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Meeting Report: Mechanism and Therapeutic Reversal of Immune Suppression in Cancer

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Introduction

The last decades were characterized by substantial progress in our understanding of the role of the immune system in tumor progression. This raised high expectations that immunotherapy would provide a breakthrough in cancer treatment. However, these expectations have not yet materialized. It became increasingly clear that tumor induced abnormalities in the immune system not only hamper natural tumor immune surveillance, but also limit the effect of cancer immunotherapy. Thus, it is critically important to understand the mechanisms of tumor induced immune suppression to make any progress in this area. The conference on the "Mechanism and Therapeutic Reversal of Immune Suppression in Cancer" that took place in Clearwater Beach, Florida, USA, January 25-28, 2007 was the first of its kind to be focused entirely on the discussion of different mechanisms of immune suppression in cancer and therapeutic approaches to their correction. This conference was part of a biannual conference series on "Molecular Targets in Cancer" from H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida, Tampa, FL, USA. 249 researchers from 23 countries participated in lively discussions of basic science research as well as new developments in the clinic. This conference provided participants with the opportunity to integrate ideas in true translational fashion.

The meeting began with a discussion of the historical perspective of cancer immunotherapy from bench to bedside (1). The concept of "checkpoint blockades" was described as the body's attempt at preventing autoimmunity, and thereby thwarting attempts at harnessing the immune system in the eradication of cancer.

Cellular mechanisms of immune suppression in cancer

Suppressor cells in cancer are a heterogeneous population. Suppressor cell populations were identified as therapeutic cellular targets including myeloid derived suppressor cells (MDSC), regulatory T cells (Tregs), tumor stromal cells, natural killer T cells (NKT), endothelial cells, and B cells.

Bone-marrow-derived Gr-1$^+$CD11b$^+$immature myeloid cells, termed Myeloid Derived Suppressor Cells (MDSC), normally found in low numbers in lymphoid organs, accumulate in tumor-bearing mice with the ability to suppress T cell function (2-5). The session began with a report on a novel mechanism of direct MDSC interaction with CD8+ T cells to achieve immunosuppression. MDSC blocked the binding of specific peptides to CD8$^+$ T cells by nitrating T cell receptors (TCR), thereby impairing interaction with MHC class I:peptide

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complexes. Another population of suppressor cells, an inflammatory-type $CD11b^+IL4R\alpha^+$, are also expanded in tumor bearing mice and mediate their function via nitric oxide synthase and arginase. Phosphodiesterase-5 inhibitors alone or in combination with vaccines delayed tumor progression, down regulated MDSC, and reversed peptide-specific T cell tolerance in these mice. Data was also presented supporting the role of $CD11b^+CD14^-$ MDSC in cancer patients. Furthermore, it was theorized that MDSC "starve" T cells of arginine and that COX-2 inhibitors could decrease arginase and attenuate tumor growth in mice. It was also shown that IL-1β secreted by tumors induced accumulation of MDSC and that cross talk between MDSC and macrophages polarized immunity towards a tumor-promoting type 2 T cell response.

Intervention strategies targeting MDSC were also described. Treatment of prostate cancer patients with all trans-retinoic acid (ATRA) decreased the presence of MDSC and increased effector T cell responses. In patients with renal cell carcinoma, an inhibitor of tyrosine kinase receptors (Sunitinib) decreased the level of T regulatory cells (Tregs) as well as MDSC. A combination of Sunitinib with tumor vaccines was proposed.

Tregs, whose normal function is to prevent autoimmunity, can also function to suppress anti-tumor immunity (6). The role of $FoxP3^+$ Tregs in tumor escape was examined. Conditional FoxP3 knockout mice demonstrated that a significant number of T cells recognize self-antigen but are normally suppressed by Tregs. Removal of Tregs by FoxP3 deletion led to increased expansion and activation of $CD11c^+Cd11b^+$ dendritic cells (DC). CD40L and OX-40 expression play an important role in Treg function. Additionally, MDSC were shown to induce the development of Tregs in tumor bearing mice. MDSC from $CD40^{-/-}$ mice lost the capacity to induce Tregs, implicating CD40/CD40L interactions between the two cell types. The difference between natural versus inducible Tregs was also discussed, and it was suggested that it is the inducible population which contributes to immune suppression in cancer. It was noted that most of the therapeutic vaccines also expand Treg density and may limit the utility of vaccination. Therefore, depletion or manipulation of Tregs in combination with vaccination may be required.

