Dartmouth-Hitchcock

THE HITCHCOCK FOUNDATION
1 Medical Center Drive
Lebanon, NH 03756-0001
Phone (603) 653-1231
Fax (603) 653-1111
Michael.Shoob@Dartmouth.EDU

APPLICATION FOR RESEARCH GRANT

TITLE OF RESEARCH: **The role of vitiligo in curative melanoma immunotherapy**

Amount requested **$30,000** Dates of study: Begin **11/1/11** (m/d/y) End **10/31/12** (m/d/y)

PRINCIPAL INVESTIGATOR

Name **Mary Jo Turk**

Title **Assistant Professor**

Department **Microbiology and Immunology and the Norris Cotton Cancer Center**

Name of Department Chair **William Green**

Institution **Dartmouth Medical School**

Mailing address of research office **One Medical Center Drive, Rubin Building 732**

Telephone No. **653-3549**

Percent of time to be devoted to project **15% Effort**

CO-INVESTIGATOR(S)

Name	Title	% Time
Andrea Boni, MD	**Pathology Resident**	**3%**

I have read and fully understand the terms and conditions of this application.

Signature _____
Applicant

Approved by _____
Department Chair Signature (Blue Ink)

NOTE: Human Subjects or Institutional Animal Care and Use Committee endorsement must be on file before funds will be released. See page 5, sections D and E for details.

LAY LANGUAGE SUMMARY

The newest, most promising immunotherapies for cancer involve a technique known as adoptive T cell therapy (ACT). Patients treated by ACT receive infusions of large numbers of T cells that are capable of killing tumor cells all over the body. In certain patients, ACT has been shown to eliminate advanced metastatic disease. However the reasons for success of ACT in some patients, but not others, are not well understood. ACT has been most well studied in patients with melanoma. Melanoma is a cancer of the pigment-producing cells in our skin, called melanocytes. Interestingly, melanoma patients that respond best to ACT have a high chance of developing white patches of skin; a condition called vitiligo. Vitiligo is evidence that T cells are cross-reacting with normal melanocytes, which share many proteins with cancerous melanoma cells. Vitiligo is an otherwise harmless autoimmune condition, which has long been viewed as a side-effect of effective immunotherapies for melanoma. However, recent studies from our laboratory now provide compelling evidence that vitiligo can also directly support T cell responses to melanoma. Based on this, we hypothesize that vitiligo is a key contributing factor to the effectiveness of adoptive T cell therapy against melanoma. To test this hypothesis, we will investigate adoptive T cell therapies for melanoma in mice that lack melanocytes, and therefore cannot develop vitiligo. These studies are expected to yield important mechanistic insight into the factors supporting the most potent new immunotherapies for cancer.

ABSTRACT

A link between tumor immunity and autoimmunity has been recognized for over 30 years, although the exact relationship between these two phenomena has remained evasive. We have recently discovered that melanocyte destruction is *required* for the maintenance of existing CD8 memory T cell responses to melanoma. Our work shows that vitiligo maintains melanoma/melanocyte Ag-specific memory T cells that do not become functionally exhausted >600 days after priming, and provide long-lived tumor protection. These studies establish a causal relationship between autoimmunity and long-lived anti-tumor immunity. However the relevance of these findings for melanoma patients receiving contemporary immunotherapies is not yet known. Adoptive T cell therapies (ACT) have demonstrated a remarkable ability to induce melanoma regression and profound vitiligo in human patients and mouse models, although vitiligo has been largely overlooked as a side effect of therapy. The proposed studies will now determine the extent to which autoimmune melanocyte destruction supports the curative effects of ACT, and the long-term persistence of transferred T cells. To uncouple tumor immunity and autoimmunity, we will employ the melanocyte-deficient W^{sh} mouse model, recently validated in our laboratory. Studies will exploit the B16 transplantable melanoma model, as well as the *Braf*^{V600E}/*Pten*$^{-/-}$ (Braf/Pten) inducible model of metastatic melanoma. Studies in Braf/Pten tumor-bearing mice, will also investigate the role of vitiligo in hosts receiving the $Braf^{V600E}$-inhibitor PLX4032, a drug that has revolutionized the treatment of metastatic melanoma patients just in the past year. These studies will provide critical new insights into the autoimmune requirements of curative cancer immunotherapies.

SPECIFIC AIMS

The autoimmune destruction of melanocytes (i.e. vitiligo) has long been recognized as a positive prognostic factor for melanoma patients.[1-3] Despite this, vitiligo has repeatedly been disregarded as an unimportant "side effect" of effective melanoma immunotherapies. Our recent work now challenges this notion by demonstrating that autoimmune melanocyte destruction is actually required for the maintenance of long-lived memory CD8 T cell responses to shared melanoma/melanocyte antigens in mice.[4] We have shown that treatment of B16 melanoma tumor-bearing mice to deplete immunosuppressive regulatory T cells (T_{reg}), followed by surgery to excise primary tumors, leads to the development of protective CD8 T cell memory against melanoma/melanocyte antigens, but only as a result of coincident CD8 T cell-mediated melanocyte destruction.[4, 5] These studies reveal that melanocyte antigen provided in the context of autoimmune vitiligo is a fundamental requirement for maintaining functional, non-exhausted, memory T cell responses to melanoma.[4] However the broad applicability of this finding to patients receiving immunotherapy for melanoma is not yet known.

The adoptive transfer of CD8 T cells is currently one of the most promising immunological therapies for cancer, and has demonstrated impressive efficacy in a subset of melanoma patients. Importantly, certain melanoma patients that respond well to adoptive T cell therapy (ACT) also develop autoimmune vitiligo. This association is mirrored in mouse models, where adoptive T cell therapy yields durable cure of established B16 melanoma tumors, exclusively accompanied by profound and wide-spread vitiligo. Based on our preliminary studies, we now *hypothesize that vitiligo is a key requirement for curing melanonoma tumors in hosts receiving adoptive T cell therapy*. To test this hypothesis, we will determine whether curative memory T cell responses to melanoma are lost in hosts that cannot develop vitiligo.

Specific Aim 1: Determine if vitiligo is required for curative adoptive T cell therapy in the B16 melanoma model. The B16 model is, by far, the most widely used melanoma model in immune competent mice (C57BL/6 origin). B16 tumors are poorly-immunogenic and highly-aggressive, and have served as the gold-standard in melanoma tumor immunotherapy studies for nearly 30 years. We will treat B16 tumors with melanoma antigen-specific CD8 T cells taken from pmel T cell receptor transgenic mice. Pmel CD8 T cells recognize both melanoma cells and melanocytes through their specificity for the melanocyte differentiation antigen gp100 (the pmel T cell receptor recognizes $gp100_{25\text{-}33}$ presented in the context of D^b).[6] Pmel cells have been shown to induce rejection of large established B16 melanoma tumors, concurrent with the development of widespread vitiligo in wild-type C57BL/6 mice.[6] However the importance of vitiligo for therapeutic durability is not known. To address this, we will use pmel cells to treat B16 melanoma tumors in our newly-validated model of melanocyte-deficient W^{sh} mice, which are unable to develop vitiligo.[4] In melanocyte-deficient mice, it is expected that pmel T cells will initially reject B16 melanoma tumors, but then fail to survive and mediate durable tumor cure. These findings would reveal a previously unrecognized need for normal melanocytes and vitiligo to support curative adoptive T cell therapy of melanoma.

Specific Aim 2: Determine if vitiligo is required for curative adoptive T cell therapy in the Braf/Pten melanoma model. Transplantable tumor models, although the standard for many years, have also been criticized for their lack of clinical relevance. This Aim will employ the genetic Braf/Pten model of inducible, autochthonous, metastatic melanoma, which our laboratory has recently bred onto a C57BL/6 background. In Braf/Pten mice, inducible melanocyte-specific expression of the oncogene $Braf^{V600E}$, coupled with loss of the tumor suppressor *Pten*, results in localized out-growth of melanoma in the dermis, followed by spontaneous metastasis to lymph nodes and lungs.[7] Because $Braf^{V600E}$ expression and Pten loss have both been identified in humans with melanoma[8], this is arguably the most clinically-relevant model of melanoma currently available. Furthermore, our preliminary data demonstrate that the $Braf^{V600E}$ inhibitor PLX4032, a molecularly targeted drug that has recently revolutionized treatment for patients with metastatic melanoma[9], can efficiently arrest growth of Braf/Pten tumors *in vivo*. We now propose to optimize combination treatment of Braf/Pten tumors with pmel CD8 T cells, in conjunction with the Braf inhibitor PLX4032, to durably cure metastatic Braf/Pten tumors. To determine the role of vitiligo in supporting these protective T cell responses, therapy will be administered to melanocyte-deficient mice that cannot develop vitiligo, as described for Specific Aim 1. These studies will reveal whether ACT and Braf inhibition can function synergistically to treat aggressive metastatic melanoma, and will further define the role of vitiligo in this combined therapy setting.

