Published in final edited form as:

Cancer J. 2010 ; 16(4): 311–317. doi:10.1097/PPO.0b013e3181eb3381.

Immune Regulatory Antibodies:

Are They the Next Advance?

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Abstract

During the past decade, new insights into the mechanisms by which T-cell activation and proliferation are regulated have led to the identification of checkpoint proteins that either up- or down-modulate T-cell reactivity. In the presence of active malignancy, pathophysiologic inhibition of T-cell activity may predominate over stimulation. A number of antibodies have been generated that can block inhibitory checkpoint proteins or promote the activity of activating molecules. In murine models, their use alone or with a vaccine strategy has resulted in regression of poorly immunogenic tumors and cures of established tumors. The prototypical immune regulatory antibodies are those directed against cytotoxic T-lymphocyte antigen-4, a molecule present on activated T cells. In this review, the preclinical rationale and clinical experience with 2 anticytotoxic T-lymphocyte antigen-4 antibodies are extensively discussed, demonstrating that abrogation of an immune inhibitory molecule can result in significant regression of tumors and long-lasting responses. The unique kinetics of antitumor response and the characteristic immune-related side effects of ipilimumab are also discussed. This clinical efficacy of this promising antitumor agent has been evaluated in 2 randomized phase III trials, whose results are eagerly awaited. Programmed death (PD)-1 is another immune inhibitory molecule against which an abrogating human antibody has been prepared. Initial preclinical testing with anti-PD-1 and anti-PD-L1 has shown encouraging results. Stimulatory molecules such as CD40, 41-BB, and OX-40 are also targets for antibody binding and activation, not blockade, and early dose ranging trials with antibodies against all 3 have shown that they can mediate regression of tumors, albeit with their own spectrum of side effects that are different from those that occur with abrogation of immune inhibition.

Keywords

antibody; T cell; immune; checkpoint; stimulation

CTLA-4: PRECLINICAL DATA

Cytotoxic T-lymphocyte antigen (CTLA)-4, a member of the CD28:B7 immunoglobulin superfamily, is normally expressed at low levels on the surface of naive effector T cells (Teffs) and regulatory T cells (Tregs) and exists in prepackaged vesicles inside the cytosol.¹ When there is a strong or long-lasting stimulus to the naive T cell through the T-cell receptor (TCR), CTLA-4 is recruited to the cell surface and is released in the immunologic synapse.² CTLA-4 then competes with CD28 for CD80/CD86,^{3–5} effectively shutting off TCR signaling.⁶ Although CTLA-4 translocation to the cell membrane has been shown to depend on many events downstream of TCR signaling (reviewed in Ref. 7), it is not yet entirely clear how CTLA-4 mediates TCR signaling shut down on binding to CD80/CD86.

The importance of CTLA-4 control of T-cell activation was originally demonstrated by studies showing that blockade of CTLA-4:B7 interactions enhanced T-cell responses in vitro.⁸ CTLA-4^{–/–} mice die within 3 to 4 weeks of birth because of diffuse lymphoproliferation and exhibit fatal tissue destruction in multiple organs, whereas CTLA-4/B7-1/B7-2 triple knockout mice lack lymphoproliferative disease. Taken together, these data confirm the nonredundant role for CTLA-4 in inhibiting T-cell expansion.^{9,10}

Both CD4⁺ and CD8⁺ T cells lacking CTLA-4 in vitro and in vivo exhibit higher proliferative potential and an activated phenotype.^{11–16} Interestingly, lack of CTLA-4 had a more dramatic effect on the proliferation of CD4⁺ T cells in vivo, resulting in a shift of the CD4:CD8 ratio toward CD4⁺ T cells. In addition, CD4⁺ T cells were required for the massive infiltration of peripheral organs, and CD8⁺ T-cell activation was entirely CD4⁺ T-cell dependent.¹¹ Data from experiments with CTLA-4-blocking antibodies consistently showed that CTLA-4 engagement induced peripheral CD4⁺ T-cell tolerance¹⁷ and regulated CD4⁺ T-cell activation by modulating cell cycle progression.^{14–15}

