Meeting Report

Cancer Immunology Research

Translating Science into Survival: Report on the Inaugural International Cancer Immunotherapy Conference

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Abstract

The inaugural International Cancer Immunotherapy Conference, cohosted by the Cancer Research Institute (CRI), the American Association for Cancer Research (AACR), the Association for Cancer Immunotherapy (CIMT), and the European Academy of Tumor Immunology (EATI), was held in New York City on September 16–19, 2015. The conference brought together nearly 1,400 scientists, clinicians, regulators, patient advocates, and other stakeholders to discuss the latest scientific developments in cancer immunology and immunotherapy, as well as the regulatory hurdles facing new drug development. This conference report summarizes the main themes that emerged during the 4-day meeting. *Cancer Immunol Res; 4(1); 3–11. ©2016 AACR.*

Introduction

As interest in cancer immunotherapy has accumulated, so have the number of scientific meetings devoted to its study. To coordinate efforts and reduce duplication, four leading nonprofit organizations hosted a joint scientific meeting September 16–20, 2015 in New York City. Titled "Translating Science into Survival," the conference reflected the combined efforts of the Cancer Research Institute (CRI), the American Association for Cancer Research (AACR), the Association for Cancer Immunotherapy (CIMT), and the European Academy of Tumor Immunology (EATI). The conference took place over 4 days and was attended by nearly 1,400 individuals. There were 11 speaker sessions, two roundtable discussions, and two poster sessions. Topics spanned the full spectrum from basic research in tumor immunology to regulatory challenges facing new immunotherapy drug development.

Specific topics covered included immune regulation of T-cell responsiveness, genomic methods for identifying tumor antigens, the tumor microenvironment, adoptive cell therapies, checkpoint blockade, biomarkers, combinatory treatments, and the microbiome. In this meeting report, we review several of the main themes and areas of interest that emerged during the 4-day meeting, with a focus on emerging areas of inquiry.

T-cell Armies

The conference began with a keynote lecture by Steven Rosenberg, of the Surgery Branch of the National Cancer Institute (Bethesda, MD). For the past 20 years, Rosenberg has been honing an approach to immunotherapy called adoptive cell therapy (ACT; ref. 1). In this approach, tumor-infiltrating lymphocytes (TIL) are removed from a patient's tumor, the cells are grown in the laboratory with IL2, and then upon expansion the cells are given back to the patient. When the approach works, it works very well: Among 93 patients with advanced melanoma treated this way, 20 (22%) had complete responses, and 19 have been in remission "well beyond 10 years," Rosenberg said in his keynote lecture.

More than 1,300 scientists attended the CRI–CIMT–EATI–AACR Joint Meeting. Photo courtesy of CRI.

In recent years, Rosenberg has worked to improve the approach and expand its applicability. The biggest hurdle was determining what antigens were being expressed by the tumor cells such that TILs could recognize them. Using deep exome sequencing, Rosenberg was able to identify these specific antigens and perform ACT with just those T cells that uniquely recognize those specific antigens. This approach is allowing Rosenberg to expand ACT to cancer types besides melanoma, including common epithelial cancers like stomach, colorectal, esophageal, and pancreatic cancers.

Rosenberg presented the case of the first nonmelanoma patient treated this way, a 45-year-old woman with advanced

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cholangiosarcoma, a cancer of the bile ducts, who also had lung metastases. After she received the treatment, the patient's cancer regressed completely and she is currently cancer-free.

ACT approaches were the focus of several talks over the 4-day conference, including chimeric antigen receptor (CAR) T-cell therapy. CAR therapy has been used by multiple groups to treat different forms of leukemia. Its efficacy is greatest for acute lymphoblastic leukemia (ALL), where response rates reach as high as 90%, but it is also effective against chronic lymphocytic leukemia (CLL), in which response rates tend to hover around 60%.

Not all CARs are the same in terms of how they are built, and there seem to be important therapeutic consequences as a result. Carl June and colleagues at the University of Pennsylvania (Philadelphia, PA) have shown that in CLL patients, treatment with CAR T cells made with a 4-1BB domain can persist in patients for at least 4 years. This duration is much longer than that for CARs made with a CD28 domain, which seem to persist around 30 days. This difference in persistence does not appear to matter for the treatment of ALL, but for CLL it makes a substantial difference. June has found a direct correlation between persistence of the CAR cells and therapeutic response in patients with CLL. In his talk, he presented laboratory findings suggesting that one reason 4-1BB CAR T cells might stick around longer is that they rely on mitochondrial oxidative phosphorylation rather than glycolysis.

Other researchers presented work they are doing in order to improve the effectiveness of CAR therapy. Ingunn Stromnes, a CRI postdoctoral fellow at the University of Washington (Seattle, WA), is using a genetically engineered mouse model of pancreatic cancer (KPC) to study immunotherapy with genetically engineered T cells. Stromnes used genetic engineering techniques to introduce a mutation into a mesothelin-specific T-cell receptor (TCR). The mutation enhances the TCR's binding to mesothelin. Stromnes demonstrated that when these T cells were infused into the KPC mice, the cells trafficked to the tumor and began to proliferate, but then began to die—an effect related perhaps to the extreme immunosuppressive environment of pancreatic cancer tumors. To partially combat this problem, she experimented with performing multiple sequential infusions. She found that the newly infused cells also work for a time and then undergo apoptosis, but before they do so they are able to double the survival time of the mice.