BACKGROUND AND SIGNIFICANCE

Melanoma is a significant clinical problem. Both incidence and death rates for melanoma have been rapidly climbing over the past 35 years.[10] With 70,000 estimated new cases in 2011, melanoma represents almost 5% of cancers in the United States.[10] The major curative treatment for solid cancers is surgery, although post-surgical tumor recurrence and metastasis remain formidable problems. For melanoma, even stage II (non-metastatic) disease recurs frequently, carrying a 30-40% mortality rate over 5 years.[11]

For patients with metastatic melanoma, IL-2 and dacarbazine have historically been the only viable treatment options, with response rates ranging only from 10-20%.[12] However, a better understanding of the genetic basis of melanoma has more recently lead to the development of effective molecularly-targeted therapies for melanoma. The most promising of these drugs targets the $Braf^{V600E}$ mutation, found in as many of 60% of all melanomas.[8] The $Braf^{V600E}$-specific inhibitor PLX4032 induces metastatic melanoma regression in a remarkable 81% of patients harboring this mutation.[9, 13] One downfall of Braf-targeted therapy is the eventual development of resistance, usually taking place within the first year of treatment.[13] Because of this, it has become clear that Braf-inhibitors will need to be combined with other types of therapy to ensure long-lasting melanoma control.

Mouse models of melanoma immunotherapy. Melanoma has historically been the most widely used model for the study of immunotherapy. Studies with the transplantable B16 mouse melanoma model have demonstrated that CD8 T cells respond to defined MHC-restricted epitopes from a family of melanoma-expressed melanocyte differentiation antigens including tyrosinase and related proteins TRP-2 and gp100.[14] These proteins are involved in melanin synthesis and are expressed by both malignant melanoma cells and normal melanocytes.[15] The creation of pmel T cell receptor (TCR) transgenic mice, in which all CD8 T cells respond to the MHC I-restricted epitope $gp100_{25\text{-}33}$, has enabled the treatment of established B16 melanoma in mice by adoptive T cell transfer.[6] Pmel T cells also express the congenic marker Thy1.1, enabling their sensitive tracking by flow cytometry.

More recently, the Braf/Pten model of inducible metastatic melanoma has been generated by our collaborator, M. Bosenberg, at Yale (see letter).[7] In the Braf/Pten melanoma model, tamoxifen-inducible, tyrosinase-driven $Braf^{V600E}$ expression, and loss of Pten, results in localized development of metastatic melanoma in the dermis.[7] Braf/Pten tumors express the $Braf^{V600E}$ mutation, and our preliminary data demonstrate that tumor growth can be arrested by the $Braf^{V600E}$-specific inhibitor PLX4032. Importantly, the $Braf^{V600E}$ mutation is only harbored by cancer cells, and work by our collaborator Andrea Boni has shown that PLX4032 treatment increases melanoma cell susceptibility to cytotoxic T cell killing *in vitro*.[16] Based on this, the present studies will combine PLX4032 treatment with adoptive T cell therapy in the Braf/Pten tumor model.

Tumor immunity and autoimmunity are closely linked. Vitiligo, or the autoimmune destruction of melanocytes, is a positive prognostic factor for melanoma patients.[2, 3, 17] The condition affects ~3% of melanoma patients [17], although its incidence is increased by immunotherapies that improve survival, including adoptive T cell therapy [18, 19], and high-dose IL-2.[20] Melanoma-associated vitiligo is manifested as depigmented patches of skin or hair, which are infiltrated with melanoma/melanocyte antigen-specific CD8 T cells.[21, 22] This has lead to the assumption that vitiligo, in humans, is mediated predominantly by CD8 T cells. In mouse studies, adoptive T cell therapies and vaccines that induce robust CD8 T cell responses to melanocyte antigens expressed by B16 melanoma also often result in vitiligo.[23] These findings suggest that vigorous, protective T cell responses to melanoma can cross-react with melanocytes, thereby causing autoimmunity. However, until recently, studies had failed to distinguish whether vitiligo itself was critical for the development of immune responses to melanoma.

Vitiligo supports memory CD8 T cell responses to shared melanoma/melanocyte antigens. The generation of memory T cell responses to tumor antigens is a fundamental goal of tumor immunotherapy.[24, 25] Memory CD8 T cells can control large established tumors [26, 27] and provide long-lived protection following surgery.[5] However, because most tumor antigens are also self-antigens, the requirements for generating long-lived and functional memory T cell responses to tumors have remained elusive. Most of our knowledge of T cell memory derives from studies of pathogens, in which target antigens are foreign proteins that are cleared by the immune system.

We have recently made major progress in the field by showing that memory CD8 T cells responses to the antigens gp100 and TRP-2 cannot be maintained in the absence of vitiligo.[4] Our studies show that vitiligo-affected mice maintain robust populations of functional, pmel CD8 T cells (transgenic T cells specific for $gp100_{25\text{-}33}$), for longer than 600 days following their initial encounter with melanoma.[4] These cells acquire and maintain an activated, but non-exhausted, effector memory (T_{EM}) phenotype, characterized by the expression of high levels of CD44, but low levels of CD62L, and the ability to produce IFN-γ but not IL-2.[4] In contrast, melanocyte deficient *c-Kit-W^{sh}* (W^{sh}) mice that cannot develop vitiligo, develop vanishingly small populations of memory T cells with a more quiescent central memory T_{CM} phenotype ($CD44^{hi}$ $CD62L^{hi}$).[4] Despite this, transfer of pmel T cells from melanocyte-deficient mice into wild-type mice with vitiligo restores the robust T_{EM} response, demonstrating that memory can be restored by the re-introduction of vitiligo.[4] Furthermore, provision of gp100 antigen to melanocyte-deficient W^{sh} mice also rescues robust T_{EM} responses.[4] These studies collectively show that melanocyte antigen liberated by vitiligo supports robust, functional CD8 T cell responses to shared melanocyte/melanoma antigens (Figure 1). These published experiments were conducted in mice that were surgically cured of B16 melanoma tumors. However the importance of vitiligo in hosts receiving curative melanoma immunotherapies is not yet known.

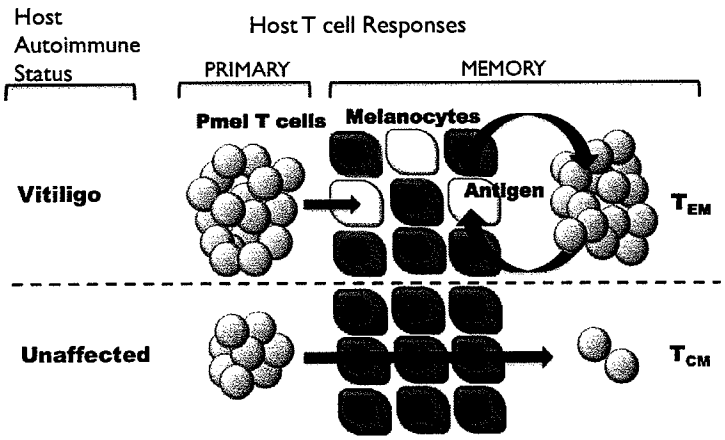


Figure 1. Model depicting the influence of melanocyte destruction (vitiligo) on long-lived CD8 T cell responses to melanoma/melanocyte antigens.

