(4) CO-STIMULATORY AND VARIED AGENTS

Anti-Interleukin-10
Presenter: Theresa Whiteside, Ph.D.

According to Dr. Whiteside, much is known about IL-10, and antibodies to IL-10 are already used to treat systemic lupus erythematosus (SLE) and rheumatoid arthritis. Nevertheless, only preclinical data are available regarding its effects in cancer.

The potential clinical use of IL-10 antibodies in cancer treatment would be based on neutralization of IL-10, which is known to exert direct growth-inhibitory effects on tumor cells *in vitro* and *in vivo,* to serve as a growth factor for B lymphoma and melanoma cells, and to both stimulate and suppress immune cells. IL-10 is produced by tumor cells, B-cells, tumor-associated macrophages, tumor-infiltrating lymphocytes, and Tregs in tumors or the blood of cancer patients.

This cytokine is pluripotent, signaling through STAT1 and STAT3 in most cells, but also involving other pathways. *In vitro,* antibodies to IL-10 sensitize tumors to chemotherapeutic drugs. IL-10 may be anti-apoptotic, perhaps by modulating BCL2.

In a murine lupus model, constant IL-10 antibody administration protected the animals from autoimmune effects and prolonged survival, whereas IL-10 accelerated the onset of autoimmunity.

Dr. Whiteside summarized clinical experience with anti–IL-10 antibodies. In a pilot study, murine antibodies were given to six steroid-dependent SLE patients for 21 days. No serious adverse events were reported, and clinical improvement was observed in all patients. Monoclonal antibody levels remained higher during treatment than levels of IL-10, suggesting that endogenous IL-10 was being neutralized. Although the patient IL-10 levels remained higher after therapy than those of normal subjects, they were lower than at baseline.

The potential for humanized, clinical-grade anti–IL-10 could involve many different settings and tumor types. Such antibodies could be used in multiple therapy regimens. Many independent clinical investigators would likely be interested in having access to them.

It might first be necessary to separate anti–IL-10 immunosuppressive effects from its immunostimulatory activities before contemplating the use of antagonists. Theoretically, anti–IL-10 could be used to sensitize resistant tumors to chemotherapeutic drugs. Other potential uses include elimination of Tregs (which produce a great deal of IL-10), direct inhibition of tumor proliferation, up-regulation of antigen process in APCs, down-regulation of tumor-associated inflammation, and elimination of tumor escape. Dr. Whiteside noted that DCs produce a great deal of IL-10 and they might contribute to the development of Tregs. The use of antibodies might defuse the activity of the IL-10–producing DCs.

Discussion

Dr. Berzofsky pointed out that one of the important functions of IL-10 is to block IL-12 production by dendritic cells, so blockade of IL-10 would be expected to increase IL-12 and interferon-gamma production and thus the stimulation of Th1 cells. Anne O'Garra has described a type of Tregs that make and also respond to IL-10. She and Giorgio Trinchieri have found that anti–IL-10R is effective at potentiating a vaccine. Dr. Berzofsky also mentioned that he had observed an ability of IL-10 *in vitro* to stimulate CTLs.

Dr. Pardoll said that this is an interesting but complex agent, and he asked if anyone has investigated the role of IL-10 in Treg suppression of antitumor activity. IL-10 blockade diminishes the Treg effect. Dr. Whiteside said that this question has been studied *in vitro* but not *in vivo.* Several participants asked whether anyone has looked at the IL-10 message in Tregs in, for example, ovarian cancer. Dr. Palucka was particularly interested to know if such studies have been done with antigen-specific Tregs. No one was aware of any such studies. Dr. Whiteside spoke about expression of IL-10 by tumor-infiltrating lymphocytes from human tumors. Dr. Pardoll said that anti–IL-10 has some potential but more investigation is needed.

Dr. Amy Rosenberg said that anti–IL-10, at least in the pilot study, appears to decrease autoimmunity; however, in a cancer-therapy setting, an autoimmune response would be desirable. She asked why this agent would be worth pursuing. She mentioned that a STAT3 knockout in CD4+ cells abrogates autoimmunity in the EAE model. Dr. Pardoll said that just because the antibody abrogates autoimmunity does not necessary imply that it will eliminate antitumor activity, but it does raise questions.