The role of CTLA-4 in CD8⁺ T-cell responses was also investigated, and adoptive transfer experiments with pmel-1 TCR transgenic mice, specific for the self antigen gp100 showed that CTLA-4 did not have a direct intrinsic effect on CD8⁺ T cells. In fact, pmel-1 CTLA-4^{–/–} mice developed autoimmune hypopigmentation in a CD4⁺ T-cell-dependent manner.¹⁸ Although CTLA-4 has a limited role in regulating immune responses by CD8⁺ T cells, experiments with 2CT TCR transgenic T cells showed that secondary CD8⁺ T-cell responses significantly increased in CTLA-4 deficient mice, suggesting that CTLA-4 may function in regulating memory responses.¹²

CTLA-4 also plays a role in regulating the suppressive function of Tregs. Conditional knockout mice lacking CTLA-4 in the CD4⁺Foxp3⁺ Treg cell compartment developed systemic lymphoproliferation, indicating that CTLA-4 deficiency in Foxp3⁺ T cells is sufficient to destabilize immune homeostasis.¹⁹ The exact mechanism by which CTLA-4 negatively regulates the immune system is unknown. Tregs expressing CTLA-4 may inhibit Teffs through the release of immunosuppressive cytokines or may modulate the expression of other cells, which in turn inhibit Teffs. Data have shown that CTLA-4-positive TCR transgenic T cells do not have long-term interactions with antigen-presenting cells (APCs) in the presence of antigen in vitro and in vivo,²⁰ suggesting that dendritic cells may be

important to induce or maintain CTLA-4-dependent Treg-mediated immune suppression. Similarly, CTLA-4 expression on Foxp3⁺ Tregs mediates down-regulation of CD80 and CD86 on the splenic dendritic cells in vitro.¹⁹ These data suggest that CTLA-4-dependent suppression by Tregs may be mediated, at least in part, by APCs such as dendritic cells.

PRECLINICAL STUDIES USING CTLA-4 BLOCKING ANTIBODIES

Monotherapy

Treatment with anti-CTLA-4-blocking antibody as a single agent causes the regression of tumors in transplantable tumor models of colon carcinoma (51BLim10), fibrosarcoma (Sa1N and CSA1M), ovarian carcinoma (OV-HM), and prostate cancer (TRAMPC1) and also provided protection to subsequent challenge with the same tumor.^{21–23} However, anti-CTLA-4-blocking antibody alone is unable to induce regression of established poorly immunogenic tumors.²⁴

CTLA-4 and Active or Passive Immunization

The combination of anti-CTLA-4 with granulocyte/macrophage colony-stimulating factor (GM-CSF)-expressing tumor vaccines resulted in the regression of the poorly immunogenic SM1 mammary tumor,²⁵ B16 melanoma,²⁴ and transgenic adenocarcinoma of the mouse prostate model of prostate cancer.²⁶ In these preclinical models, treatment with anti-CTLA-4- and a GM-CSF-expressing vaccine synergized to promote an immune response capable of causing regression of established tumors while neither treatment alone was sufficient. The nature of the immune response varied depending on the tumor type. For example, CD8⁺ T cells, natural killer (NK) cells, perforin and Fas/Fas ligand interactions, but not CD4⁺ T cells, and tumor necrosis factor (TNF)- α were required to reject B16 tumors,²⁷ whereas both CD4⁺ and CD8⁺ T-cell responses promoted rejection of the SM1 mammary tumor.²⁵ CTLA-4 blockade also synergized with other vaccination strategies, including tyrosine-related protein-2 peptide in cytosine gua-nine-oligodeoxynucleotide adjuvant,²⁸ tyrosine-related protein-2, gp100, and prostate-specific membrane antigen xenogeneic DNA vaccines²⁹ for melanoma and prostate cancer.