Adoptive T cell therapy (ACT) is a promising new strategy for treating metastatic melanoma. Adoptive T cell therapy (ACT) has demonstrated impressive efficacy in recent clinical trials in melanoma patients.[20] Objective clinical response rates of 51% have been reported in melanoma patients receiving lymphoablative chemotherapy [28], and as high as 72% in patients receiving myeloablative conditioning.[29] Adoptive T cell therapy with TCR Tg CD8 T cells also induces dramatic B16 tumor regression in the mouse B16 melanoma.[6] Importantly, in both mice [6, 30, 31] and humans[18, 19], response to ACT is associated with the development of vitiligo. Vitiligo has historically been viewed as a side-effect or even an adverse effect of therapy. The proposed Aims will now determine to what extent vitiligo supports the curative effects of ACT. Our newly validated W^{sh} model of melanocyte deficiency will, for the first time, enable the uncoupling of tumor immunity and autoimmunity in hosts receiving curative immunotherapies.

INNOVATION

Concepts: This work will test the provocative hypothesis that autoimmunity is required for the curative efficacy of tumor adoptive T cell therapies.

Tools: These studies will make extensive use of the $TyrCreER^{t}$::$Braf^{CA}$::$Pten^{lox/lox}$ (Braf/Pten) model of inducible melanoma, a gift from our collaborator M. Bosenberg.[7] Because *$Braf^{V600E}$* mutation and *Pten* loss contribute to melanoma tumorigenesis in humans, this is arguably the most clinically relevant model of mouse melanoma currently available.[32] To our knowledge, we are the first laboratory to have reassembled this model on a C57BL/6 background, and we have already demonstrated that pmel T cells are capable of responding to these tumors (see preliminary data). These studies also rely upon the *c-Kit^{Wsh}* (W^{sh}) model of melanocyte deficiency, which our laboratory recently established for the study of immune responses to melanoma.[4] This is currently the only known viable/fertile model of melanocyte deficiency [33], and will be crucial for uncoupling tumor immunity and autoimmunity.

Clinical Practice: Because vitiligo has previously been viewed as only a side-effect of immunotherapies in humans, our findings would revolutionize current thinking of what is required for the durable cure of cancer. This work would thus aid in the design and assessment of future melanoma clinical trials.

APPROACH

Specific Aim 1: Determine if vitiligo is required for curative adoptive T cell therapy in the B16 melanoma model.

Rationale: Pmel T cells have been used extensively for effective CD8 T cell therapy of B16 melanoma and the concurrent induction of profound vitiligo.[6, 34-37] One of the most potent combination therapies involves pmel T cells, vaccination, and supportive treatment with IL-2, anti-CD4 to deplete regulatory T cells, and lipopolysaccharide (LPS).[37] A modification of this published regime, developed by our collaborator C. Paulos (see letter) provides durable cure of large established B16 melanomas (Figure 2), and induces robust vitiligo. We will now perform this protocol in melanocyte deficient W^{sh} mice bearing B16 tumors to determine the contribution of melanocyte destruction to the efficacy and durability of treatment.

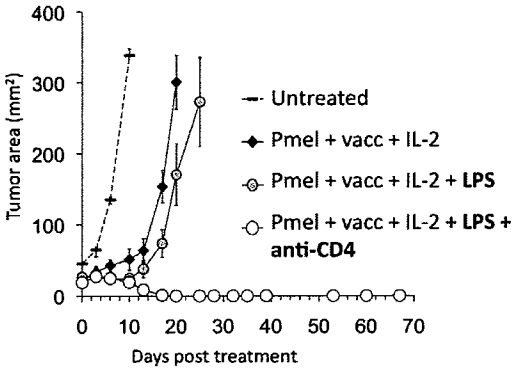


Figure 2. Curative pmel adoptive T cell therapy of B16 melanoma in wild-type mice. Mice with ~7x7 mm B16 tumors received 10^6 in-vitro activated pmel cells (day 0), hgp100-encoding fowlpox vaccine (day 0), IL-2 (2x daily, on days 0, 1, 2), 5ug ultrapure LPS (day 1), and anti-CD4 (day -1, 1, 3). Tumor growth was measured daily. Data courtesy of our collaborator C. Paulos.

Experimental design and expected results: As we have published, wild-type bone marrow chimeric (BMC) W^{sh} mice will be used as a melanocyte-deficient, vitiligo-insufficient mouse model for these experiments.[4] Bone marrow reconstitution will be used to circumvent mast cell defects that have been described in the W^{sh} strain.[38] Combination therapy (Figure 2, open circles) will be administered to wild-type or W^{sh} mice bearing established ~7mm B16 tumors. Pmel T cell population characteristics and tumor growth will be assessed throughout the following weeks. Tumor diameters will be measured thrice weekly (B16 is well encapsulated in the dermis, and non-metastatic). *Ten mice per group will be used to achieve 80% power to detect a mean difference of 2.6 mm in tumor diameters between the groups using a two-sided two-sample t-test.* Pmel T cells will be followed in blood, lymph nodes, and spleens by staining CD8 T cells for the congenic marker Thy1.1.

It is expected that early pmel T cell responses (days 5-15 post-treatment) will be similar in wild-type and W^{sh} mice with regards to population size and activation status (based on CD44, CD62L, and CD69 expression, and IFN-γ production). Accordingly, kinetics of early B16 tumor regression should be similar in both groups. This would confirm that melanocytes do not influence early priming events or early CD8 T cell effector function. However, differences are expected after the development of vitiligo, during the memory phase of the response (days 30-80). Whereas wild-type mice should remain tumor-free, it is expected that tumors will recur in W^{sh} hosts, indicating that destruction of host melanocytes is required for long-term cure. When pmel cells are assessed > 30d post-treatment, we expect to identify large populations with a $CD62L^{low}$ T_{EM} phenotype in wild-type mice, but very small (or undetectable) populations with a T_{CM} phenotype in W^{sh} mice. These findings would establish that melanocyte destruction is required to maintain curative CD8 T cell responses.

Pitfalls and Alternatives: We are confident that wild-type bone marrow reconstitution of W^{sh} mice as published will effectively correct immunological defects in this strain.[33, 39] However it remains possible that reduced protection in W^{sh} mice could be due to some defect other than melanocyte deficiency. To control for this, W^{sh} mice could also be grafted with wild-type skin with the expectation that vitiligo against melanocytes in grafted skin would restore memory (R.J Noelle at Dartmouth will provide expertise for these studies).

Specific Aim 2: Determine if vitiligo is required for curative adoptive T cell therapy in the Braf/Pten melanoma model.

Rationale: To assess the impact of vitiligo in the most clinically relevant model available, we will treat established Braf/Pten tumors by adoptive T cell therapy in combination with $Braf^{V600E}$ inhibition. We have established that Braf inhibition efficiently arrests, but does not cure, Braf/Pten tumors (Figure 3). Our consultant for these studies, A. Boni, has also published that Braf inhibition enhances T cell recognition of antigen on melanoma cells *in vitro*, and does not impair T cell function[16], suggesting that adoptive T cell

therapy could be greatly improved by PLX4032 treatment. Furthermore, our preliminary data show that pmel CD8 T cells proliferate in response to Braf/Pten tumors under conditions of CD4 T cell depletion (Figure 4), which is one component of our adoptive T cell therapy (see Figure 2). The goal of these studies will be to assess whether ACT and Braf inhibition can function synergistically to treat aggressive metastatic melanoma, and to further define the role of vitiligo in this combined therapy setting.

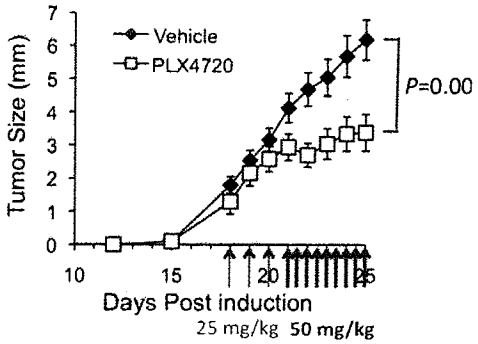


Figure 3. Effects of Braf^{V600E} inhibition on Braf/Pten melanoma growth. Melanomas were induced on day 0. Mice were either left untreated, or treated (oral gavage) with PLX4032 (research analogue), as indicated by arrows.

