Abstract
Although melanomas are substantially more immunogenic than other tumors, current immuno-
therapeutic approaches for melanoma patients have met with only limited success. Although
melanoma-specific CD8+ T-cell responses can often be generated in patients naturally or
through vaccination regimens, tumors frequently continue to grow unabated, suggesting that
tumor-specific immune responses may be actively dampened in vivo. Research over the past
decade has brought to light several mechanisms used by melanomas and other tumors to
suppress tumor-specific immune responses. These include the presence of regulatory immune
cells within the tumor microenvironment and draining lymph nodes that serve to shut down
effector T-cell function. In addition, melanoma tumors themselves express a number of soluble
and membrane-bound molecules that are responsible for inhibiting activated immune cells. The
identification of these suppressive mechanisms has provided significant opportunities for
designing novel therapeutic interventions that could augment current vaccination and adoptive
transfer approaches for treatment of melanoma.

Although melanoma is relatively immunogenic compared with other cancer types, it usually remains refractory to immuno-
logic control, despite large numbers of tumor-infiltrating lymphocytes often found naturally present in melanoma
lesions. Even in advanced melanoma, a majority of patients have circulating CD8+ T cells capable of secreting IFN-γ and
killing human leukocyte antigen (HLA)–matched or autologous tumor cells (1). High frequencies of circulating tumor
antigen–specific T cells can also be successfully generated in melanoma patients through vaccination with peptide, dendritic
cell, whole tumor cell, and viral vector-based vaccines. However, these T cells seem to be largely ineffective at inducing
disease regression or preventing progression (2–4).

It is now becoming clear that a large part of this problem is
due to events happening at the tumor site, rather than our
ability to break self-tolerance and generate effector T cells
systemically. No matter how many tumor-specific CTLs and
helper T cells we can generate through vaccination or
immunomodulation, some of which are very potent at killing
melanoma cells, many of these effector cells are essentially
“turned off” at the tumor site by a number of immunosup-
pressive mechanisms. This problem is exemplified by Zippe-
lis et al. (5), who found that stage III/IV melanoma patients
have high frequencies of in vivo primed Melan-A/MART-1–
specific CD8+ T cells; those circulating in the blood displayed
robust inflammatory and cytotoxic functions; however, CTL
isolated from tumor lesions (particularly in metastatic lymph
nodes) were functionally tolerant (5). By removing T cells
from the tumor microenvironment and expanding them
in vitro with cytokines such as interleukin 2 (IL-2), this aner-
genic phenotype can be reversed and cytolytic function
augmented; this accounts for the recent success of adoptive
T-cell therapy in melanoma using expanded tumor-infiltrating
lymphocyte populations.

Investigation of immunosuppression at the tumor site in
melanoma has gained significant ground recently through
study of animal models and analysis of human melanoma
lesions using newly identified molecular markers associated
with suppressor cell populations. These immunosuppressive
mechanisms include the action of regulatory immune cells
within the melanoma tumor microenvironment, direct im-
unosuppressive effects of melanoma tumor cells themselves
on activated, tumor-specific T cells, and indirect effects of
tumor cells on suppressing infiltrating T cells through the
activation and/or attraction of immune suppressor cells into
the tumor site.

Preclinical Evidence for a Role of CD4+ Regulatory
T Cells in Melanoma

Interest in regulatory immune cells has received a boost by
recent reports of striking clinical regressions in melanoma
patients when treatment with adoptively transferred, melanoma-
specific T cells was preceded by nonmyeloablative lympho-
depletion (6, 7). One possible mechanism underlying the
positive effect of lymphodepletion is the removal of T regulatory (Treg) and B cells. One subset of regulatory T cell, the CD4+CD25+Foxp3+ Tregs, has been particularly well studied in animal models of cancer immunotherapy, including adoptive T-cell therapy of melanoma.

