Beyond Ipilimumab: New Approaches Target the Immunological Synapse

By Ken Garber

The March 25 approval of ipilimumab (Yervoy) by the U.S. Food and Drug Administration was a milestone for the embattled field of cancer immunotherapy. As the first agent to increase overall survival in a phase III melanoma trial, ipilimumab appeared to vindicate the immune surveillance hypothesis of cancer first proposed in 1957. That hypothesis, which posits that the immune system plays a key role in keeping tumors in check, gave rise to hopes that boosting the immune system could eradicate established tumors. After three decades of almost continual futility in the clinic, ipilimumab is the first clear success: It produces durable, complete responses in a small but consistent proportion of melanoma patients.

But ipilimumab’s objective response rate is only 10%–15%, and the drug sometimes causes severe immune-related side
effects. “We’re all very excited that ipilimumab has been approved for immunotherapy,” said oncologist Suzanne Topalian, M.D., at Johns Hopkins University in Baltimore. “But we need to continue to search for other drugs in this class that may have more in the way of anti-cancer effects, and hopefully less in the way of side effects.”

To that end, several companies are developing drugs targeting molecules at the immunological synapse, the interface between T cells and antigen-presenting cells (APCs). Ipilimumab works by inhibiting one such molecule, CTLA4, a negative T-cell regulator, but other attractive targets also exist. Antibodies targeting four of them are currently in clinical trials. Although they’re mostly being tested as single agents, investigators believe their best use will be in combination with each other or with other therapies (see sidebar).

“The trick is going to be to balance clinical benefit with toxicity,” said oncologist Jedd Wolchok, M.D., Ph.D., of the Memorial Sloan–Kettering Cancer Center in New York.

**Blocking the Blockers**

The two types of targets—costimulatory molecules and immune checkpoints—act in opposite ways. To trigger an adaptive immune response, an APC first displays an antigen to T-cell receptors on neighboring T cells. After this first signal at the immunological synapse, T cells require a second, costimulatory signal from one or more receptors. Engaging the costimulatory receptors on T cells to boost immunity is one new anticancer treatment strategy. Later, once the T cell is activated against a foreign antigen, one or more negative regulators, or immune checkpoints, come into play, dampening the immune response. (Tumors can hijack this mechanism.) Blocking these immune checkpoints is the second strategy. CTLA4, ipilimumab’s target, is one checkpoint.

PD-1 is another.

PD-1 is the best validated of the new targets. In 2002, immunobiologist Leiping Chen, M.D., Ph.D., then at the Mayo Clinic in Rochester, Minn., reported that a variety of human tumors express PD-L1 (or B7-H1), the main ligand for PD-1, and that it increases apoptosis of tumor-specific T cells in vitro. Introducing PD-L1 into tumors caused them to grow in mice, where T cells had previously kept them in check. On the basis of these and other data, the biotech company Medarex sponsored a clinical study of an anti–PD-1 antibody beginning in 2006. A single dose of this antibody, now known as BMS-936558, given to heavily pretreated patients with metastatic disease, generated some anti-tumor activity, including in lung and colorectal cancer. Those tumor types “have responded very poorly to a variety of other immunotherapies that have been tried over the past 30 years,” said Topalian. “So to us, this was a remarkable finding.” Further doses led to durable responses in three of 39 patients, two of whom are continuing off treatment without tumor progression.

A second study, using bi-weekly dosing of the antibody, began in 2008. Of the first 46 metastatic melanoma patients treated, 15 achieved a partial response by RECIST (Response Evaluation Criteria in Solid Tumors) criteria. “Although some of these patients . . . have progressed since that time, many of them are still responding a year hence,” reported oncologist Mario Szolcl, M.D., of Yale University in New Haven, Conn., at the 2011 American Association for Cancer Research annual meeting. In the same study, the drug appeared to have a similar response rate in renal cell carcinoma. This study did not randomize patients to other treatments, so patient selection bias could be skewing results, and overall patient numbers were too small for results to be more than suggestive. But the objective response rate of 33% so far is better than ipilimumab’s 10%–15% for a similar metastatic melanoma patient population, although the drugs have not been tested head to head. Side effects, including hypophysitis, hyperthyroidism and adrenalitis, pulmonary infiltrates, and fatigue, resemble those seen in the ipilimumab trials but are somewhat less severe overall, according to investigators.