Experimental Design and Expected Results: Braf/Pten tumors will be induced in wild-type or melanocyte-deficient W^{sh} mice (each reconstituted with wild-type bone marrow). Because W^{sh} mice lack melanocytes, and cannot support normal melanoma tumorigenesis, Braf/Pten skin will first be grafted onto wild-type, or W^{sh} mice, and tumors will then be induced in grafted skin by topical treatment of 4-hydroxy tamoxofen, a technique which we have recently established (Figure 5). Entire grafts will be induced with 4-OHT to prevent vitiligo against residual melanocytes in grafted skin. Once tumors are established, mice will be treated with the pmel ACT regimen described in Specific Aim 1 (pmel cells + vaccine + IL-2 + anti-CD4 + LPS), either alone, or simultaneously with PLX4032. PLX4032 will be administered by gavage, twice daily for 10 days, beginning 2 days prior to T cell transfer. Primary tumors will be measured thrice weekly, and sentinel mice will be sacrificed to evaluate pigmented metastases in lymph nodes and lung. Tumor growth and statistical differences will be assessed as described for Specific Aim 1. The following results are expected with regards to early tumor growth in wild-type mice: (1) PLX4032 alone will arrest tumor growth, (2) ACT alone will induce significant tumor regression, and (3) combination of both therapies will induce complete tumor regression. In wild-type versus W^{sh} mice, significant differences in early primary tumor control are not expected, indicating no role for melanocyte destruction in supporting early therapeutic T cell responses. At later time points (>30 days post treatment), the following results are expected: (1) Mice receiving PLX4032 alone will resume normal tumor growth after treatment (2) ACT alone will promote significant long-term control of metastatic melanoma, (3) ACT + PLX4032 will result in durable melanoma cure. Importantly, at later time points, tumors are expected to recur in W^{sh} mice treated by ACT with or without PLX4032, confirming a requirement for vitiligo in supporting long-lived CD8 T cell responses. In additional studies, we will investigate if PLX4032 treatment boosts early pmel T cell responses and the development of vitiligo. This would further suggest that targeted inhibition of Braf^{V600E} can cross-prime T cell responses to dying melanoma cells *in vivo*, and/or enhance melanoma cell susceptibility to T cell killing; mechanisms that could be formally tested in subsequent experiments.

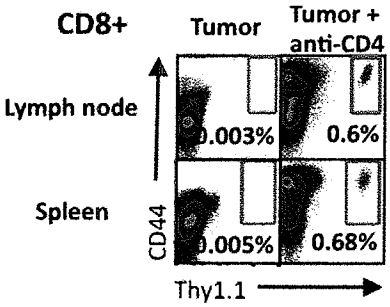


Figure 4. Pmel cells respond to Braf/Pten tumors upon depletion of CD4+ T cells. Braf/Pten mice (C57BL/6 background) with induced melanoma tumors received 10^5 naïve pmel cells and no treatment (left) or anti-CD4 treatment (right). Antigen-experienced (CD44^{hi}) pmel cells (Thy1.1$^+$) were detected in lymphoid tissues 30 days following tumor induction.

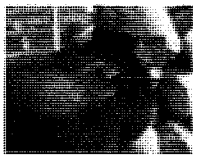


Figure 5. Growth of Braf/Pten tumor within grafted skin. Skin from Braf/Pten mice (mixed background) was grafted onto *RAG*$^{-/-}$ recipients, then induced with 4-hydroxytamofen, intradermally. Arrow indicates tumor; dotted line indicates border of grafted skin.

Pitfalls and Alternatives: Because immunotherapy has not yet demonstrated efficacy in the Braf/Pten model, timing and dosing may need to be modified. In follow-up studies, to mimic PLX4032 drug resistance[13], inhibitor treatment will first be stopped and then ACT will be administered beginning ~2 days later.

In Conclusion, this work is expected to provide a new understanding of mechanisms underlying adoptive T cell therapy for melanoma. These findings could have transformative effects on the way that clinical cancer immunotherapy trials are designed and conducted. Furthermore, studies in Braf/Pten tumor-bearing mice will provide important preliminary data establishing this model for the future investigation of melanoma immunotherapies and the role of vitiligo.

REFERENCES

1. Quaglino P, Marenco F, Osella-Abate S, Cappello N, Ortoncelli M, Salomone B, Fierro MT, Savoia P, Bernengo MG: Vitiligo is an independent favourable prognostic factor in stage III and IV metastatic melanoma patients: results from a single-institution hospital-based observational cohort study, Ann Oncol 21:409-414
2. Bystryn JC, Rigel D, Friedman RJ, Kopf A: Prognostic significance of hypopigmentation in malignant melanoma, Arch Dermatol 1987, 123:1053-1055
3. Nordlund JJ, Kirkwood JM, Forget BM, Milton G, Albert DM, Lerner AB: Vitiligo in patients with metastatic melanoma: a good prognostic sign, J Am Acad Dermatol 1983, 9:689-696
4. Byrne KT, Cote AL, Zhang P, Steinberg S, Allie R, Zhang W, Ernstoff MS, Usherwood EJ, **Turk MJ**: Autoimmune melanocyte destruction is required for robust CD8 memory T cell responses to mouse melanoma., J Clin Invest 2011, 121: 1797-809.
5. Zhang P, Cote AL, de Vries VC, Usherwood EJ, **Turk MJ:** Induction of postsurgical tumor immunity and T-cell memory by a poorly immunogenic tumor, Cancer Res 2007, 67:6468-6476
6. Overwijk WW, Theoret MR, Finkelstein SE, Surman DR, de Jong LA, Vyth-Dreese FA, Dellemijn TA, Antony PA, Spiess PJ, Palmer DC, Heimann DM, Klebanoff CA, Yu Z, Hwang LN, Feigenbaum L, Kruisbeek AM, Rosenberg SA, Restifo NP: Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells, J Exp Med 2003, 198:569-580
7. Dankort D, Curley DP, Cartlidge RA, Nelson B, Karnezis AN, Damsky WE, Jr., You MJ, DePinho RA, McMahon M, Bosenberg M: Braf(V600E) cooperates with Pten loss to induce metastatic melanoma, Nat Genet 2009, 41:544-552
8. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA: Mutations of the BRAF gene in human cancer, Nature 2002, 417:949-954
9. Bollag G, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, Spevak W, Zhang C, Zhang Y, Habets G, Burton EA, Wong B, Tsang G, West BL, Powell B, Shellooe R, Marimuthu A, Nguyen H, Zhang KY, Artis DR, Schlessinger J, Su F, Higgins B, Iyer R, D'Andrea K, Koehler A, Stumm M, Lin PS, Lee RJ, Grippo J, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, Chapman PB, Flaherty KT, Xu X, Nathanson KL, Nolop K: Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma, Nature 2010, 467:596-599
10. ACS: American Cancer Society: Cancer Facts and Figures 2011, 2011,
11. Meyer RG, Britten CM, Siepmann U, Petzold B, Sagban TA, Lehr HA, Weigle B, Schmitz M, Mateo L, Schmidt B, Bernhard H, Jakob T, Hein R, Schuler G, Schuler-Thurner B, Wagner SN, Drexler I, Sutter G, Arndtz N, Chaplin P, Metz J, Enk A, Huber C, Wolfel T: A phase I vaccination study with tyrosinase in patients with stage II melanoma using recombinant modified vaccinia virus Ankara (MVA-hTyr), Cancer Immunol Immunother 2005, 54:453-467
12. Mouawad R, Sebert M, Michels J, Bloch J, Spano JP, Khayat D: Treatment for metastatic malignant melanoma: old drugs and new strategies, Crit Rev Oncol Hematol 74:27-39
13. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K, Chapman PB: Inhibition of mutated, activated BRAF in metastatic melanoma, N Engl J Med 2010, 363:809-819
14. **Turk MJ**, Wolchok JD, Guevara-Patino JA, Goldberg SM, Houghton AN: Multiple pathways to tumor immunity and concomitant autoimmunity, Immunol Rev 2002, 188:122-135
15. Houghton AN, Eisinger M, Albino AP, Cairncross JG, Old LJ: Surface antigens of melanocytes and melanomas. Markers of melanocyte differentiation and melanoma subsets, J Exp Med 1982, 156:1755-1766
16. Boni A, Cogdill AP, Dang P, Udayakumar D, Njauw CN, Sloss CM, Ferrone CR, Flaherty KT, Lawrence DP, Fisher DE, Tsao H, Wargo JA: Selective BRAFV600E inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function, Cancer Res 70:5213-5219