Pancreatic cancer has been branded an immunologically "cold" tumor, with few mutations leading to few T-cell responses. However, according to Rienk Offringa, of the German Cancer Research Center (DKFZ; Heidelberg, Germany), this is an unfair characterization. Offringa finds that roughly 80% of resected pancreatic tumors do have T cells within them, implying that tumor antigens are available for T cells to target. Offringa is developing an ACT approach to pancreatic cancer similar to the one that has been used successfully in melanoma. The approach involves removing T cells from the tumor, growing them in the laboratory, and then reinfusing them into the patient. The time to do the treatment, he suggests, is at the first sign of a recurrence, as measured by a blood marker of pancreatic cancer called CA19-9.

A limitation of current CAR T-cell approaches is the intense labor involved in creating antigen-specific CARs using viral vectors. Laurence Cooper, of The University of Texas MD Anderson Cancer Center (Houston, TX) presented his work showing how T cells can be engineered to express CARs or genetically modified TCRs using a transposon/transposase system called Sleeping Beauty (SB). The SB system combines the advantages of cost and simplicity of naked DNA with the efficiency associated with recombinant retroviruses (2). This nonviral approach to manufacturing CARs and TCRs, Cooper suggested, may be a way to bring this powerful personalized form of immunotherapy to more patients.

Antigens, Which Antigens?

For decades, a major focus of research in cancer immunology has been on searching for unique tumor antigens that could be targeted with immunotherapies. The first tumor-specific antigens were identified in the early 1990s (3). These are proteins such as MAGE and NY-ESO-1 that are commonly found in cancer cells but not normal adult body cells, with the exception of cells in the testes. Once these antigens were identified, the race was on to use them to make therapeutic cancer vaccines. Unfortunately, despite some anecdotal successes, several large clinical trials (MAGRIT) failed to show that vaccines made with shared tumor antigens improved survival for patients with cancer.

In recent years, the focus of attention has shifted from shared tumor antigens to unique antigens that are specific to an individual patient's tumor—so-called neoantigens. While it has been known for a long time that cancers had these unique genetic fingerprints, they were not considered good candidates for vaccines because they are so idiosyncratic; they are different for every tumor and every patient. Trying to identify them to make a vaccine would mean genetically sequencing each patient's entire genome to identify these few bits of relevant genetic information—truly like finding a needle in a haystack. The technology simply did not exist to make this a realistic approach to vaccination.

Recent advances in genomics and next-generation sequencing have made the search for unique antigens much more feasible. Robert Schreiber, of Washington University School of Medicine (St. Louis, MO), discussed the approach that he and his colleagues have used to identify tumor-specific mutant antigens (TSMA). They use exome sequencing and epitope-prediction techniques to identify the TSMA that are likely to be processed and presented by MHC proteins. Schreiber and his team have shown that they can make a long peptide vaccine with these neoantigens that is effective at curing MCA sarcoma mice of their tumors (4). Clinical trials of the approach are currently under way at a number of institutions.

In his talk, Schreiber also presented new data that shed light on the requirements for effective personalized vaccines. Using an oncogene-driven sarcoma derived from mice expressing a mutant activated form of Kras and lacking p53 (KP sarcoma), he showed that tumors from these mice are not immunogenic, they do not respond to checkpoint blockade, and they do not express neoantigens as shown by exome sequencing and epitope prediction methods. Schreiber wanted to determine if forced expression of an MHC-I TSMA, such as mutant spectrin, would render these tumors immunogenic. Surprisingly, they were not. However, forced expression of both MHC-I and MHC-II antigens rendered the tumors strongly immunogenic and resulted in rejection, revealing a need for both types of MHC presentation for effective vaccination in this model.

Several other talks also focused on identifying cancer antigens and using them in vaccines. Hans-Georg Rammensee, of the University of Tübingen (Tübingen, Germany), gave a talk that focused on identifying appropriate cancer antigens in cancers that are less prone to having widespread genetic abnormalities compared with melanoma, lung cancer, or chemically induced

Joint meeting leaders: Guido Kroemer (EATI), Margaret Foti (AACR), Jill O'Donnell-Tormey (CRI), and Christoph Huber (CIMT). Photo courtesy of AACR.

tumors, such as MCA sarcoma. For example, what does the neoantigen landscape look like in ovarian, liver, or kidney cancers? Rammensee and his team found that neoantigens that could be recognized by the immune system were exceedingly rare or nonexistent in these tumor types. They therefore focused their attention elsewhere—on nonmutated germline antigens that were abundant in tumors but restricted in normal tissues. These sorts of antigens, they found, were much more common. As to how to explain the restriction of these nonmutated germline antigens to tumors, Rammensee suggested that they may represent alternatively spliced mRNAs that make unique proteins differing from patient to patient. He argued that these sorts of antigens, too, might be useful targets for personalized immunotherapy, besides tumor-specific neoantigens.