Early experiments spearheaded by North et al. (8) clearly showed a dominant role of cyclophosphamide and radiation-sensitive CD4+ Tregs, then called suppressor T cells, in the transferable inhibition of spontaneous antitumor immunity. With recent developments improving our understanding of the identity and function of Treg cells (9, 10), these and some other decades-old observations have been explained. Turk et al. (11) showed that CD4+CD25+ Tregs in mice bearing established B16 melanoma tumors suppressed the ability of tumor-specific, CD8+ T cells to protect against a second melanoma tumor challenge. Administration of an agonistic antibody against glucocorticoid-induced tumor necrosis factor receptor, known to counter Treg-mediated suppression, led to enhanced tumor regression (12, 13). One important finding from this study was that although depletion of CD4+CD25+ Tregs augmented protective immunity, this augmentation was dependent on the presence of CD4+CD25− effector T cells. This suggests that complete CD4+ T-cell depletion is not the preferred method of eliminating CD4+CD25+ Tregs.

These findings were confirmed and extended in a careful study of the individual contributions of CD4+CD25+ Tregs and CD4+CD25− effector T cells during therapeutic T-cell transfer and vaccination (14). Here, Antony et al. (15) used the pmel-1 transgenic mouse strain in which nearly all CD8+ T cells recognize a peptide from the murine and human melanoma self-antigen, gp100. Using a suboptimal modification of a previously reported treatment regimen (16), they showed that adoptive transfer of pmel-1 CD8+ T cells followed by gp100 vaccination and IL-2 administration caused significant but incomplete tumor inhibition. The authors then showed that the complete absence of host CD4+ T cells, but not CD8+ T cells, greatly enhanced the activity of this treatment. However, when IL-2 administration was withheld, the benefit of CD4+ T-cell depletion was abrogated. Adoptive transfer of selected T-cell populations into T-cell-deficient recipient mice then revealed two critical requirements for successful immunotherapy by adoptive transfer and vaccination: (a) absence of CD4+CD25+ Tregs; and (b) IL-2, either produced endogenously by CD4+CD25− effector T cells or administered exogenously (16). These results help to explain the positive effect of prior lymphodepletion on the expansion and antitumor effect, in mouse and humans of adoptively transferred CD8+ T cells and IL-2 (6, 17–19).

Other studies have shown that elimination of Tregs can significantly improve the outcome of cancer immunotherapy in preclinical models. For example, Sutmuller et al. (20) showed that therapeutic whole-cell vaccination against melanoma was significantly more effective upon depletion of CD4+CD25+ Tregs with an anti-CD25 monoclonal antibody. They also showed that Treg depletion with anti-CD25 antibody carries an inherent risk of depleting tumor-specific effector CD4+ and possibly CD8+ T cells (both of which temporarily up-regulate CD25 upon activation), thus negatively affecting treatment efficacy. The beneficial effects of Treg depletion with anti-CD25 antibody has since been confirmed by others (21–23), but it seems that CD25 may not be the optimal therapeutic target because it is also expressed on effector T cells. In addition, CD25-targeted therapy may not eliminate Tregs as efficiently in humans as it does in mice (24).

Other therapeutic interventions that have been shown to reduce or eliminate the immunosuppressive activity of Tregs in mice are the administration of antibodies against either glucocorticoid-induced tumor necrosis factor receptor (12, 13) or LAG-3 (25). Stimulation through glucocorticoid-induced tumor necrosis factor receptor may render effector T cells insensitive to Treg-mediated suppression, whereas the mechanism underlying the effects of LAG-3-blockade is unknown. Finally, a recent report identified synthetic and natural Toll-like receptor-8 ligands that could directly reverse Treg inhibitory activity in vitro and in vivo (26). Together, these approaches may begin to form an array of therapeutic interventions to more precisely silence or eliminate “bad” CD4+ Tregs while sparing “good” tumor-specific, CD4+ effector T cells.