17. Quaglino P, Marenco F, Osella-Abate S, Cappello N, Ortoncelli M, Salomone B, Fierro MT, Savoia P, Bernengo MG: Vitiligo is an independent favourable prognostic factor in stage III and IV metastatic melanoma patients: results from a single-institution hospital-based observational cohort study, Ann Oncol 2010, 21:409-414
18. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, Kammula US, Royal RE, Sherry RM, Wunderlich JR, Lee CC, Restifo NP, Schwarz SL, Cogdill AP, Bishop RJ, Kim H, Brewer CC, Rudy SF, VanWaes C, Davis JL, Mathur A, Ripley RT, Nathan DA, Laurencot CM, Rosenberg SA: Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen, Blood 2009, 114:535-546
19. Yeh S, Karne NK, Kerkar SP, Heller CK, Palmer DC, Johnson LA, Li Z, Bishop RJ, Wong WT, Sherry RM, Yang JC, Dudley ME, Restifo NP, Rosenberg SA, Nussenblatt RB: Ocular and systemic autoimmunity after successful tumor-infiltrating lymphocyte immunotherapy for recurrent, metastatic melanoma, Ophthalmology 2009, 116:981-989 e981
20. Phan GQ, Attia P, Steinberg SM, White DE, Rosenberg SA: Factors associated with response to high-dose interleukin-2 in patients with metastatic melanoma, J Clin Oncol 2001, 19:3477-3482
21. Le Gal FA, Avril MF, Bosq J, Lefebvre P, Deschemin JC, Andrieu M, Dore MX, Guillet JG: Direct evidence to support the role of antigen-specific CD8(+) T cells in melanoma-associated vitiligo, J Invest Dermatol 2001, 117:1464-1470
22. Jacobs JF, Aarntzen EH, Sibelt LA, Blokx WA, Boullart AC, Gerritsen MJ, Hoogerbrugge PM, Figdor CG, Adema GJ, Punt CJ, de Vries IJ: Vaccine-specific local T cell reactivity in immunotherapy-associated vitiligo in melanoma patients, Cancer Immunol Immunother 2009, 58:145-151
23. Guevara-Patino JA, Engelhorn ME, **Turk MJ**, Liu C, Duan F, Rizzuto G, Cohen AD, Merghoub T, Wolchok JD, Houghton AN: Optimization of a self antigen for presentation of multiple epitopes in cancer immunity, J Clin Invest 2006, 116:1382-1390
24. Cote AL, Usherwood EJ, **Turk MJ**: Tumor-specific T-cell memory: clearing the regulatory T-cell hurdle, Cancer Res 2008, 68:1614-1617
25. Klebanoff CA, Gattinoni L, Restifo NP: CD8+ T-cell memory in tumor immunology and immunotherapy, Immunol Rev 2006, 211:214-224
26. Gattinoni L, Zhong XS, Palmer DC, Ji Y, Hinrichs CS, Yu Z, Wrzesinski C, Boni A, Cassard L, Garvin LM, Paulos CM, Muranski P, Restifo NP: Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells, Nat Med 2009, 15:808-813
27. Klebanoff CA, Gattinoni L, Torabi-Parizi P, Kerstann K, Cardones AR, Finkelstein SE, Palmer DC, Antony PA, Hwang ST, Rosenberg SA, Waldmann TA, Restifo NP: Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells, Proc Natl Acad Sci U S A 2005, 102:9571-9576
28. Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, Royal RE, Kammula U, White DE, Mavroukakis SA, Rogers LJ, Gracia GJ, Jones SA, Mangiameli DP, Pelletier MM, Gea-Banacloche J, Robinson MR, Berman DM, Filie AC, Abati A, Rosenberg SA: Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma, J Clin Oncol 2005, 23:2346-2357
29. Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, Kammula U, Robbins PF, Huang J, Citrin DE, Leitman SF, Wunderlich J, Restifo NP, Thomasian A, Downey SG, Smith FO, Klapper J, Morton K, Laurencot C, White DE, Rosenberg SA: Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens, J Clin Oncol 2008, 26:5233-5239
30. Palmer DC, Chan CC, Gattinoni L, Wrzesinski C, Paulos CM, Hinrichs CS, Powell DJ, Jr., Klebanoff CA, Finkelstein SE, Fariss RN, Yu Z, Nussenblatt RB, Rosenberg SA, Restifo NP: Effective tumor treatment targeting a melanoma/melanocyte-associated antigen triggers severe ocular autoimmunity, Proc Natl Acad Sci U S A 2008, 105:8061-8066
31. Ha SP, Klemen ND, Kinnebrew GH, Brandmaier AG, Marsh J, Hangoc G, Palmer DC, Restifo NP, Cornetta K, Broxmeyer HE, Touloukian CE: Transplantation of mouse HSCs genetically modified to express a CD4-restricted TCR results in long-term immunity that destroys tumors and initiates spontaneous autoimmunity, J Clin Invest 120:4273-4288
32. Herlyn M, Fukunaga-Kalabis M: What is a good model for melanoma?, J Invest Dermatol 130:911-912

33. Cable J, Jackson IJ, Steel KP: Mutations at the W locus affect survival of neural crest-derived melanocytes in the mouse, Mech Dev 1995, 50:139-150
34. Wrzesinski C, Paulos CM, Kaiser A, Muranski P, Palmer DC, Gattinoni L, Yu Z, Rosenberg SA, Restifo NP: Increased intensity lymphodepletion enhances tumor treatment efficacy of adoptively transferred tumor-specific T cells, J Immunother 33:1-7
35. Wrzesinski C, Paulos CM, Gattinoni L, Palmer DC, Kaiser A, Yu Z, Rosenberg SA, Restifo NP: Hematopoietic stem cells promote the expansion and function of adoptively transferred antitumor CD8 T cells, J Clin Invest 2007, 117:492-501
36. Gattinoni L, Finkelstein SE, Klebanoff CA, Antony PA, Palmer DC, Spiess PJ, Hwang LN, Yu Z, Wrzesinski C, Heimann DM, Surh CD, Rosenberg SA, Restifo NP: Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells, J Exp Med 2005, 202:907-912
37. Paulos CM, Wrzesinski C, Kaiser A, Hinrichs CS, Chieppa M, Cassard L, Palmer DC, Boni A, Muranski P, Yu Z, Gattinoni L, Antony PA, Rosenberg SA, Restifo NP: Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells via TLR4 signaling, J Clin Invest 2007, 117:2197-2204
38. Grimbaldeston MA, Chen CC, Piliponsky AM, Tsai M, Tam SY, Galli SJ: Mast cell-deficient W-sash c-kit mutant Kit W-sh/W-sh mice as a model for investigating mast cell biology in vivo, Am J Pathol 2005, 167:835-848
39. Lu LF, Lind EF, Gondek DC, Bennett KA, Gleeson MW, Pino-Lagos K, Scott ZA, Coyle AJ, Reed JL, Van Snick J, Strom TB, Zheng XX, Noelle RJ: Mast cells are essential intermediaries in regulatory T-cell tolerance, Nature 2006, 442:997-1002

FACILITIES

Laboratory: Dr. Turk's laboratory is located on the 7th floor of the Rubin Building within the Norris Cotton Cancer Center at the Dartmouth Hitchcock Medical Center. The Turk laboratory occupies 550 square feet of open laboratory space, including a tissue culture alcove, and bench and desk space for 8 investigators.

Animal: An extensive and comprehensive animal barrier facility is located on the lower level of the adjacent Borwell Building. The Turk Laboratory has exclusive use of a separate room within the barrier, with capacity to house over 700 mice in isolated ventilator caging units. This room has been furnished with a ventilated sterile hood and an isofluorane anesthesia machine. The facility is AAALAC accredited and is directed by Dr. Jack Hoopes, DVM.

Computer: The Turk laboratory is equipped with 5 computers (2 PC, 3 Macintosh), and 2 HP Laserjet printers. Flow-Jo and Sigmaplot software, and extensive inter-college computer network for literature searches and electronic communications are available on all computers.

Office: Dr. Turk occupies a 150 sq. ft. office. In addition, there is a departmental office for her administrative and secretarial staff.

