(3) ANTI-CHECKPOINT AND VARIED AGENTS

Agonist Anti-GITR Ligand and Monoclonal Antibody
Alan Houghton, M.D.

Glucocorticoid-induced TNF (tumor necrosis factor) receptor (GITR) family–related protein is constitutively expressed at high levels by Tregs and minimally by naïve CD4+ and CD8+ T cells. It is up-regulated following T-cell activation. Signaling through GITR abrogates Treg suppressive activity *in vitro* and is co-stimulatory for effector CD4+ and CD8+ T cells. GITR signaling enhances tumor immunity and rejects tumors.

GITR ligation promotes immune responses to cancer antigens by suppressing Tregs and co-stimulating effector T cells. It directly induces cancer immunity and synergizes with anti–CTLA-4 blockade therapy. Additionally, anti-GITR agonists can augment cancer immunity in combination with vaccines against cancer antigens.

These agents offer some potential for development because the preclinical data show some efficacy. The direct tumor effect of the antibody or the ligand also synergizes CD4+ blockade. Studies in animal models have shown that the agonist can exacerbate autoimmunity, e.g., colitis, arthritis, vitiligo, and atopy.

Dr. Houghton envisions that the agent(s) could be used as systemic therapy alone or in combinations, and might have application across multiple tumor types. They might also be used with vaccines, CTLA-4 blockade, or chemotherapy.

Discussion

Dr. Pardoll commented on the interesting point of how much of anti-GITR action is directed toward Tregs and how much toward the effector cells to make them resistant to Treg inhibition. This agent might help elicit information about the importance of Tregs in blunting antitumor activity. Dr. Pardoll also mentioned denileukin diftitox, wondering why it kills CD25+ cells very effectively *in vitro* but not *in vivo*. Dr. Mackall explained that a progenitor population of CD25– cells refills the niche within 10 days or so; therefore the drug does not eliminate this cell subpopulation.

Dr. Schlom agreed that anti-GITR is an interesting agent, although no clinical data are available. One Boston firm is developing an anti-GITR antibody. Academic investigators are developing the ligand, and others may be developing the antibody. Dr. Pardoll observed that this agent has not been used in human trials at all, although reasonable evidence in mice indicates that it enhances immune responses. It is not clear how much of the effect is due to Treg inhibition and how much is action on effector cells.

Others commented on the difficulty of killing Tregs and a possible role for agonist anti-GITR as a means of priming Tregs for death. Dr. Houghton said that he has unpublished data from mouse studies showing that both mechanisms are operative. Dr. Berzofsky asked about which cells become resistant to Treg suppressive activity in response to the agonist. Dr. Houghton said both

CD4+ and CD8+ T cells are affected. Dr. Schlom added that this was demonstrated in Dr. Sakaguchi's lab.

Dr. Houghton noted that two potential agents exist: agonist anti-GITR ligand and the monoclonal antibody. Developmental work on fusion constructs is ongoing in Japan and Australia. These agents could be very interesting, according to Dr. Schlom, because of their Treg inhibition effect. Agents that can inhibit Tregs should have high priority.

One participant observed that the agent would have to be given almost continuously. Another opined that giving it with chemotherapy or anti–CTLA-4 would be intriguing avenues of research.

Dr. Disis said that her group has done a great deal of work with immunotoxins. It would be significant to have an agent that interferes with Treg suppression. Having to give chronic antibody would not constitute a barrier so long as the effect is maintained and the treatment is of low toxicity.

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Anti-OX40 Ligand and Monoclonal Antibody

Presenter: Alan Houghton, M.D.

OX40 (CD134) is a co-stimulatory receptor for CD4+ and CD8+ T cells. It is involved in signaling for T-cell survival, generation of memory T cells, and reactivation of memory T-cell responses. One interesting property of OX40 is that its signaling seems to inhibit Tregs *in vitro*.

Preclinical work raised the safety issues of autoimmune sequelae and exacerbation of atopy. A study in rhesus macaques showed that the agent was generally well tolerated. Enlarged lymph nodes (gut) and splenomegaly resolved over 28 days. Increased antibody titers and T-cell responses against simian immunodeficiency virus gp130 were observed after immunization.