Dr. Berzofsky asked why anti–IL-10 receptor is not on the list. It might be better to block the receptor. Dr. Cheever said that it was not submitted as a candidate to the Web site. Nevertheless, this might be a pathway worth investigating.

Dr. Disis said it appears that the candidate agents fall into two categories: those with interesting but scant data and those with a sizeable amount of preclinical and clinical data. Anti–IL-10 falls into the former group.

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Anti–LAG-3 and sLAG-3
Presenter: Elizabeth Jaffee, M.D.

Lymphocyte activation gene-3 (LAG-3 or CD223) is a negative regulator of activated T cells. Little is known about anti–LAG-3 or soluble LAG-3 fragment (sLAG), although they are very interesting agents. Only a few groups have been studying them. A colleague of Dr. Jaffee's at Johns Hopkins has shown that the agent has cell-intrinsic function and seems to signal through erk. LAG-3 is expressed on activated natural killer and T cells, but not on resting lymphocytes. It is selectively up-regulated on Tregs and is involved in mediating Treg function in murine models. sLAG-3 is released by activated T cells and is found in serum.

Rat anti-mouse LAG-3 blocks LAG-3 function without interfering with its ability to bind to MHC class II molecules *in vitro.* It blocks Treg activity *in vitro* and enhances T-cell expansion *in vivo.* It has a potential role as a check inhibitor by blocking Tregs. Anti–LAG-3 has been shown in two tumor models to block Treg activity.

sLAG-3 has a role in T-cell migration. It has been used in two phase I studies. Because it induces secretion of certain chemokines and Th1 cytokines needed for DC migration to secondary lymphoid organs, it could be a candidate adjuvant for cancer vaccines.

Two phase I studies have assessed safety and T-cell responses using sLAG-3 (IMP321) as an adjuvant to influenza or hepatitis B vaccines. In the influenza vaccine study, 40 normal volunteers were randomly assigned to receive flu vaccine in one of three doses of sLAG as adjuvant or a saline control. No differences were seen in post-vaccination humoral responses measured at day 29 or 57. The subjects who received the sLAG adjuvant had higher levels of Th1-type flu-specific CD4+ T-cell responses, however. sLAG-3 was well tolerated and is currently being evaluated in a phase I trials in metastatic renal cell carcinoma, breast carcinoma, and disease-free melanoma patients.

sLAG-3 is being produced by a company in France. It might have some potential as a cancer vaccine adjuvant for priming the immune response. Anti–LAG-3 has shown some activity in preclinical models as a checkpoint inhibitor, but would probably be better used in combination with a vaccine. Anti–LAG-3 appears to be more interesting but it has not been tested in cancer models. More data are needed about this molecule.

Discussion

Dr. Disis said that the lack of difference between the groups in the influenza vaccine study seems to indicate that sLAG does not hold a great deal of interest. She suggested eliminating sLAG from consideration but retaining the antibody. Dr. Pardoll indicated that another group did not find any evidence that LAG-3 can activate DCs.

Most agreed that LAG-3 seems to be at a "more primitive level." Others mentioned the negative prognostic value of elevated IL-10 and receptor blockade.

By voice acclamation, the participants determined the priority ranking of the varied agents to be anti–IL-10 and/or IL-10 receptor, anti–LAG-3, sLAG-3.

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Anti–Transforming Growth Factor (TGF)-beta

Presenter: Frank Calzone, Ph.D.

According to Dr. Calzone, SMAD-dependent TGF-beta signaling is well understood, although alternative signaling is not. Any antibody or TGF receptor II–based therapeutic should neutralize TGF-beta without cross-reacting with latent ligand. Dr. Calzone provided a list of various TGF-beta–targeted inhibitors and described preclinical experience with using them as cancer immunotherapy or as direct antitumor agents.