Immune Monitoring Laboratory: Also located on the 7th floor of the Rubin building, and providing resources to all laboratories in the Cancer Center, this laboratory maintains a Zeiss ELISPOT reader, a Miltenyi autoMACS, Luminex cytokine array reader, and a MACS Quant 7 color flow cytometer. A full time faculty director and 2 technicians staff this facility. *This facility will be used extensively for the proposed studies.*

DART-mouse speed congenic facility at Dartmouth is available for DNA micro-satellite analysis, and consultation regarding breeding strategies. *This facility will be used to aid with breeding strategies described in the proposed research.*

The Dartmouth Medical Center's molecular genetics center provides state of the art facilities for making oligonucleotides and peptides at a reduced cost compared to commercial sources. The institution maintains a machine shop and an electronics shop with capabilities for fabrication and repair of equipment, devices, and instruments.

Dartmouth Advanced Imaging Center: Located at the Dartmouth-Hitchcock Medical Center, the facility is dedicated to medical imaging research in small and large animals as well as humans. Modalities include a new whole body 3.0 Tesla MRI Philips magnetic resonance imaging system, and hybrid MRI-Near Infrared imaging, with micro-imaging capabilities. *This resource will be used for tracking metastatic tumor growth in the proposed studies.*

Contribution of scientific environment to probability of success: The Microbiology and Immunology Department at Dartmouth Medical School is a vibrant community of researchers in the fields of cellular and molecular immunology and disease pathogenesis. In addition to the above resources, this project will benefit from strong collaborative interactions between the Turk laboratory and the laboratories of Ed Usherwood and Randy Noelle. We also have ongoing research interactions with the clinical melanoma program at DHMC, lead by Marc Ernstoff, which will enable future clinical translation of our findings.

Yale University

Marcus Bosenberg, MD, PhD
Associate Professor of
Dermatology and Pathology
Yale University School of Medicine
P.O. Box 208059
New Haven, CT 06520-8059

Campus address:
LMP 5038A
15 York Street
203 737-3484 (phone)
203 737-4719 (fax)
Marcus.Bosenberg@yale.edu

February 22, 2011

Mary Jo Turk, Ph.D.
Assistant Professor of Microbiology and Immunology
Dartmouth Medical School
Norris Cotton Cancer Center
Rubin Building, Room 732, HB7937
One Medical Center Drive
Lebanon, New Hampshire 03756

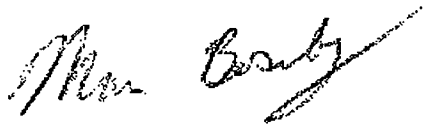
Dear Mary Jo,

I would like to express my enthusiasm to collaborate with you to study T cell memory and the role of autoimmune vitiligo in mice with inducible melanoma. Our laboratory has already provided you with our novel model of inducible metastatic melanoma: *Tyr::CreER^{T} Braf^{CA} Pten$^{lox/lox}$* mice. In this model Cre-lox technology allows the induced expression of mutant *Braf^{V600E}* (mutated in over half of human melanomas) and the loss of the *Pten* tumor suppressor gene specifically in melanocytes. Induction of primary tumors in the skin results in subsequent metastasis to lymph nodes, as well as micro metastases to the lung. Mice develop aggressive melanonoma that closely models human disease. Specifics of this model were recently published in *Nature Genetics* (Dankort et al.).

We have yet to address questions of endogenous CD8 T cell responses in these mice, and are looking to do so in collaboration with your laboratory. I am happy to work with you closely to translate this model to your laboratory, and to provide ongoing intellectual support and input as needed. Our lab is also willing to provide ongoing support with melanoma pathology.

I look forward to collaborating with you on these exciting and timely studies.

Sincerely,



Marcus Bosenberg, MD, PhD

MUSC
MEDICAL UNIVERSITY
of SOUTH CAROLINA

Microbiology & Immunology

86 Jonathan Lucas Street
HCC-612, MSC 509
Charleston SC 29425-5090
Tel 843 792 5342
Fax 843 792 9588

www.musc.edu

Mary Jo Turk
Assistant Professor of Microbiology and Immunology
Dartmouth Medical School
One Medical Center Drive
Lebanon, NH 03756

Dear Mary Jo,

I am writing to express my willingness and enthusiasm to collaborate on your studies to investigate the role of vitiligo in CD8 adoptive T cell therapy. Your recent work highlights the central importance of melanocyte destruction in the maintenance of CD8 T cell responses to melanoma. Based on these findings is of utmost importance to determine the influence of vitiligo on curative melanoma immunotherapies.

As you know, while working as a post-doc in the laboratory of Nick Restifo, I previously demonstrated the efficacy of pmel adoptive cell therapy in combination with vaccine, IL-2, and host conditioning regimens of anti-CD4, anti-NK.1.1, and LPS (published in JCI, 2007). I have subsequently generated unpublished data showing that dramatic cure of B16 melanoma can be achieved without NK depletion, but with more prolonged dosing of anti-CD4. These mice develop extensive vitiligo, although the contribution of vitiligo to the response is not known. I share these data with you for your grant submission, to establish that such a therapeutic outcome will be achievable in mice with established B16.

I intend to work with you, in collaboration with Nick Restifo, to establish this therapeutic model in your laboratory at Dartmouth. I understand that you already maintain a colony of pmel mice, and have already worked extensively with anti-CD4 treatment. We will aid expertise and guidance with respect to pmel cell culture, vaccination with hgp100 fowlpox vaccine, and treatment scheduling. If necessary, we are also happy to host one of your graduate students in our new laboratory at the Medical University of South Carolina, to train her in the required techniques.

These studies will be a continuation of our strong and ongoing collaboration, which has spanned the past twelve years, beginning with our thesis work together. I sincerely appreciate this continued opportunity to work with you, and I look forward to addressing this very important question in tumor immunology.

With warmest regards,

Chrystal M. Paulos, Ph.D.
Assistant Professor of Microbiology and Immunology

Program Director/Principal Investigator (Last, First, Middle): Turk, Mary Jo

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 11/1/11 THROUGH 10/31/12

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Turk, Mary Jo	PD/PI	n.a.						
Boni, Andrea	Co-investigator	n.a.						
Steinberg, Shannon	Grad student	n.a.						
SUBTOTALS →								0

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* Mice $5,000 Antibodies $5,000 Drug (PLX4032) $5,000 Cell culture reagents, disposables, plastics, misc reagents $2,000	17,000
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)* Animal Care $10,000 Flow Cytometry Instrument Time $3,000	13,000
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*	$ 30,000
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD	$ 30,000

BUDGET JUSTIFICATION

Personnel:

Mary Jo Turk, Ph.D., Principal Investigator (15% effort; no salary requested). Dr. Turk will be responsible for planning and overseeing all experiments, conducting weekly lab meetings, preparing data for publication, and presenting data at international meetings. She will also conduct experimental procedures as needed. She will oversee and guide this project.

Andrea Boni, M.D., Co-Investigator (3% effort; no salary requested). Dr. Boni will be involved in the planning of experiments as they relate to the use of CD8 T cells for adoptive therapy of melanoma. He will also provide expertise in the use of of Braf inhibitors to treat melanoma. He will attend Turk lab meetings and provide ongoing guidance for this project.

Shannon Steinberg, Graduate Research Assistant (25% effort; no salary requested). Ms. Steinberg, currently a third year graduate student in the Turk laboratory, will be responsible for planning and performing experiments, analyzing data, preparing data for publication, and presenting data at international meetings. She has already generated key preliminary data presented in this application. Ms. Steinberg has experience with all of the procedures outlined in this proposal including flow cytometry, adoptive T cell transfer, tumor induction, and depigmentation scoring.

Supplies:

Mice: $5,000. We estimate purchasing ~30 C57BL/6 mice per month at a cost of $15 each from NCI.

Antibodies: $5,000. Antibodies *for vivo* depletions will be produced and purified in-house. Antibodies for flow cytometry will be purchased from standard vendors.

Drugs: $5,000. PLX4032 will be purchased from Chemitek ($2700 for 1g), and ultrapure LPS will be purchased from Invivogen.

Cell culture reagents, disposables, plastics, and miscellaneous reagents: $2,000. This amount will cover media and additives including serum and peptides, as well as tissue culture flasks, pipettes, tubes, etc.

Miscellaneous

Animal Care: $10,000. Per-diem charges are based upon costs set by the Dartmouth Animal Facility ($0.62 / day, per cage of 4 mice). Because we are studying memory T cell function, certain mice will require housing as long as 120 days. In addition to purchased mice, we will be breeding and maintaining transgenic Braf/Pten mice and anticipate housing ~100 mice at any given time.