Finally, cancer therapies employing the use of adoptively transferred tumor-reactive T cells have shown encouraging clinical activity. Combining CTLA-4 blockade with the adoptive transfer of CD4⁺ T cells recognizing a melanoma differentiation antigen resulted in an expansion of Teff and an increase in the antitumor immune response.³⁰

CTLA-4 and Radiotherapy or Chemotherapy

Combining anti-CTLA-4 with ionizing radiation has been shown to augment immune responses in the 4T1 and TSA mammary tumor and MCA38 colon carcinoma models.³¹ These 2 treatments resulted in a systemic antitumor effect that inhibited the growth of a distant tumor outside the radiation field, suggesting that the combination of CTLA-4 blockade and local radiation enhanced a tumor-specific immune response after release of antigen by the radiation. In addition, combining anti-CTLA-4 treatment with a subtherapeutic dose of melphalan induced regression of tumors in mice bearing the MOPC-315 transplantable plasmacytoma tumor. A possible mechanism underlying the

antitumor effects may be that anti-CTLA-4 synergized with chemotherapy to target multiple immunoregulatory pathways.^{32,33} Alternatively, cytotoxic chemotherapy, similar to radiotherapy, could lead to antigen recognition by immunogenic cell death.

CLINICAL EXPERIENCE

The clinical application of immunomodulatory antibodies has generated a new approach to systemic anticancer therapy. Given the fundamental differences from cytotoxic chemotherapy, it was expected to observe differences in the kinetics of the antitumor response, duration of response, and immune-related adverse events (irAEs), compared with the more recognized patterns of response and toxicity observed with cytotoxic therapies.

Two CTLA-4-blocking monoclonal antibodies are currently available in clinical development including ipilimumab (MDX-010, Bristol Myers-Squibb, Princeton, NJ) and tremelimumab (CP-675206, Pfizer, New York, NY).^{34–37} Both antibodies were developed in mice tg for human immunoglobulin genes, thereby producing “fully human” antibodies against CTLA-4. These agents have been most extensively studied in melanoma; however, there have also been durable responses noted in prostate, ovarian, breast, and renal cell cancer (RCC).^{38–40} Although early studies with tremelimumab demonstrated similar patterns of efficacy and irAEs as ipilimumab, to date, the greatest clinical experience has been with ipilimumab.^{34,41–43} Clinical development of tremelimumab was interrupted 2 years ago after a randomized phase 3 clinical trial failed to show a survival difference in patients treated with tremelimumab or dacarbazine/temozolomide. It should be noted that the dose and schedule for tremelimumab are quite different from ipilimumab, and an unintentional crossover from chemotherapy to compassionate use ipilimumab may have confounded the results of the phase 3 trial. It has recently been announced that an additional phase 3 trial of tremelimumab may be conducted in melanoma patients selected on the basis of a predictive biomarker. Given the more abundant clinical information available about ipilimumab in recent years, the remainder of this review will focus primarily on ipilimumab.

CTLA-4 Monotherapy

Preclinical studies had suggested a synergistic relationship between tumor vaccination and anti-CTLA-4 therapy. Therefore, in 3 separate initial pilot clinical trials, 79 metastatic melanoma or advanced ovarian cancer patients were treated with ipilimumab either with combined or prior tumor vaccination. There was evidence of response in 20% of the patients, which prompted further studies.^{35,44,45} Subsequently, there was a dose–response relationship demonstrated in a double-blind phase II trial of ipilimumab with 3 dose levels (0.3, 3, or 10 mg/kg) administered as a monotherapy to 217 unresectable stage III/IV melanoma patients. The 10 mg/kg cohort had the greatest response rate at 11% with a median overall survival of 14 months.⁴⁶ In an additional phase II trial of ipilimumab in advanced stage melanoma patients progressing on prior therapies, 155 patients were treated with 10 mg/kg of ipilimumab, and the best overall response rate was shown to be 5.8%.⁴⁷ Currently, 2 separate phase III registration studies are under analysis with ipilimumab in both first-line and second-line therapy for metastatic melanoma. The results of the phase III trial in second-line treatment for unresectable stages II and IV melanoma, in which patients were randomly allocated in a 3:1:1 ratio to receive ipilimumab at 3 mg/kg plus a peptide

