

# Cancer Immunology and Immunotherapy: Taking a Place in Mainstream Oncology Keystone Symposia Meeting Summary

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## Abstract:

The Keystone Symposia conference on *Cancer Immunology and Immunotherapy: Taking a Place in Mainstream Oncology* was held at the Fairmont Chateau in Whistler, British Columbia, Canada on March 19-23, 2017. The conference brought together a sold-out audience of 654 scientists, clinicians, and others from both academia and industry to discuss the latest developments in cancer immunology and immunotherapy. This meeting report summarizes the main themes that emerged during the four day conference.

## Introduction:

The field of cancer immunotherapy continues to garner increased interest as attested to by the rapidity with which the symposium on *Cancer Immunology and Immunotherapy: Taking a Place in Mainstream Oncology* reached its maximum attendance capacity. The symposium focused on improving cancer immunotherapies, many of which have resulted in durable responses for increasing numbers of patients. The four-day gathering also provided a forum for discussions and potential collaborations between basic or translational scientists and clinical investigators from both academia and industry. The major topics presented included (I) personalized therapies using tumor-specific neoantigen vaccines, (II) adoptive cell therapy (ACT) engineering to improve the functionality and persistence of T cells, (III) protein and antibody therapies to modulate endogenous antitumor immunity, (IV) combinatorial immunotherapies including merging conventional treatments with immunotherapy, and (V) new technologies to improve immunomonitoring by identifying molecular targets and assessing therapeutic efficacy.

Dr. Glenn Dranoff (Novartis, Cambridge, MA) delivered the first keynote address. Dranoff pioneered the use of GVAX, a vaccine comprised of irradiated, autologous tumor cells engineered to secrete the cytokine GM-CSF (1), showing that GVAX can lead to tumor destruction in some patients. Notwithstanding the ability of GM-CSF to enhance antitumor immunity, under some circumstances it may contribute to immune suppression. Dranoff demonstrated a critical role for GM-CSF signaling-induced expression of the transcription factor PPAR- $\gamma$  in regulating myeloid cells. Loss of PPAR- $\gamma$  in the myeloid lineage reduced the efficacy of GVAX, which was associated with a reduced ratio of intratumoral effector CD8<sup>+</sup> T cells to Foxp3<sup>+</sup> T regulatory cells (Tregs). In contrast, PPAR- $\gamma$  agonists increased the ratio of intratumoral CD8<sup>+</sup> T cells to Tregs when given with GVAX. He concluded his talk with a brief summary of multiple combination early phase clinical trials either in progress or upcoming at Novartis.

Dr. Andreas G. Plückthun (University of Zürich, Switzerland), who pioneered the use of Designed Ankyrin Repeat Proteins (DARPs), genetically engineered antibody mimetics (2), gave the second keynote address. He described work developing a HER2 DARPin that demonstrated efficacy with no toxicity in a mouse breast cancer model. He also described the use of DARPs in immuno-oncology, revealing that anti-4-1BB DARPin given in combination with anti-PD-1 and an anti-VEGF DARPin had significant antitumor effects in preclinical models. He also developed methods to cloak DARPin-*Pseudomonas aeruginosa* exotoxin fusions by addition of polyethylene glycol moieties, increasing the half-life, reducing immunogenicity, and improving

selectivity. He concluded by proposing that these systems have the potential to overcome barriers for the safe, therapeutic use of toxins alone or in combination with immunotherapy to modulate targets beyond the reach of current drugs.

## **Checkpoint Blockade Therapy: Mechanisms of Action and Immune Resistance**

The development and subsequent FDA approval in 2011 of the immune checkpoint antibody ipilimumab (anti-CTLA-4) for melanoma was perhaps the most important clinical advance in cancer immunotherapy to date (3). Dr. Jim Allison (MD Anderson Cancer Center, Houston, TX), who pioneered the use of checkpoint blockade therapy (CBT) (4), gave an overview of the number of CBT antibodies recently garnering FDA approval for many cancers. Despite this, Allison concedes we still lack a fundamental understanding of the mechanisms that underlie CBT-induced tumor regression. He then presented work whereby mass cytometry was used to interrogate tumor-infiltrating cells, comparing mouse tumors with different sensitivities to CBT. These studies revealed differences between intratumoral T cells from anti-CTLA-4 or anti-PD-1 treated mice. Specifically, anti-PD-1 induced expansion and activation of CD8<sup>+</sup> T cells with a dysfunctional phenotype marked by TIM-3 and PD-1 expression, whereas anti-CTLA-4 expanded an ICOS<sup>+</sup>PD-1<sup>+</sup> Th1-like CD4<sup>+</sup> T cell population and CD8<sup>+</sup> T cells. Interestingly, the phenotypic changes were similar between CBT resistant and sensitive tumors.