Flow Cytometry: $2,000. We anticipate using ~1h of instrument time per week, at a cost of $50/hr

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Turk, Mary Jo	Assistant Professor of Microbiology and Immunology
eRA COMMONS USER NAME (credential, e.g., agency login) Mjturk	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
John Carroll University, Cleveland, OH	B.S.	05/95	Chemistry
Purdue University, West Lafayette, IN	Ph.D.	02/01	Biochemistry
Memorial Sloan-Kettering Cancer Center, NY	Postdoctoral	09/04	Immunology

A. Personal Statement

I first began to appreciate the intricate link between tumor immunity and autoimmunity while training in tumor immunology with Alan Houghton at Memorial Sloan-Kettering Cancer Center. Here my research focused on endogenous CD8 T cell responses to poorly-immunogenic B16 melanoma. My work was among the first to define a role for $CD4^+CD25^+$ T_{reg} in preventing host T cell immunity to cancer. My research also involved extensive study of DNA vaccines against TRP-1 and other melanocyte differentiation antigens, which are now an essential tool for the proposed studies. After establishing my own laboratory at Dartmouth Medical School, I have focused more intensely on long-lived CD8 memory T cell responses to cancer; a clinically critical but understudied area of tumor immunology. Our work established that T_{reg} depletion during primary melanoma growth, followed by surgical tumor excision, drives the development of long-lived and protective CD8 T cell memory. We have more recently discovered that autoimmune vitiligo, which also develops as a result of T_{reg} depletion and melanoma excision, is an absolute requirement for the maintenance of this memory response. These findings define a new relationship between autoimmunity and tumor immunity, and form the basis for the proposed studies. My background in melanoma tumor immunology and autoimmune vitiligo, as well as my track record of groundbreaking studies in the field of T cell memory to cancer, provide me with the unique ability to lead the proposed research successfully. I look forward to undertaking this exciting project, which represents the next crucial step in our understanding of the interplay between tumor immunity and autoimmunity.

B. Positions and Honors

Positions and Employment

1992–1995 Chemist/Technician, NASA Glen Research Center, Cleveland, OH
1995–1997 Teaching Assistant, Department of Chemistry, Purdue University,West Lafayette, IN
1996–2001 Research Assistant, Departmtent of Chemistry, Purdue University, West Lafayette, IN
2001–2004 Research Fellow, Memorial Sloan Kettering Cancer Center, New York, NY
2004- Assistant Professor, Department of Microbiology and Immunology, Dartmouth Medical School, Lebanon, NH

Other Experience and Professional Memberships

2009- Member, American Association of Immunologists

Awards and Honors

1997-1999 National Institutes of Health Chemical Pharmacology Training Grant
2002-2004 National Institutes of Health Immunology Training Grant
2006 Concern Foundation Conquer Cancer Now Award
2006 Melanoma Research Foundation New Investigator Award

C. Selected Peer-reviewed Publications (Selected from 28 peer-reviewed publications)

Most relevant to the current application:

1. **Turk M.J.**, Guevara-Patino J.A., Rizutto G.A., and Houghton A.N. (2004). Concomitant tumor immunity to a poorly immunogenic melanoma is prevented by regulatory T cells. *Journal of Experimental Medicine*, 200(6), 771-82.
2. Guevara-Patino J.A., Engelhorn M.E., **Turk M.J.**, Lu C., Duan F., Rizzuto G., Cohen A.D., Merghoub T., Wolchok J.D., Houghton A.N. (2006). Optimization of a self-antigen for presentation of multiple epitopes in cancer immunity. *Journal of Clinical Investigation,* 116(5), 1382-90.
3. Zhang P.S., Cote A.L., de Vries V.C., Usherwood E.J., and **Turk M.J.** (2007). Induction of post-surgical tumor immunity and T cell memory by a poorly-immunogenic tumor. *Cancer Research,* 67(13), 6468-76. PMCID: PMC2564800.
4. Côté AL, Usherwood EJ, **Turk M.J.** (2008). Tumor-specific T-cell memory: clearing the regulatory T-cell hurdle (Review). *Cancer Research*, 68(6), 1614-7. PMCID: PMC2682528.
5. Zhang W., Zhang T., **Turk M.J.**, and Usherwood E.J. (2010). A persistent virus vector confers superior anti-tumor immunity, compared with a non-persistent vector. *Cancer Immunology and Immunotherapy,* 59 (5), 707-13. PMCID: PMC2838928.
6. Collison L.W., Chaturvedi V., Henderson A.L., Giacomin P. R., Guy C., Bankoti J., Finkelstein D., Forbes K., Workman C. J., Brown S.A., Rehg J.E., Jones M. L., Ni H., Artis D., **Turk M.J.**, and Vignali D. A. A. (2010). Interleukin-35-mediated induction of a novel regulatory T cell population. *Nature Immunology,* 11 (12), 1093-101. PMCID: PMC3008395.
7. Côté A.L., Zhang P., O'Sullivan J.A., Jacobs V.L., Clemis C.R., Sakaguchi S., Guevara-Patiño J.A., and **Turk M.J.** (2011). Stimulation of the glucocorticoid-induced TNFR family-related receptor (GITR) on CD8 T cells induces protective and high-avidity T cell responses to tumor-specific antigens. *Journal of Immunology*, 186(1), 275-83. NIHMSID: 274939.
8. Byrne K.T.*, Côtè A.L.*, Zhang P., Steinberg S., Guo Y., Allie R., Zhang W., Ernstoff M.S., Usherwood E.J., and **Turk M.J.** (In Press). Autoimmune melanocyte destruction is required for robust CD8 memory T cell responses to mouse melanoma. *Journal of Clinical Investigation, In press.*

Additional recent publications of importance to the field (in chronological order):

9. Cohen A.D., Diab A., Perales M-A., Wolchok J.D., Rizzuto G., Huggins D., Liu C., **Turk M.J.**, Sakaguchi S., and Houghton A.N. (2006). Agonist anti-GITR antibody enhances DNA vaccine-induced CD8+ T cell responses and tumor immunity against melanoma. *Cancer Research*, 66 (9), 4904-12. PMCID: PMC2242844
10. Ferrone C.R., Perales M.A., Goldberg S.M., Somberg C.J., Hirschhorn-Cymerman D., Gregor P.D., **Turk M.J.**, Ramirez-Montagut T., Gold J.S., Houghton A.N., Wolchok J.D. (2006). Adjuvanticity of plasmid DNA encoding cytokines fused to immunoglobulin Fc domains. *Clinical Cancer Research*, 12(18), 5511-9.
11. Bak S.P., Alonso A., **Turk M.J.**, and Berwin B. (2008). Murine ovarian cancer vascular leukocytes require arginase-1 activity for T cell suppression. *Mollecular Immunology*, 46 (2), 258-68. PMCID: PMC2613193
12. Ahonen C.L., Wasiuk A., Fuse S., Turk M.J., Ernstoff M.S., Suriawinata, J.D. Gorham, R.M. Kedl RM, Usherwood E.J., and Noelle R. J. (2008). Enhanced efficacy and reduced toxicity of multifactorial adjuvants compared to unitary adjuvants as cancer vaccines. *Blood,* 111(6), 3116-25. PMCID: PMC2265452
13. Huarte E., Fisher J., **Turk M.J.**, Mellinger D., Foster C., Wolf B., Meehan K.R., Fadul C.E., and Ernstoff M.S. (2009). Ex vivo expansion of tumor specific lymphocytes with IL-15 and IL-21 for adoptive immunotherapy in melanoma. *Cancer Letters*, 285 (1): 80-8.
14. Nesbeth Y., Scarlett U., Cubillos-Ruiz J., Martinez D., Engle X., **Turk M.J.**, and Conejo-Garcia J.R. (2009). CCL5-Mediated Endogenous Antitumor Immunity Elicited by Adoptively Transferred Lymphocytes and Dendritic Cell Depletion. *Cancer Research,* 69(15), 6331-8. PMCID: PMC2755640
15. Gunturu K.S., Meehan K.R., Mackenzie T.A., Crocenzi T.S., McDermott D., Usherwood E.J., Margolin K.A., Crosby N.A., Atkins M.B., **Turk M.J.**, Ahonen C., Fuse S., Clark J.I., Fisher J.L., Noelle R.J., and Ernstoff M.S. (2010). Cytokine working group study of lymphodepleting chemotherapy, interleukin-2, and granulocyte-macrophage colony-stimulating factor in patients with metastatic melanoma: clinical outcomes and peripheral-blood cell recovery. *Journal of Clinical Oncology*, 28(7), 1196-202. PMCID: PMC2834469

