Perspective

Immunotherapy earns its spot in the ranks of cancer therapy

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Since it became clear that all cancer cells express tumor-specific and tumor-selective antigens generated by genetic alterations and epigenetic dysregulation, the immunology community has embraced the possibility of designing therapies to induce targeted antitumor immune responses. The potential therapeutic specificity and efficacy of such treatments are obvious to anyone who studies the exquisite specificity and cytotoxic potency of immune responses. However, the value assigned to a therapeutic modality by the oncology community at large does not depend on scientific principle; all that matters is how patients respond. The bar for the ultimate acceptance of a therapy requires more than anecdotal clinical responses; rather, the major modalities of cancer therapeutics, including surgery, chemotherapy, radiation therapy, and, more recently, drugs targeting oncogenes, have earned their place only after producing dramatic frequent clinical responses or demonstrating statistically significant survival benefits in large randomized phase 3 clinical trials, leading to FDA approval. Although tumor-targeted antibodies have certainly cleared this bar, immunotherapies aimed at harnessing antitumor cellular responses have not—until now.

Cancer vaccines finally gain FDA approval

Despite encouraging anecdotal reports and promising Phase 1 and 2 clinical trials, skepticism of the clinical value of T cell–targeted immunotherapies escalated among oncologists over the last decade. This was largely caused by a string of randomized phase 3 clinical trials in which the vaccinated group failed to demonstrate statistically significant survival benefit (Eggermont, 2009). Immunologists pointed to the frequent induction of tumor-specific T cell responses as evidence of vaccine activity. However, for oncologists, the patient survival data defined these trials as the ultimate negative result. This string of negative results was broken in 2010 by a Phase 3 trial of sipuleucel-T (also called Provenge, produced by the Dendreon Corp.), which was originally touted as a DC vaccine for prostate cancer (Kantoff et al., 2010). Whether sipuleucel-T is actually a DC vaccine is uncertain, as the product is produced by incubating the patient’s unfractionated peripheral blood mononuclear cells with a fusion protein linking granulocyte-macrophage colony-stimulating factor to a prostate cancer antigen termed prostatic acid phosphatase (PAP). Whatever grows during this incubation period is intravenously injected back into the patient. No published data clearly demonstrate that the final infused product contains significant numbers of PAP-loaded DCs, or that sipuleucel-T administration activates PAP-specific T cells in patients. Despite these myriad mechanistic uncertainties, the randomized phase 3 trial demonstrated a statistically significant survival benefit of nearly 4 mo for vaccinated versus unvaccinated groups, which is equivalent to the benefit derived from other drugs approved for advanced prostate cancer. Sipuleucel-T received FDA approval for marketing, and as such is generally considered to be the first therapeutic cancer vaccine to reach this critical milestone. Although this success was a major step forward for cancer immunotherapy, the reaction to it has been tempered, in part because the response rate to sipuleucel-T (defined as a ≥50% decrease in serum prostate-specific antigen level, a rough marker for disease burden) is virtually zero. In addition, there is no statistical effect on time-to-progression, defined as the time between initiation of therapy and clear progression of the disease relative to untreated patients. Thus, although the FDA...
Checkpoint blockade: the game changer

The game changer for cancer immunotherapy has now arrived, and it is in the form of antibodies that block inhibitory receptors on immune effector cells, often referred to as immune checkpoints. Indeed, this is an emerging story of the triumph of basic science over a terrible disease, a victory for which the collective immunology field can take great pride. The immune system contains hundreds of feedback inhibitory loops that regulate the amplitude of induced responses, minimize collateral tissue damage during infections, and generate and maintain self-tolerance. The most therapeutically accessible checkpoints are inhibitory receptors on lymphocytes and their ligands (either membrane or secreted), as these molecules can be blocked specifically with antibodies. The two checkpoint receptors that have been most actively studied in the context of clinical cancer immunotherapy are CTLA-4 and PD-1. CTLA-4 is expressed exclusively on T cells. Although PD-1 is also expressed on B cells and natural killer (NK) cells, its major inhibitory functions have been elucidated in the context of T cell responses (Linsley et al., 1990, 1991; Ishida et al., 1992; Greenwald et al., 2005; Okazaki and Honjo, 2007). In addition to their function in dampening effector T cell responses, both CTLA-4 and PD-1 (as well as other checkpoint receptors, such as LAG-3) are highly expressed on regulatory T (T reg) cells, and in fact directly promote T reg cell–mediated suppression of effector immune responses (Huang et al., 2004; Wing et al., 2008; Peggs et al., 2009; Francisco et al., 2009). Thus, these checkpoint receptors play multiple roles on distinct cell types to qualitatively and quantitatively regulate immunity (Fig. 1).