vaccine, ipilimumab alone, or the peptide vaccine alone as a control arm, demonstrated that overall survival was superior in either ipilimumab arm compared with the control arm.⁴⁸ The median overall survival rates for the 2 ipilimumab arms were 10.0 and 10.1 months, compared with 6.4 months for the control arm. Hazard ratios for survival were 0.68 and 0.66 for the 2 ipilimumab arms compared with vaccine alone, indicating that ipilimumab significantly prolonged overall survival in patients who had failed a prior first-line therapy for unresectable stages II and IV disease. These data are the first to show a survival advantage in a phase III controlled, randomized trial in unresectable melanoma. Ipilimumab has also been studied in the adjuvant setting for stage II through IV resected melanoma patients with no evidence of disease. Currently, there is an ongoing phase III double-blind, placebo-controlled adjuvant ipilimumab trial for stage III melanoma patients without evidence of disease, sponsored by European Organization for the Research and Treatment of Cancer.

Novel Criteria for Antitumor Response to Ipilimumab and Increased Duration of Response

The kinetics and duration of response with anti-CTLA-4 therapy seem to differ from traditional treatments, particularly chemotherapy. In a recent phase II trial of ipilimumab in stage IV melanoma, the 1-year survival rate was 47.2%, and the 2-year survival rate was 32.8%, which seem quite favorable compared with a historical median survival of 6 to 9 months with metastatic disease.⁴⁷ A set of novel immune-related response criteria (irRC) was recently proposed because of the consistent observation of late responses with ipilimumab, sometimes occurring 5 to 6 months after initiating treatment, in contrast to chemotherapy where direct cytotoxic effects can be observed rapidly, and late effects are rare. The delayed action of anti-CTLA-4 therapy may be related to the time needed for specific activation of the immune system to recognize antigens expressed by individual tumors or a function of the kinetics regarding immune-mediated tumor destruction. In addition, radiologic evidence of progression of disease at early time points may reflect a heterogeneous mixture of inflammation, edema, and lymphocytic infiltration as opposed to true increase in tumor volume. In response to these observations, a set of novel irRC have been developed recently. To develop the irRC, phase II clinical trial data from the ipilimumab program were used to define 4 patterns of response: (1) decrease in baseline lesions without evidence of new lesions; (2) durable stable disease with possible slow, steady decline in tumor burden; (3) response of tumor volume after initial increase in total tumor burden; and (4) response in the presence of new lesions. All these response patterns were associated with long-term survival, comparable with that of patients who responded using modified World Health Organization criteria at week 12 after initiation of therapy.⁴⁹

Immune-Related Adverse Events

Not unexpectedly, anti-CTLA-4 antibody therapy has been associated with a unique set of irAEs including diarrhea, colitis, rash, and endocrinopathies.⁵⁰ A clinical trial conducted to determine whether prophylactic nonabsorbed corticosteroid (budesonide) could reduce the diarrhea did not reveal a difference in rates of grade II or greater diarrhea.⁵¹ Fortunately, most irAEs are easily managed with simple algorithms and corticosteroids, and those few patients with steroid-refractory disease respond to TNF-blocking agents or mycophenolate.

Intriguingly, despite the rapid resolution of symptoms from irAEs using immunosuppressive medications, such drugs do not seem to temper antitumor effects.

Combinations With Standard Therapies

Ipilimumab has been studied in combination with either high-dose interleukin (IL)-2 or traditional chemotherapies. Overall, there has been no demonstration of deleterious effects in combination with IL-2 or dacarbazine. However, combination with IL-2 did not seem to be synergistic, with 3 CRs and 5 PRs in 36 patients treated with 0.1 to 3 mg/kg of ipilimumab every 3 weeks combined with IL-2.⁵² This is in contrast to combination with dacarbazine, where a 31.4% disease control rate was noted in the combination arm of ipilimumab and dacarbazine, whereas a 21.6% disease control rate was noted in the ipilimumab alone arm.⁵³ These data have been presented in an abstract form, and there is currently an additional ongoing phase III clinical trial combining ipilimumab with chemotherapy compared with chemotherapy alone based on those data.