Coley Lectures

Every year for the past 40 years, CRI has given an award to scientists in honor of the late William B. Coley, considered the father of cancer immunotherapy. The winners of the award give a lecture at CRI's annual symposium, highlighting the research for which they are being honored. This year's winners were Alexander (Sasha) Rudensky, of Memorial Sloan Kettering Cancer Center (New York, NY), and Glenn Dranoff, of Novartis Biomedical Institutes (Cambridge, MA).

Rudensky is the 2015 winner of the William B. Coley Award for Distinguished Research in Basic Immunology. In his Coley lecture, Rudensky discussed his laboratory's work on regulatory T (Treg) cells, which play an important role in shutting down immune responses once a dangerous microbe or cancer cell has been eliminated. Rudensky's group was one of three that, in 2003, showed that a molecular switch called Foxp3 was the main driver of Treg development (5). Since then, Rudensky's team has further elucidated how the transcription of Foxp3 is controlled and contributes to the functionality of Tregs, especially through key DNA enhancers. The critical importance of this switch is demonstrated by a naturally occurring genetic disease called IPEX, in which the *Foxp3* gene is mutated. People with this condition develop severe, life-threatening autoimmunity. A similar condition, called scurfy, occurs in mice. In recent years, Tregs at the site of a tumor have emerged as an important target for immunotherapies, as evidenced by several other presentations given at this symposium.

Glenn Dranoff is the 2015 recipient of the William B. Coley Award for Distinguished Research in Tumor Immunology. Over his long career, primarily at the Dana-Farber Cancer Institute and Harvard Medical School in Boston, Dranoff has made many important contributions to the field. In his Coley lecture, he gave a broad overview of his major research preoccupations, which he acknowledged could be traced back to work he did in the early 1990s on the therapeutic cancer vaccine that came to be known as GVAX. Back then, Dranoff and his colleagues "stumbled" on the finding that the cytokine GM-CSF was distinct in its ability to strengthen antitumor immunity in mice; it was the most potent molecule of more than 30 tested. The finding drew attention to the role of dendritic cells in tumor immunity because it was at this same time that Ralph Steinman was showing that GM-CSF was a key growth factor for dendritic cells. These twin observations helped to garner support for immunotherapies, such as GVAX, designed to stimulate antigen presentation by dendritic cells.

Dranoff showed that a cancer vaccine made from whole, irradiated tumor cells that had been genetically engineered to produce GM-CSF could effectively treat cancer in mice (6). In addition to performing this basic research, Dranoff was also a key player in the clinical testing of GVAX in humans. A phase I trial was conducted in the late 1990s in patients with metastatic melanoma (7). Biopsies from patients indicated the vaccine was highly effective at triggering an immune response in the form of T and B cells moving into the tumors. Unfortunately, most patients did not experience lasting therapeutic benefit. However, Dranoff pointed out that a subset of the patients who received the vaccine are still alive and free of disease 20 years later. Therefore, in at least some cases, the vaccine demonstrated meaningful clinical benefit in the absence of any toxicity, which in itself is an important finding.

Inject Locally, Treat Globally

The hope of immunotherapy is that once a local immune response against cancer is initiated, it will continue to fight cancer elsewhere in the body. Several investigators presented talks focused on using various vaccination strategies to jumpstart such a systemic response.

Ron Levy, of Stanford University (Stanford, CA), presented work on an approach he calls *in situ* vaccination, which he and others are using to treat patients with lymphoma. In this approach, CpG is injected locally into lymphoma tumors along with a checkpoint blockade antibody. Unmethylated CG dinucleotides are present at high frequency in prokaryotic DNA and are detected by immune cells that express TLR9. CpG therefore serves as an effective adjuvant. By generating a strong immune response in the immediate context of the tumor, this approach promotes an immune response against released tumor antigens.

Levy presented the case of a 38-year-old man with follicular lymphoma who experienced complete regression of his cancer after local injection of CpG. Importantly, this approach treats not only the tumor at the site of injection; it also generates a systemic immune response that can kill cancer elsewhere in the body.

Using mouse models, Levy has tested this approach with a variety of different checkpoint antibodies given along with CpG,

and anti-OX40 appears to have the best efficacy. Levy believes the antibodies are acting to eliminate Tregs at the site of the tumor, which then permits an immune response to be initiated and act systemically.

Cornelis (Kees) Melief, of Leiden University Medical Center (Leiden, the Netherlands), opened his talk with a helpful reminder: "The cause of cancer dictates the nature of immunogenicity." In other words, different cancers are more or less recognizable by the immune system depending on how the cancer cells initially acquired their transformed phenotype. For example, some of the most effective immunotherapies work against virus-caused cancers, as viral antigens are clearly distinguishable from self-antigens. In two cases of common virus-caused cancers—liver cancer and cervical cancers—very effective preventive cancer vaccines are available; by vaccinating against the virus—hepatitis B virus (HBV) or human papillomavirus (HPV), respectively—one effectively prevents the development of cancer.

