

Special Conference on Tumor Immunology and Immunotherapy: A New Chapter

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Abstract

The overall objective of the fifth American Association for Cancer Research Special Conference, "Tumor Immunology and Immunotherapy: A New Chapter," organized by the Cancer Immunology Working Group, was to highlight multidisciplinary approaches of immunotherapy and mechanisms related to the ability of immunotherapy to fight established tumors. With the FDA approval of sipuleucel-T, ipilimumab (anti-CTLA-4; Bristol-Myers Squibb), and the two anti-PD-1 antibodies, pembrolizumab (formerly MK-3475 or lambrolizumab; Merck) and nivolumab (Bristol-Myers Squibb), immunotherapy has become a mainstream treatment option for some cancers. Many of the data presented at the conference and reviewed in this article showcase the progress made in determining the mechanistic reasons for the success of some treat-

ments and the mechanisms associated with tolerance within the tumor microenvironment, both of which are potential targets for immunotherapy. In addition to combination and multimodal therapies, improvements in existing therapies will be needed to overcome the numerous ways that tumor-specific tolerance thwarts the immune system. This conference built upon the success of the 2012 conference and focused on seven progressing and/or emerging areas—new combination therapies, combination therapies and vaccine improvement, mechanisms of antibody therapy, factors in the tumor microenvironment affecting the immune response, the microbiomes effect on cancer and immunotherapy, metabolism in immunotherapy, and adoptive T-cell therapy. *Cancer Immunol Res*; 3(6):590–7. ©2015 AACR.

Introduction

The American Association for Cancer Research (AACR) Special Conference on "Tumor Immunology and Immunotherapy: A New Chapter" was held from December 1–4, 2014, in Disney's Contemporary Resort, Orlando, Florida. The 4-day conference was attended by over 500 participants from around the world. The conference was presented in conjunction with the AACR Cancer Immunology Working Group, and the four co-chairpersons were Nina Bhardwaj (Mount Sinai Medical Center, New York, NY), Robert H. Vonderheide (University of Pennsylvania Abramson Cancer Center, Philadelphia, PA), Stanley R. Riddell (University of Washington Fred Hutchinson Cancer Research Center, Seattle, WA), and Cynthia L. Sears (Johns Hopkins University, Baltimore, MD). The conference was organized to showcase the progress made in determining the mech-

anistic reasons for the success of some treatments and the mechanisms associated with tolerance within the tumor microenvironment (TME), both of which are potential targets for immunotherapy. In addition to combination and multimodal therapies, improvements in existing therapies are needed to overcome the numerous ways that tumor-specific tolerance thwarts the immune system. The meeting focused on progress generated and/or emerging areas since the 2012 AACR Special Conference on Tumor Immunology in Miami, Florida. This meeting summary is presented in seven sections that highlight, respectively, new combination therapies, combination therapies and vaccine improvement, mechanisms of antibody therapy, factors in the TME affecting the immune response, the microbiome's effect on cancer and immunotherapy, metabolism in immunotherapy, and adoptive T-cell therapy.

Combination Therapies: Radiation Biology, Chemotherapy, and Immunotherapy

Radiotherapy has long been used as a single and local therapy. It is most effective when used following surgical resection of primary tumors, but once tumor recurs, the effectiveness of radiotherapy decreases. From an immunologic perspective, radiation causes necrosis, or danger signal-inducing cell death, which can lead to suboptimal activation of the immune system through antigen processing and presentation of the destroyed tumors.

Many current studies are focusing on the combination of radiation and immunotherapeutic approaches to more fully stimulate the immune system. Non-Hodgkin lymphoma (NHL) is readily treatable with existing first-line therapy; however, upon recurrence, NHL is often resistant to treatment. Joshua Brody

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presented results from their phase I/II studies using Toll-like receptor (TLR)-based vaccination to induce regression of B-cell lymphoma (1). NHL tumors express TLR9 and can be activated with intratumoral injection of CpG. Activation of NHL and intratumoral dendritic cells (DC) with CpG led to T-cell activation as evidenced by the upregulation of CD137. When two doses of 2-Gy radiation were added to the CpG treatment, long-lasting remissions were elicited in several patients, with their tumor-reactive T cells expressing CD137 and CD45RO. Booster vaccinations result in faster responses, indicating that the tumor-specific responses are memory responses. Results from this group's concurrent murine studies using intratumoral Flt-3 to activate CD103⁺ DCs have led to clinical studies using Flt-3, poly-IC, and radiation. This treatment regimen specifically activated tumoral DCs (not peripheral DCs or the tumoral myeloid subsets), with increased expression of BDCA-1 and BDCA-2 DC subsets expressing CD1c and CD141. Repeated treatments led to a shift from central to effector memory T cells.