Clinical development is in early phases. A phase I study of a mouse monoclonal antibody is ongoing at the Providence Cancer Center, Portland, Oregon. Elizabeth Jaffee, M.D., said her group has studied the agent in combination with GVAX. It seems to prolong the survival of CD8+ T cells, but does not enhance the non-immunodominant epitope. This would be one of multiple combinations that could act in synergy, but anti-OX40 alone does not have much activity. A human antibody was being developed by a company in the United Kingdom, but the intellectual property is currently owned by a holding company in Bermuda.

Dr. Houghton suggested that giving the agent after chemotherapy might be a useful approach. Activity was observed in a mouse model using such a regimen.

Discussion

Dr. Palucka cautioned that because OX40 is in the Th2 pathway, it would be important to look for late-onset events.

Dr. Urba informed the group that his institution is involved in the clinical trial of the monoclonal antibody. Private funds were raised to make a murine GMP antibody. Human monoclonal antibodies are being stored by the company that owns the intellectual property, but they are not being released to allow investigator-initiated research. The murine antibody has been well tolerated. Three dose levels are being tested. He mentioned skewing of Th1/Th2 responses. The investigators have seen evidence of both CD4+ and CD8+ T cells in the peripheral blood. It appears to have a survival-enhancing effect on both types. No subjects have yet met the criteria for partial response. The mouse antibody disappears quite rapidly; to be useful in the long run,

the product would have to be a humanized antibody. Several agonistic antibodies are available that are fully human. Dr. Urba said that his group had no success trying to procure the clone in order to produce it.

By voice acclamation, the participants ranked the first two anti-checkpoint agents thus:

1. Anti-GITR
2. Anti-OX40

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Anti-Cytotoxic T Lymphocyte–Associated Antigen-4 (CTLA-4, CD152)
Presenter: Steve Rosenberg, M.D., Ph.D.

CTLA-4, according to Dr. Rosenberg, is an inducible receptor that is engaged by the B7 family of ligands and inhibits CD4+ and CD8+ T-cell activation. By blocking the negative signals of CTLA-4, the antibody can augment and prolong T-cell immune responses. In animal models, anti–CTLA-4 antibody can induce tumor rejection in immunogenic tumors, and in combination

with antitumor vaccination, can induce rejection of minimally immunogenic tumors. Knockout mice lacking CTLA-4 develop lymphoproliferative disease.

Preclinical studies have shown that combinations of anti–CTLA-4 and vaccines are more effective in tumor prevention than they are in models of more advanced disease, although they can slow tumor growth. No evidence of autoimmunity has been found in monkeys given ipilimumab.

Dr. Rosenberg highlighted the clinical experience with this agent. A clinical trial of anti–CTLA-4 in metastatic melanoma patients achieved an objective response rate of 17% by RECIST or WHO criteria. The responses were highly durable; some complete responses have gone beyond 4 years with regression at nearly every metastatic site, including the brain. However, 36% of subjects experienced grade III/IV autoimmune toxicity (colitis, 17%; hypophysitis, 9%). The objective response rate was highly correlated with autoimmunity. Most of the significant autoimmune events could be effectively treated, but hypophysitis would likely limit the use of anti–CTLA-4 as a first-line drug because it would require lifelong treatment with steroids. Steroid treatment, however, did not appear to reverse the antitumor effect; those patients had the same durability of response. Interestingly, prior therapy with interferon alpha-2b was associated with decreased survival (12.4 vs. 18.2 months).

Only three immunotherapies have been shown to effectively lead to tumor regression by RECIST/WHO criteria; anti–CTLA-4 is one of them. Dr. Rosenberg posited that this is a very active and valuable agent that holds promise for patients with metastatic melanoma.

Anti–CTLA-4 is being produced by Bristol-Myers Squibb and Pfizer. It is likely to be approved by the FDA.

Discussion

Dr. Weber noted that he will serve as principal investigator on a 121-patient phase II trial of this agent. The spectrum of toxicity for anti–CTLA-4 varies with tumor type. With sarcoma, for example, unusual late responses have been observed. It offers great potential for combination therapies.

Dr. Rosenberg referred to a paper in PNAS by Dranoff. No evidence has been seen to suggest that the response rate to anti–CTLA-4 was greater when given with a peptide vaccine than without. It appears, therefore, that it does not act as an effective adjuvant.