PD-1, understandably, has become a popular target. Besides the antibody from Medarex (now part of Bristol-Myers Squibb), two other anti–PD-1 antibodies are in clinical development, along with an

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**Monoclonal Antibodies Targeting Immunological Synapse in Cancer**

<table>
<thead>
<tr>
<th>Company</th>
<th>Agent</th>
<th>Mechanism</th>
<th>Status</th>
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</thead>
<tbody>
<tr>
<td>Bristol-Myers Squibb</td>
<td>Ipilimumab (Yervoy)</td>
<td>CTLA4 blockade</td>
<td>Approved for melanoma; phase III in prostate cancer</td>
</tr>
<tr>
<td>Bristol-Myers Squibb</td>
<td>BMS-663513</td>
<td>CD137 (4-1BB) activation</td>
<td>Phase II in melanoma complete; three other trials terminated</td>
</tr>
<tr>
<td>Bristol-Myers Squibb</td>
<td>BMS-936559 (MDX-1106, ONO-4538)</td>
<td>PD-1 blockade</td>
<td>Phase I for several cancers</td>
</tr>
<tr>
<td>Curetech Ltd. (Yavneh, Israel)</td>
<td>CT-011</td>
<td>PD-1 blockade</td>
<td>Phase II in multiple myeloma, lymphoma, colorectal cancer, pancreatic cancer</td>
</tr>
<tr>
<td>Merck</td>
<td>MK-3475 (SCH 900475)</td>
<td>PD-1 blockade</td>
<td>Phase I</td>
</tr>
<tr>
<td>Bristol-Myers Squibb</td>
<td>BMS-936559 (MDX-1105-01)</td>
<td>PD-L1 (B7-H1) blockade</td>
<td>Phase I</td>
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<tr>
<td>Tolerx</td>
<td>TRX518</td>
<td>GITR activation</td>
<td>Phase I on temporary hold</td>
</tr>
<tr>
<td>Pfizer</td>
<td>CP-870893</td>
<td>CD40 activation</td>
<td>Phase I in pancreatic cancer and melanoma</td>
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<tr>
<td>Portland Providence Medical Center</td>
<td>Agonist anti-OX40</td>
<td>OX40 activation</td>
<td>Phase I in prostate cancer</td>
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antibody against the PD-L1 ligand (see table). Targeting the ligand, in addition to shutting down the PD-1 checkpoint, could have a direct antitumor effect, because many tumor cells express the ligand.

One limitation of targeting a single immune checkpoint is that tumor cells can compensate by upregulating another one. “One of the early events that happens after a patient is given anti-CTLA4 is upregulation of PD-1 on their T cells,” said Wolchok. A combination trial of anti-PD-1 with ipilimumab is now under way. Investigators, mindful of the risk of severe autoimmunity, are starting with low doses of both antibodies. “We’re going very slowly and very carefully,” said Wolchok.

Turning On the Stimulators
In contrast to PD-1’s inhibitory role in T cells, four other new targets play a costimulatory role, and researchers have designed agonist (activating) antibodies for them. The first to enter the clinic, in 2006, was an antibody to the OX40 costimulatory receptor. OX40 is not involved in effector T-cell activation but rather promotes T-cell survival and expansion. Giving tumor-bearing mice agonist anti-OX40 antibodies delays tumor growth and improves survival compared with untreated mice, and the treatment confers long-lasting tumor-specific immunity.

In the clinical study, based at the Portland Providence Medical Center in Oregon, patients received three infusions of anti-OX40 within a week. (The study involved no follow-up doses because patients develop an immune response to the drug, a mouse antibody.) Nine of 27 patients experienced minor tumor shrinkage, although none met RECIST criteria for objective responses. “This [finding] was pretty positive, given that it was a mouse antibody and we were just giving one dose,” said immunologist Andrew Weinberg, Ph.D., whose group developed the drug. Treatment caused T cells to proliferate and to display markers suggesting enhanced activity. The Portland group is now testing the drug in prostate cancer in combination with chemotherapy and radiation, and more trials are planned. Eventually they intend to test humanized anti-OX40 antibodies that Agonox, a spinoff biotech company, is developing.

GITR is another new costimulatory target. Like OX40, GITR is expressed after T-cell activation and enhances T-cell function and survival. GITR also affects regulatory T cells (Tregs), a specialized T-cell lineage that downregulates cellular immunity. Although some studies show that GITR activates Tregs—not a good thing in cancer—Wolchok and others have shown in mouse models that an agonist anti-GITR antibody decreases the number of Tregs in the tumor, thus boosting anti-tumor immunity. So one result of targeting GITR might be a change in the ratio of effector T cells to Tregs in tumors to favor a robust immune response.

