2007 iSBTc Annual Meeting Program Summary

by James C. Yang, MD, Meeting Organizer

The 22nd Annual Meeting of the International Society for Biological Therapy of Cancer (iSBTc) was convened at the Seaport Convention Center in Boston, Massachusetts from November 2 – November 4, 2007. The Annual Meeting was well attended, drawing an audience of 421 people.

As preludes, an educational "Workshop on Combination Biological Therapies of Cancer" and a "Primer on Tumor Immunology and Biological Therapy" were conducted on November 1. The Workshop investigated the scientific, methodological and regulatory issues associated with developing biological treatments that utilize multiple agents in a rational manner. The Primer drew on world-class volunteer faculty who covered a multitude of topics ranging from vaccines and tumor antigens to innate immunity and angiogenesis.

The Annual Meeting was opened by iSBTc President, Jon M. Wigginton, MD, who introduced Ernest C. Borden, MD, from the Cleveland Clinic Foundation. Dr. Borden was selected to deliver this year's Richard V. Smalley Memorial Lectureship. This annual award commemorates the life and contributions of Richard V. Smalley, MD, who was a founding member of iSBTc and a lifelong member and officer of the organization. In a clinical and scientific career spanning four decades, Dr. Borden has been intimately involved in the biology and clinical application of interferons. He delivered an overview of interferons on their 50th birthday, and by describing new findings on the epigenetic control of the interferon response, showed that the story on these proteins is not nearly complete.

The first Plenary Session focused on adoptive transfer of immune cells as therapy for cancer, titled "Adoptive Cellular Therapy: Sustained Regression of Large Tumor Burdens with *In Vitro* Expanded T-Cells." Described here was the experience of the National Cancer Institute's (NCI) Surgery Branch of administering cultured tumor infiltrating lymphocytes to patients with metastatic melanoma after preparatory lymphodepletion. Recipient lymphodepletion can enhance T-cell transfer by eliminating T-regulatory cells, stimulating homeostatic cytokines and depleting non-specific resident immune cells that compete for those cytokines. Simple cytoxan and fludarabine prior to TIL and IL2 resulted in partial or complete responses in 21 of 43 patients treated, while adding 1200 cGy of whole body radiation produced 18 responses in 25 patients (72%). After adoptive cell transfer, median response durations were a year or more and some responses were ongoing beyond four years with no further therapy.

Michel Sadelain, MD, PhD, from Memorial Sloan Kettering Cancer Center in New York, NY, discussed the construction of chimeric receptors for T-cells using retroviral gene therapy with a talk titled, "Targeting Tumors with Genetically Enhanced T Lymphocytes." These combinations of single chain antibodies, costimulatory modules (eg. from CD28) and signal transduction chains such as CD3-zeta, can be introduced into T-cells and confer recognition of tumors reflecting the specificity of the parental antibody. Dr. Sadelain then showed that the ligands for costimulatory receptors can be provided in "cis" or "trans" by transducing the T-cells themselves with the genes encoding these ligands. In a model of established human tumor xenographs in immunodeficient mice, he demonstrated potent and specific tumor regression induced by transferring human T-cells engineered to express these chimeric T-cell receptors and the ligands CD80 (B7.1) and CD137L (4-1BBL).

In the third invited lecture, Michael C.V. Jensen, MD, from the City of Hope and Beckman Research Institute, showed alternative ways to engineer T-cells to produce tumor recognition and to optimize anti-tumor activity in a talk titled, "Engineering Cytolytic Effector Cells for Glioma Immunotherapy Using Gene Insertion and

Zinc Finger Nuclease Genomic Editing." Working with a chimeric receptor where a modified IL-13 serves as the extracellular binding moiety coupled to cytoplasmic CD3-zeta for signal transduction, this effort is targeting glioblastoma multiforme (GBM), a tumor characterized by high expression of IL-13R-alpha2 (with early clinical trials underway). Because T-cell-toxic glucocorticoids are widely used in patients with GBM, another innovation is to render gene-engineered T-cells resistant to glucocorticoids by disrupting their glucocorticoid receptor. This is being accomplished by targeting the endogenous gene with zinc finger nucleases in concert with inserting the novel chimeric receptor to produce T-cells resistant to glucocorticoids and redirected to recognize GBM.