Prostate cancer is generally resistant to ipilimumab. Dr. Padmanee Sharma (MD Anderson Cancer Center, Houston, TX) presented intriguing data that may explain this observation. Similar to what she previously described (5, 6), increased numbers of ICOS<sup>+</sup> T cells were found in prostate tumors post ipilimumab; however, clinical response was not robust. Analysis revealed upregulation of PD-L1 as well as VISTA on predominantly distinct subsets of tumor infiltrating macrophages after ipilimumab suggesting PD-L1 and VISTA may act as a compensatory inhibitory pathway in this setting. She referenced work by Dr. Randolph Noelle (Dartmouth College, Lebanon, NH) on the immune inhibitory effects of VISTA (7). Dr. Noelle described studies using a mouse tumor model whereby delayed treatment with anti-VISTA blocking antibody and/or anti-PD-1/CTLA-4 led to a mix of responders and nonresponders. Noelle saw increased T cell proliferation with lower expression of immune inhibitory receptors in the responders. Further analysis revealed that anti-VISTA therapy altered the tumor myeloid compartment, allowing for improved antitumor immune responses. This work has led to an anti-VISTA phase I clinical trial. Additionally, anti-VISTA may synergize in certain cancers with other immunotherapies, as suggested by Sharma.

Turning to other mechanisms of immune resistance, Dr. Antoni Ribas (UCLA, Los Angeles, CA) focused on acquired immune resistance, whereby relapse occurs after initially responding to CBT. He pointed out that loss of IFN $\gamma$  signaling in mouse tumor models results in immune resistance (8-10). He then described two clinical cases of late acquired resistance to anti-PD-1 therapy in lung cancer where loss-of-function mutations in JAK1 or JAK2 were acquired that allowed cancer cells to avoid the anti-proliferative effects of IFN $\gamma$  (11). Ribas also referred to work presented by Sharma, demonstrating that ipilimumab nonresponders had an enriched frequency of mutations in IFN $\gamma$  pathway genes (12). Dr. Nicholas Restifo (NCI, Bethesda, MD) described a CRISPR/Cas9 screen used to identify genes regulating tumor sensitivity to killing by cytolytic T cells (CTL). Confirmation for one of the validated candidates (APLNR) came from studies showing that tumor expression of APLNR was critical for efficient clearing of mouse B16 tumors.

In contrast to acquired immune resistance, pancreatic cancer represents natural resistance to immunotherapy, as described by Dr. Elizabeth Jaffee (Johns Hopkins University, Baltimore, MA). She emphasized that pancreatic tumors have relatively few effector T cells so not only must the desmoplastic stromal barrier be broken down, but induction of effector T cells is critical. Jaffee presented data from a pancreatic cancer clinical trial of GVAX alone or with chemotherapy, noting that GVAX altered the intratumoral cell composition, finding fewer Tregs and more CD8<sup>+</sup> T cells in nonaggregate intratumoral areas (13). GVAX also increased IDO and PD-L1 expression in the tumor, making combination with IDO inhibitors

or anti-PD-1/ PD-L1 an attractive strategy. Jaffee's group is also pursuing vaccination approaches targeting specific tumor antigens with a modified *Listeria* vaccine.

Immunotherapies such as CBT have the potential to synergize with conventional therapies such as radiation. Dr. Sandra Demaria (Weill Cornell Medicine, Cornell University New York, NY) previously showed (14) that targeted tumor irradiation delivered in a few fractions of 6 to 8 Gy each plus CBT induced systemic antitumor immunity in carcinomas resistant to CBT alone. She presented new data that induction of T cell responses capable of mediating abscopal effects requires cancer cell-intrinsic type I IFN pathway activation by radiation. The latter is regulated by the DNA exonuclease Trex1, which was induced by high-dose radiation, abrogating the benefit of combination therapy.

Several talks focused on targeting other immune checkpoints and costimulatory moieties. Dr. Ana Anderson (Dana-Farber, Boston, MA) presented data demonstrating antitumor activity of TIM-3 antibody blockade, further characterizing ligands of TIM-3 and optimal antibodies to target this pathway. Dr. Andrew Weinberg (Providence Cancer Research Center, Portland, Oregon) showed compelling data demonstrating remarkable efficacy of agonist antibodies to the costimulatory molecule OX40 in some models.