One participant noted that studies have been limited to metastatic melanoma and renal cell carcinoma. Some anecdotal evidence suggests possible action in prostate cancer, but the agent might not have activity in other cancers. Another person asked if this gap is attributable to a lack of data or publications.

Dr. Pardoll noted that anti–CTLA-4 will probably be approved for melanoma, but he speculated that it might be interesting to study in combinations or in other tumors. Superb preclinical data have been published. Unpublished data show evidence of synergy in animals using anti-PD1 and

anti–CTLA-4 antibodies. Other unpublished data showed that among 25 prostate cancer patients treated with anti–CTLA-4, clinical responses were observed in 2 or 3, whereas when it was given with GVAX, clinical responses were seen in 5 or 6 of 25. He would like to see more anti–CTLA-4 available for such studies. The reality is that Bristol-Myers Squibb is working to get the drug approved. Off-label use might interfere with that process.

On the question of assigning priorities, Dr. Cheever said this is a valuable agent being used broadly. More than 1,700 patients have been treated with the antibody. Anti–CTLA-4 appears to be on the path to approval. When approved, the only barrier to inhibit its use in studies would be its cost. Thus, despite substantial interest in the agent by workshop participants, it will not be ranked on the priority list. It is being presented primarily because it has shown immunologic and therapeutic effectiveness and if approved, will be "first in class" for immunologic checkpoint antibodies.

Dr. Jesus Gomez-Navarro said that kinetic parameters are very important because they help investigators find ways to use anticancer agents in better ways. He advocated placing anti–CTLA-4 in its own special category.

Dr. Calzone said that anti–CTLA-4 is "a toehold for therapy" and suggested that anti-PD1 might enhance its effect.

No references were provided.

Anti-Programmed Death-1 (PD-1)
Presenter: Jeffrey Weber, M.D., Ph.D.

Structurally related to CTLA-4 and CD28, PD-1 is a receptor that is a member of the immunoglobulin superfamily and that binds to its ligands, PDL1 and PDL2. PD-1 is up-regulated on activated T and B cells and monocytes. It binds to PDL1 on T and B cells, macrophages, and DCs, as well as on parenchymal and tumor cells. PDL2 is present only on DCs and macrophages.

PD-1 is a negative regulator of T-cell function and is implicated in tolerance induction in mice. PDL1 expression by tumors appears to protect them from immune attack by cytotoxic T lymphocytes (CTLs); therefore, PDL1 expression on many human tumors is associated with a poor prognosis. This is not true for PDL2, however. Blockade of PDL1 and PD-1 in murine tumor models leads to long-lasting tumor regression.

Abrogation of PD-1 in humans increases the numbers of functional cytokine-secreting CTLs. Hamanishi (2007) published a study showing that ovarian cancer patients who had greater levels of PD ligands (especially PDL1) had better survival rates than those who expressed little or no PD ligand. Other data presented by Dr. Weber demonstrated that treatment with anti–PD-1 antibody increased the number of melanoma-specific CTLs. He noted that the effect was not one of diminished apoptosis, but rather, of increased proliferation.

A phase I trial (first-in-human) is under way in colon cancer patients that will continue to MTD. No significant or dose-limiting toxicities have been observed thus far. A phase II study will commence after the MTD is defined and toxicities are assessed.

Preclinical data suggest that squamous esophageal, colon, lung, and ovarian cancers, as well as melanoma, because they express high levels of PDL1, could be targets for interruption of the PD-1/PDL1 axis. Promising avenues of research include use of anti–PD-1 alone or in combination with a vaccine or anti–CTLA-4. Based on experimental data, the combination of anti–PD-1 and anti–CTLA-4 might be a way to generate T cells for promoting an antitumor effect. If PD-1 is shown to be as common on activated tumor-specific T cells as is suspected, then T-cell "exhaustion" (Ahmed, 2006) might be a common immunosuppression mechanism in melanoma and other cancers. PD-1 abrogation could prove to be an important way to dis-inhibit antitumor T-cell immunity.

Anti–PDL1 antibody with blockade at the tumor site might be a useful approach, although the antibody would have to be able to penetrate the tumor to a great extent. However, anti–PDL-1 could possibly alter parenchymal tissue and increase its recognition, leading to autoimmunity.

