

# Tumor Immunology and Immunotherapy Program



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## Overview

The overall goal of the Tumor Immunology and Immunotherapy Program is to understand and utilize the ability of the immune system to prevent, diagnose and treat human cancers. The scientific approach to achieve this goal is to: 1) Characterize the specific interactions between tumor and immune cells that lead to tumor recognition and tumor rejection (or failure to do so); 2) Define the broader host-tumor immune interactions that modulate tumor cell biology and the anti-cancer immune response; and 3) Translate this understanding to the human immune system for clinical application, and conversely analyze the clinical and population correlates of this application to discover the mechanistic underpinnings of tumor-immune system interactions.

Research in the Tumor Immunology and Immunotherapy Program is organized into three distinct but interrelated themes:

1. Mechanisms of immunological tumor rejection
2. Microenvironment and host-tumor interactions, and
3. Immunotherapy and clinical discovery.

These themes represent a progression from examination of the discrete interactions between tumor and immune effector cells, to the broader range of immunological interactions that influence both tumor cell biology and anti-tumor immunity, and finally to the translation of these basic studies into the human cancer arena. Research also flows in the opposite direction from clinical discovery to develop a mechanistic understanding of the significant findings observed in clinical trials and population studies.

## Themes

### Theme 1: MECHANISMS OF IMMUNOLOGICAL TUMOR REJECTION

Research in this theme focuses on the specific interaction involving tumor presentation of antigen and CD8 CTL activation. Studies aim to characterize novel tumor antigens, the genetic regulation and molecular mechanisms for processing and presentation of antigens, and the structural basis of defects in histocompatibility antigens expression by malignant cells.

**Antigen Processing Machinery Cellular Immunity and Prognosis:** Studies by Drs. Ferrone, Bangia, Repasky, and Tomasi have focused on how antigen processing and presentation is lost in cancers. This group has established that downregulation of MHC class I and the antigen processing machinery (APM) in laryngeal squamous cell carcinoma and melanoma cells from stage III and IV patients is significantly associated with reduced patient survival. Complementary studies by Drs. Odunsi and Ambrosone (CPPS) in a large population study in epithelial ovarian cancer found that intraepithelial CD8<sup>+</sup> tumor infiltrating lymphocytes and high CD8<sup>+</sup>/Treg ratio are associated with improved survival in epithelial ovarian cancer patients. (Ogino *et al.*, *Cancer Res* 2006; 66:9281; Anichini *et al.*, *Cancer Res* 2006; 66:6405; Sato *et al.*, *Proc Natl Acad Sci USA* 2005; 102:18538)

### Theme 2: MICROENVIRONMENT AND HOST-TUMOR INTERACTIONS

Research in this theme seeks to define and understand the role of the interactions between the tumor cells and the surrounding microenvironment on tumor cell survival, as well as modulation of the host anti-tumor immune responses.

**Thermotherapy and Microenvironment Modifications:** Dr. Evans and her group have established that lymphocytes traffic poorly to tumor sites under steady-state conditions, providing one explanation for the failure of the immune system to limit tumor. Fever-range whole body hyperthermia in mice increased trafficking of circulating naive and central memory CD4 and CD8 T lymphocytes across vascular check points. Systemic thermal therapy (core temperature elevated to 39.5 °C) strongly upregulated the intravascular density of ICAM-1 on tumor vessels and high endothelial venules (HEVs) of lymphoid

organs, causing a 5-fold and 2-fold increase, respectively, in ICAM-1-dependent homing of CD8 T cells. Normal vessels of extra-lymphoid organs (heart, kidney, liver, pancreas) were not responsive to thermal therapy. The site-specific nature of the thermal response serves to increase the probability that antigen-restricted cytolytic CD8 T cells will encounter tumor targets within tissues. Intravital microscopy revealed a marked increase in the frequency of T cells undergoing ICAM-1-dependent firm adherence in both HEVs and tumor microvessels; firm sticking interactions were not detected in normal vessels under normothermic or hyperthermia conditions. (Chen *et al.*, *Cancer Immunol Immunoth* 2006 55:299, Chen *et al.*, *Nat Immunol* 2006; 7:1299; Chen *et al.*, *Immunity* 2004; 20:59)