Such inhibitors, however, pose some cancer risks. Inhibiting the SMAD pathway could increase risk of carcinomas that might become apparent long after drug approval and wide clinical acceptance. As evidence, Dr. Calzone pointed out that TGF-beta receptor-I and -II, as well as SMAD4, are frequently inactivated by mutation in human pancreatic and biliary cancers. Also, experimentally, TGF-beta is a potent, negative regulator of epithelial cell proliferation (normal cells and non-aggressive cancers).

A number of antibodies have been raised against TGF-beta. Dr. Calzone pointed out several reasons why selecting an antibody would be preferable to the huFc receptor-II. Most importantly, process development for an antibody is well-defined with high yields (1 g/L) readily achievable. Antibodies have a better pharmacokinetic profile than the receptor drugs. Safety events associated with TBR immune recognition are rare but potentially significant.

A phase I cancer study of the antibody (GC-1008 manufactured by Genzyme/AstraZeneca) is under way, whereas no human data are available on the huFc receptor-II. No results from the study have been published yet. The trial has the objective of assessing MTD and safety in patients with locally advanced metastatic renal cell carcinoma or malignant melanoma. Another phase I study by AstraZeneca has been completed, enrolling 45 patients with early stage, diffuse, cutaneous systemic sclerosis. More serious adverse events were reported in the treatment group, but the antibody was generally well tolerated, and the adverse events were manageable. No efficacy was shown.

Among the contemplated uses of anti–TGF-beta would be as a single agent to amplify or unmask natural immunosurveillance, as an agent to enhance T-cell adoptive immunotherapy in cancer, or to amplify the efficacy of an anticancer vaccine aimed at inducing CTL-mediated tumor regression. A clinical study of TGF beta blockade would require special expertise because this treatment mode could have multiple effects on tumors (stroma, tumor, Tregs). The situation would be very complicated.

Dr. Calzone suggested that pan-specific TGF-beta neutralizing offers more opportunity to demonstrate efficacy, and this seems more critical than safety given the available clinical data. Any trial should generate detailed information on the response of T-cell subsets to make the connection between TGF blockade and tumor immunobiology *versus* direct antitumor activity or stroma-mediated tumor inhibition.

Discussion

The participants discussed which agents are in development and their proposed uses. Some discussion ensued about Genentech's activities in this area and the focus on using the agent for various aspects of fibrosis, e.g., to prevent scarring or collagen deposition.

Dr. Berzofsky reported that some preclinical work was done in his lab on the immunoregulatory pathway in which natural killer T cells (NKT) induce myeloid cells to make TGF-beta that inhibited CTL-mediated tumor immunosurveillance. In at least three tumor models, his group was able to reduce or eliminate metastases or tumor recurrence. The participants agreed that

having an agent to target both the NKT pathway and the Treg pathway would be very exciting. Dr. Berzofsky is running the first-in-human trial together with Dr. John Morris of the Metabolism Branch, NCI, in melanoma or renal cell carcinoma patients. The study has four sites, with NCI as the lead site. It is a dose-escalation trial; several dose cohorts are already completed. The investigators are looking at effects on T-cell response and biomarkers. The primary goal is safety and ascertainment of the MTD, which has not yet been reached.

Dr. Pardoll said that TGF is an attractive target. These studies should provide a sense for the extent to which these effects are immunologic versus non-immunologic. It would be important to look in a neo-adjuvant setting. A significant body of preclinical data supports the rationale for use of anti–TGF-beta. The time would seem to be right to bring TGF beta blockers into the clinic. Several participants agreed with the latter statement.

Dr. Cheever said that it was difficult to know how to rank these related agents. Some "heavy hitters" are involved with development and testing and thus the agents are likely to be broadly available for testing. Scientific interest in TGF-beta blockade is great. The participants generally recognized that clinical advancement of TGF-beta neutralizing antibodies (and TBR kinase inhibitors) for the treatment of fibrosis and cancer is being addressed by biotech (Genzyme) and pharma (Lilly). Immediate access to these drugs and funding for clinical trials in tumor immunology may be difficult.