Regulatory Immune Cells in Human Melanoma

Although animal models have clearly shown a role for CD4+ Tregs in the inhibition of antitumor immunity, clinical studies have shown that CD4+ Treg cells may also play a significant role in human melanoma. The concept of Treg subsets residing intratumorally or systemically in cancer patients is not new, as reports on the presence of “suppressive” CD4+ T-cell clones in melanoma first appeared in the late 1980s. In 1989, Mukherji et al. (27) reported on a population of suppressive, tumor-reactive CD4+ T-cell clones from peripheral blood and lymph node metastases of melanoma patients. These suppressor cells were reactivated in vitro by autologous and allogeneic melanoma cells in an HLA-DR–restricted manner. This group later showed that the decline in antigen-specific CD8+ tumor-infiltrating lymphocyte activity in melanoma patients repeatedly vaccinated with dendritic cell pulsed with MART-1 and MAGE-1 peptides was correlated with an expansion of CD4+CD25+ IL-10–producing cells (28). Wang et al. (10, 29) have followed up on these observations by identifying the antigen specificity of two CD4+ Treg cell clones isolated from human melanoma lesions. One Treg clone reacted to the tumor antigen LAGE1, whereas another clone was found to be reactive against ARCT1. These results suggest that vaccination may lead to the expansion of melanoma antigen-specific CD4+ Treg cells, possibly due to the release of antigens from dying tumor cells. This, coupled with recent reports showing that high-dose IL-2 therapy can expand the population of circulating CD4+CD25+Foxp3+ Treg cells (30), indicates that tracking Tregs both systemically and intratumorally should be considered for all future melanoma vaccine and cell therapy trials.

In addition to the classic CD4+CD25+ Treg cells, whose mechanism of suppression depends on direct target cell contact, another population of CD4+ suppressor T cells, Tr1 cells, work largely through the secretion of high amounts of IL-10 and transforming growth factor-β (31). In a study of 12 patients undergoing curative resections for metastatic melanoma, Viguier et al. (32) found that Foxp3-expressing CD4+CD25high cells were overrepresented in metastatic lymph node and inhibited the function of tumor-infiltrating lymphocytes. These Treg cells displayed a polyclonal T-cell receptor Vβ repertoire and inhibited CD4+CD25− and CD8+ T-cell responses in a contact-dependent fashion. However, in some
cases, Tr1-like cell activity associated with IL-10 and transforming growth factor-β secretion was also found arising in both the CD4+CD25+ and CD4+CD25− subsets (32). Thus, both flavors of regulatory CD4+ T cells may arise in melanoma lesions, and it will be important to decipher whether they have unique or overlapping functions in suppressing antitumor CD8+ T-cell responses at different stages or locations of disease. It will also be important to identify the origin of tumor antigen-specific CD4+ Tregs; that is, whether they arise from natural Tregs that differentiate in the thymus or from CD4+CD25− naïve T cells that “immune deviate” into Tregs during tumor evolution.

The high concentration of Tregs in lymph node metastasis is also intriguing. It suggests that tumor spread into the lymph node might induce the recruitment or induction of regulatory CD4+ cells into the growing tumor mass, which are then activated against unique or shared tumor antigens. The activated Treg cells could then conceivably spread to tumors at other sites in the body, including the primary site. It will be important to determine if Treg function is specifically initiated in lymph node metastasis because it may help to explain why lymph node spread is such a bad prognostic marker for melanoma and other solid tumors.