In addition to stimulating immune responses, the combination of radiotherapy and immunotherapy may also serve to reinvigorate preexisting tumor-specific CD8⁺ T-cell populations. In their poster, Andrew Rech and colleagues show that in a murine model of melanoma (B16-F10), the combination of single-dose radiation and CTLA-4 blockade resulted in modest tumor regressions. Further investigation of nonresponding mice revealed a population of functionally exhausted CD8⁺ T cells expressing eomesodermin (Eomes), T-bet, and PD-1, similar to a population of CD8⁺ T cells that they found to infiltrate melanoma metastases in patients (2). Studies of chronic viral infections have shown that Eomes^{hi}PD-1^{hi} cells are highly cytotoxic, but these cells are terminally differentiated and are derived from the T-bet^{hi} cell-progenitor pool (3). T-bet^{hi} cells are PD-1^{int} and as such are restored by PD-1/PD-L1 blockade to fully functional effector CD8⁺ T cells (4). Targeting the T cells susceptible to reinvigoration in melanoma-bearing mice using a triple combination of radiation, anti-CTLA-4, and anti-PD-L1 (Radvax) drove tumor regressions in 80% of mice (2). Strikingly, the proportions of Eomes^{hi}PD-1^{hi} (exhausted) versus Ki67⁺Granzyme B⁺ (reinvigorated) CD8⁺ T cells were predictive of Radvax responses in B16 melanoma and correlated with long-term survival in patients with melanoma treated with the combined regimen of radiation and anti-CTLA-4 (2).

Recent studies in triple-negative breast cancer have demonstrated that radiation increased the effectiveness of anti-CTLA-4 treatment. Sandra Demaria showed in the 4T1 model that anti-CTLA-4 treatment by itself was not effective; however, adding radiotherapy not only led to the destruction of the radiation-treated primary lesion, but also primed a CD8⁺ T-cell response that was able to destroy metastases. The T-cell effect was CXCR6-dependent and abrogated in CXCR6-knockout mice. Given that multiple cancers express the CXCR6 ligand CXCL16, which could be induced by radiotherapy (5), these results may lead to treatments that enhance the effect of radiation and anti-CTLA-4. Furthermore, the combination of radiation and anti-CTLA-4 led to multiple beneficial effects. In one study, the combination led to a broader T-cell repertoire. Anti-CTLA-4 drove the expansion of the most frequent tumor-infiltrating lymphocyte (TIL) clonotypes, while radiation treatment broadened the repertoire. The combination of anti-CTLA-4 and radiation has been shown to lead to newer TIL specificities than radiation treatment alone. Fractionated radiation also led to altered IFN response gene

expression, which could be enhanced by blocking TGF β , as radiation-induced TGF β hinders T-cell activation through reactive oxygen species (ROS; ref. 6). In the clinical setting, five 6-Gy doses of radiotherapy in conjunction with ipilimumab for treatment of non-small cell lung cancer (NSCLC) metastasized to the liver showed an abscopal effect that included increased T cells and decreased tumor load in lymph nodes (7).

In order to maximize the effectiveness of checkpoint blockade inhibition, it will be important to understand where anti-CTLA-4 and anti-PD-1 will be most effective depending on the state of the immune response in relation to the level of immunoeediting, i.e., is the tumor edited or privileged? Robert Vonderheide discussed this concept in the context of clinical data demonstrating that melanoma patients treated with the combination of anti-CD40 and anti-CTLA-4, and then given palliative radiation at one site, showed an abscopal effect and elimination of the previously expanding tumors. A phase I clinical trial in multiple tumor types using fractionated radiation at one site and systemic ipilimumab showed abscopal effects in stage IV melanoma and an objective response rate of 21%. Follow-up studies using B16 melanoma showed similar results with abscopal effects seen 17% of the time when radiation of one tumor site was combined with systemic anti-CTLA-4 treatment. Resistance in distant tumors was linked to PD-L1 expression in the TME. Combining radiotherapy, anti-CTLA-4, and anti-PD-1 or anti-PD-L1 led to a control rate of 80% of distant tumors. Increased percentages of CD8⁺ and decreased FoxP3⁺ regulatory T cells (Treg) were seen with radiotherapy increasing the diversity of T-cell receptors (TCR) in the abscopal tumor (2).

Gemcitabine and nanoparticle-bound Taxol (nab-paclitaxel) are chemotherapeutic standards of care in the clinic, but they do not lead to long-term cures for pancreas cancer. Katelyn T. Byrne showed that, in a mouse model of pancreas cancer, agonistic anti-CD40 treatment leads to increased survival, in part, by activating monocytes within the TME. When used to treat KPC-derived tumors, the combination treatment of gemcitabine/nab-paclitaxel/anti-CD40 led to a significant survival advantage and some long-term cures compared with that of individual treatments, with 50% of mice showing tumor regression. This combination therapy activated both CD4⁺ and CD8⁺ T cells, significantly reduced the number of CD4⁺ Tregs in the TME, and activated the antigen-presenting cells (APC) in both the tumor and draining lymph nodes. This treatment not only highlights the benefit of combined therapy but also shows that T cells can be activated against even the most immunosuppressive tumors.