Dr. Berzofsky said that the primary sponsor of his trial is Genzyme, which owns GC1008. He posited that it would be important to test the agent in multiple cancers, but the theoretical risk of exacerbating the disease has caused some foot dragging. Trying it in combination with cancer vaccines (e.g., prostate cancer vaccine) would also be a very interesting avenue of research. The pharmaceutical companies would probably be most interested in developing it as a single agent, but immunologists would probably like to try it in combinations or as an adjuvant.

The participants expressed greater interest in the antibody than in the receptor. By voice acclamation, the participants determined the priority ranking of the varied agents to be anti–TGF-beta, anti–IL-10 and/or IL-10 receptor, anti–LAG-3, sLAG-3, TGF-beta receptor.

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CD40 Agonists

Presenter: Paul Sondel, M.D., Ph.D.

The two agents considered in this category are an agonistic recombinant CD40 ligand trimer and a fully human and selective CD40 agonist monoclonal antibody. The target is the CD40 receptor itself. The goal of using the agonist is to provide pharmacologically the signal that is physiologically given by the ligand on the surface of CD40+ helper T cells, thereby helping antigen-presenting cells (APCs) perform better, and activating any population of cells bearing CD40 molecules on their surface.

Dr. Sondel described the main ways the agonist works in preclinical models: through APC activation and induction of T-cell immunity or by direct tumor inhibition (especially in CD40-bearing B-cell lymphomas). CD40 agonists can also affect tumors not expressing CD40 through other mechanisms, such as an anti-angiogenic effects or induction of antitumor innate immunity. Preclinical studies identified cytokine release syndrome as a toxicity problem.

Dr. Sondel described available unpublished and published data on clinical experience, mostly based on the fully human monoclonal antibody. One phase I trial enrolled 29 patients with melanoma or other solid tumors. Four subjects had measurable objective responses by RECIST criteria. Most showed up-regulation of the CD86 co-stimulatory molecule. In one well-studied case, tumor-specific T cells were induced. Cytokine response syndrome and liver/hematologic toxicity were reported.

The other molecule that has been tested is the recombinant human CD40 ligand trimer. The initial phase I study showed 2 partial responses out of 32 solid tumors or non-Hodgkin lymphoma. Some 76% of patients had decreases from baseline in the percentage of circulating CD19 B cells on day 5, possibly related to the peripheral clearance of these CD40+ cells by binding to the ligand. The percentage of CD4+ T cells increased during this time in 81% of treated patients.

Dr. Sondel speculated that these agents could be used as monotherapy for induction of innate and adoptive immunity to CD40+ and CD40– tumors; they might also be used as single agents for direct inhibition of CD40-expressing tumors, which includes up to 70% of solid tumors. CD40 agonists have excellent potential for combination therapy with other anticancer treatments,

including chemotherapy, radiotherapy, cancer vaccines, toll-like receptor agonists, cytokines, and TNF receptor–family agonists.

It appears, however, that no compelling need exists to produce the monoclonal antibodies because the pharmaceutical industry (Pfizer) is already involved and appears willing to provide them for investigator-initiated research. The recombinant trimeric ligand was being developed by Immunex-Amgen, but is no longer; therefore, it may be a candidate for NCI production or distribution.

Discussion

Dr. Tom Waldmann discussed the potential for desirable effects involving combination of CD40 agonistic therapy with IL-15, which may lead to important effects not mediated by IL2. However, IL-15 has a short half life, and the reagent is not very effective in the absence of IL-15R alpha. By giving anti-CD40 ligand, the IL-15 receptor alpha subunit is induced on DCs and IL-15 bound to this receptor is recycled, its biological activity is increased, and its effects are prolonged, possibly for 3 weeks. Thus an added benefit of CD40 ligation would be the enhancement of treatment with IL-15.

The CD40 signal is a very important and effective activator of DCs. Drs. Berzofsky and Mackall have experience using CD40 ligand for maturing human DCs, but it has been unavailable since it became the intellectual property of Amgen.