Last year, Wolchok’s group launched a clinical study of an agonist anti-GITR antibody in melanoma. Four patients have been treated to date, each with a single dose of antibody. “We’ve not come across any serious safety issues,” said Wolchok. But the trial was put on hold in March when Tolerx, the biotech company making the antibody, suffered a major business setback. (An unrelated antibody failed in a late-stage diabetes trial.) Tolerx is in the process of selling the anti-GITR antibody to another company. Once that process is complete, Wolchok expects the trial to resume.

Finding Winning Combinations
Although the FDA approved ipilimumab as single-drug therapy for melanoma, “the name of the game now is going to be combination therapies,” said Suzanne Topalian, M.D., of Johns Hopkins. A trial of ipilimumab with an experimental anti–PD-1 antibody (see story) is just the first of many.

The potential combinations are dizzying. Besides combining two immune checkpoint inhibitors, each could be used together with a costimulation activator. Other options include cancer vaccines, adoptive cellular immunotherapies, or kinase inhibitors. Even chemotherapy makes sense as a way to unmask tumor antigens to a boosted immune system. “There aren’t enough people or days or patients to test all the very interesting things that can be done,” said oncologist Mario Sznol, M.D., of Yale University at a recent grand rounds talk at Yale. “One of the things that we need to do is figure out what are the critical nonredundant pathways and target those, so we don’t waste a lot of time inhibiting two targets that are in the same pathway.”

Predicting which patients are likely to respond to immunotherapy is another crucial task that has barely begun. For example, no one knows why some patients respond dramatically to ipilimumab or anti–PD-1 antibodies, whereas most patients have no objective tumor responses at all. Efforts to identify predictive markers are under way, but the task is daunting. In a phone interview, Sznol said that an immunotherapy biomarker “probably depends on things that the tumor does, things that the infiltrating cells do, things about your prior immune system, maybe even some genetic component . . . [and on] where you are in the stage of the disease. It’s very complex. So I would imagine that we’ll never find one single thing that’s going to be 100% predictive.”
expresses the ligand, not vice versa. Receptor binding greatly enhances the APC’s ability to present antigen and activate T cells against the foreign target. Studies dating back to 1999 show that CD40 activation promotes antitumor immunity.

In the last few years, more than 100 cancer patients have received Pfizer’s agonistic anti-CD40 monoclonal antibody. Results for a pancreatic cancer trial appeared in the journal Science in March. Four of 21 patients receiving antibody treatment in combination with gemcitabine had partial responses by RECIST criteria. “But when we looked at biopsy samples or surgical samples obtained from patients whose tumors were regressing, we didn’t see infiltrating T cells,” said trial investigator Robert Vonderheide, M.D., Ph.D., at the University of Pennsylvania. This was a mystery. What was CD40 doing if not mobilizing T cells against the tumor?

The surprising answer, which emerged from a genetically engineered mouse model of pancreatic cancer, was that the antibody causes macrophages, not T cells, to kill tumor cells and the surrounding stroma. Macrophages in tumors usually promote tumor growth. But the anti-CD40 antibody, Vonderheide concluded, re-educates these macrophages to kill tumor and stromal cells. Other trials of the Pfizer antibody are under way or planned.

Despite such encouraging results, researchers remain wary because targeting the immunological synapse can backfire. In 2006, an agonist antibody against the costimulatory receptor CD28 caused a massive cytokine storm and multi-organ failure in six healthy volunteers. (They survived, but with lasting organ damage.) Just this year, Bristol-Myers Squibb suspended trials of its agonistic antibody against yet another costimulatory receptor, 4-1BB, because some patients suffered liver problems, including a high incidence of grade 4 hepatitis.

More such setbacks may occur. But ipilimumab’s success has restored confidence to the field, which now awaits the results of the ongoing clinical studies for the new agents. “The basic science has delivered; the translation has happened,” Vonderheide said.

Dr. Topalian’s laboratory receives research support from Bristol-Myers Squibb. Dr. Weinberg has an ownership interest in Agonox LLC. Dr. Wolchok is a consultant to Bristol-Myers Squibb, and his laboratory receives research support from the company and from Novartis. Dr. Vonderheide’s laboratory has received research support from Pfizer. Dr. Sznol is a consultant for Bristol-Myers Squibb.

© Oxford University Press 2011. DOI: 10.1093/jnci/djr281