Histone deacetylases (HDAC) have recently shown promise as targets for epigenetically reprogramming tumors and the immune system. Andressa Laino described the effects of inhibiting HDAC6 as a potential tumor therapy. Inhibiting HDAC6 led to increased MHC expression in melanoma cell lines and effects on cells of the immune system. In HDAC6-knockout mice, B16 melanoma grows more slowly, and when coupled with TriVax vaccination (peptide, anti-CD40, and poly-IC), the T-cell contraction phase is reduced, leading to greater T-cell persistence. Chemical inhibition of HDAC6 reduced the production of IL10 by DCs in response to lipopolysaccharide stimulation and increased the proportion of central memory T cells that were generated. *In vitro* studies showed that human T cells increased their IFN γ and CD107a production in response to HDAC6 inhibition.

Combination Therapy and Vaccine Improvement

Even though the progress in increasing T-cell responses by increasing costimulation has been substantial, it is also important to increase the efficacy and understand the mechanism of immunotherapeutic vaccines. Familial and genetic predispositions to cancer have been well characterized. Driver mutations have been targeted prophylactically with success in murine models of cancer, yet trials of this kind in humans need to be done (8). Olivera Finn described a clinical trial targeting the MUC1 antigen prophylactically in early-stage adenoma in colon cancer. Patients at high risk of developing adenoma or having an increased risk of adenoma recurrence were vaccinated with a 100-mer MUC1 peptide in an adjuvant and tested for MUC1 antibody responses. Approximately 50% of vaccinated patients showed a long-term anti-MUC1 response, with 23% of patients showing a reduction in adenomas over 3 years. Patients who did not respond to the vaccine showed an increased number of circulating myeloid-derived suppressor cells (MDSC) prior to vaccination, suggesting that it might be helpful to prescreen patients who could benefit from this or other therapies (9).

Vaccines are most often used to treat established tumors. Whole-cell vaccines using GVAX (GM-CSF-secreting cell-based vaccine) and cyclophosphamide (Cy) to deplete Tregs to treat patients with pancreatic adenocarcinoma (PDA) showed promise in phase I trials. Analysis of PDA tissues surgically resected 2 weeks after Cy/GVAX treatment shows the development of tertiary lymphoid structures within the tumors (10). Microdissection of these aggregates showed that patients with an increase in markers of Th17 cells and decreased Treg and PD-L1 signatures showed improved survival. Also interesting was the fact that GVAX increased the level of membranous PD-L1 in PDA, leading to work in preclinical models showing that anti-PD-1, anti-PD-L1, and TGF β inhibitor therapy can enhance the efficacy of GVAX in the Panc02 tumor system (11). Clinical trials using GVAX and checkpoint inhibitors have also shown success. A phase I trial combining GVAX and ipilimumab in previously treated pancreatic cancer patients showed increased survival and anti-mesothelin CD8⁺ T-cell responses in patients receiving the combination compared with that in controls (12); these results have led to an ongoing phase II trial. Combining vaccines in a prime-boost regimen has also shown promise. Phase I trial data combining primary Cy/GVAX vaccination followed by boosting with human mesothelin containing *Listeria*-based vaccine also showed a dramatic increase in survival compared with patients who received Cy/GVAX treatments alone (13). Together, these studies demonstrated the potential of combining checkpoint blockade inhibition with vaccine regimens to improve vaccine efficacy.

GVAX targeting of melanoma has also shown success. Clinical trials using autologous GVAX combined with ipilimumab demonstrated the utility of these vaccines. GVAX-based melanoma vaccines increased the level of T-cell infiltrate in melanoma from 3% to approximately 60%. Importantly, in patients who had prior treatment with GVAX, ipilimumab elicited immune responses that correlated with an increased ratio of CD8⁺:Treg (14). However, GVAX has the drawback that it could induce myeloid cells, which are beneficial because they remove apoptotic cells, but myeloid cells produce TGF β and induce Tregs, both of which can promote tumor growth. Data from preclinical studies using a dominant-negative mutant of the MFG-E8 protein demonstrated

that inhibiting this protein can reduce the induction of suppression (15). Studies to block MFG-E8 are now in the clinical-planning stages. The combined regimen of anti-CTLA-4 and melanoma GVAX showed a second beneficial effect in that it elicits tumor vasculopathy in long-term survivors. This vasculopathy is the result of antibody responses against angiogenic proteins, including VEGF and angiopoietins 1 and 2, leading to reduced macrophage infiltration. Further targeting of angiogenesis using ipilimumab and bevacizumab (anti-VEGF) led to the development of blood vessels within the tumor that resemble high endothelial venules (HEV) and increased T-cell infiltration (16, 17). Monoclonal antibodies developed from the sera of long-term survivors are now being produced for clinical trials (18).