Two concurrent sessions were also conducted in the afternoon. One concerned new roles for cytokines in the host response to cancer and inflammation and the other was directed at angiogenesis and vascular therapies.

In the angiogenesis session, Wadih Arap, MD, PhD, from the MD Anderson Cancer Center in Houston, Texas, gave a wide-ranging talk on molecular addressins, with an emphasis on the tumor vascular endothelium, titled, "Ligand-Directed Targeting and Molecular Genetic Imaging in Diseases with an Angiogenesis Component." Dr. Arap explained that these molecular addressins can mediate binding to specific organs and cell types. These specific peptide sequences can be utilized to deliver cells, therapeutic molecules or radiation to target tissues and tumors.

Luigi Naldini, PhD, who directs an extensive program in gene therapy at the San Raffaele Foundation in Milan, Italy, used lentiviral manipulation to investigate the function of a novel subset of monocytes which express the angiopoetin receptor Tie2. His talk was called, "Tie2-Expressing Monocytes: a Novel Lineage of Proangiogenic Cells." Use of Tie2-driven gene constructs in transplanted bone marrow showed that these cells home to tumors and play a critical role in tumor angiogenesis. Furthermore, he showed that this cell population could not only be a target for therapy, but through more innovative gene manipulations could also serve to deliver therapeutics to tumor.

The concurrent session on cytokines opened with Michael T. Lotze, MD, from the University of Pittsburgh Cancer Institute, and his discussion on the increasingly complex and often paradoxical impact of inflammation on cancer and its effects on cell death pathways and antigen presentation. Dr. Lotze's talk was titled, "Interleukin 1 Family Members Regulate Tumor Immunity in Response to Damage Associated Molecular Pattern Molecules (DAMPs)." Potential roles of novel members of the extended IL-1 family of cytokines were reviewed, and the key influence and pleotropic effects of the molecular danger signal, HMGB1 were emphasized.

The second day of the meeting opened with the Keynote Address by Carl H. June, MD, of the University of Pennsylvania, titled, "Adoptive T-Cell Therapy: Grand Challenges and Opportunities." Dr. June pursued the use of genetically-modified T-cells, adoptively transferred for the treatment of cancer and viral infections. In a talk which interdigitated basic science and early clinical trials, Dr. June showed that gene manipulation of in vitro cultured T-cells is a reality and represents a powerful means of attacking disease by directly manipulating the immune cells responding to cancer and pathogens. A portion of the presentation showed the benefit of driving transferred T-cells in vivo with appropriate patient vaccination. In another trial using a lenteviral vector encoding an anti-sense sequence targeting HIV *env* (conditionally expressed under *tat* and *rev* control so that the antisense is upregulated upon HIV infection), CD4 cells were transduced and adoptively transferred to patients with HIV infection. Comparisons of single versus multiple sequential T-cell transfers showed greater persistence (for months in some cases) of the genetically modified T-cells with some evidence of improvement in viral load and immune parameters. An early experience with adoptive cell transfer in conjunction with autologous stem cell transplant in patients with multiple myeloma was also presented, further showing that host lymphodepletion and in vivo vaccination can enhance lymphocyte persistence.

One of the concurrent afternoon sessions on November 3, chaired by Paul F. Robbins, PhD, from the National Cancer Institute, dealt with tumor recognition. As might be expected, this covered diverse topics from T-cell

therapies targeting viral antigens and virally-induced malignancies, to modulators of T-cell reactivity. In a talk titled, "Therapeutically Accessible Molecules That Regulate the Regulators," Drew M. Pardoll, MD, PhD, from the John Hopkins University School of Medicine, provided evidence for a functional role of the LAG-3 protein, a molecule that is up-regulated on anergic as well as Treg cells, in inhibiting T-cell activation. Injection ProHAXTramp mice with an anti-LAG-3 antibody in conjunction with a VAC-HA immunization promoted the activation of endogenous HA-reactive CD8+ T cells, which are normally tolerized due to expression of HA on prostate tumors that develop in these mice. In addition, transduction of LAG-3 appeared to convert CD4+/CD25- T-cells into Treg cells.