**The Inflammatory Milieu:** Inflammation has been called the soil in which carcinogenesis takes root; inflammation is characteristically mediated by the immune system. The Lung Inflammation Group was formed in 2007 as a new TII initiative to focus efforts on understanding the interaction between smoking, microbial elements, immune-mediated chronic inflammation and lung cancer development and progression. The group consists of Drs. Baumann, Demant (GN), Lau, Pauly, Segal, and Thanavala. Members of this group share the common interest in how chronic inflammation in the lung can contribute to development/progression of lung carcinoma. Each member brings a distinct expertise, model system and reagents that facilitate multiple collaborations

### Theme 3: IMMUNOTHERAPY AND CLINICAL DISCOVERY

Research in this theme utilizes the information derived from the other two themes in order to develop and characterize molecular and immunologic strategies for manipulating the innate and adaptive immune responses to malignancy. Research also focuses on clinical deployment of new immune therapeutics.

**Vaccine Development:** Dr. Odunsi determined that NY-ESO-1, a member of the "cancer-testis" (CT) family of antigens, is a suitable target for immunotherapy in ovarian cancer (Odunsi *et al.*, *Cancer Res* 2003; 63:6076). This observation was tested directly in a phase I clinical trial of immunization with an NY-ESO-1 derived peptide of dual MHC class I and II specificities, combined with incomplete Freund adjuvant (IFA). He demonstrated induction of antigen specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses in the majority of immunized patients, along with the critical role of cognate CD4<sup>+</sup> T cell help in promoting effector differentiation and sustenance of CD8<sup>+</sup> T cells. Dr. Odunsi has shown at least two mechanisms of immune escape: (i) suppression of high-avidity effector T cells by CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> regulatory T cells and (ii) development of antigen loss variants. To generate high avidity effector cells that are not suppressed by regulatory T cells, Dr. Odunsi demonstrated that a diversified prime and boost vaccination strategy in ovarian cancer patients using recombinant vaccinia and fowlpox expressing NY-ESO-1 efficiently induce Th1 effector cells of higher avidity than Treg suppressed pre-existing effectors. (Nishikawa *et al.*, *J Immunol* 2006; 176:6340)

**Antiangiogenic Agent Development:** Dr. Seon has identified endoglin (EDG; CD105), a homodimeric cell membrane antigen, and generated several anti-EDG mAbs, termed SN6 series mAbs that define different epitopes of EDG molecule. These mAbs have significant antiangiogenic activity that inhibits new tumor growth and metastasis, and appear to be more potent than (and not affected by resistance to) clinically available anti-VEGF antibodies. A human/mouse chimeric mAb, termed c-SN6j, has been tested in preclinical studies. (Shiozaki *et al.*, *Cancer Immunol Immunother* 2006; 55:140). Sufficient quantities of clinical grade SN6j have now been manufactured to initiate an investigator-initiated phase I clinical trial in refractory/relapsed solid tumors.

## Selected Scientific Accomplishments

### Naveen Bangia, PhD

*Assistant Professor, Immunology*

Involvement of normal immune cells in supporting the survival of malignant immune cells has also been identified in B cell chronic lymphocytic leukemia (B-CLL). Studies by Drs. Naveen Bangia and Chanan-Khan (MTET) reveal striking clinical efficacy of lenalidomide (a derivative of thalidomide, with unknown mechanism of action) in B-CLL. Following treatment of B-CLL patients with lenalidomide, a significant inflammatory response in tumor-involved lymph nodes ("tumor flare reaction") manifest as red, tender swollen lymph nodes, low grade fever and rash within hours of the first lenalidomide dose. The severity of the flare reaction correlating with the degree of subsequent clinical response. Correlative studies demonstrated that lenalidomide did not directly induce apoptosis in primary tumor cells *in vitro* despite potent *in vivo* anti-leukemic effects in the same patient. Prior to treatment, patient CD8<sup>+</sup> T cells actually supported the survival of B-CLL cells, and this was abrogated by lenalidomide treatment. Lenalidomide upregulated B-CLL costimulatory molecule expression and increased the number of T and NK cells in patients, as well as NK cytotoxicity against B-CLL blasts *in vitro*. These findings suggest that induction of a potent anti-tumor immune response is a major component of lenalidomide action in B-CLL, and is a rare clinical demonstration of an immunotherapy strategy that can eradicate bulky disease. Drs. Bangia and Chanan-Khan (MTET) are now determining the mechanism by which lenalidomide modulates the immune microenvironment that tips the balance in favor of inducing/uncovering robust NK and T cells anti-B-CLL immune responses. These studies also demonstrate the translation of clinical observations back to the laboratory to determine underlying mechanism.