Dr. Sondel favors the antibody because it has several important characteristics, e.g., it has action on APCs, it can be injected into tumors, and it has an effect on the innate immune system. He, therefore, advocated giving it a high priority ranking.

Dr. Weber agreed, saying that demonstration of clinical response plus a sound scientific rationale is a compelling combination.

A participant inquired about the agent's mechanism against B cells. Dr. Sondel said that it induces apoptosis via the cytokine storm. There was a brief discussion about the concomitant decrease in peripheral B cells and the possibility that this decrease is due to migration and not death.

Dr. Schlom recommended not having both anti-CD40 and the ligand at the top of the priority list. Dr. Sondel suggested both are important and have been developed separately. Because the trimeric ligand is not available, he suggested putting it at the top of the list, just above the antibody. It would be more expensive to produce than the monoclonal antibody.

By voice acclamation, the participants determined the priority ranking of the varied agents to be anti-CD40 and/or CD40L, anti–TGF-beta, anti–IL-10 and/or IL-10 receptor, anti–LAG-3, sLAG-3 (low priority), TGF-beta receptor (low priority).

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Adenovirus-CCL21

Presenter: Karolina Palucka, M.D., Ph.D.

CCL21 is a CC chemokine, also known as secondary lymphoid tissue chemokine and by several other terms. In the central nervous system, the target is CXCR3. CCL21 is expressed by high endothelial venules and in T-cell zones of spleen and lymph nodes, strongly attracting naïve T cells and mature DCs via interaction with the CCR7 target.

Human DCs transduced with advenovirus-CCL21 have been shown *in vitro* to produce large amounts of CCL21, to attract T cells and DCs, and to prime naïve T cells. In animal models, intramural injection leads to CD4- and CD8-dependent antitumor response in both localized and metastatic disease. The response is characterized by infiltration of DCs and lymphocytes within resolving primary tumors at both the local injection site and metastatic sites.

Also, CCL21-transduced DCs are effective in transgenic mice that develop bronchoalveolar carcinoma spontaneously. Other preclinical work in animals involved its use as an adjuvant for TERT-DNA vaccine in a breast cancer model, and it has shown immunologically mediated regression of pancreatic tumors in mice upon intratumoral delivery and improved survival and therapeutic efficacy of adoptive T-cell transfer in a mouse model of melanoma.

A clinical trial has been approved for non–small-cell lung cancer. The goal is to generate and manipulate the trafficking of effector cells—a very interesting strategy, according to Dr. Palucka. Chemokines are very important in anticancer effects, but there is some hesitancy about the use of viral vectors. One concern is that the T cells could be "led astray" to generate a response against the vector and not the tumor antigen. It could be a good helper effect, but the competition for antigen presentation would be worrisome with a viral vector.

Among the uses contemplated for adv-CCL21 as an adjuvant to cancer vaccines are (1) *ex vivo* transduction of cancer vaccines based on *ex vivo* DCs or cell lines, for example, GVAX; (2) *in vivo* as an adjuvant to cancer vaccines; and (3) *in vivo* for intratumoral gene therapy.

Adv-CCL21 is in production.

Discussion

Dr. Weber asked about using antigen-pulsed DCs. Dr. Palucka said that this would need more study to see what is presented. There may be no problem. One possibility would be using RNA transduction to avoid the possibility of competition for antigen presentation.

This strategy is very different from the others discussed during the course of the meeting and might be very significant. Dr. Sondel said that this approach may be the only way to pursue chemokines that could be used to attract T cells. The participants discussed the relative merit of this chemokine compared with the other molecules.

Dr. Palucka mentioned capturing antigens *in situ* rather than loading them *ex vivo.*

Dr. Berzofsky suggested that this chemokine might also attract central memory cells as well as naïve T cells. Dr. Palucka agreed with this. Another participant suggested using an avipox vector, which is not immunogenic.

The RAID program is already making this agent for two individuals. It would likely be possible to manufacture additional quantities to carry out a few more studies. For that reason, some participants thought that adv-CCL21 should probably have a relatively high priority.