Discussion

Dr. Urba pointed out that this agent is quite promising, and he reiterated that phase I trials are taking place at Detroit, Henry Ford, Johns Hopkins, and a site in North Carolina. Twelve subjects with five cancer types have been accrued. No adverse events have been reported yet. Preliminary findings were reported at the Special Programs of Research Excellence (SPORE) meeting. Dr. Rosenberg commented that the effectiveness of IL-2 and other nonspecific kinds of immunotherapies would depend on the ability to unmask native antitumor responses to the cancers being treated. It is not clear that such mechanisms exist outside of melanoma or renal cell carcinoma.

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B7-H1 Antagonist

Presenter: Walter Urba, M.D., Ph.D.

This anti-checkpoint agent is an antibody to B7-H1, a PD-1 ligand. PD-1 is expressed on activated CD4+ and CD8+ T cells, as well as natural killer cells and monocytes.

The more B7-H1 expressed, the worse the prognosis. Normally it is a negative regulator in that it inhibits T-cell proliferation and cytokine production. B7-H1 expression is increased by interferon-gamma. Blockade of B7-H1/PD-1 enhances T-cell immunity. Blockade with anti–PD-1 is not exactly the same as blockade of B7-H1.

Preclinical studies indicate that blockade enhances autoimmunity in models of diabetes mellitus, colitis, and experimental autoimmune encephalomyelitis. Blockade also disrupts fetal-maternal tolerance, resulting in an increased abortion rate. Minimal effects are seen in murine tumor models when anti–B7-H1 is administered alone; it is most active when combined with other immunotherapy (e.g., anti-CD137).

One interesting area is T-cell exhaustion. Endogenous responses might be exhausted, but immunotherapy might be able to resurrect a response that is present, albeit limited. B7-H1 antagonist might be useful *ex vivo* to develop active T cells for adoptive therapy. Also, it might have activity as a single agent or in combination with vaccines or other immunomodulators. The antagonist appears to also have potential as a prognostic or predictive tool.

Anti–B7-H1 would likely be useful in several different areas of research, especially in comparison with anti–PD-1, which blocks the other end of the B7-H1 pathway.

Discussion

Dr. Pardoll commented on the nonequivalence of anti–PD-1 and anti–B7-H1 and offered several possible explanations. He mentioned several investigators' work in the area, including Chen and Freeman. The anti–B7-H4 enhances responses more than anti–PD-1 antibodies. Lieping did a comparison in knockout mice and found greater enhancement of immunization-induced responses in the B7-H4 knockouts. The cardiac toxicity reported with troponin has not been

reproduced in PD-1 knockout mice. The Medarex antibodies' optimal blocking in *in vitro* assays is similar. Anti–PD-1 and anti–B7-H4 are interesting, but not equivalent, antibodies.

Medarex is interested in marketing the antibody, but it is not in active development because of the company's involvement in the anti–PD-1 trial.

One participant commented that NCI might not be able to intervene to procure this agent because it would go against NIH policy. It is not clear that the barrier could be surmounted with these Medarex products. The chances of obtaining anti–PD-1 seem slim because of intellectual property issues.

Dr. Jaffee agreed that the preclinical data are very impressive. The target is expressed on some tumors.

For the purpose of priority ranking, the participants decided to consider anti–PD-1 and anti–B7-H1 as a single entity because they are similar.

By voice acclamation, the participants determined the priority ranking of the anti-checkpoint agents to be anti–PD1 and/or anti–B7-H1, anti-GITR, anti-OX40.

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B7-H4 Antagonist

Presenter: Walter Urba, M.D., Ph.D.

Dr. Urba explained that this anti-checkpoint agent is an antibody to B7-H4. Its target (B7-H4) has a structure similar to B7-1,2, but it lacks binding sequences for CTLA-4 or CD28. It is expressed on multiple non-lymphoid tissues and is highly expressed in a variety of cancers. It is also expressed on activated T cells, B cells, DCs, monocytes, and particularly on tumor-associated macrophages. B7-H4 binds to an unknown receptor borne on activated but not naïve T cells, thereby negatively regulating T-cell immunity in peripheral tissues. Antibody blockade increases allogenic CTL activity.

Some interesting preclinical work has been done. Tregs enable antigen-presenting cell-suppressive activity by increasing B7-H4 expression—a process that is IL-10 dependent. When B7-H4 is depleted, the suppressive activity of Treg-conditioned antigen-presenting cells is reduced. B7-H4 blockade increases T-cell proliferation and reduced tumor volumes *in vivo.*

Human anti–B7-H4 has been produced by Medarex, but no clinical data are available. The company's development plan is unclear.