Overcoming suppressive factors in the TME is also an activity desired for tumor vaccines. Matrix metalloproteinase 2 (MMP2) is secreted by tumor cells and is important for metastasis formation; however, it also affects the immune system by changing DC phenotypes. MMP2 can bind to TLR2 on DCs, leading to reduced IL12 secretion and upregulation of OX-40L, resulting in Th2 priming (19). Injecting poly-IC intratumorally overcame the MMP2 effect by leading to IFN γ production. *Listeria* vaccine also reversed the effects of MMP2 by restoring IL12 production in human DCs *in vitro*. Natural killer (NK) cells also show signs of exhaustion in patients with advanced-stage melanoma. Exhausted NK cells have reduced killing activity, reduced IFN γ and IL2 production, increased KIR 3DL1 expression, and increased proliferation. TIM-3 is upregulated on exhausted NK cells from melanoma patients compared with those from healthy donors. TIM-3 blockade reversed NK-exhaustion (20).

Peptide vaccines are potentially easier to produce and administer than other types of vaccines; however, their success depends on the adjuvants and costimulation that can be made available. Esteban Celis described TriVax, the combination of anti-CD40, poly-IC or CpG, and peptide, given intravenously as a potential therapeutic approach. Preclinical studies using TRP-2 peptide showed efficacy in the B16 model of melanoma and in a lung cancer model. The effectiveness of this regimen depended on perforin and type I IFN expression; however, the combination showed some toxicities. When BiVax (poly-IC and peptide) was tested, success was seen using human papillomavirus (HPV) peptides against the TC1 tumor, but not using TRP-2 peptides to vaccinate against melanoma. Peptide structure was found to be a contributing factor, as HPV peptide is hydrophobic at the 3' end, whereas TRP-2 is not. Palmitolating the 3' end of the TRP-2 peptides led to increased efficacy when used with BiVax, confirming that the peptide structure is a factor in its immunogenicity (21).

Mechanisms of Antibody Therapy

With the success of antibody therapy specific to coinhibitory molecules (checkpoint blockade of CTLA-4, PD-1, and PD-L1), a recent focus has been the use and improvement of immunostimulatory antibodies such as anti-CD40 and anti-4-1BB (22–24). In mouse models, checkpoint blockade and immunostimulatory antibodies can have similar positive outcomes, yet when brought to the clinic, checkpoint blockade antibodies perform better. Recent studies have tested whether the antibody structure may explain the functional differences between the two types of antibodies. *In vitro* studies demonstrated that anti-CD20 worked

better as an IgG2a isotype compared with anti-CD40 that worked better as an IgG1. The difference was that anti-CD20 works through an antibody-dependent cellular cytotoxicity (ADCC)-dependent mechanism and requires FcR activation, whereas anti-CD40, as an agonistic antibody, requires cross-linking through the nonactivating FcRIIb receptor. One possible explanation for the lower efficacy of agonistic antibodies is that there are few antibodies in the clinic that preferentially bind to the nonactivating FcRIIb receptor. Recent studies from White and colleagues demonstrated how the fine structure of an antibody affects its agonistic ability. Two subtypes of human IgG2 exist, h2 and h1. The h2 subtype leads to better agonistic activity than h1, with the critical differences lying in the arrangement of the disulfide bridges of the hinge region (25). These results open up new avenues for engineering better agonistic antibodies for the clinic.

Glycoengineering is used to alter the effects of antibody therapy. GA101 (obinutuzumab) binds to CD20 similarly as does rituximab, though GA101 is a type II antibody with reduced fucosylation that increases its affinity for human FcγRIIIa. Because of the slightly different binding site and glycoengineering, GA101 led to less CD20 internalization compared with rituximab (26, 27). In addition, GA101 led to increases in multiple effector functions, including nitric oxide release in macrophages, activation and phagocytosis in polymorphonuclear cells (PMN), and NK-cell degranulation (28). Furthermore, GA101 activation of NK cells is not inhibited by KIR-HLA interactions compared with rituximab (29,30). Preliminary results from clinical studies have demonstrated that GA101 leads to increased depletion of malignant and normal B cells.