### James Clements, PhD

*Assistant Professor, Immunology*

Tumor infiltration by innate immune cells (in particular myeloid cells such as tumor infiltrating macrophages) has been shown to be an integral step in establishing an inflammatory and pro-tumor microenvironment. Furthermore, it has been shown that tumor-derived factors inhibit myeloid differentiation in animal models and cancer patients, blocking the generation of antigen presenting cells (in particular dendritic cells (DC)) and leading to an accumulation of immature myeloid cells which themselves are immunosuppressive. Both effects may play a significant role in the immunosuppressed state seen

in cancer patients. James Clements, PhD is examining mechanisms involved in myeloid cell migration and adhesion to the extracellular matrix (ECM), events that are essential for tumor infiltration by these cells. He has found that SLP-76 and Vav1 deficient dendritic cells have significant defects in integrin-dependent signaling pathways and manifest poor adhesion to components of the extracellular matrix and abnormalities in migration.

### Sharon S. Evans, PhD

*Professor, Immunology*

Sharon S. Evans, PhD in collaboration with Heinz Baumann, PhD has developed a highly productive program on the role of fever-range thermal stress in dynamically regulating lymphocyte – endothelial adhesion, a critical interface controlling lymphocytes trafficking into tissues. This group has established that lymphocytes traffic poorly to tumor sites under steady-state conditions, providing one explanation for the failure of the immune system to limit tumor progression. Over the past 5 years, Dr. Evans has dissected the thermal sensitivity of several major leukocyte adhesion molecules located both on the lymphocyte effector cells and also on the endothelial surface. Fever-range whole body hyperthermia in mice increased trafficking of circulating naive and central memory CD4 and CD8 T lymphocytes across vascular check points. Systemic thermal therapy (core temperature elevated to 39.5 °C) strongly upregulated the intravascular density of ICAM-1 on tumor vessels and high endothelial venules (HEVs) of lymphoid organs, causing a 5-fold and 2-fold increase, respectively, in ICAM-1-dependent homing of CD8 T cells.

In sharp contrast, normal vessels of extra-lymphoid organs (heart, kidney, liver, pancreas) were not responsive to thermal therapy as indicated by a failure to increase ICAM-1 intravascular display or improve ICAM-1-dependent CD8 T cell trafficking. The site-specific nature of the thermal response serves to increase the probability that antigen-restricted cytolytic CD8 T cells will encounter tumor targets within tissues. Intravital microscopy revealed a marked increase in the frequency of T cells undergoing ICAM-1-dependent firm adherence in both HEVs and tumor microvessels; firm sticking interactions were not detected in normal vessels under normothermic or hyperthermia conditions. Thermal stress improved the initial ICAM-1-independent tethering and rolling interactions of lymphocytes in tumor microvessels; these interactions were not altered by heat in HEVs or normal vessels. These studies identified a non-redundant role for the inflammatory cytokine IL-6, acting

in conjunction with a soluble form of the IL-6 receptor, in promoting ICAM-1-dependent trafficking of CD8 T cells in response to thermal stress. These data support the hypothesis that the unique cytokine-rich microenvironment of tumor tissues and lymphoid organs can be exploited therapeutically by inflammatory cues (e.g., systemic thermal therapy) to mobilize the recruitment of immune effector cells to sites that are relevant for the development of anti-tumor immunity.

### Kelvin P. Lee, MD

*Jacobs Family Chair in Immunology  
Vice Chair, Medicine*

Kelvin Lee, MD has been examining the molecular and signaling events by which tumors block myeloid dendritic cell differentiation. His group has shown that signal transduction through protein kinase C  $\beta$ II (PKC  $\beta$ II) plays a central role in initiating the DC differentiation in myeloid progenitors. They have subsequently found that tumor-secreted factors suppress regulate PKC  $\beta$ II gene expression *in vitro* and *in vivo*, which block the ability of these myeloid progenitors undergoing DC differentiation.