## **Responders and Nonresponders**

One indicator of response to CBT is the T cell infiltrate prior to treatment, with inflamed tumors being more likely to respond (15). Dr. Tom Gajewski (University of Chicago, Chicago, IL) described studies showing exclusion of T cells by  $\beta$ -catenin expressed in mouse melanomas that had constitutively active Braf and deleted PTEN (16). The exclusion from the tumor of transferred antigen-specific CTLs was associated with a lack of Batf3<sup>+</sup> dendritic cells (DCs). In human melanomas, PTEN loss was associated with a non-inflamed phenotype and resistance to anti-PD-1. Additionally, about half of non-inflamed melanomas displayed active  $\beta$ -catenin signaling. He commented that therapies aimed at recruiting DCs that provide T-cell chemokines into non-inflamed tumors could improve responses to CBT.

Although the T-cell tumor infiltrate shows correlations with response, better predictive indicators are needed to stratify patients who most likely will benefit from immunotherapy. Dr. Ira Mellman (Genentech, South San Francisco, CA) pointed out that for many tumors, expression of PD-L1 on tumor cells is less predictive of response to anti-PD-1/PD-L1 than expression on tumor infiltrating immune cells, consistent with recent reports (17-19). Mellman then presented intriguing data showing that PD-1 engagement acts primarily on costimulation. It dephosphorylates CD28 to a much greater degree than CD3 in a reconstituted model of PD-1 in a cell membrane (20). He also showed data demonstrating that combinations of chemotherapy and/or targeted therapies like MEK inhibitors may extend the benefit of anti-PD-1/PD-L1 and concluded by summarizing work being done at Genentech targeting multiple arms of the cancer-immunity cycle.

## **Adoptive Cell Therapy**

Several groups presented findings on ACT approaches, including chimeric antigen receptor (CAR) T cell therapy (21) and ACT with T-cell receptor (TCR)-engineered T cells(22). Dr. Philip Greenberg (University of Washington, Seattle, WA) focused on how to build better T cells for durable antitumor immunity (22). He described clinical studies of high risk acute myeloid leukemia (AML) patients treated with WT1-specific TCR transgenic CD8<sup>+</sup> T cells. Clinically, there was early evidence of efficacy but no apparent increase in survival. Greenberg is exploring further modulations of transgenic T cells by adding costimulatory endodomains to inhibitory ectodomains. Greenberg also presented intriguing data showing that tumor antigens processed by the immunoproteasome differed from those processed by the constitutive proteasome. Although this phenomenon had been previously described (23), Greenberg found a transgenic TCR that recognized a unique WT1 epitope processed differently that, importantly, recognized some patient tumors better than the WT1 transgenic TCR used in the trial. He concluded by describing successful efforts to make CD4<sup>+</sup> TCR transgenic T cells for ACT.

CAR therapy has shown remarkable efficacy in some cancers such as acute lymphoblastic leukemia (ALL) (24). Dr. Carl June (University of Pennsylvania, Philadelphia, PA) continued his work towards improving next-generation CAR-T cells. CAR-T cells made with a 4-1BB domain can last in patients for at least 4 years as opposed to those made with the CD28 domain, which persist for about one month. This persistence seems to be particularly important in treating chronic lymphoid leukemia. Dr. Michel Sadelain (Memorial Sloan Kettering, New York, NY) continued with the theme of improving CAR-T cells showing that targeting CARs to the T cell receptor  $\alpha$  constant (*TRAC*) locus using CRISPR/Cas9 enhanced T cell potency and outperformed conventional CAR-T cells in a mouse model of ALL (25). Dr. Stanley Riddell (Fred Hutchinson, Seattle, WA) spoke about ACT in both hematologic and solid cancers. He began by describing ACT in preclinical models in which cell products containing both CD4<sup>+</sup> and CD8<sup>+</sup> CAR-T cells were more effective than either subset alone. Riddell also elaborated on a recently initiated CAR trial in non-small cell lung cancer patients. Although there were no complete responders, shrinkage of subcutaneous tumor nodules and lymph nodes was noted, with no toxicity. Riddell speculated that combining CAR-T cells with strategies to overcome the immunosuppressive microenvironment may be necessary to improve efficacy.