Malcolm K. Brenner, MD, PhD, from the Baylor College of Medicine in Houston, Texas, described immunotherapeutic strategies being used to target EBV-associated malignancies arising in a variety of disease settings in a talk titled, "Adoptive Immunotherapy of Malignancies with Gene Modified, EBV-Specific, Cytotoxic T-Cell Lines." Although specific T-cell lines generated using in vitro tumor stimulation was generally readily attained in patients with post-transplant lymphomas, the poor immunogenicity of the LMP1, LMP2 and EBNA1 antigens expressed on other tumors such as Hodgkin's lymphoma, NHL and Burkitt's lymphoma necessitated the use of recombinant Adenovirus constructs expressing these proteins to effectively prime antigen reactive T-cells. In addition, the preliminary results of a clinical trial evaluating genetic modification of either primary T cells or EBV CTL with a chimeric antigen receptor (CAR) reactive with GD2 protein expressed on the surface of a variety of tumors were presented. In this trial, neuroblastomas patients who received CAR-modified CTL appeared to demonstrate higher levels of persistence than patients receiving CAR-modified normal T-cells. However, the overall levels of persistence of either of the transduced cells were relative low and no clinical responses were reported.

Bryon D. Johnson, PhD, from the Medical College of Wisconsin, provided evidence of a role for tumor-derived MIF in inhibiting T-cell function in a talk titled, "Tumor-Derived Macrophage Migration Inhibitory Factor (MIF) Inhibits Immune Reactivity to Neuroblastoma *In Vivo*." Injection of neuroblastomas expressing lower levels of MIF as a result of transduction with a shRNAi construct were less tumorogenic and appeared to lead to the induction of enhanced anti-tumor immunity. Preliminary results indicated that part of the mechanism of action of tumor derived MIF may be through the induction of T-cell apoptosis.

In his talk titled, "5-aza-2'- Deoxycytidine Treatment Increases Expression of Tumor Associated Antigens in Human Melanoma," Timothy Haggerty, PhD, from Cytocure, LLC of Beverly, Massachusetts, presented studies demonstrating that treatment of tumor cells with 5-aza-2'-deoxycytidine (5aza) and IFN-β up-regulated the expression of the melanoma antigens MART-1 and gp100, as well as HLA class I in multiple tumor cell lines. In addition, a combination of 5aza with IFN-b appeared to further up-regulate class I and antigen expression in some tumor cell lines.

In the other concurrent session chaired by Elizabeth A. Repasky, PhD, from the Roswell Park Cancer Institute in Buffalo, New York, and Brian I. Rini, MD, of the Cleveland Clinic Taussig Cancer Center, the anti-CTLA4 antibody, CP-675,206 (tremilimumab) was discussed in the context of colorectal cancer and melanoma. Although well tolerated in heavily-treated patients with colorectal cancer, there was only sporadic activity. In patients with melanoma, there was not only more clinical anti-tumor activity, but more immune-related adverse events, with a trend towards longer survival in patients who had such events. In his talk, "Novel Agents in Renal Cell Carcinoma: Challenges and Opportunities in Capitalizing on Active Therapy," Dr. Rini gave a comprehensive overview of the new agents for renal cancer, reviewing the underlying biology as well as the results of Phase II and Phase III trials of sunitinib, sorafenib, bevacizumab and temserolimus. In discussing the current and future clinical strategies for these agents, he discussed their sequential use and possible combinations, both of which appear promising.

The Presidential Session at the end of the day was again a highlight of the meeting with selected young investigators presenting their work. Amy Wesa, PhD, from the University of Pittsburgh, showed that the deficit of TH1-type CD4+ cells reactive with the melanoma associated antigens EphA2 or MAGE-A6 in melanoma patients was associated with a pro-apoptotic phenotype compared to CD4 cells specific for

influenza in her talk, ""Apoptosis of Circulating Tumor-Antigen Specific TH1 CD4+ T-Cells from Melanoma Patients."