Finally, also worth considering is the potential role of immunosuppressive immature myeloid cells and plasmacytoid dendritic cell in melanoma (33, 34). Immature myeloid cells (Lin−, HLA class IIlow) and immature granulocytes have been found in breast, prostate, renal, and lung cancer, with the latter producing high quantities of the enzyme arginase (Arg1; refs. 35–37). Immature myeloid cells are a heterogeneous population of cells associated with different stages of myeloid cell differentiation with low or absent HLA class II expression (35, 38). Overall, these cells are associated with a dysregulated cytokine environment in patients, especially those with epithelial tumors secreting granulocyte macrophage colony-stimulating factor and vascular endothelial growth factor (38). Apart from producing reactive oxygen intermediates inhibiting T-cell activation and promoting apoptosis, immature myeloid cells also act as tryptophan and arginine sinks through the action of 2,3-dioxygenase and arginase (34, 37, 39). Depletion of these amino acids severely impairs the expression of key signaling proteins in T cells, such as the T-cell receptor ζ chain of the T-cell receptor complex. Plasmacytoid dendritic cells have similarly been implicated in many different tumor types, especially in breast cancer where the presence of these cells is correlated with increased IL-10 production by CD4+ T cells and with poorer prognosis (40). Although virally activated plasmacytoid dendritic cells are capable of producing large amounts of type I IFNs, which can enhance inflammation and adaptive immunity, in the tumor microenvironment immature plasmacytoid dendritic cell precursors may pervade and play a immunosuppressive role. The role of immature myeloid cells and plasmacytoid dendritic cell in human melanoma is largely unknown, and it remains to be determined how they contribute to intratumoral immunosuppression or activation.

Immunosuppression by Tumor-Derived Mediators

Another source of immunosuppressive activity originates directly from the tumor cells themselves. This is illustrated effectively by the murine melanoma B16, which, in addition to being poorly recognized by T cells due to low expression of MHC class I molecules, has evolved multiple mechanisms that actively suppress immunity through the production of a host of soluble and membrane-bound factors. For example, B16 expresses galectin-1, which can interact with poly-N-acetylated-lactosamine–containing molecules on T cells and extracellular matrix components, inducing T-cell growth arrest and apoptosis, blockade of inflammatory cytokine production, and T-cell adhesion (41). B16 melanoma can also induce the local inhibition of T-cell immunity through production of the enzyme arginase (42). In addition, B16 cells also express constitutively active signal transducers and activators of transcription 3 (STAT3), resulting in the production of soluble factors that inhibit the maturation of dendritic cells, thus limiting the priming of tumor-specific T cells in the tumor-draining lymph node (43). All of these mechanisms have also been described in melanoma, including suppressive cytokines, release of nitric oxide through induction of inducible nitric oxide synthase in melanoma (44), and the release of soluble ligands inhibiting natural killer and CTL function (reviewed in ref. 45).

Human melanomas have for many years been shown to produce IL-10 that results originally from altered metabolism in melanocytes induced by UV-B irradiation. IL-10 production by tumors, along with other immunosuppressive factors, such as vascular endothelial growth factor, seems to be regulated by a high constitutive STAT3 phosphorylation and activation in tumor cells. Vascular endothelial growth factor inhibits dendritic cell differentiation from CD34+ progenitor cells, whereas IL-10 acts later by inhibiting normal dendritic cell maturation pathways and inducing the generation of tolerizing dendritic cell that skew CD4+ and CD8+ T-cell responses toward a Th2 or Tc2 pattern of cytokine production, limiting cytolytic capacity (46, 47). IL-10–treated dendritic cell can also induce T-cell unresponsiveness (anergy) associated with increased CTLA-4 expression on T cells (48, 49).

Another important cytokine-inhibiting antitumor T-cell activity is transforming growth factor-β. Transforming growth factor-β can specifically inhibit the expression of five cytolytic gene products in tumor-associated CTL, namely, perforin, granzyme A, granzyme B, Fas ligand, and IFN-γ (50). Although the inhibitory effects of transforming growth factor-β are well established in mouse models, its role in human melanoma has not yet been firmly established.