Recent clinical results demonstrated significant therapeutic efficacy of antibody-mediated blockade of these pathways in patients with advanced cancer, and herald the potential realization of cancer immunology’s greatest aspiration: to create sustained immune responses that successfully battle the disease long after completion of the therapeutic intervention. Importantly, the evidence for clinical efficacy of anti–PD-1 in melanoma and renal cancer, which arrived shortly after anti–CTLA-4 demonstrated clinical efficacy in melanoma, indicates that the anti–CTLA-4 results are not a one-off for immunotherapy. Furthermore, CTLA-4 and PD-1 signal through completely different mechanisms (Parry et al., 2005) and play very distinct roles in regulating T cell responses. This suggests that blockade of these pathways will not be simply redundant. It may be possible to define patients who would selectively respond to blockade of one or the other pathway or possibly to combination blockade. Indeed, given the number of defined immune modulatory receptors under active study, it is likely that many more opportunities to enhance and refine cancer immunotherapy exist and are ready to be exploited.

Clinical success of CTLA-4 blockade

Despite persistent uncertainty as to the exact mechanisms by which CTLA-4 down-modulates T cell responses (Schneider et al., 2002, 2006; Riley et al., 2002; Qureshi et al., 2011), there is consensus on its central role in limiting the amplitude of T cell activation. CTLA-4 primarily counteracts the co-stimulatory activity of CD28 (Rudd et al., 2009). CD28 and CTLA-4 share identical ligands (CD80 and CD86; Linsley et al., 1991; Azuma et al., 1993; Freeman et al., 1993; Hathcock et al., 1993). Because CTLA-4 has a much higher overall affinity for both ligands (Linsley et al., 1994), its expression on the surface of T cells dampens activation of T cells by both out-competing CD28 for binding to CD80 and CD86 and by actively delivering inhibitory signals to the T cell. Physiologically, the major role of CTLA-4 is in the initial activation stages of both naive and memory T cells. The stronger the stimulation through the
T cell receptor, the greater the amount of CTLA-4 that is expressed on the T cell surface (Egen and Allison, 2002). CTLA-4 therefore acts as a signal rectifier to maintain a consistent level of T cell activation in the face of widely varying concentrations and affinities of ligand for the T cell receptor. The dramatic immunological phenotype of CTLA-4 knockout mice—death in 3 wk from destructive lymphoid infiltration into multiple organs (Tivol et al., 1995; Waterhouse et al., 1995)—attests to its critical role as a regulator of T cell–dependent immune responses.

