Res*. 2007; 13:6681–6688. [PubMed: 17982122]
73. Beck KE, et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. *J Clin Oncol*. 2006; 24:2283–2289. [PubMed: 16710025]
74. Blansfield JA, et al. Cytotoxic T-lymphocyte-associated antigen-4 blockade can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. *J Immunother*. 2005; 28:593–598. [PubMed: 16224277]
75. Phan GQ, et al. Immunization of patients with metastatic melanoma using both class I-and class II-restricted peptides from melanoma-associated antigens. *J Immunother*. 2003; 26:349–356. [PubMed: 12843797]
76. Weber JS, et al. Phase I/II study of ipilimumab for patients with metastatic melanoma. *J Clin Oncol*. 2008; 26:5950–5956. [PubMed: 19018089]
77. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity*. 1999; 11:141–151. [PubMed: 10485649]
78. Nishimura H, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science*. 2001; 291:319–322. [PubMed: 11209085]
79. Diehl L, et al. CD40 activation in vivo overcomes peptide-induced peripheral cytotoxic T-lymphocyte tolerance and augments anti-tumor vaccine efficacy. *Nat Med*. 1999; 5:774–779. [PubMed: 10395322]
80. Lenahan C, Avigan D. Dendritic cell defects in patients with cancer: mechanisms and significance. *Breast Cancer Res*. 2006; 8:101. [PubMed: 16469120]
81. Ruter J, Antonia SJ, Burris HA, Huhn RD, Vonderheide RH. Immune modulation with weekly dosing of an agonist CD40 antibody in a phase I study of patients with advanced solid tumors. *Cancer Biol Ther*. 2010; 10:983–993. [PubMed: 20855968]
82. Vonderheide RH, et al. Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. *J Clin Oncol*. 2007; 25:876–883. [PubMed: 17327609]
83. Beatty GL, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science*. 2011; 331:1612–1616. [PubMed: 21436454]
84. Advani R, et al. Phase I study of the humanized anti-CD40 monoclonal antibody dacetuzumab in refractory or recurrent non-Hodgkin's lymphoma. *J Clin Oncol*. 2009; 27:4371–4377. [PubMed: 19636010]

85. May KF Jr, Chen L, Zheng P, Liu Y. Anti-4-1BB monoclonal antibody enhances rejection of large tumor burden by promoting survival but not clonal expansion of tumor-specific CD8+ T cells. *Cancer Res.* 2002; 62:3459–3465. [PubMed: 12067989]
86. Sznol MHF, Margolin K, et al. Phase I study of BMS-663513, a fully human anti-CD137 agonist monoclonal antibody, in patients (pts) with advanced cancer (CA). *J Clin Oncol.* 2008; 26(20 suppl):abstract 3007.
87. Gurney AL, et al. Identification of a new member of the tumor necrosis factor family and its receptor, a human ortholog of mouse GITR. *Curr Biol.* 1999; 9:215–218. [PubMed: 10074428]
88. Tone M, et al. Mouse glucocorticoid-induced tumor necrosis factor receptor ligand is costimulatory for T cells. *Proc Natl Acad Sci U S A.* 2003; 100:15059–15064. [PubMed: 14608036]
89. Turk MJ, et al. Concomitant tumor immunity to a poorly immunogenic melanoma is prevented by regulatory T cells. *J Exp Med.* 2004; 200:771–782. [PubMed: 15381730]
90. Cohen AD, et al. Agonist anti-GITR antibody enhances vaccine-induced CD8(+) T-cell responses and tumor immunity. *Cancer Res.* 2006; 66:4904–4912. [PubMed: 16651447]
91. Cohen AD, et al. Agonist anti-GITR monoclonal antibody induces melanoma tumor immunity in mice by altering regulatory T cell stability and intra-tumor accumulation. *PLoS One.* 2010; 5:e10436. [PubMed: 20454651]
92. Evans DE, Prell RA, Thalhoffer CJ, Hurwitz AA, Weinberg AD. Engagement of OX40 enhances antigen-specific CD4(+) T cell mobilization/memory development and humoral immunity: comparison of alphaOX-40 with alphaCTLA-4. *J Immunol.* 2001; 167:6804–6811. [PubMed: 11739496]
93. Weinberg AD, et al. Engagement of the OX-40 receptor in vivo enhances antitumor immunity. *J Immunol.* 2000; 164:2160–2169. [PubMed: 10657670]
94. Redmond WL, Gough MJ, Charbonneau B, Ratliff TL, Weinberg AD. Defects in the acquisition of CD8 T cell effector function after priming with tumor or soluble antigen can be overcome by the addition of an OX40 agonist. *J Immunol.* 2007; 179:7244–7253. [PubMed: 18025166]
95. Jensen SM, et al. Signaling through OX40 enhances antitumor immunity. *Semin Oncol.* 2010; 37:524–532. [PubMed: 21074068]
96. Gough MJ, et al. Adjuvant therapy with agonistic antibodies to CD134 (OX40) increases local control after surgical or radiation therapy of cancer in mice. *J Immunother.* 2010; 33:798–809. [PubMed: 20842057]
97. Petty JK, He K, Corless CL, Vetto JT, Weinberg AD. Survival in human colorectal cancer correlates with expression of the T-cell costimulatory molecule OX-40 (CD134). *Am J Surg.* 2002; 183:512–518. [PubMed: 12034383]
98. Kovacsovics-Bankowski MWE, Floyd K, et al. Increased CD4 and CD8 memory T cell proliferation following anti-OX40 administration to cancer patients: immunologic assessment of a phase I clinical trial. *ISBtC.* 2009