Clinical trials that target Tregs were discussed in several presentations. Data was presented from a phase III clinical trial that targets cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) expressed on Tregs, and from a phase II trial using anti CTLA-4 combined with peptide vaccination. Clinical responses were correlated with immune breakthrough events. PD-1 is also expressed on Tregs and is the focus of phase I trials. A Phase I trial to deplete Tregs using Ontak in combination with vaccination in ovarian cancer was also described. Tregs were depleted in 6 of 7 patients, however concurrent depletion of T effector cells was noted as well. Additionally, Ontak was used in combined with a dendritic cell vaccine in renal carcinoma patients. The level of Tregs was reduced, although it rebounded after the end of treatment.

In addition to MDSC and Tregs, other cell types were identified that modulate immune responses. Tumor stroma can persist even after removal of tumor cells and can contribute to immunosuppression (7). The importance of targeting tumor stroma antigens, which can be released after chemotherapy or radiation, was discussed. In a mouse model, $CD8^+$ T cells recognized antigen-loaded stromal cells but not cancer cells. A novel role for natural killer T cells (NKT) in tumor tolerance was also discussed (8). A subset of NKT1 cells was identified, which recognizes CD1d and aids in tumor rejection while its counterpart, NKT2, which recognizes sulfatide, aids in tumor recurrence. Additionally, a unique form of tolerance in $CD8^+$ T cells due to B cells was described, in a process that uncouples the TCR from downstream signaling events. Finally, the imbalance between antigen presenting cells, co-stimulatory molecules B7-H1, B7- H4, and Tregs was discussed.

Molecular mechanisms of immune suppression in cancer

Molecular targets regulating immune suppression included nitric oxide synthase, arginase, indoleamine 2,3 dioxygenase (IDO), signal transducers and activators of transcription (STAT), and others.

IDO, an enzyme necessary for tryptophan degradation, is highly expressed in many cancers (9). Mouse models were presented where immunization effectively eliminated IDO negative tumors, but transfection of IDO into these tumor lines allowed for tumor escape due to suppression of antigen specific T cells. The IDO inhibitor, 1-methyl-L-tryptophan, partially restored CTL responses. The role of IDO in DC immunosuppression was addressed in several reports. 1-methyl-L-tryptophan could restore positive DC interaction with T cells. A hypothesis was presented that activated Tregs are inducing IDO in dendritic cells. Additionally, Bin1 regulates IDO expression via the STAT/NF-κB pathway by suppressing tumor growth. Hence, the use of IDO inhibitors, and manipulating IDO pathways, may offer novel approaches in the clinic.

Both STAT-1 and STAT-3 signaling have been implicated in tumor development (10,11). Tumor escape mechanisms in STAT-$1^{-/-}$ mice, which develop spontaneous tumors similar to human ductal breast carcinoma, were reported. It was also shown that STAT3 might trap NF-κB in the nucleus thereby redirecting transcription from immunostimulatory genes towards oncogenes. Abnormal STAT-3 signaling was identified in the inhibitory effects of IL-10 on DC maturation and migration. Silencing STAT-3 with short hairpin RNAs led to restoration of a normal DC stimulatory phenotype. Overexpression of STAT-3 in DCs impaired $CD4^+$ T cell function, whereas STAT-3 $^{-/-}$ DCs reversed T cell tolerance. Small molecule inhibition of STAT-3 using curcubitacin analogues broke tolerance, further implicating STAT-3 as a negative regulator.

In addressing the tumor microenvironment, it was noted that loss of chemokines prevents DCs from homing to the tumor site. It was also reported that alteration of phospholipid expression on the tumor cell membrane could reduce the immunosuppressive effect of the tumor on DC survival and function. A new method of altering the tumor microenvironment and thereby attract lymphoid cells, by delivering LIGHT (a member of the TNF ligand superfamily) to mouse tumors was described. Treatment, alone or in combination with vaccination, led to T cell and dendritic cell infiltration into the tumor, and subsequent tumor rejection. Data was also presented on silencing of suppressor of cytokine signaling (SOCS1) in dendritic cells. These cells were able to break tolerance in mouse models and allowed vaccination to reject tumors.

Boosting vaccination

Emerging evidence of the immunosuppressive nature of the tumor microenvironment has led to new strategies to enhance vaccine therapy of cancer. It was reported that combining peptide vaccination with CPG and $CD25^+$ Treg depletion completely abolished established tumors in mice. Epitope spreading was suggested in this model. Additionally, a “super booster” was presented which combined peptide vaccination with toll-like receptor agonists and CD40 antibodies. CpG-ODN fused with anti-Her-2/neu antibody also induced tumor rejection in Her-2neu mice. Additionally, the results of a Phase II clinical trial of combination vaccine/adoptive T cell transfer for renal cell carcinoma were reported. Patients, prevaccinated with lethally irradiated tumor cells, followed by CD3 antibody-expanded autologous post-vaccine T cells, showed complete or partial responses for greater than 4 years. The next step will utilize CD28 and CD3 antibodies for better T cell expansion.