However, the immunogenicity of virally caused cancers also makes them good targets for therapeutic immunotherapies—ones designed to treat rather than prevent the cancer. On this front, Sjoerd van der Burg, also of Leiden University, presented work on a vaccine made from long peptides derived from HPV. Van der Burg had shown previously that such a vaccine can effectively treat early-stage cervical cancer. However, in patients with late-stage disease, the vaccine typically is not effective, probably because these patients are highly immunosuppressed. Within the immunosuppressive tumor microenvironment are myeloid-derived suppressor cells (MDSC). These cells normally play a protective role, preventing excess inflammation and autoimmunity, but they can be a major liability in the context of cancer. In many cases, well-developed tumors have found a way to co-opt the suppressive powers of these cells to their own benefit.

Interestingly, van der Burg and others have found that some types of chemotherapy can preferentially decrease MDSCs in the tumor environment, allowing the HPV vaccine to trigger a more powerful immune response. For that reason, a clinical trial was started testing the concurrent administration of the vaccine along with chemotherapy for patients with advanced cervical cancer.

Another cancer-associated virus is Epstein-Barr virus (EBV), which is known to cause lymphoma, nasopharyngeal cancer, and other cancers. Upwards of 90% of individuals are infected with this virus at some point, but their immune system keeps it safely under control. However, for patients that undergo lymphodepletion, typically in preparation for a bone marrow transplant used to cure many hematologic diseases, dormant EBV can become reactivated and lead to lymphoma. Helen Heslop, of Baylor College of Medicine (Houston, TX), has succeeded in greatly reducing the likelihood of this happening by giving the patient virus-specific T cells from a matched donor. These T cells are able to restore EBV immunity in a very high percentage of cases. Heslop has also developed an off-the-shelf version of the virus-specific T cells and has recently begun experimenting with combining virus-specific T cells with CAR technology to deal with persistence problems that sometimes affect CAR T cells.

Responders and Nonresponders

Checkpoint blockade, particularly anti-CTLA-4, anti-PD-1, and anti-PD-L1 therapy, has shown remarkable activity across multiple tumor types. Yet not everyone responds to these therapies, and predictive biomarkers to enable patient selection have remained elusive. Ira Mellman of Genentech (South San Francisco, CA) noted that only 10% to 30% of patients typically respond to checkpoint blockade therapy. A pressing goal for the field, therefore, is to identify which patients are most likely to respond to these agents and determine how to improve treatment options for the nonresponders. Mellman focused on PD-L1 staining in the tumor environment as a potentially predictive biomarker. Conventional wisdom in the field is that tumors with high expression of PD-L1 will tend to respond better to anti-PD-1/anti-PD-L1 treatments. However, Mellman pointed out that for many tumors, PD-L1 staining on tumor cells is actually less predictive than staining on immune cells in the tumor. This result is a bit perplexing given the popular view that PD-1/PD-L1 checkpoint inhibitors work by blocking cancer cells from engaging PD-1 on T cells.

Taking this clinical finding back to the laboratory, Mellman then investigated mechanistically how PD-1 signaling works. Using a reconstituted model of PD-1 proteins in a cell membrane, he found evidence that PD-1 signals act predominantly on costimulatory molecules like CD28 rather than on the T-cell receptor. This may explain why PD-L1 staining on immune cells better predicts responses to anti-PD-1 therapy in some types of cancer. These immune cells, which include MDSC, may provide a negative costimulatory signal to T cells, holding them in check. PD-1 checkpoint inhibitors interrupt this negative signal, allowing T cells to become activated.

Despite lingering questions about the biology of PD-L1, there is emerging evidence that PD-L1 can be a useful biomarker for making clinical decisions, at least in some indications. As Jedd Wolchok (Memorial Sloan Kettering Cancer Center, New York, NY) reported, based on results from a large phase III clinical trial (NCT01844505), PD-L1–negative melanoma patients might do better with the combination of ipilimumab and nivolumab compared with the monotherapy alone, whereas patients who are PD-L1 positive might do just as well (but with fewer toxicities) with the monotherapy alone (8).

Continuing the theme of responders and nonresponders, Pam Sharma, of MD Anderson Cancer Center, presented work examining tumor samples obtained from patients undergoing surgery as a means to understand mechanistically why some patients fail to respond. Using bladder cancer tissues, she and others were able to identify a role for ICOS in predicting good responses to treatment with ipilimumab. Bladder cancer patients treated with ipilimumab who responded well tended to have higher ICOS expression, raising the possibility that activating this pathway in nonresponders may be a way to improve responsiveness to therapy.

Based on mutational load, some types of tumors are naturally more prone to immune recognition than others. Melanoma is one such "hot" tumor, with many mutations, whereas prostate cancer is on the "colder" end of the spectrum, with many fewer mutations. Can a cold tumor be converted into a hot tumor? Sharma showed that, indeed, treatment of prostate cancer patients with ipilimumab causes immune cells to traffic into tumors. However, no patients treated this way had a complete response as a result. Using surgical biospecimens, Sharma was able to pinpoint the reason why: The tumors upregulated other inhibitory immune pathways. This result raises the possibility that combination immune checkpoint blockade might benefit patients with prostate cancer.