The agent could have broad applicability in various cancer types. It might be used as a single agent or in combination with vaccines or other immunomodulatory agents. It would likely be useful for multiple investigators.

Dr. Urba said that both B7-H1 and B7-H4 antagonists would be potentially beneficial. B7-H1 blockade has more supporting preclinical data, but B7-H4 blockade offers the benefit of possibly interfering with Treg function. Dr. Pardoll pointed out that in contrast to other B7 agents, B7-H4 is inhibitory in all systems.

Eugene Kwan published data on a set of patients with renal cell carcinomas. Those with higher H4 expression had worse prognoses and those with high expression of both H1 and H4 had the worst prognoses, suggesting a possible synergistic effect. Might it be possible to try using a knockout as a surrogate for H4 suppression and the antibody for H1 blockade?

It was noted that Dr. Lieping Chen voted by proxy for anti–B7-H1 to have a high priority in the rankings. Dr. Cheever noted the lack of data supporting its potential value.

By voice acclamation, the participants determined the priority ranking of the anti-checkpoint agents to be anti-PD1 and/or anti–B7-H1, anti-GITR, anti-OX40, anti–B7-H4.

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Human Agonistic Anti–4-1BB (Anti-CD137) Antibody
Kim Margolin, M.D.

This agent is a non-blocking functional monoclonal antibody to CD137/4-1BB. Its actions are co-stimulatory, anti-apoptotic, and proliferative. Its target, CD137, is a member of the TNF superfamily of receptors and is present on activated T cells, natural killer cells, and natural killer T cells. The receptor is not present on tumors.

Interaction of the agent with its target enhances activation. Interferon-gamma plays an essential role. *In vitro* preclinical studies have shown co-stimulation of T cells suboptimally stimulated with anti-CD3. Also, when given with simian immunodeficiency virus vaccine (gag DNA), anti–4-1BB enhanced the cellular response. Antitumor effects have been observed with murine anti–4-1BB in various *in vivo* tumor models.

Preclinical toxicity studies in mice suggested a predominance of natural killer T cells in the liver (e.g., hepatic necrosis, elevated transaminases). In monkey models, occasional mild colitis was observed, possibly related to the high number of activated lymphocytes in intestinal mucosa.

A phase I, first-in-human study is ongoing and is being expanded to a phase II trial involving a single agent and exposure to four doses. The subjects are advanced cancer patients with a variety of tumors. Toxicities have consisted of faint skin rash, mild neutropenia, and hepatoxicity (likely to be dose limiting).

A phase I trial of anti–4-1BB in combination with paclitaxel and carboplatin is accruing, and another one involving radiotherapy or chemoradiotherapy is being planned. Other future possibilities include using anti–4-1BB as part of antigen-specific strategies, combinations with cytokines, and screening for a possible role in autoimmune modulation, perhaps in combination with checkpoint blockade (e.g., anti–CTLA-4A).

Dr. Urba noted that anti–4-1BB is an interesting agent for which a significant body of data exists, based on human studies.

It appears that the manufacturer is gearing up for demand via letters of intent. The phase I studies will provide the necessary data on multi-dosing and effects in different histologies. Anti–4-1BB is a promising antibody without severe toxicities. At the recent SPORE meeting, it was reported that the antibody could either co-stimulate or deplete Tregs, depending on the model used.

The participants discussed a company called GTC, which is making a chimeric antibody with one of Lieping Chen's monoclonal antibodies. GTC makes transgenic goats that secrete antibodies in their milk.

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Discussion of Anti-Checkpoint and Varied Agent Prioritization

The participants speculated that some sources of anti–4-1BB are likely to become available soon to the investigator community, whereas anti-GITR is unlikely to. By voice acclamation, the priority ranking of all the anti-checkpoint agents and varied agents discussed in this group was determined to be:

1. Anti–PD-1 and/or anti–B7-H1
2. Anti–4-1BB
3. Anti-GITR
4. Anti-OX40
5. Anti–B7-H4

* Anti–CTLA-4 was of high interest, but was not ranked because registration/approval is likely to occur in the near term.