## Cancer Vaccines

Vaccination against infectious disease is widely considered the most impactful biomedical advance in history. However, therapeutic cancer vaccine successes have been limited. The ability to rapidly identify tumor-specific mutant neoantigens has, in part, reignited cancer vaccine enthusiasm (26-29). Dr. Robert Schreiber (Washington University, Saint Louis, MO) began by showing that recognition of neoantigens could drive cancer immunoediting of highly immunogenic unedited tumors (30). This was among the first studies to use genomic sequencing and epitope prediction algorithms to identify mutant neoantigens. He went on to show that similar approaches could be used to identify neoantigenic targets of T cells activated by CBT in edited, progressively growing tumors, and that neoantigen synthetic long peptide (SLP) vaccines could therapeutically induce tumor regression in mice (31). For one mouse tumor line, reverting two dominant mutant neoantigens to their nonimmunogenic wild-type sequences via CRISPR/Cas9 allowed for the uncovering of additional subdominant neoantigens. Schreiber suggested the current ease, speed, and cost effectiveness of neoantigen identification and their tumor-specific expression makes neoantigen vaccines an attractive and potentially safer immunotherapy.

Following a similar theme as Schreiber, Dr. Catherine Wu (Dana-Farber Cancer Institute, Boston, MA) presented exciting early results from an SLP neoantigen vaccine trial. Six melanoma patients received personalized neoantigen SLP vaccines and poly-ICLC. Almost all peptide pools induced responses comprised of both CD8<sup>+</sup> and CD4<sup>+</sup> neoantigen-specific T cells that were not detectable before vaccination. Two of the patients experienced recurrence and when subsequently treated with anti-PD-1 their tumors regressed. Wu also described efforts to identify peptides bound to MHC class I using single-allele peptide sequencing by mass spectrometry. Using this information, a new antigen predictor algorithm was developed that performed two- to three-fold better when compared to current methods (32).

In contrast to SLP vaccines, Dr. Ugur Sahin (TRON, Mainz, Germany) described a different vaccine approach (RNA liposome complexes) to target tumor antigens. This RNA vaccine greatly expanded antigen-specific T cells in mice and, in the case of neoantigens, about a third of the mutations in the vaccine were recognized with most being restricted to MHC class II. He further showed that MHC class II neoepitopes confer CD40L-mediated help, remodel tumor microenvironment and induce antigen spread. He then discussed results from an ongoing vaccine clinical trial in melanoma. Eight of 13 patients receiving vaccines experienced stable disease, although one patient who progressed responded to subsequent treatment with anti-PD-1. Another displayed temporary disease control but eventually progressed and was found to have acquired a loss of  $\beta_2M$  and thus MHC class I.

Dr. Connie Trimble (Johns Hopkins University, Baltimore, MD) presented phase I clinical trial data testing a heterologous DNA<sub>E7</sub>-prime, recombinant Vaccinia<sub>E6E7</sub> boost vaccination regimen, with and without topical imiquimod, in patients with HPV16-associated cervical intraepithelial neoplasia, prior to planned

standard therapeutic resection. Vaccination peripherally was followed by striking changes in the lesion microenvironment, including the appearance of tertiary lymphoid structures, clonally expanded TCRs, and an activated, Th1 phenotype. Vaccinated subjects who had histologic regression also had viral clearance. Clinical and immunologic endpoints from cohorts who underwent both therapeutic vaccination and topical imiquimod are maturing.

### **Activating and Suppressive Intratumoral Immune Cells**

Innate and adaptive immune cells in the tumor microenvironment may not only fail to control, but often promote tumor growth. Using an inducible breast cancer mouse model, Dr. Alexander Rudensky (Memorial Sloan Kettering, New York, NY) found increased numbers of Tregs in tumor bearing mice (33). Whereas defective extrathymic generation of Tregs did not affect tumor progression, knocking out the Foxp3 regulatory element required for maintenance during division of differentiated Treg cells resulted in attenuated tumor growth and metastasis. Rudensky also described work revealing a role for amphiregulin in Tregs and CD4<sup>+</sup> T cells in the lung. Conditional knockout of amphiregulin in all T cells or Tregs alone reduced tumor volume without affecting the number or function of T cells. Taken together with other work (34), Rudensky remarked that tumor-infiltrating CD4<sup>+</sup> Tregs and non-Tregs have distinct function in promoting tumor growth, independent of their enhancement or suppression of antitumor immune response.