The results from DC vaccines in melanoma patients were discussed and it was suggested that Langerhans cells might be a better target for stimulating $CD8^+$ T cells. The different mechanisms by which DCs and Langerhans cells process antigen were addressed and suggested that targeting the phosphoinositol-3 kinase pathway could boost vaccination attempts. In a Phase I trial, agonist CD40 antibodies were used to boost APC responses, resulting in partial responses in 4 of 32 patients and stable disease in 7 patients. The main adverse effect was cytokine release syndrome indicating immune stimulation. Naturally occurring immunity against the tumor antigens MUC-1 and Cyclin B1 was also explored (12). Though MUC-1 has been used in vaccination protocols, the success rate is only 20% in cancer patients. However, there is evidence for natural development of anti-MUC-1 antibodies, and thus there is potential for a protective vaccine targeting this antigen, thereby providing a natural boost against cancer development.

Synergism between vaccination and conventional chemo/radiation therapies

Though initially counterintuitive, immunosuppressive chemotherapy and radiation treatments nevertheless seem to synergize with vaccination to produce a greater therapeutic benefit than either approach alone. A method was introduced that led to tumor rejection through small molecule inhibition of TGFβ receptor kinase (SM16, BiogenIdec). Combination of SM16 with either adenovirally delivered IFNβ or lymphocyte adoptive transfer in mice showed synergistic effects, as SM16 altered the tumor microenvironment and allowed for enhanced efficacy of the other treatments. Gemcitabine was also used to alter the tumor microenvironment, and combined with anti-GITR antibodies plus immunization against Her-2/Neu in mice, broke tolerance to stimulate tumor antigen specific CD8+ CTL.

It was reported that a p53 DC vaccine combined with traditional chemotherapy in a Phase I/II trial for small cell lung cancer resulted in a significant increase in median survival and responses to second line chemotherapy compared to historical controls with chemotherapy alone (13). The next aim will be to combine vaccination with ATRA to target MDSC. Another trial used granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting allogeneic pancreatic tumor cells as a vaccine, with some pancreatic cancer patients also receiving radiation or chemotherapy (14). Median survival was significantly increased compared to chemotherapy regimens alone. Mesothelin was identified as a target antigen from these trials.

Round Table Discussion and Summary

The meeting closed with a lively round table discussion targeting several key topics. The antigenicity/immunogenicity of tumors was described as typically weak, and therefore has limited the efficacy of vaccination protocols. Future protocols should target multiple epitopes in order to enhance immune responses, and to combine different treatment paradigms to further boost efficacy. Patients, which have experienced remission after vaccination, represent an opportunity to explore the immune response to tumor associated antigens. Additionally, the role of the various arms of immune suppression in cancer was discussed and much remains to be learned about the heterogeneous population of suppressor cells, as well as their function in various tissue types and cancers. However, there is a clear need for clinical testing of some of the therapeutic strategies to reverse tumor-induced immune suppression. Finally, how should we proceed with vaccinating patients? It was agreed that timing and choice of combination therapies are key to remission and cure of cancer.

Acknowledgements

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APPENDIX

List of speakers and titles of their presentation in alphabetical order

Conference Chair – *Dmitry Gabrilovich (H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL)*

Keynote speaker - Checkpoint Blockade in Cancer Immunotherapy. *James P. Allison, M.D, Memorial Sloan Kettering, New York, NY*

Speakers:

TGF-Beta Inhibition Augments Immunogene Therapy and Adoptive Transfer of Lymphocytes *Steven Albelda, MD, University of Pennsylvania, Philadelphia*

A Dendritic Cell Vaccine with p53 as the Tumor Antigen for Small Cell Lung Cancer *Scott Antonia, M.D., H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL*

Inflammatory Monocytes Induced by Tumors Alter T lymphocyte Responsiveness through Larginine Metabolism *Vincenzo Bronte, M.D., Padua University, Padua, Italy*

Adoptive T Cell Therapy of Renal Cell Cancer *Alfred Chang, M.D., University of Michigan, Ann Arbor, MI*

Dual Roles of CD40 in the Development of Tumor-Specific T-Regulatory Cells and its Potential Use in Immune Therapy for Cancer *Shu-Hsia Chen, PhD Mount Sinai School of Medicine, New York City*

Antigen Presentation Attenuators and Anti-Tumor Immunity *Si-Yi Chen, M.D., Ph.D., Baylor University, Houston, TX*

The B7 Family Molecules in Cancer Evasion *Lieping Chen, M.D., Ph.D., Johns Hopkins University, Baltimore, MD*

Inhibiting T Reg Activity in Tumor Bearers *Mario P. Colombo, Ph.D., Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy*