In her talk, "Priming and Selection of Allorestricted, Peptide-Specific T-Cells for Adoptive Therapy of Tumors," Susanne Wilde, of the Institute of Molecular Immunology in Munich, Germany, exploited a strategy to circumvent central thymic deletion to identify high-affinity T-cells recognizing the melanoma-associated self-antigen tyrosinase. She used HLA-A2+ dendritic cells transfected with RNA encoding tyrosinase and stimulated a non-HLA-A2 T-cell responder repertoire *in vitro*. This responder population was not subjected to thymic deletion of A2-restricted tyrosinase-reactive clones because it was not HLA-A2+, but stochastically contains T-cells that could respond to an allogeneic tyrosinase epitope presented by A2. Dr. Wilde indeed found A2-restricted tyrosinase recognition and those T-cell clones proved to be of higher affinity than clones obtained from an HLA-A2+ repertoire by similar methods.

Talya Schwarzberg, MD, of the Beth Israel-Deaconess Medical Center in Boston, Massachusetts, reviewed the clinical experience with high-dose IL-2 in patients with metastatic renal cell cancer who had also undergone any of a number of prior VEGF-targeted anti-angiogenic therapies (AAT). Dr. Schwarzberg's talk was called, "Retrospective Analysis of Interleukin-2 Therapy in Patients with Metastatic Renal Cell Carcinoma Who Had Received Prior Antiangiogenic Therapy." In a group of 16 patients, the initial targeted therapies included bevacizumab, sunitinib and sorafenib, alone or in combination. Fifteen of these patients were able to move on to high-dose IL-2 after not responding to AAT, but 37% were not able to receive the second of two intended cycles of IL-2, primarily for a number of severe cardiac toxicities. This study indicates that the strategy of sequential treatments with AAT followed by IL-2 should be further studied and perhaps entertained more cautiously.

In the fourth presentation, titled, "CD4+CD25highFoxp3+ T Regulatory Cells Kill Autologous CD8(+) and CD4(+) T-Cells Using GranzymeB and Fas/FasL-Dediated Pathways," Laura Strauss, PhD, of the Hillman Cancer Center in Pittsburgh, compared the cytocidal interactions between T-regulatory cells and CD8+ or CD4+ cells from normal volunteers and patients with head and neck cancer. She found that T-reg from patients caused CD8 cell apoptosis but not T-reg from normal donors. In turn, T-reg were subject to attack from CD4 cells but protected by the addition of high concentrations of IL-2. Ultimately, the judging panel felt compelled to award Presidential Awards to the presentations of both Drs. Wesa and Wilde.

The last day of the meeting began with a summary of the 2006 iSBTc "Mini-Symposium on Biological Effects of Targeted Therapies," which had been held in Los Angeles in October, 2006. Michael B. Atkins, MD, from the Beth Israel Deaconess Medical Center in Boston, presented the results of that symposium, emphasizing both the promise of targeted therapies and the deficiencies in our present understanding of these agents. This was followed by Martin "Mac" A. Cheever, MD, of the Fred Hutchinson Cancer Research Center, who summarized a ranked list of immunological agents generated by an NCI Workshop in July 2007, titled, "NCI Immunotherapy Agent Workshop." This Workshop was convened to determine which agents had the highest potential for cancer therapy. From 124 initial candidates, thirty were selected, led by IL-15, PD-1/PD1L inhibitors, IL-12 and CD40 pathway agonists.

Later that day, there was a session on cancer vaccines and then a panel presentation on the scientific, regulatory, and approval issues of the agent sepuleucel-T for prostate cancer. In the vaccine session, Joseph W. Fay, MD, of Baylor Medical Center in Dallas, presented an overview of 64 patients with metastatic melanoma who were treated with dendritic cells loaded with either melanoma-associated peptide antigens or cultured melanoma tumor lines under a series of consecutive protocols in his talk, "Long-Term Survival in Patients with Metastatic Melanoma Vaccinated with Melanoma-Antigen Loaded Dendritic Cells." In long-term follow up, an objective response rate of 11% was seen (2 PRs and 5CRs), with most responses showing durations of years. Factors associated with long-term survival were M1a disease (cutaneous and nodal metastases only), no tumor growth while on protocol, and a normal pre-treatment LDH.