Dr. Creekmore pointed out that genetic stability is another potential problem with virus-vectored agents.

By voice acclamation, the participants determined the priority ranking of the varied agents to be anti-CD40 and/or CD40 ligand, anti-TGF-beta, anti–IL-10 and/or IL-10 receptor, adv-CCL21, anti–LAG-3, sLAG-3 (low priority), TGF-beta receptor (low priority).

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LIGHT

Presenter: Drew Pardoll, M.D., Ph.D.

LIGHT, another TNF superfamily member, is part of a complex receptor-ligand network comprising 10 or so molecules, Genome Database designation TNFSF14. It was discovered by Lieping Chen. LIGHT binds to three molecules, complicating its potential use in treatment. It clearly has co-stimulatory activity on T cells through expression of herpes virus entry mediator (HVEM). It mediates some of its antitumor activity through the lymphotoxin-beta receptor by apoptotic activity. LIGHT-HVEM interactions mediate graft-versus-host disease (GVHD). LIGHT also has antitumor effects, as evidenced in preclinical studies, but it is difficult to ascertain which receptor is involved.

Dr. Pardoll is not aware of any clinical data. He posited that soluble LIGHT might be used for systemic administration alone or in combination with vaccines. Some studies have shown that LIGHT can be introduced via a vector for transduction of tumor cells. Anti-LIGHT antibodies (or anti-HVEM) could be used to treat GVHD. Potentially, LIGHT could be useful for any cancer type as an adjunct to vaccination or for adoptive CD8+ cell transfer. Another possibility would be paracrine administration via direct injection into tumors or transduced tumor vaccines. He suggested that LIGHT should be lower on the list of priorities due to its complexity and the lack of supporting clinical data. Soluble LIGHT would probably be the most interesting form for future study.

Dr. Schlom reported some preclinical work done in his lab that involved development of avipox-vectored LIGHT; it worked extremely well in that form although its activity was not compared with that of soluble LIGHT. Its use is very complicated because the receptor is down-regulated on fully activated cells.

The participants agreed that data are scarce about how LIGHT relates to cancer pathology; therefore, it should be low on the list. Monkey studies would be in order. The agent has a great deal of bioactivity, but more data are needed about the correlation between LIGHT

concentrations and inflammatory conditions. Again, it would be necessary to investigate the question of local administration or administration with vaccine.

By voice acclamation, the participants determined the priority ranking of the varied agents to be anti-CD40 and/or CD40 ligand, anti–TGF-beta, anti–IL-10 and/or IL-10 receptor, adv-CCL21, LIGHT and/or LIGHT vector, anti–LAG-3, sLAG-3 (low priority), TGF-beta receptor (low priority).

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1-Methyl Tryptophan

Presenter: George Prendergast, Ph.D.

Dr. Prendergast declared a potential conflict of interest stemming from a personal interest and his consulting work with a company moving this agent into the clinic. The organizing committee requested that he present information on the molecule because of his unique expertise in this area.

1-methyl tryptophan is a simple, small molecule that inhibits the immunosuppressive enzyme IDO, as well as IDO2. IDO suppresses T-cell activation via tryptophan catabolism, thereby limiting antigen-induced T cell activation and mediating immunosuppression in cancer. IDO is highly expressed in tumor cells and plasmacytoid DCs in tumor-draining lymph nodes. The IDO

knockout mouse is resistant to inflammatory carcinogenesis and is viable, fertile, and without autoimmune disease.

1-methyl tryptophan has been widely studied as a D+L racemic mixture, with the D stereoisomer being more biologically active. The D isomer has an outstanding pharmacokinetic and toxicity profile in mouse, rat, and dog models, displaying significant stability in plasma with a half-life of about 8 hours. Notably, the L isomer is a stronger inhibitor of IDO whereas D has more activity against IDO2. Some compelling biochemical evidence suggests that the D isomer blocks IDO2 better than IDO; therefore, IDO2 may be a relevant target *in vivo.*

The D isomer has been selected for clinical translation by NewLink Genetics Corporation and NCI.