Factors in the TME Affecting the Immune Response

This year's conference focused more specifically on what in the TME leads to immune suppression. In some cases, exclusion of relevant immune cells from the microenvironment, as opposed to tolerance, may be a reason therapies fail. Doug Fearon used the KPC mouse model of PDA as an example of immune-cell exclusion. The KPC model of PDA in which p53 is lost can induce CD8⁺ T cells specific for shared antigens, yet it is resistant to anti-CTLA-4 and anti-PD-L1 treatment due to T-cell exclusion. PDA tumors contain cancer-associated fibroblasts (CAF) expressing high levels of fibroblast-activating protein (FAP; ref. 31). Using DTR-FAP mice, they showed that tumor control by CD8⁺ T cells begins quickly when FAP⁺ fibroblasts are eliminated. One potential mechanism for exclusion is the production of CXCL12 by FAP⁺ CAFs. CXCL12 binds to CXCR4 receptors, which are expressed on cancer cells and CD11b⁺ and CD3⁺ cells. Continuous infusion of the CXCR4 antagonist AMD3100 combined with anti-PD-L1 led to T-cell trafficking into the tumor with tumor destruction starting 24 hours after treatment (32). Future studies in the Fearon laboratory will address why PDAs, but not PanINs, bind to CXCL12, and the mechanism of T-cell exclusion. A clinical trial to assess the potential effect of AMD3100 in the TME is in progress.

Inadequate priming of T cells by intratumoral DCs is another potential target for correction by immunotherapy. Adjuvants for stimulating DCs within the TME could use the tumor as an *in situ* source of vaccine. Adjuvants that activate the stimulator of interferon genes (STING) are being developed by Aduro Biotech, Inc. STING recognizes bacterial cyclic di-nucleotides (CDN), and

it has been used as an effective adjuvant in mice. Investigators at Aduro Biotech, Inc., have developed ADUS100, a CDN that activates all five human STING gene products with promising results in murine tumor models. Intratumoral injection of ADUS100 into primary tumors inhibited the growth of B16 melanoma and metastasis, and increased the AH1 tumor-antigen response in the CT26 colon cancer model. MCP, TNF, and IL6 were induced in the TME as well as higher numbers of neutrophils. Combined with radiotherapy, ADUS100 induced TNF-dependent blood vessel changes within the tumor and systemic T-cell responses in the Panc02 model that were able to control secondary tumor growth (abscopal effect). Compared with TLR agonists, engineered CDNs were more effective in preclinical studies, and may have significant clinical benefits (33–35).

B cells have been shown to mediate immunosuppression within the TME. In the opening plenary session, Michael Karin presented his group's findings in castration-resistant prostate cancer, in which B cells are recruited to the tumor site via CXCL13, produced by α -SMA⁺ cells responding to hypoxic conditions in the TME (36). Upon further analysis, they observed that B cells produce lymphotoxin B, which drives IKK- α -dependent progression of prostate tumors (37). B cells play a critical role in promoting transgenic adenocarcinoma mouse prostate (TRAMP) tumor progression after treatment with chemotherapies (such as oxaliplatin) capable of driving immunogenic cell death. In these settings, oxaliplatin induced B cells to undergo class-switching to IgA⁺ plasma cells in a TGF β -dependent manner. Plasma cells in the TME produced IL10, expressed FasL, and were the major source of PD-L1 in prostate cancer. As a result, chemotherapy had no effect on tumor progression, as the B cells inhibited T-cell production of IFN γ and perforin. However, when oxaliplatin was used in TRAMP-bearing B cell-deficient mice, or combined with anti-CD20 or anti-PD-L1, T-cell function was restored and tumor growth was reduced.

The inflammatory state of the TME is a prognostic indicator of how well a patient will respond to immunotherapy. In melanoma, patients with a preinflamed TME (PD-L1 and IDO expression, Treg infiltration) have a better long-term prognosis when treated with melanoma vaccines or ipilimumab. In these tumors, the suppressive factors found are actually the result of activated T cells in the microenvironment (38). In tumors with a "noninflamed" phenotype, T cells are essentially excluded. Anergy is a T-cell intrinsic mechanism that could explain noninflamed tumors. Thomas Gawjewski presented ongoing studies using Affymetrix and ChIP sequencing of T cells precipitated with anti-Egr-2, a mediator of T-cell anergy, and showed that Treg-attracting chemokines CCL1 and CCL22 were upregulated in these T cells. CTLA-4/PD-L1, CTLA-4/IDO, and/or PD-L1/IDO combination therapies that may reverse anergy were successfully tested in B16 melanoma, yet the question remained whether the T cells were reactivated or were new emigrants. Using FTY720 blockade, which keeps T cells in the lymph nodes, tumors still shrank, indicating that the effects were due to reactivation of intratumoral T cells.