CTLA-4 was the first immune regulatory receptor to be targeted for clinical immunotherapy. Blockade of CTLA-4 as a general strategy was initially questioned because there is no tumor specificity of expression of CTLA-4 ligands, and because the dramatic autoimmune/hyperimmune phenotype of CTLA-4 knockout mice predicted a high degree of immune toxicity associated with blockade of this receptor. Nonetheless, pioneering preclinical studies with anti–CTLA-4 antibodies demonstrated that CTLA-4 could be partially blocked, leading to significant antitumor responses without the immune toxicities observed in CTLA-4 knockout mice (Leach et al., 1996). Two fully human anti–CTLA-4 antibodies, ipilimumab (developed by Medarex/Bristol-Myers Squibb) and tremelimunab (developed by Pfizer, recently sublicensed by MedImmune), began clinical testing in 2000. As doses of each antibody were escalated, a consistent pattern was observed in patients with advanced melanoma. Both antibodies produced true objective clinical responses (defined formally by shrinkage in cross-sectional area of radiologically measurable tumors by ≥30% with no new metastases or growth of defined metastases relative to clinical trial entry) in 10–12% of patients, but also produced immune-related toxicities involving various tissue sites in 25–30% of patients (Hodi et al., 2003; Phan et al., 2003; Ribas et al., 2005; Beck et al., 2006). From an immunologist’s perspective, this result was quite significant because it demonstrated that mouse models, if integrated with an understanding of the biology of this pathway, were indeed somewhat predictive of clinical outcomes. However, the anti–CTLA-4 therapeutic index (ratio of clinical benefit to toxicity) was limited, and the ultimate fate of CTLA-4 blockade hung in the balance for a decade as clinical trials proceeded. The first randomized phase 3 clinical trial, performed by Pfizer using the anti–CTLA-4 antibody tremelimunab in patients with advanced melanoma, was a negative result. No survival benefit was seen in the antibody-treated group, and the program was abruptly terminated (Ribas 2010).

However, Medarex, in collaboration with Bristol-Myers Squibb, persisted in development of their anti–CTLA-4 antibody, ipilimumab (now called Yervoy). They evaluated different doses and schedules, and defined algorithms for improved clinical management of the immune toxicities (using steroids and TNF blockers). Interestingly, although there is evidence that clinical responses might be associated with immune-related adverse events (Downey et al., 2007), this correlation is highly imperfect, suggesting that antitumor responses could potentially be dissociated from collateral tissue destruction. These carefully implemented elements of clinical drug development resulted in a major triumph announced in 2010. In a randomized clinical trial of patients with advanced melanoma assigned to receive a melanoma-specific gp100 peptide vaccine alone, gp100 vaccine plus anti–CTLA-4, or anti–CTLA-4 alone, there was a 3.5 mo survival benefit for patients in both groups receiving anti–CTLA-4 (with or without the gp100 peptide vaccine) compared with the group receiving peptide vaccine alone (Hodi et al., 2010). Ipilimumab thus became the first therapy in history to demonstrate a statistically significant survival benefit for patients with metastatic melanoma in a randomized clinical trial. More impressive than the mean survival benefit was the difference in the “tail” of the survival curves. Whereas the peptide vaccine alone group had only ~5% of patients surviving after 2 yr from entry into the study, nearly 20% of the anti–CTLA-4–treated groups showed long-term (>2 yr) survival. Interestingly, the proportion of long-term survivors is higher than the proportion of formal objective clinical responders. Furthermore, the increased proportion of long-term survivors occurred despite the very limited administration of ipilimumab (only 4 doses) in this trial. This finding lends credence to the concept that immune-based therapies might be able to prolong patient survival even in the absence of significant levels of tumor shrinkage on radiological scans, and to keep tumors in check long after completion of the therapy. This notion was in fact put forward to explain the survival benefit in sipuleucel-T–treated prostate cancer patients in the absence of objective tumor regressions or changes in time-to-progression (see above).

Another important feature of the anti–CTLA-4 clinical responses is their kinetics. Responses in a number of patients were quite delayed (up to 6 mo after treatment initiation) and, in some cases, lesions actually appeared to get larger on CT or MRI scans before ultimately regressing. In some cases, retreatment of clinical responders that subsequently relapsed re-induced clinical responses. There is evidence that, even before radiological responses, amplification of T cell responses to tumor antigens such as Ny-ESO-1 or induction of ICOS expression on T cells after anti–CTLA-4 treatment may predict a positive therapeutic effect (Liakou et al., 2008; Yuan et al., 2011). Taken together, these findings demonstrate that the rules for evaluating responses to immunotherapy might be quite different than those for evaluating conventional chemotherapy or oncogenic pathway-targeted molecules, which tend to induce faster but more short-lived responses (Hoos et al., 2010).

