Cancer Immunotherapy Out of the Gate: The 22nd Annual Cancer Research Institute International Immunotherapy Symposium

Abstract

The 22nd annual Cancer Research Institute (CRI) International Immunotherapy Symposium was held from October 5–8, 2014, in New York City. Titled "Cancer Immunotherapy: Out of the Gate," the symposium began with a Cancer Immunotherapy Consortium satellite meeting focused on issues in immunotherapy drug development, followed by five speaker sessions and a poster session devoted to basic and clinical cancer immunology research. The second annual William B. Coley lecture was delivered by Lieping Chen, one of the four recipients of the 2014 William B. Coley Award for Distinguished Research in Tumor Immunology; the other three recipients were Gordon Freeman, Tasuku Honjo, and Arlene Sharpe. Prominent themes of the conference were the use of genomic technologies to identify neoantigens and the emergence of new immune modulatory molecules, beyond CTLA-4 and PD-1/PD-L1, as new therapeutic targets for immunotherapy. Cancer Immunol Res; 3(5); 444–8. ©2015 AACR.

Introduction

Immunotherapy is increasingly recognized as a promising approach to treat many cancers, including those that are not generally responsive to conventional therapies like chemotherapy. In 2013, *Science* magazine designated cancer immunotherapy the "Breakthrough of the Year," in recognition of the important progress in cancer treatment being made with this approach. The use of checkpoint blockade inhibitors and chimeric antigen receptor (CAR)—modified T cells, in particular, has sparked widespread interest in the field, owing to the impressive results emerging from clinical trials using these agents (1, 2). Based on these results, in the past year, the FDA granted breakthrough status to several of these agents, and in late 2014 approved two PD-1–blocking antibodies, pembrolizumab and nivolumab, for the treatment of advanced melanoma that has failed prior treatment; in March 2015, the FDA approved nivolumab for the treatment of advanced squamous non–small cell lung cancer (NSCLC).

Reflecting the optimism surrounding this rapid progress, the Cancer Research Institute (CRI) titled its 22nd annual symposium "Cancer Immunotherapy: Out of the Gate." The meeting took place from October 5–8, 2014, in New York City and was attended by over 600 scientists, including basic, translational, and clinical researchers from academia and industry. The symposium began with a Cancer Immunotherapy Consortium (CIC) satellite meeting devoted to issues in immunotherapy drug development. Following this satellite meeting were five speaker sessions and a poster session devoted to basic and clinical research in cancer immunology. The keynote address—the second annual William B. Coley lecture—was delivered by Lieping Chen, one of the four recipients of the 2014 William B. Coley Award for Distinguished Research in Tumor Immunology; the other three recipients were Gordon Freeman, Tasuku Honjo, and Arlene Sharpe. Dr. Chen discussed the discovery of PD-1 as an immune checkpoint that could be targeted with immunotherapy.

In this meeting report, we review several prominent themes that arose from the 4-day-long symposium, with a focus on emerging areas of inquiry.

Accelerating Approvals

The CRI Cancer Immunotherapy Consortium (CIC) is a collaborative think tank comprising stakeholders from academia, industry, regulatory agencies, and patient interest groups. Each year, the CIC meets to discuss ways to overcome hurdles affecting immunotherapy drug development. Part I of the CIC satellite meeting was devoted to the topic of "de-risking immunotherapy." De-risking in this context means reducing the likelihood of late-stage clinical trial failures, which add to the cost of research and development and delay new drug approvals. Presentations focused on the role of preclinical mouse models in immunotherapy development, looking at both their powers and limitations. While mouse models are clearly useful for dissecting basic aspects of immune biology, they are not always good predictors of clinical responses in humans, in part because mice and humans have very different pharmacodynamics. For that reason, the typical research sequence of beginning with mouse models and moving through to clinical trials in humans may not always be the best approach. It was suggested that more back and forth between the preclinical and clinical testing—including using mouse models to test hypotheses generated in the clinic—would improve the overall drug development process.