It has been hypothesized that preexisting T-cell response is a predictor of success with anti-PD-1 therapy. Antoni Ribas presented results from a study from his research group analyzing and comparing pretreatment and posttreatment tumor samples showing that success of anti-PD-1 therapy could be predicted when there was a preexisting population of T cells

around and in the tumor. These investigators showed that PD-L1 is upregulated in the TME and that the T-cell population was more clonal based on TCR V β usage and likely to be more tumor specific (39). These T cells also proliferated inside the TME, emphasizing the importance of a preexisting antitumor response (40).

Another factor in the TME that affects immunosurveillance and could be a target for immunotherapy is the heat-shock protein (HSP) pathway (41). CD91 is a key binder of HSPs in the immunosurveillance process and recently has been shown to be an important factor for priming an early T-cell response. Robert Binder presented results showing that mice immunized with HSPs could reject subsequent tumor challenges, yet CD91-knockout mice were unable to do so and, in general, have a decreased ability to control tumor growth in a CD8⁺ T cell-dependent manner. Further studies showed that expression of receptor-associated protein (RAP), an inhibitor of CD91 and cross-presentation by DCs, on methylcholanthrene-induced tumors increased the rate of tumor growth (42). Some human colon cancers are found to overexpress RAP, providing a possible mechanism by which to escape immunosurveillance, making RAP a potential target for immunotherapy. Although approximately 10 to 12 g of HSPs is produced by early-stage tumors, this may not be enough to overcome immune tolerance. Large doses of tumor-derived HSPs or targeting RAP may be a way to overcome these tolerance mechanisms.

Inflammation in the nascent tumor site has long been implicated in tumor development and progression, and a Th17 signature in colorectal cancer patients correlates with decreased disease-free survival (43). In the CPC-APC (Apc^{F/F}, Cdx2^{Cre}) mouse model of spontaneous colorectal cancer, IL17RA has been knocked out specifically in transformed enterocytes. As a result, tumor incidence was significantly reduced, largely due to reduction in malignant cell proliferation (44). Although antibody blockade of IL17A before overt tumor development reduces spontaneous tumor incidence and burden, antibody therapy at later stages has no effect on tumor size unless combined with 5-fluorouracil, suggesting it as a therapeutic combination for patients with colorectal cancer.

MDSCs can be a major suppressive factor within the TME, and signals that lead to MDSC accumulation are attractive targets for therapy. Building on previous studies showing that IFN regulatory factor-8 (IRF-8)-deficient mice develop greater numbers of MDSCs (45), Paschall and colleagues analyzed the mechanism of IRF-8 action. Using conditional knockouts of IRF-8, they determined that IRF-8 was not cell intrinsically active, but controlled the production of GM-CSF in T cells and tumor cells. Adoptive transfer of IRF-8 T cells was sufficient to increase MDSC production, which was abrogated when GM-CSF was knocked out. In tumor-bearing mice, however, T cells are not the primary source of GM-CSF, but tumor cells are, as silencing of IRF-8 in tumors led to increased GM-CSF and MDSC production, making IRF-8 a potential target for immunotherapy (46).

DCs, in general, are critical APCs but are more so within the TME. Miranda L. Broz presented the work from the Krummel group that described a rare DC population residing within the TME that is critical for tumor rejection. These DCs represent a CD11c⁺ CD103⁺ population that relies on GM-CSF and the transcription factors Batf3, IRF-8, and Zbtb46 for their development. Although found in the TME, these CCR7⁺ DCs readily traffic to the lymph node to stimulate T-cell responses. Depletion

of these CCR7⁺ DCs abrogated tumor rejection in the EG7.1/OT-1 model system. Importantly, increased numbers of these rare DCs in 12 human tumor types correlated with a better prognosis, adding to their use as a prognostic biomarker and potential therapeutic target (47).

Signatures of T-cell suppression within the TME could be attractive targets of immunotherapy. Using the CT26 model of colon cancer, Katherine A Waugh presented work from Jill Slansky's group that compared the genetic signatures of peripheral versus AH1-specific TILs. Results from these preliminary studies showed some expected findings such as downregulation of the genes encoding TCR and CD28, and the upregulation of genes encoding TGF β and PD-1 within the TME. Pathway analysis indicated a role for the E2F family of transcription factors and the methyltransferase PRMT1. PRMT1 knockdown studies in AH1-specific T cells showed increased proliferation compared with that in control T cells, indicating a role for PRMT1 in T-cell suppression. Current studies are focusing on the effects of these molecules on high- versus low-avidity tumor-specific T cells.