Human melanoma cells can also express and secrete bioactive soluble Fas ligand, APO2-ligand/tumor necrosis factor–related apoptosis-inducing ligand, and NKG2D-ligand/MIC-A/B that either block natural killer and CTL-activating receptors (soluble tumor necrosis factor–related apoptosis-inducing ligand and MIC-A/B) or can induce the apoptosis of activated human T cells (Fas ligand; refs. 51–54). Soluble NKG2D-L expression is enhanced by proinflammatory cytokines like IFN-γ and represents, together with other IFN-γ–mediated negative regulators, how the local intratumoral immune response may lead to rebound suppressive effects in tumor cells. Although the role of Fas ligand and tumor necrosis factor–related apoptosis-inducing ligand in “immune privilege” of tumors has been questioned because of the shown proinflammatory role of the Fas pathway (51), recent studies have found that human melanoma cells contain Fas ligand in...
chronically activated CD8+ T cells during chronic viral (68, 69). Recently, the PD-1 pathway has been shown to be described B7 molecules, such as B7S1 (71), on tumor cells activation, the effects of blocking B7-H1 and other newly metastasis and enhanced CD8+ T-cell responses against CT26 s.c. tumors to the liver. Similar results were obtained using B16 melanoma in C57BL/6 mice and facilitates the spread of PD-1–deficient mice, Iwai et al. (68, 69) showed that PD-1 facilitates T-cell apoptosis in the presence of IL-10. Using inhibits effector T-cell restimulation and CTL function and the programmed death (PD) receptor PD-1 on activated effector T cells (67). PD-1 has been found to be expressed on chronically activated “exhausted” CTL, and its ligation inhibits effector T-cell restimulation and CTL function and facilitates T-cell apoptosis in the presence of IL-10. Using PD-1-deficient mice, Iwai et al. (68, 69) showed that PD-1 signaling on host T cells attenuates T-cell responses against B16 melanoma in C57BL/6 mice and facilitates the spread of s.c. tumors to the liver. Similar results were obtained using the CT26 colon cancer model in BALB/c mice, where the same group showed that blockade of PD-1 signaling using anti–PD-1 monoclonal antibody injection prevented liver metastasis and enhanced CD8+ T-cell responses against CT26 (68, 69). Recently, the PD-1 pathway has been shown to be especially critical in inducing the unresponsiveness of chronically activated CD8+ T cells during chronic viral infection in mice (70). Blockade of PD-1 can overcome this CD8+ effector cell “exhaustion.” Because tumor-infiltrating effector cells and effector memory cells also undergo chronic activation, the effects of blocking B7-H1 and other newly described B7 molecules, such as B7S1 (71), on tumor cells and inhibitory myeloid cells will need to be investigated.

Where Do We Go from Here?

The wide spectrum of immune evasion strategies used by human cancers suggests that effective immunotherapies will require multifaceted approaches to circumvent the dominant mechanisms of immunosuppression present within individual cancer patients. To accomplish this, we need to develop standardized methods that can reliably assess the mechanisms of suppression within the tumor microenvironment so that novel treatments can be both designed and administered in a rational manner.

As mentioned, nonmyeloablative lymphodepletion regimens have proved to be beneficial in augmenting antitumor responses following adoptive T-cell transfer (6, 7). However, it will be important to develop new clinical reagents that will more precisely eliminate specific regulatory immune cell subsets in vivo, without harming beneficial immune effector cells. The usefulness of CD4+CD25+Foxp3+ Treg cell depletion using anti-CD25 and Ontak (IL-2-diphtheria toxin conjugate) is controversial, with some studies showing no benefit (24) in tumor antigen–vaccinated melanoma patients and others showing marked enhancement of tumor-specific CTL responses in vaccinated renal cell cancer patients (72). More clinical trials are needed to address this question, along with whether agents like cyclophosphamide, touted to deplete Treg cells, can act synergistically with these other agents. Both the dose and timing of application of these and other agents need to be addressed when examining their effects on the intratumoral T-cell response in melanoma.

Combinations of Treg cell inhibitors with agonists of tumor necrosis factor receptor family costimulatory molecules, like OX40 and 4-1BB, also hold promise to further enhance the survival and effector function of CD8+ and CD4+ T cells in melanoma lesions and inhibit Treg cell function (73–75). In addition, Toll-like receptor ligands may hold much promise for inhibiting the function of Treg cells (26). Thus, combinatorial regimens using the correct adjuvant combination with modulators boosting initial tumor-associated antigen–specific T-cell expansion may prove to be a more effective strategy to elicit antitumor T-cell activation. However, additional agents may still be required that overcome effector T-cell suppression and antigen-presenting cell dysfunction inside the tumor by either shutting off suppression or making T cells resistant to inhibition through enhanced intratumoral costimulation and effector cell survival. This latter intervention will also require a thorough characterization of negative costimulatory pathways acting on effector cells and effector memory cells within tumors that shut off Treg cells.