There were also lively discussions of how to get the most out of phase I studies. Potential strategies discussed included: the incorporation of meaningful study endpoints appropriate for small nonrandomized trials; the use of relevant biomarkers to help determine whether a particular combination is more effective than monotherapy; more systematically collecting bioinformatics data; and testing the right combination in the appropriate cohorts. With respect to this last point, Robert Vonderheide, of the...
University of Pennsylvania, proposed that it is "okay to say the 'V' word" (meaning vaccine) and called attention to the importance of considering the need for T-cell priming in certain indications, such as pancreatic cancer (3).

Part II of the satellite meeting was focused on evaluating new surrogate endpoints for cancer immunotherapy trials. Because the mechanism of action of immunotherapies is different from that of conventional chemotherapy, the time course and patterns of response may also differ. Therefore, the commonly used surrogate endpoint criteria currently in use (i.e., RECIST and WHO), which were developed during the chemotherapy era, may not adequately capture benefits from immunotherapies. This realization provided the justification for the development of immune-related response criteria (4), which were used to evaluate the effectiveness of the first checkpoint inhibitor, ipilimumab (anti-CTLA-4).

With data now available on several additional checkpoint inhibitors, including nivolumab, tremelimunab, pembrolizumab, MEDI4736, and MPDL3280A, it is possible to further evaluate immunotherapy-specific surrogate endpoints. Several such endpoints were discussed, including milestone survival, stable disease, progression-free survival, immunotherapy patterns of response, and objective response rate. White papers discussing both parts of the CIC satellite meeting are currently in preparation.

Identifying Neoantigens

In order for the immune system to kill cancer cells, it must be able to recognize and target cancer-associated or cancer-specific antigens. What these antigens are in any particular patient, however, has remained largely a mystery. Though several shared cancer-associated antigens have been identified, including MAGE and NY-ESO-1, studies have failed to show conclusively that these particular antigens are the ones responsible for a therapeutic response to immunotherapy in a given patient (5).

In addition to shared tumor antigens, human tumors also contain many hundreds of mutational "neoantigens." This repertoire of neoantigens has been called the "antigenome" (6). Because these mutated proteins are foreign to the immune system, they are likely to be an important source of immunogenicity underlying therapeutic response to checkpoint blockade therapy (7). However, the vast majority of these neoantigens are specific to each patient. Until recently, such neoantigens were seen as an unlikely basis from which to construct cancer immunotherapies—specifically, vaccines—because each immunotherapy would in essence need to be tailored to each specific patient.

Current research is devoted to using genomic methods to identify tumor-specific mutant antigens (TSSMA) that may be used in personalized vaccines to augment a patient's immunity (8). Robert Schreiber, of Washington University in St. Louis, and Matthew Gubin, a postdoctoral fellow in the Schreiber laboratory, shared results obtained using exome sequencing and Matthew Gubin, a postdoctoral fellow in the Schreiber laboratory, shared results obtained using exome sequencing and Matthew Gubin, a postdoctoral fellow in the Schreiber laboratory, shared results obtained using exome sequencing and epitope prediction technologies, such as second/next-generation sequencing and So-called peptide-exchange technology, in which large quantities of MHC complexes are generated in the presence of a peptide ligand that cleaves upon UV light exposure. This technology allows the production of collections of hundreds or thousands of different peptide–MHC complexes to evaluate T-cell reactivity against potential neoantigens (6). Because the number of biologic samples is generally insufficient to screen all potential cancer-antigen epitopes in separate assays, Schumacher and colleagues developed a multiplexed flow cytometry method, which allows the analysis of 28 different T-cell populations in a single sample. In this strategy, termed "combinatorial coding," each peptide–MHC complex is conjugated to a set of fluorochromes, each individual fluorochrome can be used in many color combinations, and antigen-specific T cells can be identified by the color code that they carry. Using this technology, Schumacher and colleagues analyzed tumor-infiltrating lymphocytes (TIL) from melanoma patients treated with checkpoint blockade immunotherapy and showed that cytotoxic T-cell reactivity against neoantigens was found in 6 of 8 melanoma patients. Furthermore, using a different method, the group also showed helper T-cell reactivity against neoantigens in 4 of 5 melanoma patients (10). These
results demonstrate that the DNA damage that is present in human melanoma frequently leads to a T-cell response against mutant antigens. These investigators also showed that neoantigen-enriched TILs from a human melanoma mediated superior tumor control against melanoma outgrowth in immune-deficient NSG mice bearing the autologous tumor. More recently, using the above genomic sequencing methods, they showed that neoantigen-specific T-cell responses can be induced by PD-1 blockade in NSCLC (11).