The Microbiome's Effect on Cancer and Immunotherapy

The side effects of altering the gut microbiome through the use of antibiotics have been known for years; however, recently, the role of the gut microbiome has begun to be understood in cancer therapy. There is evidence that during chemotherapy, bacterial products from the gut are translocated to the lymph nodes and modulate the antitumor effect of cyclophosphamide (48). Translocated Gram⁺ bacterial products led to the generation of Th17 and Th1 cells that aided in antitumor responses. In mice treated with antibiotics designed to kill Gram⁺ bacteria, tumors lost their sensitivity to cyclophosphamide treatment. The role of gut microbiota in conjunction with anti-CTLA-4 treatment was studied, as colitis is one of the most common side effects of anti-CTLA-4 treatment. More recent studies have shown that in germ-free mice, the antitumor effect of anti-CTLA-4 is abrogated; however, if mice are reconstituted with *Bacteriodes fragilis*, the positive effects of anti-CTLA-4 treatment are regained. IL12-producing CD103⁺CD11b DCs activated through TLR2 and TLR4 mediated part of the *Bacteriodes*/anti-CTLA-4 effect as neutralizing IL12 or knocking out the TLRs abrogated the therapeutic effect. Antigen-specific T cells were also necessary because splenic T cells from mice primed with *Bacteriodes*-pulsed DCs adoptively transferred into germ-free mice can also restore anti-CTLA-4 sensitivity. Interestingly, *Escherichia coli* is associated with the toxic inflammatory effects of anti-CTLA-4 treatment. These studies open the door for potentially engineering the gut microbiome to enhance current immunotherapeutic approaches (48).

The gut microbiome is especially linked to colon cancer. Wendy Garrett presented results from her laboratory using genomic sequencing of colon tumors and showed that *Fusobacterium* (Fuso) has an increased association with colon carcinomas compared with other gut microbial species (49, 50). To test whether Fuso was a causal agent, germ-free APCmin^{+/-} mice were reconstituted with Fuso or control bacteria. Fuso-reconstituted mice showed increased tumor incidence, but interestingly, they did not show an increase in inflammation. Fuso was associated with higher numbers of CD11b⁺ cells, as

well as CCL2, in both mouse and human tumors. In mouse studies, knocking down CCL2 led to reduced tumor growth, whereas injecting CCL2 directly into CT26 tumors led to increased tumor growth and an influx of MDSCs, which may aid in tumor growth (50). Other studies have analyzed the microbiome associated with colon cancer. Colon carcinomas of the ascending colon were associated nearly 100% of the time with biofilms compared with carcinomas of the descending colon (13%). These biofilms invade the mucous layer and the tumor and are associated with decreased E-cadherin expression, increased IL6 expression, and activated Stat3 in colonic epithelial cells (51). No one bacterial species dominated the biofilm. Interestingly, N1,N12-diacetylspermine, a metabolite that no bacteria species can make by itself, is increased in the biofilms, indicating that multiple bacterial species may collaborate to associate with colon carcinoma.

Metabolism in Immunotherapy

One recent focus in oncology has been the targeting of tumor metabolism as a means of treatment. Several studies reported that patients under stress conditions have poorer outcomes (52, 53). Elizabeth Repasky presented studies in murine models that tested what aspects of the immune system are affected by thermal stress (54), given that at IACUC-mandated standard temperatures (20°C–26°C; ST), mice are technically under cold-stress compared with higher (~30°C) thermo-neutral temperature (TT). She showed that mice housed at ST were less able to control Panc02 tumors compared with mice housed under TT conditions, as well as mice housed in ST that were given cisplatin. Increases in MDSCs and Tregs were found in ST mice compared with animals housed in TT (55, 56). A possible mechanism for the susceptibility of ST-housed mice is the production of norepinephrine as administration of propranolol, a β -blocker, led to the reversal of the ST susceptibility to tumor challenge (52). Conversely, in murine models of graft-versus-host disease (GVHD), ST-housed animals were less susceptible to GVHD induction than their TT-housed counterparts; however, administration of propranolol exacerbated GVHD symptoms in ST-housed animals (Leigh and Repasky, manuscript submitted). This finding is clinically relevant because β -blockers are given to patients who undergo bone marrow transplants. Overall, these findings highlight a potential consideration for future immunotherapy.

The metabolic activity of T cells is an important consideration in immunotherapy, as T cells are activated and go from a nutrient-rich environment in the lymph node to the comparably nutrient-poor environment of tumors. Using labeled glucose or glutamine and mass spectrometry analysis to study ATP metabolism, it has been found that less than 20% of glucose in Th1 CD4⁺ and CD8⁺ T cells went into the tricarboxylic acid cycle (TCA) cycle, but that the conversion of glutamine to glutamate, which subsequently entered the TCA cycle, was the major source of ATP (57). Studies using glutamine depletion *in vitro* showed that reduced glutamine level led to lowered oxidative phosphorylation and ATP production (58). Signaling through the AMP kinase was found to be one mechanism by which T cells respond to changes in nutrient availability (59). Russell Jones presented preliminary results from animal studies in which T cells have AMP-activated protein kinase knocked out

and demonstrated that these animals have lower T-cell numbers, IFN γ production, and tumor clearance in response to *Listeria* challenge.