Mary L. Disis, MD, of the University of Washington, presented work identifying IGFR family members as attractive targets for immunotherapy, particularly in breast cancer. In her talk, "Insulin like Growth Factor Receptor Family Members: Essential Tumor Antigens in Breast Cancer," she identified a series of Class II presented peptides from IGFR proteins and postulated that T-cell repertoires to these epitopes may exist because of similarities to antigens from known human pathogens. Finally, using breast tumors derived from the neu transgenic mouse model, it was shown that immunotherapy targeting IGFBP-2 slowed the growth of tumors compared to controls. In another presentation, Craig L. Slingluff, Jr., MD, from the University of Virginia, described an interesting morphological finding at the site of peptide vaccinations in IFA in his talk, "Features of Lympoid Neogenesis After Dermal Injection of Incomplete Freund's Adjuvant with or without Peptides." By biopsing sites of vaccination, he found that lymphoid neogenesis occurred in the skin, recapitulating some of the structural features of lymph nodes and hypothesized that this may be one mechanism by which T-cells are activated by this route of repeated vaccination.

The last session of the meeting was a "Hot Topic" discussion on the biology, development, and clinical testing of the product sipuleucel-T for androgen independent prostate cancer titled, "Hot Topic Session: The Dendreon Debate." Sipuleucel-T is an autologous product, prepared by leukapheresing a patient with prostate cancer, and shipping it to central facilities run by the Dendreon Corporation in Seattle, Washington. There it is enriched for antigen presenting cells by simple one-step buoyant density centrifugation and then incubated with a fusion protein of prostatic acid phosphatase and gm-CSF for approximately 40 hours (no additional cytokines are used), and then shipped back to the local MD for infusion. David L. Urdal, PhD, the Chief Scientific Officer for the Dendreon Corporation, described its clinical development and the randomized trials which led to its submission for licensing. These trials used time to progression as their primary endpoint, which did not reach statistical significance in any trial. An analysis of survival in the 127 patients in D9901 (45 randomized to placebo) showed a significant increase in survival, although that was not a primary pre-stated endpoint. A second trial of 98 patients again showed trends towards improved time to progression and survival but neither was statistically significant.

Several other speakers with expertise in drug approval, statistics, dendritic cell biology, and GU oncology reviewed the sipuleucel-T story from each of their persectives. These talks were quite valuable because they objectively reviewed the scientific and clinical data, which is not nearly as well known as some of the controversies and heated opinions about sipuleucel-T. Although some of those did arise during this very timely session, the last word appears to be held by a 500-patient randomized trial that has recently completed accrual, and whose outcome will likely trump any further discussion of the results and merits of the prior smaller trials. In general, the vast majority of participants left with a better understanding of the data and issues concerning sipuleucel-T than with which they arrived.

In summary, one of the main themes of the 2007 iSBTc Annual Meeting appeared to be an increased awareness of the tumor microenvironment and, in particular, its immunosuppressive influences. There were several groups exploring the use of adoptively transferred in vitro-expanded T-cells as one means of circumventing some of these local obstacles to effective cancer immunotherapy. There was continued interest in the burgeoning family of co-stimulatory receptors and ligands and the equally expanding list of inhibitory co-receptors. Finally, as a trend across all these areas of interest, there was increased emphasis and progress on taking many of these concepts to clinical trials, where evidence of clinical activity has indeed been seen.

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The International Society for Biological Therapy of Cancer (iSBTc) Annual Meeting offers delegates an international forum where immunologic and biologic approaches to cancer treatment are showcased, discussed, and critically evaluated. Several features of the Annual Meeting are exhibit showcases, oral abstract presentations, poster presentations/viewings, Keynote Addresses, and Award presentations. For more information on the iSBTc Annual Meeting and the society in general, please check out our website at www.iSBTc.org.