Responders and Nonresponders

An urgent question facing investigators is why some patients respond well to immunotherapies while others do not. For example, only 20% of melanoma patients respond to ipilimumab (anti–CTLA-4) as monotherapy (12). Exome sequencing is being used to unravel this mystery as well. Alexandra Snyder, of Memorial Sloan Kettering Cancer Center (MSKCC), working collaboratively with colleagues from the laboratories of Timothy Chan and Jedd Wolchok, used this method to study the genetic landscape of anti–CTLA-4 responders versus nonresponders. It has been speculated for some time that one of the reasons that melanoma is often responsive to immunotherapy is because these cancers contain many genetic mutations (caused by UV radiation from sunlight; ref. 13). Snyder and colleagues wanted to know if it was the total number of mutations that made the difference, or whether a small number of particularly powerful mutations were most important to success of therapy. Using exome sequencing on matched tumor and blood samples from melanoma patients who were treated with ipilimumab or tremelimumab, they found that mutational load was correlated with clinical benefit, but that this alone was not sufficient to predict being a “responder” to CTLA-4 blockade. Snyder and colleagues were able to identify a common “molecular signature” of neoantigen substrings that were found in anti–CTLA-4 responders versus nonresponders. Intriguingly, the neoantigens they identified created peptide sequences that were very similar to sequences found in common infectious diseases, such as tuberculosis, Streptococcus pyogenes infection, and yellow fever virus. Their results raise the tantalizing possibility that melanoma can elicit an immune response by activating the immune memory that we have generated to infectious diseases. These results were published in the New England Journal of Medicine (14).

New Immune Targets

Several talks at the meeting were devoted to exploring recent progress using PD-1/PD-L1 checkpoint inhibitors to treat a variety of cancers, including lung, colorectal, stomach, and liver cancer. Roy Herbst of Yale School of Medicine presented results from clinical trials of anti–PD-1/PD-L1 antibodies in patients with NSCLC. Although 20% to 25% of patients receiving these treatments have objective responses—some of which are quite durable—most patients do not respond, indicating a great need to identify biomarkers of successful response. Although not a perfect correlation, results indicate that level of PD-L1 expression (both before and during treatment) correlates with response to anti–PD-1/PD-L1 therapy. Interestingly, in a large trial of MPDL3280A (anti–PD-L1; Genentech), PD-L1 expression on tumor-infiltrating immune cells was even more predictive of response than PD-L1 expression on tumor cells (15). Herbst and colleagues argued that more detailed studies of the tumor microenvironment (TME) are necessary to understand why PD-1/PD-L1 inhibitors work in some cases but not others.

Neil Segal, of MSKCC, discussed early results of checkpoint inhibitor immunotherapy for gastrointestinal cancers, including colorectal, stomach, liver, and pancreatic cancer. Available evidence suggests that these cancers may be appropriate targets of immunotherapy.

On the basic research front, Kristen Pauken, a postdoctoral fellow in the laboratory of E. John Wherry, at the University of Pennsylvania, presented data on PD-1 blockade and T-cell exhaustion. She showed that antiviral CD8+ T cells reinvigorated by PD-1 blockade regained the ability to persist in the complete absence of antigen, suggesting that PD-1 blockade is capable of restoring properties of antigen-independent renewal. Pauken and colleagues have identified a potential predictive biomarker for effective response to PD-1 that consisted of high coexpression of PD-1 and the transcription factor Eomesoderm.