Dr. Lee's lab is also investigating the role of dendritic cells in actually supporting the survival of blood cancers, specifically the plasma cell malignancy multiple myeloma. Essential interactions between multiple myeloma (MM) cells and bone marrow (BM) stroma that are vital to MM survival, proliferation and chemotherapeutic resistance represent potentially novel therapeutic targets. Molecular and cellular characterization of these interactions are hindered by the complexity of the BM microenvironment. They have taken the approach of identifying pro-survival myeloma cell surface receptors and identifying corresponding ligands on BM stromal cells. Using this approach, the Lee Lab recently reported that in myeloma cells, direct activation of MM-CD28 transduces a pro-survival signal - consistent with previous clinical studies correlating MM-CD28 expression with disease progression and poor prognosis. They have discovered that the CD28 ligand (CD86) expressing counterparts were the myeloma cells themselves (CD86 has been separately reported to be a poor prognostic marker in myeloma) and dendritic cells (DC). The Lee Lab and others have found that DC selectively accumulate within the myeloma infiltrates of patient bone marrow *in vivo* and protect MM cells from death signals when co-cultured *in vitro*. Also, "backsignaling" via CD80/CD86 induces significant DC induction of the immunosuppressive tryptophan-catabolizing enzyme indoleamine 2, 3 dioxygenase (IDO) as well as the essential MM growth factor

IL-6. This has led to the hypothesis that dendritic cells are an essential component of the immunological microenvironment supporting myeloma cell survival as well as suppressing potential anti-myeloma immune responses. In this context their recent findings demonstrate that lenalidomide, which is thought to have activity in multiple myeloma through disruption of the MM microenvironment (via largely uncharacterized mechanisms), significantly disrupts DC differentiation from myeloid precursors.

### Kunle A. Odunsi, MD, PhD

*Professor, Gynecologic Oncology*

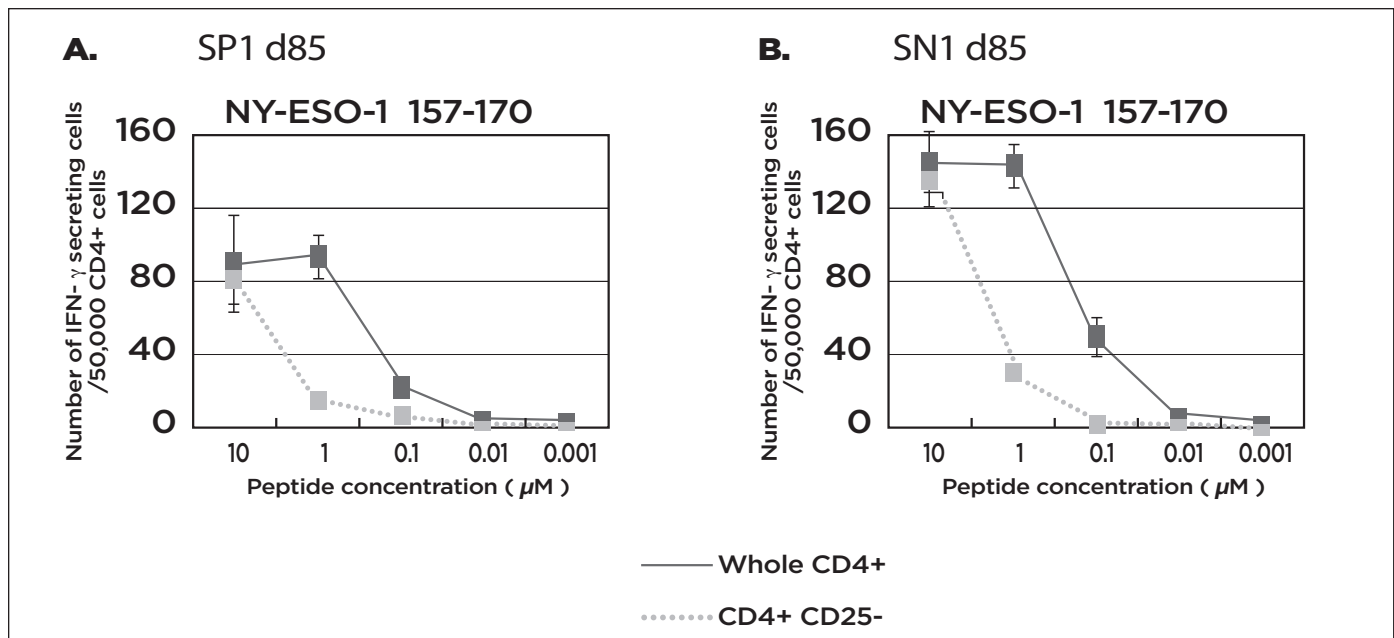
Drs. Odunsi and Karpf (MTET) have examined the tumor expression of NY-ESO-1 in ovarian patients following vaccination with the NY-ESO-1 peptide ESO<sub>157-170</sub>, and have found that 50% of the patients with recurrent/progressive disease had lost tumor expression of NY-ESO-1. However, *in vitro* treatment of ovarian cancer cells with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (decitabine) resulted in *re-expression* of the NY-ESO-1 gene, and these results are the basis for a newly initiated phase 1 trial of decitabine NY-ESO-1-based vaccine therapy in patients with recurrent epithelial ovarian cancer. In a second example, Drs. Ferrone and Karpf have found that DNA methylation is also a key regulator of HMW-MAA expression by human melanoma cells (Luo W *et al.*, *Oncogene* 2006; 25:2873) (RO1 CA105500). Together, these findings suggest that manipulating the epigenetic regulation of tumor antigen expression through molecular therapeutics (as has been done to enhance MHC/APM expression) may effectively enhance active immunotherapeutic strategies. The investigators involved in the study of cancer vaccines have focused on target antigen identification (antigen discovery), antigen characterization (extensive analysis at mRNA and protein level in tumors), immunogenicity (analysis of pre-existing or spontaneous immunity to the antigen and definition of MHC-restricted epitopes) and early phase clinical trials of vaccine reagents (peptides, proteins) and adjuvants.