Blockade of the PD-1 pathway
On the heels of the ipilimumab success came impressive early clinical data from blockade of the inhibitory receptor PD-1. In contrast to CTLA-4, the major role of PD-1 is to limit the activity of T cells (and likely NK cells) in the peripheral tissues during inflammatory responses to infection and to limit autoimmunity (Ozakazi and Honjo, 2007; Keir et al., 2008; Nishimura et al., 1999, 2001). Overexpression of PD-1 in the context of chronic infection contributes to failure to clear...
immune responses directed at genetically or epigenetically destructive potential of T and NK cells at these sites. PD-L1 is up-regulated on many cell types in response to proinflammatory cytokines (particularly interferons), whereas PD-L2 is up-regulated on DCs and macrophages in response to different proinflammatory cytokines (Shin et al., 2005; Wilke et al., 2011). PD-1 is expressed in varying amounts on activated T cells and NK cells; thus, the co-expression of ligand and receptor in inflamed tissue mitigates the collateral tissue-destructive potential of T and NK cells at these sites.

PD-L1 is frequently up-regulated on different types of tumor cells, where it inhibits local antitumor T cell responses (Dong et al., 2002; Zou and Chen, 2008). In addition, PD-1 is expressed on the majority of tumor infiltrating lymphocytes (Sfanos et al., 2009; Ahmadzadeh et al., 2009). Together, these observations suggest that antibody-mediated blockade of this pathway may enhance intratumoral immune responses. This notion was indeed validated through many mouse studies demonstrating enhanced antitumor immunity after antibody-mediated blockade of PD-1 or its ligands (Dong et al., 2002; Iwai et al., 2002; Blank et al., 2004; Thompson et al., 2004). Furthermore, the relatively mild phenotypes of mice lacking PD-1, PD-L1, or PD-L2 suggest that blockade of this pathway may result in less collateral immune toxicity than CTLA-4 blockade; this hypothesis appears to have been supported by clinical trial data (see first paragraph, following page). PD-L1 and PD-L2 knockout mice display virtually no phenotype unless challenged with an infection or crossed onto an autoimmune prone background (Dong et al., 2004; Shin et al., 2005; Keir et al., 2006). PD-1 knockout mice generally begin to develop tissue-specific and strain-specific autoimmune syndromes after 9 mo of age (Nishimura et al., 1999, 2001). However, the increased amplitude and rapidity of onset of tissue inflammation and destruction in autoimmune prone strains crossed to PD-1 or PD-1 ligand knockout mice predicts that patients with underlying autoimmune or inflammatory processes might be susceptible to immune toxicities upon PD-1 pathway blockade.

Optimal application of PD-1 pathway blockers requires an understanding of the signals that induce expression of PD-1 ligands on tumor cells and myeloid cells within the tumor microenvironment. It has been suggested that in some tumor cells, PD-L1 expression is driven by constitutively active oncogenic signaling pathways. Indeed, the PI3kinase–AKT pathway, commonly activated in many different tumor types, can induce PD-L1 expression in glioblastomas (Parsa et al., 2007). Similarly, constitutive ALK signaling, which is observed in certain lymphomas and occasionally in lung cancer, can drive PD-L1 expression via STAT3 signaling (Marz et al., 2008).