Jerome Galon, of INSERM (Paris, France), is developing a better prognostic model of colorectal cancer progression called

Immunoscore. Unlike the current TNM classification scheme, which is based solely on tumor characteristics, the Immunoscore is based on what Galon refers to as the immune contexture—the identity, density, functional orientation, and location of immune cells in the tumor. Galon cited existing evidence that Immunoscore is a better prognostic model than TNM, and a worldwide consortium is currently validating the prognostic value of the Immunoscore model.

Assay Harmonization

Part of what makes a predictive biomarker a useful tool is its likelihood of being both quantifiable and highly objective. The issue of companion diagnostic variability and how to tackle it was the subject of a panel discussion sponsored by the Cancer Immunotherapy Consortium with individuals from academia, regulatory agencies, and industry. The focus of the panel was on the use of PD-L1 as a biomarker and companion diagnostic testing for immunotherapy patients. Recent data suggest that the expression of PD-L1 correlates with improved response to checkpoint blockade, thereby leading to the value of PD-L1 as a predictive biomarker (9–15). In response to these data, several different biopharma companies have developed their own assays for measuring PD-L1 expression. Bristol-Myers Squibb developed an automated assay (using the 28-8 clone, an antibody for PD-L1) that defined >5% tumor cell membranous expression of PD-L1 as "PD-L1 positive" (16, 17). Genentech/Roche developed their own assay based on PD-L1 expression on immune infiltrating cells, which they state leads to enhanced responses to atezolizumab (14). Merck developed an assay for pembrolizumab based on PD-L1 positivity on tumor cells with surface expression (>1%; ref. 18). Researchers at Merck also looked at PD-L1 expression on immune cells with the use of pembrolizumab in other indications (19). Last year, the FDA finalized its companion diagnostic guidelines in which it stated its preference for simultaneous development of companion diagnostic tests in order to identify the best responding population to immunotherapy.

The existence of multiple diagnostic assays, from multiple companies, raises several scientific and practical issues. First and foremost are differences in how each company is performing its assay. There are several potential sources of variability, including (i) PD-L1 expression on tumor cells vs. immune cells; (ii) cellular distribution of the staining (membrane vs. cytoplasmic); (iii) quantifying percent positive (manual vs. automated); (iv) tissue acquisition (biopsy, surgical resection, fresh tissue vs. formalin fixed, length of type in fixation agent, sectioning and embedding); (v) antibody usage (variety of different antibodies used across companies have different specificities); and (vi) training of the pathologist (subjectivity). Moreover, with multiple anti-PD-1 and anti-PD-L1 drugs entering the market, this creates issues for hospitals: Should hospitals adopt multiple diagnostic tests of PD-L1 for each patient? This would be a costly endeavor. Furthermore, financial costs aside, it will be virtually impossible in some circumstances to collect enough tissue to enable testing across multiple platforms.

Realizing some of the confounding factors in the use of PD-L1 as a predictive biomarker, the four major biopharma companies that are currently codeveloping PD-1 pathway drugs and their own investigational use assay for PD-L1 expression have convened in recent months to address these issues. One way in which the various stakeholders are trying to address the issue of assay harmonization is to determine the differences between each company's PD-L1 test. Abigail McElhinny from Ventana Medical Systems/Roche Diagnostics (Tucson, AZ) discussed The Blueprint Project, a collaboration among Bristol-Myers Squibb, Merck, AstraZeneca/MedImmune, Roche/Genentech, Dako/Agilent, Ventana, AACR, and the International Society of Lung Cancer Foundation, to promote cross-industry collaboration on the PD-L1 diagnostic assays in use. All six companies will collaborate on a trial in one indication, non–small cell lung cancer, to compare differences in the immunohistochemistry tests used for PD-L1, with the primary goal being to provide information to the medical community about the various differences in the PD-L1 assays.

While the panel focused primarily on PD-L1 as a biomarker, some participants called for a more systematic approach to selection of patient populations for treatment. As Paul Tumeh from UCLA (Los Angeles, CA) pointed out, there is a large body of data to suggest that preexisting immune infiltration in tumors is correlated with response to immunotherapies (20–22). Furthermore, the mutational landscape has also been correlated with response to checkpoint blockade (23, 24). Perhaps, as Robert Anders from Johns Hopkins (Baltimore, MD) and others alluded to at the meeting, in the future the best way to choose which patients may respond appropriately to immunotherapy will take into account all of these factors.

Isotype Matters

Checkpoint antibodies targeting the CTLA-4 and PD-1 pathways have demonstrated tremendous success in the clinic. Although it is not entirely clear precisely how these antibodies work, it is widely believed that they function by targeting both immune effector cells, such as $CD8^+$ T cells, and Tregs. Checkpoint antibodies can function by either triggering Fc receptor–mediated antibody-dependent cell-mediated cytotoxicity (ADCC) thereby depleting the target cell population, or by blocking receptor and ligand engagement, thereby altering the behavior of the target cell population. Other immune-modulatory antibodies, such as agonist antibodies targeting activating receptors, can be just as effective as checkpoint blockade in animal models, yet their impact in the clinic has not been as robust.