Cancer vaccine approaches must stimulate robust, sustained, and integrated cellular and humoral immunity to cancer antigens. An example is represented by Dr. Odunsi's strategy in the development of cancer vaccines for epithelial ovarian cancer. In a large population study conducted in collaboration with Christine Ambrosone, PhD (CPPS), he found that intraepithelial CD8<sup>+</sup> tumor infiltrating lymphocytes and high CD8<sup>+</sup>/Treg ratio are associated with improved survival in epithelial ovarian cancer patients. These results indicate that efforts to stimulate

and/or augment anti-tumor immunity in ovarian cancer may be beneficial. Dr. Odunsi conducted an extensive search for target antigens that could be suitable for immunotherapy in ovarian cancer, focusing on the family of “cancer-testis” (CT) antigens. In a series of recent publications, Dr. Odunsi’s group has shown that the majority of ovarian tumors express at least one of these antigens, providing a framework for a multi-antigen vaccination strategy that may increase the diversity of the anti-tumor immune response, and counteract immune escape due to antigen loss variants.

To test whether providing cognate helper CD4<sup>+</sup> T cells would enhance the anti-tumor immune response in ovarian cancer, Dr. Odunsi conducted a phase I clinical trial in ovarian cancer of immunization with an NY-ESO-1 derived peptide of dual MHC class I and II specificities, combined with incomplete Freund adjuvant (IFA). He demonstrated induction of antigen specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses in the majority of immunized patients. Further, he showed the critical role of

CD4<sup>+</sup> T cell help in promoting effector differentiation and sustenance of CD8<sup>+</sup> T cells. In patients that relapse in spite of induction of T cell responses, Dr. Odunsi has shown at least two mechanisms of immune escape: development of antigen loss variants, and suppression of high-avidity effector T cells by CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells (see graph below). Subsequently, Dr. Odunsi has shown that diversified prime and boost vaccination using recombinant vaccinia and fowlpox expressing NY-ESO-1 efficiently induce antibody, CD4<sup>+</sup> and CD8<sup>+</sup> anti-tumor immune responses in ovarian cancer patients. Moreover, vaccine elicited Th1 effector cells were of higher avidity than Treg suppressed pre-existing effectors, which may enhance anti-tumor efficacy. Importantly, the immunization strategy appears to be associated with prolongation of disease free survival. In collaboration with Dr. Shrikant, Dr. Odunsi is testing and optimizing strategies to overcome the *in vivo* tolerogenic constraints of Tregs in a murine model of ovarian cancer.



#### Tregs suppress high-avidity pre-existing NY-ESO-1-specific CD4<sup>+</sup> Th1 cell precursors *in vitro*.

Whole CD4<sup>+</sup> T cells or CD4<sup>+</sup> depleted of Tregs were isolated from PBMC on day 85 of immunization with NY-ESO-1 DP4 peptide 157-170 (ESO157-170) and cultured with APC pulsed with ESO157-170. Fifteen to 20 days later, avidity of induced NY-ESO-1-specific Th1 cells was analyzed by ELISPOT assay using APC pulsed with graded doses of peptide. Preexisting NY-ESO-1157-170-specific T cells from patient SP1 were high-avidity, and could recognize as little as 0.1 $\mu$ M of peptide in the presence or absence of CD4<sup>+</sup>CD25<sup>+</sup> T cells (Figure a). In contrast, peptide vaccine-induced NY-ESO-1157-170-specific T cells derived from patient SN1 had much lower avidity compared to naturally occurring suppressed NY-ESO-1157-170-specific T cell precursors elicited from CD4<sup>+</sup>CD25<sup>-</sup> T cell population (Figure b). These results indicated that peptide vaccination did not overcome the Treg suppression of high-avidity pre-existing NY-ESO-1157-170-specific CD4<sup>+</sup> T cell precursors but rather expanded a repertoire of low-avidity CD4<sup>+</sup> T cells that are less sensitive to Tregs.