Adoptive T-cell Therapy

Given the number of known shared tumor antigens and recent advances in genetics, adoptive T-cell therapy is becoming a more viable option for treating established cancers. The Wilms tumor antigen-1 (WT1) is overexpressed in leukemic stem cells and has been targeted by transferring donor-derived WT1-specific T cells into patients with a high probability of relapse. Chapuis and colleagues (60) performed dose-escalation studies to demonstrate that this therapy is safe and leads to prolonged survival. The question remained whether TCRs could be engineered with higher affinities for the target peptide:HLA-A0201 complex, leading to better therapeutic outcomes. Preclinical studies targeting WT1 with T cells engineered to have higher affinity TCRs showed that this treatment does not lead to autoimmunity even though the WT1 antigen is expressed in the thymus, laying the groundwork for further engineering of TCRs for adoptive transfer (61). Philip Greenberg presented preliminary results from a clinical trial in which an engineered TCR with the highest affinity for the WT1 peptide:HLA-A0201 complex was transferred into recipient T cells and reinfused into patients, showing promising results, including many patients in complete remissions. These results have led to studies targeting various HLA alleles and different cancers, including acute myeloid leukemia, pancreatic ductal adenocarcinoma, and ovarian cancer. Ingunn Stromnes presented preclinical data using a mesothelin-specific TCR in the KPC model of pancreas cancer and showed that a single infusion of transferred T cells upregulated inhibitory markers, including TIM-3, Lag-3, and PD-1; however, multiple infusions of engineered T cells prolonged the survival of KPC mice.

Classically, adoptive T-cell therapy has targeted overexpressed antigens especially in melanoma. With advances in genomic sequencing, it is potentially possible to target patient- and tumor-specific mutations. Studies using exome-sequencing to identify tumor-specific mutations, tandem mini-genes expressing tumor-specific mutations, and autologous patient APCs enabled the identification and isolation of TILs from melanoma that recognize the tumor-specific mutations. These TILs were expanded *ex vivo* and transferred back into patients with some success (62). Given that melanomas contain on average 200 mutations, compared with epithelial cancers, which may express 30 or fewer mutations, this new technique was used to isolate mutation-specific T cells from a patient with cholangiocarcinoma whose tumor contained only 26 mutations. The disease was stabilized using CD4⁺ T cells that were specific for erbb2-interacting protein (ERBB2IP), demonstrating that this method has the potential to more specifically target multiple types of cancer (63).

The metabolic state of adoptively transferred T cells is also a key factor in their success as a therapy. Memory T cells have the ability to self-renew and have different metabolic characteristics than their naïve counterparts. The Akt pathway has been shown to affect the metabolism of CD8⁺ T cells differently than other T-cell subsets and cell types (64). Human and murine CD8⁺ T cells cultured under adoptive transfer conditions in the presence of an Akt inhibitor showed an increase in

fatty acid metabolism, increased persistence and IFN production *in vivo*, and expression of memory T-cell markers (65). In a mouse model of melanoma, transgenic CD8⁺ T cells cultured with an Akt inhibitor were able to control tumor growth better than untreated control T cells. Further studies and clinical trials with Akt inhibition and adoptively transferred T cells will be needed to confirm these results.

Chimeric antigen receptors (CAR) are another mode of adoptive T-cell therapy; studies of CARs that target CD19 on the surface of malignant cells have shown success in treating chronic lymphocytic leukemia (CLL) and acute lymphocytic leukemia (ALL). In previous phase I studies with CARs, 73% of patients with CLL and 93% of patients with ALL have shown some levels of response. CAR treatment has potential side effects, including macrophage-activation syndrome as a result of increased IL6 production and cytokine-release syndrome; the presence of IL6 correlates with initial disease burden and can be treated with tocilizumab (an antibody against the IL6 receptor; ref. 66). Myeloma has been considered CD19 negative. However, Yangbing Zhao presented results from preliminary studies testing anti-CD19 CAR treatment that have shown promise. Three patients who relapsed after their second stem cell transplant have been treated, with some patients showing reduced IgA levels more than 129 days after treatment.

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Summary

The status of immunotherapy as a cancer treatment has increased rapidly compared with standard treatments. Our knowledge of T-cell activation and tolerance, antibody effector mechanisms, tumor genetics, and cancer biology has led to the improvement of cancer therapies for patients with previously terminal cancers. This AACR special conference highlighted the progress and promise of combination therapies, building on the success of single agents in clinical trials, our understanding of more specific mechanisms of tolerance, improvements in antibody development and function, adjuvant and adoptive T-cell therapy, and new factors that affect the cells of the immune system. The research presented at this conference will be the basis for the next set of advances in cancer immunotherapy.

Disclosure of Potential Conflicts of Interest

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