Dr. Prendergast described the preclinical experience with 1-methyl tryptophan. Work with IDO knockout mice offers an initial genetic validation in cancer. When subjected to a classical protocol of inflammatory skin carcinogenesis, wild-type mice developed tumors whereas the knockout mice were resistant to tumor formation. Other mouse models involving grafted tumors or transgenic, "immuno-edited" tumors showed that 1-methyl tryptophan limited tumor growth and reduced tumor size in combination with cytotoxic chemotherapy. In such experiments, antitumor activity was CD4+ T cell–dependent. The D isoform has better antitumor activity than the L isoform in most models. IDO knockout abolishes the antitumor effect.

Dr. Prendergast spoke about the IDO2 gene in the human genome, which was discovered only recently. The IDO2 is situated immediately downstream of IDO but was not recognized previously due to mis-annotations in the human genome database. Although little is known about IDO2 as yet, there are two genetic polymorphisms in the coding region of the human enzyme that abolish its activity. Interestingly, these polymorphisms occur widely in heterozygous and homozygous configurations, suggesting that IDO2 activity varies widely in human populations. If, as Dr. Prendergast hypothesizes, IDO2 is targeted by D-1-methyl tryptophan, then these IDO2 polymorphisms might affect clinical applications by abolishing the target.

The IND is in place for a traditional dose escalation phase I study. Possible safety concerns include eosinophilia-myalgia syndrome, autoimmunity due to "learned" tolerance, and susceptibility to *Toxoplasma gondii* infection. Dr. Prendergast noted that none of these problems have been observed in animal studies.

In terms of contemplated uses, Dr. Prendergast suggested that the agent could be used as a general adjuvant for cancer therapy that acts to relieve a mechanism of tumor immune suppression. It could be combined with cytotoxic chemotherapy, tumor vaccines, toll-like receptor agonists (e.g., CpG), radiotherapy, monoclonal antibodies, or drugs that target other mechanisms of immune suppression (e.g., OX40, PDL-1).

The NCI has D-1-methyl tryptophan. Its synthesis is straightforward and relatively inexpensive. NewLink has prepared a lot for the phase I clinical trial and will be synthesizing new lots. The agent should be widely available within a year or so.

Discussion

In response to a participant's question about whether 1-methyl tryptophan treatment would be applicable in all tumors or only patients with tumors that overexpress IDO, Dr. Prendergast explained that it might be applicable in all tumors because IDO is also thought to participate in immunosuppression via expression in antigen-presenting cells present in tumor-draining lymph nodes. He also commented that the pharmacodynamics of an IDO inhibitor could be determined in a straightforward manner by evaluating the ratio of tryptophan to kynurenine, the product of the IDO reaction, in blood. An assay method to determine kynurenine levels from blood using LC/MS/MS is being used by the investigators.

Another participant asked about the phase I trial and whether the investigators plan to monitor single-nucleotide polymorphisms (SNPs). Dr. Prendergast said there is an SNP that occurs in IDO-1, but it is not widely present in the human population.

Dr. Schlom suggested that 1-methyl tryptophan may be a perfect agent to investigate using the cell search machine. The cells could be isolated and examined for IDO. He inquired whether IDO in serum correlates with tumor burden, noting that a discord exists between levels of carcinogenic antigen (CEA) and tumor burden. Dr. Prendergast said that IDO enzyme cannot be found in blood, but that kynurenine can be measured.

Dr. Prendergast mentioned arginase as another enzyme whose activity is associated with immune suppression, saying that the literature is very interesting, but the picture is more complex.

Dr. Palucka commented on the relevance to DCs. IDO expression in DCs is associated with a suppressive function they manifest in the context of antigen presentation to T cells.

By voice acclamation, the participants determined the priority ranking of the varied agents to be anti-CD40 and/or CD40 ligand, anti–TGF-beta, 1-methyl tryptophan, anti–IL-10 and/or IL-10 receptor, adv-CCL21, LIGHT and/or LIGHT vector, anti–LAG-3, sLAG-3 (low priority), TGF-beta receptor (low priority).

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