Other Malignancies

Although the vast clinical experience of CTLA-4 blockade has been with melanoma, there is an increasing evidence of efficacy in other malignancies. Decreases of prostate-specific antigen and regression of visceral metastasis have been noted in prostate cancer patients treated with combined ipilimumab and GM-CSF therapy. Notably, similar irAEs seen with ipilimumab-treated melanoma patients have also been noted in the prostate cancer patients including diarrhea and endocrinopathy.⁵⁴ Although most trials have been performed in solid malignancies, treatment in non-Hodgkin lymphoma patients and relapsed allogeneic transplant patients has demonstrated some early evidence of clinical benefit. Interestingly, in the allogeneic transplant patients, no exacerbation of graft versus host disease was noted.^{55,56}

Other Novel Immune Regulatory Antibodies

Programmed death (PD)-1 is a regulatory molecule that is expressed on activated T and B cells and monocytes.^{57,58} Its ligands, known as PD-L1 and PD-L2, or B7-H1 and B7-H2, are expressed on APCs, tumor cells, placental, and nonhematopoietic cells found in an inflammatory microenvironment.⁵⁹ PD-1 is expressed on T regulatory cells, also interacts with dendritic cells and NK T cells, and is associated with angiogenesis.^{60–63} PD-L1 is an immune modulating molecule that is often aberrantly expressed on tumors, which results in tumor-induced immune suppression. Expression of PD-L1 on tumors correlates with the presence of tumor-infiltrating lymphocytes (TILs) and with poor clinical outcome for a number of cancers including pancreas, ovarian, head and neck, and melanoma.^{64–66} This strongly supports the abrogation of PD-1 as a clinical strategy that would promote the function of chronically “exhausted” tumor-specific T cells, and the resulting blockade of the PD-1-PD-L1 axis may have the added bonus of diminishing tumor-induced immune suppression. PD-1 genetic knockout mice develop organ-specific autoimmunity in a strain-specific manner, consisting of autoimmune dilated cardiomyopathy or nephritis and arthritic changes.^{67,68} PD-1 is highly expressed on endogenous melanoma antigen-specific T cells, can be found on T cells induced by melanoma peptide vaccination, and is also found at high levels on TILs from melanoma patients.^{69–72} Two PD-1 antibodies have been tested in

clinical trials: MDX-1106 and CT-011. The latter is a humanized IgG1 from Curetech with a half-life in serum of 9 to 17 days, has been tested in 17 patients with hematologic malignancies, and has shown 1 complete response in lymphoma and 1 mixed response in an acute myeloid leukemia patient.⁷³ No maximum tolerated dosage (MTD) was reached with that antibody at doses up to 6 mg/kg. MDX-1106, with a half-life of 12 to 20 days in serum, is a fully human IgG4 antibody and has been tested in a phase I dose escalation trial of 39 patients with solid tumors.^{74,75} No MTD was reached at doses up to 10 mg/kg. Two objective responses and 3 mixed responses were observed with MDX-1106. PD-L1 or B7-H1 was detected on responding tumors, and there was an increase in infiltrating CD8 T cells detected after treatment in regressing lesions. Single doses of 0.3, 1, 3, and 10 mg/kg were administered without reaching dose-limiting toxicity with MDX-1106. Two durable PRs were observed in a RCC and a colon cancer patient, with 1 episode of likely immune-related colitis observed after 5 doses of drug. Grade 1 to 2 rashes, arthralgias, liver function elevations, and fatigue were observed. In a subsequent phase II study, 21 patients with treatment refractory metastatic non-small cell lung cancer, RCC, melanoma, or prostate cancer received a single infusion of MDX-1106 at 10 mg/kg.⁷⁵ Six patients received retreatment. No MDX-1106-related serious adverse events occurred. One patient developed arthritis requiring treatment, and 2 patients had asymptomatic increase of thyroid stimulating hormone. One patient with RCC had a partial response (PR) after 3 doses, lasting 5+ months. Regression of individual lesions (mixed responses) was seen in 2 melanoma patients; to date, 1 has received 7 doses of MDX-1106 over 15 months without serious toxicity. Biopsy of a regressing lymph node metastasis showed a moderately increased and selective CD8⁺ T-cell infiltrate posttreatment. The median serum t_{1/2} of MDX-1106 was 20.6 days, which was roughly 50% longer than observed for lower doses. Paradoxically, at 10 mg/kg, long-lasting receptor occupancy was seen on T cells in an in vitro assay up to 300 days after a single dose was administered, which is at variance with the noted serum half-life. Subsequent studies include expansion of phase II trials in melanoma, and combinations of CTLA-4 abrogating antibody ipilimumab and MDX-1106 based on preclinical murine data showing synergistic antitumor effects of that combination.