Several presentations focused on a new crop of targets for immune modulation. Tibor Keler, of Cellnex Therapeutics, presented results of preclinical and clinical studies of a fully human anti-CD27 agonist antibody called varilumab (“varli” for short). CD27, a member of the TNF superfamily, is a costimulatory receptor found on T cells, and its ligand CD70 is transiently expressed on matured dendritic cells (DC) and highly activated lymphocytes. Targeting this receptor with an antibody is an effective way to “step on the gas” of the immune response. Keler and colleagues found in an ongoing phase I clinical study of 80 patients that varilumab treatment was safe, well tolerated, and resulted in clear biologic effects as well as a durable complete and partial response. Varilumab is being investigated for use in combination therapy with various immune modulators and targeted therapies for a number of cancers.

Holbrook Kohrt, of the Stanford Cancer Institute, discussed preclinical results using a CD137/4-1BB–stimulating antibody in combination with tumor-targeting mAbs, such as rituximab (anti–CD20) and trastuzumab (anti–HER2), to enhance antibody-dependent cell-mediated cytoxicity (ADCC). CD137/4-1BB is a costimulatory receptor and a member of the TNF superfamily that is found on a variety of activated immune cells, including natural killer (NK) cells. CD137 is minimally expressed on resting NK cells, but is upregulated when NK cells encounter mAbs bound to tumor cells. Combining CD137 stimulation with tumor-targeting mAbs (e.g., anti–CD20 or anti–HER2) may therefore be an effective way to enhance NK-mediated ADCC. Kohrt and colleagues showed this to be the case in mouse models of lymphoma and breast cancer. Clinical trials are under way to test the combination of rituximab and anti-CD137 in patients with lymphoma.

Another new immune target is the enzyme indoleamine 2,3-dioxygenase (IDO), produced by immune cells in the TME, and also by tumor cells, in response to inflammation. IDO is believed to function as part of a “counter-regulatory” pathway that limits unchecked excessive inflammation and, therefore, serves a protective function under normal circumstances, but which may hinder effective immune responses against tumors. In response to proinflammatory signals, IDO is expressed by host DCs in the TME and the tumor-draining lymph nodes. Its downstream metabolites can activate regulatory T cells (Treg) that dampen incipient immune responses, tolerate DCs, inhibit antigen-presenting cells, and anergize effector cells. As discussed by David Munn, of Georgia Regents University, drugs that inhibit IDO may...
therefore be an effective way to "release the brakes" on the immune response to cancer. Several IDO inhibitors are being tested in clinical trials, in combination with chemotherapy and with other immunotherapies.

Modulation of Treg function was the subject of the presentation by David Schaefer, currently of Lilly Research Laboratories, based on work performed at MSKCC. He and his colleagues are focused on understanding the mechanism whereby Tregs suppress antime-lanoma immunity and how antibody targeting the glucocorti-coid-induced TNF receptor (GITR) found on Tregs can remove the suppression (16). Schaefer and colleagues used ex vivo three-dimen-sional collagen-fibrin gel cultures of B16 mouse melanoma cells to demonstrate that Tregs utilize membrane-bound TGFβ to inhibit antitumor response. Treg lineage instability, induced by in vivo treatment with anti-GITR antibody, was shown by this method to hobble the ability of Tregs to suppress effector T cells in the TME. A clinical trial of anti-GITR is currently under way.

Another novel immune target is VISTA (V-domain immuno-globulin-containing suppressor of T-cell activation), discussed by Li Wang of the Medical College of Wisconsin (17). Genetically engineered mice that lack VISTA exhibit severe inflammatory disease, indicating that VISTA is a negative immune regulator similar to CTLA-4 or PD-1. VISTA-blocking antibodies synergize with both tumor vaccines and checkpoint blockade antibodies in murine tumor models, and thus may provide a way to increase the effectiveness of vaccines and other immunotherapies.