Several speakers addressed the issue of antibody isotype and how different isotypes may affect function and therapeutic outcomes. Typically, if the purpose of the antibody is to eliminate the target cells, then a human IgG1 is used to engage activating Fcγ receptors (FcγR) to enable the initiation of immune effector mechanisms. Alternatively, if the aim of the antibody is to inhibit cell behavior by blocking receptor:ligand engagement, then a human IgG4, IgG2, or Fc null IgG1 is used that does not lead to initiation of Fc-mediated immune effector mechanisms. But there can be surprises.

Checkpoint inhibitors currently in use (both FDA approved and in development) fall into several categories with respect to the isotypes on which they are built and whether they are believed to be blocking or depleting. Two antibodies to CTLA-4 in the clinic exist: ipilimumab (Bristol-Myers Squibb), a humanized immunoglobulin 1 (IgG1), and tremelimumab (AstraZeneca/Medimmune) (IgG2). IgG1 is typically much better at inducing ADCC than IgG2 (25), Some preclinical data suggest that ipilimumab does not induce ADCC on Tregs (26), whereas other data suggest that it does (27, 28). Both ipilimumab and tremelimumab are

effective in the clinic, and both are associated with adverse events. Nivolumab (Bristol-Myers Squibb) is a humanized IgG4 that blocks PD-1 from binding to its ligand, PD-L1, and thus does not deplete PD-1$^+$ CD8$^+$ T cells. Durvalumab (AstraZeneca/Medimmune) and atezolizumab (Roche/Genentech), are both antibodies to PD-L1 on a human IgG1 framework that activates Fc receptors and depletes PD-L1$^+$ cells, presumably MDSCs. Work published by Jeffrey Ravetch's group at Rockefeller University (New York, NY) shows that the Fcγ receptor contributes to the *in vivo* activity of antibodies to PD-1 versus those to PD-L1. For example, PD-1 antibodies are FcγR independent *in vivo*, and this compromises their antitumor efficacy *in vivo*. In contrast, PD-L1 antibodies require FcγR, and their antitumor activity is due to altering and potentially depleting MDSCs within the tumor. These results have important clinical implications for the design of therapeutic antibodies (29).

Alan Korman, of Bristol-Myers Squibb (Redwood City, CA), discussed work that his laboratory has done to decipher the mechanism by which CTLA-4 antibodies achieve their therapeutic effects. His preclinical model shows that antibodies that block CTLA-4 derive much of their therapeutic benefit from selectively depleting Tregs at the tumor site, and that this effect is dependent upon the engagement of activating Fc receptors (28). Other preclinical evidence shows that Treg cell depletion is dependent on the presence of Fcγ receptor–expressing macrophages within tumor lesions, indicating that Treg cells are depleted in trans (27). Recent evidence suggests that, in humans, ipilimumab may lead to the depletion *in vitro* of Tregs via ADCC (30).

Martin Glennie from the University of Southampton (Southampton, UK) shared data demonstrating that the use of mouse models to study immune-modulating antibodies can confound our understanding of how we move the antibodies to a clinical setting. He focused on differences between mouse and human Fc receptors. The family of Fc receptors has a number of activating receptors (FcγR I, IIA, IIC, IIIA, IIB in humans and FcγR I, III, and IV in mice), as well as inhibitory receptors (FcγRIIB in humans and FcγRII and FcγRIIb in mice). It is the activating Fcγ receptors that are required for ADCC. However, for immunostimulatory antibodies, Glennie and colleagues found that the immunostimulatory properties of certain agonistic antibodies is due to the particular isotype framework in which it is made. They did this by engineering the epitope binding variable regions of the rat anti-mouse CD40 mAb onto a mouse IgG1 or mouse IgG2 constant region. When injected into mice with OVA or 4-hydroxy-3-nitrophenyl OVA, the antibody with the IgG1 backbone had immunostimulatory properties, such as CD8$^+$ T-cell stimulation, whereas the antibody with the IgG2 backbone did not. Surprisingly, the FcγRIIb interaction was required for the immunostimulatory properties, despite its usual inhibitory role in the mouse immune system. According to Glennie, the mechanism by which this occurs is through cross-linking and the creation of multimeric aggregates that can induce receptor clustering at the cell surface. One caveat with respect to the development of clinical drugs lies in the fact that the mouse and human IgG isotypes are not analogous. For example, in mice, IgG1 is the most potent agonist antibody, which binds strongly to FcγRIIb. However, no human equivalent has comparable binding capacity to FcγRIIb. Interestingly, human IgG2 has agonist activity, and Glennie and colleagues determined that this was due to an unusual hinge ring in the disulfide bonds that prevents movement and allows for the self-association of the antibodies on the cell surface that is perhaps better for downstream signaling.