PD-L1 is one of the ligands for the PD-1 receptor, and a human IgG4 antibody, MDX-1105, has been prepared against that molecule. Its use was designed to disrupt the interaction of PD-L1 on tumor cells, which for melanoma has been described as being present in up to 80% of metastatic lesions, with PD-1 on T cells. In animal models of leukemia, disruption of PD-1 and PD-L1 binding with an anti-PD-L1 antibody resulted in decreased tumor burden and prolonged survival.⁷⁶

CD40 is a member of the TNF receptor superfamily and is broadly expressed on APCs and other cells including endothelium and platelets.^{77–81} It has no intrinsic kinase or other signal transduction activity. Activation of CD40 by binding to its ligand, CD40 ligand, found on activated T cells “licenses” the APC for T-cell activation. Agonistic CD40 antibodies may substitute for T cell “help,” and in tumor-bearing hosts, they trigger effective immunity against tumor antigens. CD40 is also surprisingly expressed on many tumor cells, and its ligation may provoke tumor cell apoptosis and impaired tumor growth.

CP-870893 is a fully human IgG2 antibody that has shown high affinity binding to CD40 and has antitumor activity in human xenografts in nude mice. It has been shown to activate dendritic cells and B cells. It was tested in a dose escalation trial of a single intravenous infusion in 29 patients.⁸² The single-dose MTD was estimated at 0.2 mg/kg, with a dose-limiting cytokine-release syndrome consisting of fevers, chills, and rigors observed. This was accompanied by acute increase of serum levels of TNF- α and IL-6. Increased CD86 was observed on B cells, as was transient disappearance of CD40⁺CD19⁺ B cells from the circulation. Induction of melanoma antigen-specific T cells was seen in the absence of vaccination. A repeat phase I trial of weekly CD40 antibody infusion for up to 8 doses was performed in 27 patients. The MTD was again felt to be at 0.2 mg/kg because of cytokine-release symptoms, with 7 stable disease, and no objective responses were seen. CD86 was again found to be increased on CD19⁺ B cells. The serum half-life was estimated to be <24 hours.

Based on those promising data, a phase I carboplatinum + paclitaxel + CP-870893 trial was carried out in 33 patients. Carboplatinum + paclitaxel was given on day 1 followed by CP-870893 on days 3 or 8 intravenously. Twenty-nine patients were evaluated by response evaluation criteria in solid tumors with 6 PR (21%) and 14 stable disease (48%). By 6 hours, CD19⁺ cells had disappeared from the peripheral blood. No colitis, hypophysitis, or hepatitis was seen. Vitiligo was observed in 2 melanoma patients. Cytokine-release syndrome was again the most prominent side effect seen.