Regulatory T Cells in Cancer *Tyler Curiel, M.D., M.P.H., San Antonio Cancer Institute, San Antonio, TX*

Expression of Indoleamine 2,3-Dioxygenase by Tumor Cells as an Immune Resistance Mechanism That Can Be Fight *Benoit Van den Eynde, M.D., Ph.D., Ludwig Institute for Cancer Research, Brussels, Belgium*

Promotion of a Type-1 Cell Response in Metastatic RCC Patients by SU11248; Modulation of a T Reg Population *Jim Finke, Ph.D., Cleveland Clinic, Cleveland, OH*

Human Tumor Antigens, Tumor Imunosurveillance and Tumor Escape *Olivera J. Finn, Ph.D., University of Pittsburgh, Pittsburgh, PA*

Bringing Immune Response to Tumor Sites to Generate CTL for Metastasis *Yang-Xin Fu, MD, PhD, University of Chicago, Chicago*

T-Cell Tolerance Induced by Immature Myeloid Cells *Dmitry Gabrilovich, M.D., Ph.D., H. Lee Moffitt Cancer Center, Tampa, FL*

Tipping the Immune System Balance in Favor of Effective Anti-Cancer Therapy *Elizabeth Jaffee, M.D., Johns Hopkins University, Baltimore, MD*

Combined Chemoimmunotherapy Can Efficiently Break Self-Tolerance and Induce Anti-Tumor Immunity in a Tolerogenic Murine Tumor Model *Chang-Yui Kang, PhD, National University of Seoul, Seoul*

Molecular Mechanisms of Negative Signaling by Cancer Associated HPV in Immune Escape *W. Martin Kast, Ph.D., University of South California, Los Angeles, CA*

Disruption of Regulatory Pathways to Enhance the Efficacy of Cancer Vaccines *Sergei Kusmartsev, PhD, University of Florida, Gainesville, FL*

Regulatory T Cells and Cancer *Hy Levitsky, Johns Hopkins University, Baltimore, MD*

Targeting the Tumor Microenvironment with CpG-ODN Modifies Suppressor Cells Within the Tumor Resulting in Tumor Rejection *Joseph Lustgarten, PhD, Mayo Clinic Arizona, Scottsdale*

T Cell Regulation by Dendritic Cells Expressing Indoleamine 2.3 Dioxygenase *Andrew Mellor, Ph.D., Medical College of Georgia, Augusta, GA*

T Regulatory Cells and AICD: Two Intrinsic Mechanisms for Immune Suppression *Bijay Mukherji, University of Connecticut Health Center, Hartford, CT*

IDO, Cross-Presentation and Tolerance *David Munn, M.D., Medical College of Georgia, Augusta, GA*

Arginine Availability Regulate the Immune Response in Cancer Patients *Augusto Ochoa, M.D., Louisiana State University, New Orleans, LA*

Immune Suppression and Inflammation in Tumor Progression *Susan Ostrand-Rosenberg, Ph.D., University of Maryland, Baltimore, MD*

Dendritic Cells as Vectors and Targets in Cancer Immunotherapy *Anna Karolina Palucka, M.D., Ph.D., Baylor Institute of Immunology, Dallas, TX*

Regulation and Therapeutic Inhibition of IDO in Cancer *George C. Prendergast, PhD, Lankenau Institute for Medical Research, Wynnewood*

Regulatory T Cells *Alexander Rudensky, Ph.D., University of Washington, WA*

Programming of CD8+ T Cell Tolerance *Stephen Schoenberger, Ph.D., La Jolla Institute for Allergy & Immunology, San Diego, CA*

Cross-Targeting to Destroy the Tumor Microenvironment *Hans Schreiber, M.D., Ph.D., University of Chicago, Chicago, IL*

Cancer Immunoediting: Basic Mechanisms and Therapeutic Implications *Robert Schreiber, Ph.D., University of Washington, St. Louis, MO*

Mechanisms of Dendritic Cell Dysfunction in Cancer *Michael Shurin, M.D., University of Pittsburgh, Pittsburgh, PA*

Targeting Signaling Pathways in Antigen Presenting Cells to Overcome Tolerance to Tumor Antigens *Eduardo Sotomayor, M.D., H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL*

Autoimmunity and Vaccination in Melanoma *Jeff Weber, M.D., Ph.D., University of Southern California, Los Angeles, CA*

Role of STAT3 in mediating a cross talk between tumor and regulatory T cells *Hua Yu, PhD, Beckman Institute, Duarte, CA*

Antigen Presenting Cells in Human Tumor Microenvironment *Weiping Zou, M.D., Ph.D., University of Michigan, Ann Arbor, MI*

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