Rafi Ahmed from Emory University (Atlanta, GA) also described studies examining the role of isotype in the function of PD-1 antibodies. Using the chronic infection LCMV model, Ahmed and colleagues screened mouse PD-1 antibodies and identified several that appeared to have efficacy. They picked one of these (clone 8H3) to perform a detailed analysis utilizing repeated administration of the antibody. After repeated intraperitoneal injections, the investigators analyzed tetramer$^+$ CD8$^+$ T cells in the blood, spleen, and liver by flow cytometry and found a surprising result: Many of the antigen-specific CD8$^+$ T cells in some of the tissues (particularly liver) had disappeared 24 hours after injection. This antibody is made on a mouse IgG1 backbone, and unlike human IgG1, mouse IgG1 is historically considered a nondepleting antibody, and thus should only block the interactions of PD-1 with PD-L1. However, Ahmed's results suggest this is not always the case, as there was a 97% reduction of antigen-specific T cells in the liver, a 38% reduction in the lung, and a 75% reduction in the bone marrow. This depletion is entirely Fc dependent, since mutation of the Fcγ receptor eliminates this effect.

We are just beginning to understand the precise mechanisms by which checkpoint blockade antibodies function both in mouse models and in the clinic. The above results indicate that the antibody backbone on which these drugs are built is likely to affect whether the antibody is blocking or depleting, and that we may not always be able to predict these responses in advance.

Designing Adaptive Trials

The speed with which cancer immunotherapy drugs are moving through the pipeline and obtaining FDA approval is truly remarkable. One panel discussion during the conference pertained to how to develop immune-oncology clinical trials that allow such expedited approval. Roy Baynes from Merck Research Laboratories (Rahway, NJ) discussed the pembrolizumab phase I melanoma trial as an example of an adaptive phase I design that expedited its development. Their adaptive phase I study supported accelerated approval of pembrolizumab in the United States for patients with ipilimumab-refractory melanoma and took just 3 years from initiation to approval. Their first-in-human study began in 2011, and had a 3+3 dose-escalation design with an expansion cohort in melanoma (initial sample size of 32 patients). They used 97% power to exclude null hypotheses (10% objective response rate and 30% disease-control rate, with a one-sided *P* test done each time a cohort expanded) as well as including an interim futility analysis that was conducted after 11 patients. A striking response led them to increase the expansion cohort to 50 patients. A similar design was used for the non–small cell lung cancer cohort, in which an additional cohort was added to evaluate efficacy once activity was observed. Their example of how a small (132 patients) phase I study grew into a large (1,235 patients) phase I study demonstrates how an adaptive trial design that establishes the appropriate dose, schedule, and development of a biomarker can lead to drug approval within 3 years. The adaptive study must be designed with sufficient rigor to support regulatory filings and is an excellent way to seek accelerated approval, while avoiding multiple trials replicating earlier findings.

Dan Chen from Genentech discussed how the next wave of immunotherapies, which will most likely include combinations, will be much more difficult to move through the developmental pipeline than what we have seen recently and provided recommendations for surmounting likely hurdles. Using waterfall plots and spider plots, he pointed out that distinguishing differences in efficacy between combination immunotherapies and monotherapies could be quite challenging. He offered three ways to address this problem: (i) understanding the human biology; (ii) incorporating appropriate and meaningful endpoints; and (iii) choosing the right combination for the right patient. With the myriad possible combinations, it will be both economically and practically impossible to test every single combination. Thus, an appropriate biologic rationale is needed for combining immunotherapies. If we truly understand the biology of each individual monotherapy and have appropriate early biomarkers to assess them, then it should be possible to determine in a small early-phase trial through the use of pre- and post-biopsies and immune monitoring if there is, indeed, synergy. And meaningful endpoints, such as complete response rate, will enable identification of efficacy of the combination. Finally, given that each patient is different both phenotypically and genotypically, it is important to choose the right combination for the right patient.

Tai-Tsang Chen from Bristol-Myers Squibb discussed the mechanics of using the appropriate statistics to gauge efficacy in immunotherapy. Overall survival (the time between the start of therapy and death) remains the gold standard in oncology clinical trails to measure clinical efficacy. The most common analyses are the log-rank test and Cox regression analysis, which have maximal statistical power under the proportion hazards assumption. However, patient responses to immunotherapy may differ from responses to other chemotherapy and targeted drugs used in oncology. This is because immunotherapies target the immune system rather than the tumor and clinical effects can be delayed. Measures of efficacy, therefore, need to take into account this difference in mechanism of action and kinetics of response. Interim analysis is a strategy that necessitates careful consideration, including the timing of the interim analysis (late vs. early), the population (all of the patients or a subset of patients), and the type of analysis (superiority vs. futility analysis). It is critical in immunotherapy trials to ensure that the follow-up duration is performed and that the long-term survival effect is captured. Finally, Chen discussed how to use milestone survival, or survival probability, at one time point, as an intermediate endpoint, with overall survival used as the primary endpoint. Milestone survival analysis uses Kaplan-Meier survival probabilities at a pre-agreed time point on a first cohort of randomized patients. In this way it serves as a cross-sectional assessment of the overall survival data but at a given time point.