SGN-40 is another CD40 agonistic molecule that is a humanized IgG1 antibody. Seventeen patients were treated with escalating doses of SGN-40 ranging from 0.2 to 0.6, 1.5, 3, and 6 mg/kg in a phase I trial.⁸³ The study showed the antibody to be safe and well tolerated in a patient population with lymphoid malignancies, acute myeloid leukemia, and myeloma. No single-dose maximum tolerated dose was defined in the study. Clinical benefit was observed in 33% of the patients with one complete remission observed. Pharmacokinetic analyses show that serum C_{max} and the area under the curve of SGN-40 increased proportionally with dose. The median $t_{1/2}$ of SGN-40 ranged from 217 to 410 hours. Sustained increase in the percentage of peripheral blood CD4⁺ lymphocytes was observed up to 21 days after treatment. One complete response was observed in a lymphoma patient, and there were 5 additional patients with stable disease. Based on these preliminary data, a phase I–II clinical study evaluating the safety and efficacy of SGN-40 administered at 1.5 mg/kg in diffuse large B-cell lymphoma after autologous bone marrow transplantation has been initiated. SGN-40, now named dacetuzumab, was tested at 2 mg/kg weekly for 4 weeks in 50 patients with refractory or recurrent non-Hodgkin lymphoma, and then additional cohorts of patients received the drug with an inpatient dose escalation up to 8 mg/kg. Toxicities of grade 3 included anemia, pleural effusion, conjunctivitis, increased liver functions, and thrombocytopenia. The MTD was not established at the dose tested. Six of 50 patients had a response, with 1 complete response and 5 PR.

OX40, a member of the TNF superfamily, is a costimulatory molecule expressed transiently at the surface of CD4 and CD8 T cells on activation.^{84–87} Binding of OX40 to its ligand augments T-cell function and survival. OX40 is also expressed by CD4⁺ CD25⁺ T regulatory cells. Engagement of OX40 on T regulatory cells abrogates their suppressive

function. A murine IgG1 monoclonal antibody against OX-40 was prepared, and it was tested in a phase I dose escalation trial that included 10 patients per cohort with a doses of 0.1, 0.4, and 2 mg/kg of antibody administered on days 1, 3, and 5 for a single course of therapy. The results of the first 2 cohorts that received 0.1 and 0.4 mg/kg of antibody have been reported.⁸⁸ The overall toxicity of the murine antibody was low, and regression of tumor that did not meet the criteria for partial response was observed in 5 of 20 patients. A 2- to 3-fold increase in the proliferation of CD4⁺ CD95⁺ T cells, mostly in the FoxP3⁻ population, was seen. There was no change in the turnover of CD4⁺ FoxP3⁺ T regulatory cells. The proportion of cycling CD8⁺ CD95⁺ T cells also peaked 15 to 29 days after the administration of the antibody with a 2- to 4-fold increase of cycling cells compared with a group of 9 controls. An increased proliferation by 2- to 3-fold of non-CD3 cells, mostly NK cells, was also observed. These data indicated that an important effect of the administration of an anti-OX40 antibody was to induce proliferation of CD4⁺ T helper cells, CD8⁺ T cells, and NK cells in a dose-dependent manner. It would be of interest to determine whether the cycling CD8⁺ population was enriched for tumor-reactive T cells. Because the antibody used in these interesting studies was murine, human antimurine antibodies were inevitably induced, limiting its utility. Future studies with a chimeric or human anti-OX40 antibody are needed.

4-1BB is another member of the TNF receptor superfamily that is a marker of T-cell activation.⁸⁹⁻⁹³ It is highly but transiently elevated on activated CD8 T cells and on TIL from melanoma patients. 4-1BB mediated signaling by a monoclonal antibody enhanced antigen-specific T-cell activity, mediated regression of established murine tumors, and induced the differentiation and expansion of a polyclonal tumor antigen-specific CD8⁺ T-cell response. Anti-41-BB added to the antitumor activity of vaccines. Interestingly, the combination of anti-41-BB and anti-CTLA-4 antibody reduced the immune-related toxicity attributed to the anti-CTLA-4 antibody. An agonistic antibody against 4-1BB or CD137 has been prepared and tested in refractory cancer patients in a phase I dose escalation trial.⁹⁴ Patients received 0.3, 1, 3, 6, 10, and 15 mg/kg of anti-CD137 administered every 3 weeks for 4 injections, with retreatment for stable disease or better. An MTD was not established; primary toxicities were rash, fatigue, and pruritis, with increases of aspartate aminotransferase/alanine aminotransferase and neutropenia levels also seen. Of 47 patients with melanoma who received the anti-4-1BB agonistic antibody, 3 patients had a partial response, and 6 patients with stable disease were seen. A randomized phase II trial at doses of 1, 3, and 10 mg/kg is planned.