Likewise, it will be critical to develop clinical reagents that are capable of blocking immunosuppression at the molecular level. One candidate suppressor switch system operated through the STAT3 transcription factors has recently emerged in melanoma (76, 77). STAT3 is constitutively phosphorylated and activated in a variety of tumors, mediates the secretion of immunosuppressive cytokines, and promotes angiogenesis. Interestingly, STAT3 is also constitutively activated in dendritic cells, T cells, and natural killer cells in tumors and tumor-draining lymph node, leading to functional unresponsiveness. Kortylewski et al. (78) showed that shutting off STAT3 in these cells using a systemic STAT3 inhibitor (CPA-7), or targeted gene deletion in transgenic mice with the Cre-Lox system, restored antitumor T-cell activity correlated with restoration of normal dendritic cell function. It will be interesting to follow how the STAT3 inhibitor drug field progresses in the coming years and whether these drugs can be effectively combined with other immunomodulators, such as anti-CTLA-4 and adjuvants given during T-cell priming with tumor antigens.

In conclusion, the recent characterization of immunosuppressive mechanisms in human cancers has generated much hope and provided several new strategies with which to explore new methods of improving immunotherapy. We have come to the realization that equal effort needs to be placed on maintaining T-cell responses at the tumor site as on priming T cells and breaking tolerance. Thus, in addition to “immune-initiating”
agents that help stimulate and expand T cells, a separate group of “immune maintenance” agents need to be tested in the clinic that can facilitate T-cell survival, migration of T cells into the tumor bed, and sustain effector function at the tumor site. New clinical trials will need to more comprehensively address these two phases of the antitumor immune response.

### Open Discussion

**Dr. Atkins:** In the Cytokine Working Group led by Marc Ernstoff from Dartmouth, we’re doing a trial of lymphodepletion followed by high-dose IL-2. In the first eight patients, there have been three responses.

**Dr. Hwu:** That’s interesting to do lymphodepletion plus IL-2 alone, thinking that you have resident cells that you’re going to skew toward a T-cell population more reactive against tumor. You’re hypothesizing that the regulatory cells are going to be more sensitive to the chemotherapy than are effector cells. I’d love to see the results.

**Dr. Atkins:** It’s possible that giving IL-2 diphtheria toxin first may be an easier way of skewing the population if it were selective for regulatory cells.

**Dr. Hwu:** If you then immunize or try to stimulate effector cells in vivo, it’s still possible that you can regenerate antigen-specific regulatory cells.

**Dr. Sosman:** Is the overall impression at the National Cancer Institute that there is a difference clinically between vaccinations with and without lymphodepletion? Does CTLA-4 antibody really enhance vaccination?

**Dr. Hwu:** It was a single-arm trial with a GP-100 209-2 M peptide and a GP-100 2809 V peptide, both of which are good at immunizing. On our simplest assays, they were both already at maximum levels, and it wasn’t clear that there was a difference. I don’t know if they’ve gone back to look at more stringent assays at the NIH. There was no difference using the most sensitive assays, but I think it’s because they started with immunogens that were already strong.

**Dr. Mier:** Is there an indoleamine 2,3-dioxygenase knockout? What’s the phenotype?

**Dr. Gajewski:** There is no overt phenotype and they’re fertile. In normal wild-type pregnant female mice, if you give 1-methyltryptophan the mother rejects the fetus. But in the indoleamine 2,3-dioxygenase knockout mouse, if you give 1-methyltryptophan, they don’t reject. There is some compensatory mechanism in place that compensates for the absence of indoleamine 2,3-dioxygenase.