This analysis enables potential earlier assessment of benefit and risk and gives a greater statistical power when delayed treatment effect is present. However, some inherent challenges need to be considered, including determining the best timing for milestone survival and maintaining study integrity after milestone analysis, when milestone survival is an intermediate endpoint. The integrity of the study could diminish if milestone assessment is not performed by a third party. Novel endpoints and statistical analyses need to be employed when assessing the efficacy of cancer immunotherapy, as our traditional methods may not capture the biology of the response in patients.

An Ounce of Prevention

Much of the conference focused on using immunotherapy to treat cancer. But several investigators presented work they are doing on the other side of the equation: developing ways to prevent cancer.

First to explore the topic of prevention was Jolanda deVries, of the Centre for Molecular Life Sciences (Nijmegen, the Netherlands), who has been exploring a cancer vaccine for people with Lynch syndrome, who are at great risk of developing colorectal cancer. Lynch syndrome is an autosomal dominant inherited cancer predisposition caused by germline mutations in DNA mismatch repair genes. These mutations eventually cause colorectal cancer in approximately 80% of patients, and about 5% of patients with colorectal cancer have Lynch syndrome.

The particular vaccine approach that deVries is using involves dendritic cells. Dendritic cell vaccines have been used to treat several different types of cancer, including melanoma and prostate cancer (31). DeVries observed that melanoma patients who had T-cell infiltrates within their tumor prior to treatment with the dendritic cell vaccine had better survival outcomes. This finding suggested that other cancer types with a strong T-cell presence in tumors would be good candidates for vaccination. Like melanoma, Lynch syndrome–associated colorectal cancer is an immunologically "hot" tumor, in the sense that it contains many genetic mutations, increasing the likelihood that the immune system will recognize it.

DeVries demonstrated that a dendritic cell vaccine made from commonly mutated proteins in colorectal cancer tumors was safe, and also led to a documented rise in antigen-specific T cells in the blood of these patients. She is now conducting a clinical trial of the vaccine in patients with Lynch syndrome who do not yet have cancer (but who are very likely to develop it).

Robert Vonderheide, of the University of Pennsylvania, set out to develop what he calls a "polio vaccine" for cancer—a widely effective means to prevent cancer in at-risk populations. In order to develop such a vaccine, Vonderheide stated that he would need a "universal tumor antigen," an immunogenic protein that could be found in many, if not all, cancers. He found his putative universal tumor antigen in a molecule called telomerase reverse transcriptase (TERT), which is produced by the vast majority of human cancers, but which is not typically found in normal cells. Indeed, this enzyme is believed to be one of the molecules that can allow cancer cells to become immortal.

Vonderheide has started a clinical trial of a TERT-based preventive cancer vaccine for patients with *BRCA1/2* gene mutations. Those with *BRCA1/2* mutations have higher rates of several types of cancer, including breast, ovarian, and pancreatic cancer, and often develop these cancers at a young age. His vaccine is a DNA-based vaccine that includes a nonfunctional version of TERT as well as the gene for IL12, and he will be conducting a first-in-human trial of the vaccine in the near future. As part of the study, patients will be enrolled after surgery and any adjuvant therapy. If the trial is successful, the next goal would be to evaluate the vaccine in patients prior to surgery. Having an effective vaccine for this high-risk population would be a major therapeutic advance; for many of these individuals, the question is not will they develop cancer, but when.

Your Inner Microbes

One panel at the conference focused on the microbiome and its interactions with the immune system. As Laurence Zitvogel, of Gustave Roussy Cancer Center (Villejuif, France), pointed out, humans contain within them more bacterial cells than human cells—by an order of about 10:1. Immunologists have known for a long time that the microbes living on and in us shape the development of our immune system. What has become clear in recent years is that these microbial hitchhikers also influence the way we respond to cancer treatments. Many cancer treatments, such as chemotherapy and immunotherapy, can disrupt the delicate balance of microbes in our gut, and even the integrity of our intestinal lining. Zitvogel wanted to know if the bacterial composition of our guts can be altered in such a way that the efficacy of checkpoint blockade immunotherapy is maintained but the toxicity is limited. In her talk, she presented tantalizing findings that suggest that, indeed, bacterial species can affect both aspects of checkpoint blockade therapy. A paper addressing this issue was recently published in *Science* (32).

Jason Hudak of Harvard Medical School (Boston, MA) presented work on a clever technique he has developed for labeling different components of bacteria with different colored fluorescence. Called "click chemistry," the technique involves feeding the bacteria a synthetic compound that mimics a natural nutrient to which fluorescent chromophores can be attached. The multicolored bacteria can then be followed as they interact with immune cells, and their distinctly labeled chemicals allow for easy tracking under the microscope. The technique promises to greatly enhance our understanding of how bacteria—and the chemicals they make—can lead to inflammation and colorectal cancer.

Conclusions

The inaugural International Cancer Immunotherapy Conference, entitled "Translating Science into Survival," was a fruitful meeting of minds. Many immunologic approaches were covered, progress was noted, and holes in our current knowledge and applications thereof were exposed. The second International Cancer Immunotherapy Conference, cohosted by the same four nonprofits, will be held September 25–28, 2016, in New York City.

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