In summary, a number of pairs of costimulatory molecules have been elucidated on APC and T cells that seem to play a role in tumor-induced immune suppression. Their suppressive function can be abrogated or their stimulatory function can be amplified by the expedient of monoclonal antibodies. CTLA-4 abrogating antibodies have shown the most promise clinically and have had the most extensive testing to date, primarily in melanoma. The recent results of a second-line randomized trial of that antibody with or without a peptide vaccine compared with peptide vaccine alone met its pre-defined endpoint with a statistically significant prolongation of survival in patients who received the antibody compared with the control group that received vaccine alone. Further data are eagerly awaited from a phase III randomized trial in previously untreated patients who received

ipilimumab with dacarbazine compared with dacarbazine alone. Combination trials are planned with ipilimumab and signal transduction inhibitors, or ipilimumab and other immune modulating antibodies. The predominant side effects of ipilimumab have been novel irAEs, predominantly rashes, colitis, hypophysitis, hepatitis, and pancreatitis, which have necessitated the development of algorithms for the management of those toxicities using steroids and other immunosuppressants such as the TNF-blocker infliximab. Intriguingly, the use of immune suppression does not abrogate the antitumor activity of ipilimumab. Because clinical responses with ipilimumab have been observed with prolonged kinetics or after initial progression, the standard oncologic response evaluation criteria in solid tumors may need to be amended.

Several other immunoregulatory antibodies are in earlier stages of development but look promising. PD-1 antibody has clinical activity in a phase I trial and may act to block T-cell suppression, like ipilimumab, or could impede the interaction of PD-L1 on tumor cells with PD-1 on effector cells, or both. T regulatory cells also express PD-1 and are sensitive to the effects of PD-1 antibody, which reverses their suppression. PD-1 expressing T cells are in an “exhausted” state in HIV-related disease and in cancer. Thus, abrogation of PD-1 may work at different points in T-cell functional pathways to alter the sensitivity of tumor cells to destruction by effector cells. The modest side effects induced by treatment with PD-1 antibody strongly suggest that it has a very different mode of action than CTLA-4 antibodies. The recent report of a phase II study of PD-1 antibody showed that there were 15,146 responses, all PRs, in stage IV melanoma patients receiving 1, 3, or 10 mg/kg every 2 weeks, providing encouraging evidence of clinical activity.⁹⁵

Anti-CD40 antibodies are the prototype of the immune stimulating agonistic antibodies. The initial study with that antibody suggested that it had clinical activity, but displayed a very different half-life, a different spectrum of side effects, and that much lower doses were tolerated than ipilimumab or anti-PD-1 antibody. The presence of CD40 on B cells and monocytes also indicates that the target cells of anti-CD40 antibodies might be diverse. Encouraging signs of clinical activity using 2 completely different anti-CD40 antibodies should encourage extensive phase II testing to define proper dosing and scheduling, and appropriate combination treatment with that drug.

OX40 and 4-1BB (CD137) are additional agonistic markers of activated T cells to which monoclonal antibodies have been prepared. The earliest clinical trials of both agents show some evidence of clinical activity, although the optimal utility for those agents may be with adoptive therapy or in combination with vaccines with other immune stimulation.

Based on the available data from trials with CTLA-4-blocking antibodies, anti-PD-1 antibodies, and others, we would conclude that indeed, immune regulatory antibodies are the next therapeutic advance in cancer, and ipilimumab may represent the prototype of an entirely new series of therapeutic anticancer agents.

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