In addition to Tregs, monocytes/myeloid cells in the tumor microenvironment are often immunosuppressive (35, 36) as illustrated in talks given by Dr. Vincenzo Bronte (Verona University Hospital, Verona, Italy) and Dr. Alberto Mantovani (Humanitas Clinical Research Center, Milan, Italy). Bronte showed that upregulation of an anti-apoptotic regulator in monocytes caused them to become powerfully immunosuppressive and that chemotherapy could reverse the inhibitory activity of both mouse and human monocytes. Mantovani described tumor-associated macrophages (TAM) as exerting a dual, yin-yang influence on chemotherapy and radiotherapy, either antagonizing the antitumor activity of these treatments by orchestrating a tumor-promoting, tissue-repair response or enhancing antitumor immunity (35). He went on to describe studies uncovering a role for innate immunity in macrophage-driven tumor promotion with the discovery that PTX3 is an extrinsic oncosuppressor that dampens activation of complement, CCL2 production, and tumor-promoting macrophage recruitment (37).

Chronic inflammation can promote oncogenesis (38) while at the same time immunity can prevent cancer development as illustrated in a talk given by Dr. Michael Karin (UCSD, La Jolla, CA). He presented intriguing findings whereby in nonalcoholic steatohepatitis (NASH)-induced mouse liver cancer, IgA<sup>+</sup> plasmacytes suppress cancer immunosurveillance through PD-L1 and IL-10, acting to promote CD8<sup>+</sup> T cell dysfunction. Whereas IgA knockout mice develop fewer tumors, mice lacking CD8<sup>+</sup> T cell developed more tumors faster. Treatment with anti-PD-L1 decreased tumor load and activated liver CTLs. Karin noted that in patients, NASH results in accumulation of liver PD-L1<sup>+</sup>IgA<sup>+</sup> plasmacytes and PD-1<sup>+</sup>CD8<sup>+</sup> T cells. These novel findings reveal a potential therapeutic target for intervention in NASH patients.

### **Improving Immunomonitoring With Novel Technologies**

Dr. Lisa Coussens (Oregon Health & Science University, Portland, OR) defined how certain populations of T cells can promote tumor progression and metastasis (39). Based on her observations she is developing better methods to monitor immune responses. She described techniques, with one pixel resolution digital imaging, that allow for high density immune profiling in tissues. After validating the platform, she analyzed head and neck cancer tumors and evaluated three different clusters: lymphoid inflamed, hypoinflamed, and myeloid inflamed, demonstrating that different clusters correlated with different outcomes. She also analyzed pancreatic ductal adenocarcinoma samples and found an inverse correlation between myeloid and CD8<sup>+</sup> T cell density. In a neoadjuvant trial she found GVAX induced more PD-L1 in tumor, along with increased numbers of granzyme B<sup>+</sup> CD8<sup>+</sup> T cells as well as CD163<sup>+</sup> macrophages.

Dr. Jim Heath (Caltech, Pasadena, CA) has been developing micro and nanotechnologies that are facilitating better immunomonitoring. He described efforts to assay many proteins at the same time by placing cells into nanoliter chambers and then adding a miniature antibody array to assay for proteins. He was able to observe that the most polyfunctional T cells dominated the total immune response. He also commented that the polyfunctional strength index appeared to be uniquely prognostic for patients undergoing CAR therapy. He went on to discuss techniques that aid in the identification and detection of tumor antigen-specific T cells. This platform increased sensitivity of detection and should allow for better uncovering of tumor-specific T cells in tumor and blood where sensitivity is critical.

Dr. Garry Nolan (Stanford University, CA) has been a pioneer in the development and use of mass cytometry for high-dimensional, single-cell analysis (40). His talk focused on “system-wide order from disorder”, associated with the interaction between immune cells and cancer. He presented data from a study uncovering the cellular properties of effective immunotherapy that revealed the necessity of a systemic response for rejection (41). This response was coordinated across tissues and required for efficient tumor eradication in several preclinical models. An emergent population of CD4<sup>+</sup> T cells in the periphery conferred protection against tumors and was found to be expanded in patients responding to immunotherapy. Additionally, Nolan continues to make remarkable technological advances that will undoubtedly aid in the development of new treatments and better immunomonitoring.

## **Conclusions**

The success of current immunotherapies was highlighted throughout the meeting with the caveat that much work is still needed. However, it is becoming more apparent that we are entering a new phase, in which personalized immunotherapy, along with the ability to stratify patients who are most likely to benefit from it, and the ability to gauge which specific therapy(s) individual patients should be administered, is leading us to improved efficacy and safety for a greater number of cancer patients.

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