A potentially more common mechanism facilitating PD-L1 up-regulation on tumors reflects their adaptation to endogenous immune responses directed at genetically or epigenetically generated tumor antigens—a process we term adaptive resistance. In adaptive resistance, the tumor utilizes the natural physiology of the PD-1 pathway to protect itself from an antitumor immune response. Expression of PD-L1 as an adaptive response to antitumor immunity likely occurs because this ligand is induced on most epithelial cancers in response to both type 1 interferons and γ-interferon, similar to epithelial and stromal cells in normal tissues (Kim et al., 2005; Lee et al., 2005). In lymphoid malignancies, PD-L2 is more commonly up-regulated (Rosenwald et al., 2003), likely in response to a different set of proinflammatory cytokine signals. This mechanism represents an alternative to the conventional drug resistance mechanisms or tumor escape mechanisms that involve mutation of drug targets or strong epitopes at the genome level. Instead, adaptive resistance suggests that immune surveillance does exist even in advanced tumors but the tumor ultimately resists immune elimination by up-regulating ligands for inhibitory receptors on tumor-specific lymphocytes within the tumor microenvironment. In support of this notion, a recent study in melanoma demonstrated a very high correlation between cell surface PD-L1 expression on tumor cells and both lymphocytic infiltration and intratumoral γ-interferon expression (Taube et al., 2012). Ultimately, acquisition of immune resistance by developing tumors involves cross-talk between tumor and immune microenvironment resulting in both genetic and epigenetic alterations within the cancer cell, commonly termed immune editing (Schreiber et al., 2011).

Antibodies against PD-1 entered the clinic in 2006 and antibodies against PD-L1 entered the clinic in 2009. Although the clinical experience with anti–PD-1 antibodies is less extensive than that with anti–CTLA antibodies, the initial results look extremely promising (Brahmer et al., 2010). In the first phase 1 clinical trial with a fully human IgG4 anti–PD-1 antibody produced by Medarex (Fc engineered to eliminate any FcR binding), patients were treated with doses beginning at 0.1 mg per kilogram and escalating in half-log increments to 10 mg per kilogram. This initial phase 1 trial exclusively involved very late stage patients whose tumors had progressed despite multiple rounds of both conventional and experimental chemotherapy; nevertheless there were a number of cases of tumor regression, including mixed responses, partial responses, and a complete response. Tumor regressions were observed in four of the five malignancies examined (colon cancer, melanoma, renal cancer, and lung cancer), and were associated with significant increases in lymphocyte infiltration into metastatic tumor deposits. Because 20–40% of peripheral blood T cells express PD-1, it was possible to design a receptor occupancy assay on peripheral blood. This analysis demonstrated that between 70 and 90% of the PD-1 molecules expressed on peripheral blood lymphocytes were occupied by anti–PD-1. Surprisingly, plateau levels of receptor occupancy were achieved at doses as low as 0.3 mg per kilogram, attesting to the high affinity/avidity of the clinical antibody. In addition, high levels of receptor occupancy were maintained for as long as 90 d after cessation of antibody administration. This finding appears to be based on the high equilibrium binding of the anti–PD-1 antibody at
preexisting underlying autoimmune or inflammatory processes in the patient. Evaluation of this prediction awaits more patient experience, but right now patients with overt autoimmune syndromes are excluded from receiving the antibody.

A very interesting and potentially clinically relevant preliminary finding in the initial phase 1 trial of anti–PD-1 antibodies correlated expression of PD-L1 on the tumor with clinical responses to the antibody. Given the notion that the PD-1 pathway physiologically regulates the magnitude of immune responses in tissues, it is logical to imagine that the enhancement of antitumor immune responses upon blockade of this pathway would depend in large part on expression of a PD-1 ligand on the tumor. In nine patients for whom pretreatment biopsies were available, immunohistochemistry demonstrated three patterns of PD-L1 expression: negative, cytoplasmic only, and membrane. Indeed, it would be predicted that expression of PD-L1 exclusively in the cytoplasm would fail to activate the PD-1 pathway. Indeed, of five patients whose tumors were negative for membrane PD-L1 expression, there were no clinical responders to anti–PD-1 therapy. In contrast, 3 of 4 patients whose pretreatment tumor biopsies demonstrated membrane PD-L1 expression on ≥5% of tumor cells exhibited clinical responses to treatment with the antibody. Given the geographic heterogeneity of PD-L1 expression within a given tumor, it is somewhat surprising that even a minority of positively expressing tumor cells nevertheless predicts clinical response to PD-1 pathway blockade. If expression is indeed linked to lymphocyte activity in the tumor microenvironment (adaptive resistance), then it is