### Elizabeth A. Repasky, PhD

*The Dr. William Huebsch Professorship in Immunology*

Numerous investigators have shown that blood vessels that form within tumors are defective, resulting in leaky, non-perfused vessels and high interstitial fluid pressure, all of which contribute to poor penetration of drugs and immune effector cells and to tumor regrowth after therapy. Elizabeth Repasky, PhD oversees a successful program devoted to exploration of the role of body temperature on tumor growth, anti-tumor immunity and intra-tumoral vascular function. This research is yielding new information on the use of heat as an adjuvant for various cancer therapies, including immunotherapy. Dr. Repasky's laboratory was the first to show that several cellular and histological properties of tumors (including their growth rate, tumor vascular function and leukocytic infiltration); and activation of lymphocytes and several lymphocyte PKC isoforms are affected by fever-range, whole body hyperthermia. Her group has recently discovered that mild, whole body hyperthermia selectively enhances perfusion of tumor blood vessels and delivery of chemotherapeutic molecules.

In related investigations, Dr. Repasky's group has shown that systemic fever-range elevations in body temperature may also serve to reduce the activation threshold of immune effector cells (T cells and NK cells), and result in maturation of dendritic cells; this may help these cells to more efficiently deal with the presence of weak tumor antigens (Ostberg *et al.*, *Int J Hyperthermia* 2003; 19:520). They have shown that membrane lipid domains and positioning of NKG2D in human NK cells are correlated to enhanced cytotoxicity resulting from fever-range conditions. Circulating natural killer (NK) cells normally experience temperature gradients as they move about the body, but the onset of inflammation can expose them and their targets to elevated temperatures for several hours. Exposure of human peripheral blood NK cells and target cells to fever-range temperatures significantly enhances lysis of the target cells. Use of blocking antibodies revealed that this effect is dependent upon the function of both the activating receptor NKG2D and its ligand on tumor targets, MICA. These data reveal that NK cells and other immune cells possess thermally responsive regulatory elements that facilitate their ability to capitalize upon reciprocal stress-induced changes simultaneously occurring on target cells during inflammation and fever.

### Thomas B. Tomasi, MD, PhD

*M&T Bank Chair in Cancer Research  
Professor, Immunology*

Antigen processing and presentation by MHC in malignant cells can be restored by therapeutic manipulation. This has been the research focus of Thomas B. Tomasi, MD, PhD examining both inducible regulation by interferons as well as epigenetic regulation by DNA methylation/acetylation and micro-RNAs. Dr. Tomasi has shown that heat shock protein (Hsp) 90 is a major regulator of both type I and II interferon signaling. Janus kinases are client proteins of Hsp90 and a chaperone complex containing Hsp90, Hsp70 and Cdc37 is required for STAT-1 and STAT-2 phosphorylation. Deletion of Hsp90 with siRNA or a specific chemical inhibitor geldanamycin (GA) inhibits IFN- $\alpha$  and IFN- $\gamma$  inducible target genes including MHC class I antigens, CD40 and MHC class II antigens, while constitutive MHC class I and MHC class II antigen expression is not affected (Shang *et al.*, *J Biol Chem* 2006; 281:1876) (RO1 HD017013). At the epigenetic level, Dr. Tomasi's group has shown that treatment of J558 plasmacytoma and B16 melanoma tumors with deacetylase inhibitor enhances MHC class I antigen, MHC class II antigen and CD40 expression. These tumors then directly present antigens and become effective as an epigenetic vaccine. Surprisingly, immune gene expression (MHC and co-stimulatory) can be restored 'completely' (to B cell and macrophage levels) on several tumor cells by brief pulsing of an histone deacetylase inhibitor (HDACi) given prior to IFN- $\gamma$ . If this holds true for other cells it may be that major tumor escape mechanism that can be completely overcome by using HDACi plus cytokine, with significant implications for therapeutic strategies involving local and systemic HDACi.

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