John Stagg, of the University of Montreal Hospital Research Center, presented research on the targeting of CD73, one of the two enzymes crucial for the extracellular adenosine pathway (18). CD73 is an ecto-5'-nucleotidase expressed on various cell types, including tumor cells and immune cells, that catalyzes the dephosphorylation of AMP to adenosine. High extracellu-lar concentration of adenosine is broadly immunosuppressive, inhibiting antigen presentation and effector T-cell responses. Dr. Stagg has found that patients with triple-negative breast cancer who have high CD73 expression have a worse prognosis, providing a rationale for blocking the activity of this receptor in patients. He also demonstrated that targeting CD73 or down-stream A2A adenosine receptor synergizes with immune check-point inhibitors (19).

Increasing Immune Traffic into Tumors
Successful immunotherapy responses depend on T cells being able to infiltrate a tumor, yet many tumors are poorly infiltrated by T cells (20). Pancreatic cancer is a classic example (21). Several researchers presented work bearing on the question of how to get T cells into sites of tumor in order to stimulate an immune response. Gregory Beatty, of the University of Pennsylvania, presented work from his laboratory on the use of CAR T cells to target mesothelin, a commonly expressed protein in pancreatic ductal adenocarcinoma (PDA). One rea-son why T cells may fail to attack PDA is that these tumors may express low levels of MHC or lack sufficiently immunogenic antigens for recognition by T cells. CAR T cells do not require MHC to bind their targets and thus might be more effective in this context. Beatty showed preliminary evidence that mesothe-lin-targeting CAR T cells may be able to traffic to pancreatic tumors and elicit antitumor immune responses. However, in preclinical mouse models the efficacy of CAR T cells has been found to be influenced by the tumor microenvironment, which may explain why CAR T cells have yet to produce long-term clinical responses in solid malignancies (22). Understanding the basis for this loss of function is the focus of many laboratories.

Another way that researchers have tried to coax T cells into tumor sites is through the use of oncolytic viruses (23). Dmitriy Zamarin, of MSKCC, presented research on how such viruses can be employed to enhance the effectiveness of immunotherapies such as anti–PD-1. The Newcastle Disease Virus (NDV) is an avian virus that infects birds but causes no clinically apparent disease in humans. Zamarin and colleagues found that injecting NDVs into tumors in mice bearing B16 melanomas promoted inflammation and recruited immune cells to the site of injection. The immune cells then engulfed the tumor cells and the tumor shrank. Most interestingly, they found that NDV treatment not only had a local effect on the tumor to which it was injected, but also stimulated a systemic abscopal response; tumors at distant sites also shrunk. Specifically, the oncolytic NDVs induced immunogenic cell death of the injected tumor, eliciting tumor-specific effector T cells that could then seek out and destroy other tumors bearing those same antigens (24). Zamarin and colleagues found that distant tumors are efficiently infiltrated with activated effector T cells but not with Tregs. They have engineered a panel of recombinant NDVs expressing various costimulatory ligands and cytokines to assess their efficacy within the TME. They found that NDVs expressing immunostimulatory ligands such as ICOSL, given in combination with CTLA-4 blockade, improved survival and protected against tumor rechallenge. However, not all costimulatory ligands work in NDV.

Summary
The 22nd annual CRI International Cancer Immunotherapy Symposium was held in New York City in October 2014, and brought together 600 researchers to discuss the latest advances in cancer immunology and immunotherapy research. Immunotherapies are now recognized as a powerful way to treat several different types of cancer, and researchers both within and outside the field are optimistic about immunomodulating treatments becoming increasingly effective for a wider range of cancers. Clinical successes achieved with CTLA-4- and PD-1-blocking antibodies, several of which are now approved by the FDA, as well as with adoptive cell therapy using CAR T technology, have established immunotherapy as a "breakthrough" approach to cancer treatment, and the race is on to find additional effective approaches and targets for immunotherapy. Several new approaches and targets were a prime focus of the CRI symposium. These included developing genomic methods to identify neoantigens for use in personalized cancer vaccines, targeting additional regulatory molecules, such as CD27, IDO, GITR, VISTA, and CD73, and using oncolytic viruses to increase T-cell homing to tumors.

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