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<th>Table 1. Antagonist antibodies and drugs for immune inhibitory pathways and agonist antibodies for co-stimulatory receptors in clinical testing for cancer</th>
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<td><strong>Target</strong></td>
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*Antibody not yet validated for target specificity.*

*Antibody previously tested in melanoma patients; plans to re-initiate clinical testing after a hiatus.*
likely that a single biopsy offers a mere snapshot in time of a dynamic process within the tumor and therefore underrepresents the total PD-L1/B7-H1 expression in a tumor integrated over time. If these preliminary results hold true upon expanded analysis, it is quite possible that tumor expression of PD-L1 could be used as a biomarker to predict which patients should be treated with anti–PD-1. PD-L1 is also expressed on nontransformed cells, particularly myeloid cells, in the tumor microenvironment. Current efforts are focused on evaluating cellular patterns of PD-L1 expression within tumors to further refine the predictive value of this biomarker.

A multitude of opportunities
To date, blockers of two immune inhibitory receptors have been tested in patients with advanced cancer. Both have shown promise, indicating that this is indeed a fruitful general approach to be transferred to the clinic. The CTLA-4 and PD-1 pathways are only the beginning. A number of additional B7 family inhibitory ligands, including B7-H3 and B7-H4 (Yi and Chen 2009; He et al., 2011) are expressed on certain tumors, and their expression does not overlap with each other or with that of PD-L1. Various inhibitory receptors, including Tim3 and LAG-3, can be up-regulated on tumor-infiltrating lymphocytes, and both Tim3 and LAG-3 appear to act coordinately with PD-1. Dual blockade may enhance the activity of PD-1 pathway blocking antibodies (Sakuishi et al., 2010; Goldberg and Drake, 2011; Woo et al., 2012). In addition to secreted or membrane-bound inhibitory ligands, metabolic enzymes such as indoleamine 2,3 dioxygenase (IDO) and arginase, which are expressed by inhibitory myeloid-derived suppressor cells that commonly infiltrate tumors, can locally inhibit immune responses by depleting amino acids essential for anabolic metabolism of T cells. These enzymes can be inhibited by small molecule drugs, some of which are in clinical trials (Table 1). Similar findings on the cooperative inhibitory function of these checkpoint pathways have been reported in the context of T cell exhaustion during chronic viral infection (Blackburn et al., 2009). Indeed, blocking antibodies or drugs specific for most of the immune inhibitory pathways up-regulated in the tumor microenvironment already exist (Fig. 2). Some of these pathways likely represent important mechanisms of immune resistance, which may function codominantly or independently of the PD-1 pathway. Rigorous molecular profiling of the immune microenvironment of human cancers will be critical in defining the most promising “next generation” of immune targets for cancer immunotherapy. The ultimate goal is to tailor therapeutic combinations guided by analysis of regulatory pathways that dominate a given tumor’s microenvironment.

On the flip side, agonist antibodies specific for co-stimulatory receptors will also likely bear fruit. Some of the co-stimulatory TNFR-family members including CD137, CD27, and OX-40 appear particularly interesting based on preclinical studies (Melero et al., 1997; Evans et al., 2001; Blackburn et al., 2009), although clinical toxicities of prolonged anti-CD137 treatment suggest that it must be applied in more judicious fashion. Likewise, DCs and other myeloid cells become activated by engagement of the TNFR family member CD40, for which there are a number of agonist antibodies in development or in the clinic. The most important advance for clinical application of cancer vaccines may well take the
form of combinatorial strategies using checkpoint antagonists or co-stimulatory agonists. As mentioned above, many clinical cancer vaccines have been demonstrated to induce antitumor immune responses without evident tumor regressions (van Elsa et al., 1999). This is undoubtedly caused in part by the inability of induced T cell responses to overcome resistance barriers in tumors created by molecules such as the PD-1 ligands. Many preclinical studies have demonstrated the synergistic efficacy of vaccination and checkpoint blockers as well as co-stimulatory agonists. The future is indeed bright for this field.

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REFERENCES


Perspective