**Dr. Hwu:** It might be because there is so much redundancy in the system. You knock out only one immunoregulatory pathway and it may not affect anything.

**Dr. Gajewski:** It would be interesting to know what the compensatory mechanism is.

**Dr. Ross:** Because these regulatory mechanisms are inherently so strong, it’s easy to hypothesize that the lymphodepletion approach would just be transient. How do you maintain the therapeutic advantage over time?

**Dr. Hwu:** There are a variety of regulatory cells, and in this case we’re just getting rid of everything. But they can come back, and you can develop regulatory cells following immunization. It’s possible that we’re going to continue to develop them. We need better markers to try to get rid of those populations more specifically and also better understand the context in which the immunoregulatory cells develop. That is what we’re trying to do with our adoptive therapy studies. If we grow T cells in specific cytokines, depending on the cytokine, we may get different levels of Fox-P3. We need to understand this more to try to prevent the outgrowth of regulatory cells.

**Dr. Atkins:** With regard to the CTLA-4 antibody presentation at the American Society of Clinical Oncology meeting, Dr. Reubin from M.D. Anderson presented that patients with autoimmunity but no tumor response were shown to have increased effector T-cell PD-1 expression, suggesting that this B7H1/PD-1 interaction was an alternative regulatory pathway that prevented tumor regression despite generating autoimmune response. Because melanoma cells can express B7H1, it suggests that you may need to block both to get higher than a 15% response in that population. Can you comment on that?

**Dr. Hwu:** There were a low number of responders on this study, and from patient to patient the error bars were wide. Those data are preliminary and we still have to look at this more closely. The fact that these are two redundant pathways underscores the importance of blocking multiple pathways and we can’t ignore PD-1.

**Dr. Gajewski:** The other interesting factor is that the reproducible effect on peripheral blood lymphocytes is on CD4-positive cells rather than CD8-positive cells as a dominant effect, with a decrease in the number of regulatory phenotype CD4s and an increased number of activated effector CD4s.

**Dr. Hwu:** If you look at the CTLA-4 knockout mouse, that is also consistent because the CD4 cells are dominant. The NIH group crossed the pmel CD8+ transgenic with the CTLA-4 knockout and not a lot happened. The dominant effect of CTLA-4 may be on CD4s.

**Dr. Gajewski:** We don’t want to believe that the anti-CTLA-4 antibody is acting predominantly at the level of regulatory cells, but it’s a possibility still in the clinical setting, although it’s not in the mouse models. The anti-CTLA-4 antibody works in CD25-depleted animals. It still has therapeutic efficacy, but perhaps there is a different mechanism in the human studies.

**Dr. Hwu:** Except it’s such a strong inhibitor of the effector cell. It is likely working right on the effector cell rather than depleting regulatory cells.

**Dr. Mier:** Do you know if the Treg phenotype is malleable and reversible? In other words, what could you do to an isolated CD4, CD25 bright cell to ablate the suppressor function?

**Dr. Hwu:** We were thinking about trying to transfect T cells with small interfering RNAs against Fox-P3 to see if we turned off the Fox-P3 and reversed immunoregulation.

**Dr. Sosman:** Is there a link between CTLA-4 responses and some polymorphisms?

**Dr. Gajewski:** Jeff Weber published that there are CTLA-4 polymorphisms that are associated with autoimmunity in untranslated regions. Those autoimmune-prone polymorphisms were linked to response and autoimmunity with antibody blockade.

**Dr. Mier:** Are the polymorphisms associated with variances in expression?

**Dr. Gajewski:** They have been reported to in other studies.
References


71. Prasad D, Richards VS, Mai XM, Dong C. B7S1, a novel B7 family member that negatively regulates T cell activation. Immunity 2003;18:863–73.


Immunosuppression in Melanoma Immunotherapy: Potential Opportunities for Intervention

Gregory Lizée, Laszlo G. Radvanyi, Willem W. Overwijk, et al.