D. Research Support

Ongoing Research Support

R01 CA120777-01 Turk (PI) 7/1/06 – 5/30/11 (no cost extension)
NIH
"Mechanisms of Concomitant Tumor Immunity"
Goal: characterize mechanisms whereby progressive, poorly-immunogenic tumors prime and suppress protective T cell responses.
Role: Principle Investigator

1R43AI088948-01(SBIR) Turk (sub-award PI) 04/01/10 – 09/30/11
NIH
"Angiogenesis antagonist plus CD40-TLR agonist adjuvant combination vaccine"
Goal: to develop a combination therapy using proprietary anti-CD40/TLR adjuvant vaccine combined with a blocking Ab to the angiogenesis factor VEGFR2 as well as cyclophosphamide.
Role: Principle Investigator of Dartmouth Sub-award

R01 CA123079-01A1 Noelle (PI) 05/01/07-04/30/12
NIH
"Synergy of the innate and acquired immune responses in tumor immunology"
Goal: Evaluate the impact of combined CD40 and TLR signaling on the induction of cell -mediated immune responses,and the induction of protective anti-tumor immunity.
Role: Co-investigator

R01 AI067405-01A2 Berwin (PI) 03/15/07-02/29/12
NIH
"Mechanisms of Chaperone-Mediated Immune Responses"
Goal: Determine the basis of chaperone-mediated tumor rejection.
Role: Co-investigator

RSG-10-229-01-LIB Berwin (PI) 7/1/10 – 6/30/14
American Cancer Society
"Ovarian Cancer Inhibition by Depletion of Myeloid-Derived Suppressor Cells."
Role: Co-investigator.

1R AI -01(SBIR Phase II) Turk (sub-award PI) 01/15/11 – 01/14/14
NIH
"Preclinical Development of a Novel and Powerful Immunotherapeutic"
Goal: To optimize CD40-TLR adjuvant treatment for clinical vaccine studies in melanoma.
Role: Principle Investigator of Dartmouth Sub-award, 5% effort

1 U54 CA151662-01 Baker (PI), Fiering (project PI) 9/16/10 – 7/31/15
NIH
Center of Cancer Nanotechnology, Project 4 "Magnetic Nanoparticle Therapy for Ovarian Cancer."
Role: Co-investigator

Completed Research Support

New Investigator Award Turk (PI) 1/1/06 – 12/31/07
The Melanoma Research Foundation
Title: Generating and maintaining post-excisional immunity against poorly-immunogenic melanoma
Role: PI

P20 RR16437 COBRE Green (PI) 1/01/05 – 6/30/06
NIH/NCRR
Immune Mechanisms Controlling Inflammation and Cancer
Role: Project Leader

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Andrea Boni	Resident, Anatomic and Clinical Pathology
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Free University Vita-Salute San Raffaele, Milan, Italy	M.D.	07/2003	
Free University Vita-Salute San Raffaele, Milan, Italy	Residency	07/2003	Internal Medicine
National Cancer Institute	Postdoctoral	04/2005	Tumor Immunology
Massachusetts General Hospital/Harvard Medical School	Postdoctoral	08/2008	Tumor Immunology
Dartmouth Hitchcock Medical Center	Residency	06/2010	Pathology, Anatomical and Clinical

A. Personal Statement

My career in a research lab started in 1999 when I was a second year medical student, studying $CD8^+$ T cell polarization and its influence on dendritic cells. In 2005 I joined the laboratory of Dr. Nicholas Restifo where I established a new model for successful adoptive T cell therapy of melanoma using transgenic TRP-1-specific IL-17 producing $CD4^+$ T cells. These studies were the first to demonstrate that tumor-specific CD4 T cells are capable of regressing large established tumors. These mice developed extensive vitiligo, although the effects of vitiligo on therapy have never been explored. After completing my research at the NCI, I was recruited by Dr. Jennifer Wargo to join her new laboratory at the Massachusetts General Hospital. Here my research revealed that specific inhibition of $Braf^{V600E}$ in human melanoma cell lines, using the drug PLX4720, enhanced T cell recognition of melanoma differentiation antigens, but did not impair T cell function. These studies were the first to establish that Braf inhibition can alter the immunogenicity of melanoma cells. I am now completing my residency in Pathology at Dartmouth Hitchcock Medical Center in New Hampshire, and have re-connected with Dr. Turk and her laboratory, with whom I had previously collaborated while at the NCI. I currently attend weekly Turk Lab meetings, where I participate in general discussions and provide expertise relevant to tumor immunology and melanoma genetics. My extensive past work with melanoma-specific CD4 T cells and targeted $Braf^{V600E}$ inhibition as therapeutic treatments for melanoma make me an ideal consultant for the studies proposed in the current grant application. I look forward to continuing to work with Dr. Turk on these important and clinically significant studies throughout, and beyond, my residency tenure at Dartmouth.

B. Positions and Honors

Positions and Employment

2003–2005 Resident Physician, Internal Medicine Program, San Raffaele Hospital, Milan, Italy
2005–2008 Research Fellow, Surgery Branch, National Cancer Institute, Bethesda, MD
2008–2010 Research Fellow, Massachusetts General Hospital, Boston, MA
2010– Resident Physician, Pathology Department, Dartmouth Hitchcock Medical Center, Lebanon, NH

Other Experience and Professional Memberships

2003- Present Member, Italian College of Physicians
2006- Present Junior Member, American Society of Clinical Oncology (ASCO)
2008- Present Trainee Member, American Society for Investigative Pathology (ASIP)
2009- Present Associate Member, American Association for Cancer Research (AACR)

C. Selected Peer-reviewed Publications

Most relevant to the current application:

1. Kerkar SP, Muranski P, Kaiser A, **Andrea Boni**, Sanchez-Perez L, Yu Z, Palmer DC, Reger RN, Borman ZA, Zhang L, Morgan RA, Gattinoni L, Rosenberg SA, Trinchieri G, Restifo NP. "Tumor-specific CD8+ T cells expressing interleukin-12 eradicate established cancers in lymphodepleted hosts." Cancer Research. 2010 Sep 1;70(17):6725-34. Epub 2010 Jul 20. PMCID: PMC2935308.
2. **Andrea Boni**, Cogdill AP, Dang P, Udayakumar D, Njauw CN, Sloss CM, Ferrone CR, Flaherty KT, Lawrence DP, Fisher DE, Tsao H, Wargo JA. "Selective BRAFV600E inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function." Cancer Research. 2010 Jul 1;70(13):5213-9. Epub 2010 Jun 15.
3. Gattinoni L, Zhong X, Palmer DC, Hinrichs CS, Yu Z, Wrzesinski C, **Andrea Boni**, Cassard L, Ji Y, Church L, Paulos CM, Muranski P and Restifo NP. "Wnt signaling arrests effector T cell differentiation and generates CD8(+) memory stem cells." Nature Medicine. 2009 Jul; 15(7): 808 - 813. PMCID: PMC2707501
4. **Andrea Boni**, Muranski P, Cassard L, Wrzesinski C, Paulos CM, Palmer DC, Gattinoni L, Hinrichs CS, Chan CC, Rosenberg SA, Restifo NP. "Adoptive transfer of allogeneic tumor-specific T cells mediates effective regression of large tumors across major histocompatibility barriers." Blood. 2008 Dec 1; 112(12):4746-54. PMCID: PMC2597140
5. **Andrea Boni**, Muranski P, Antony PA, Cassard L, Irvine KR, Kaiser A, Paulos CM, Palmer DC, Touloukian CE, Ptak K, Gattinoni L, Wrzesinski C, Hinrichs CS, Kerstann K, Feigenbaum L, Chan CC, Restifo NP. "Tumor-specific Th17-polarized cells eradicate large established melanoma." Blood. 2008 Jul 15; 112(2):362-73. PMCID: PMC2442746

Additional recent publications of importance to the field (in chronological order):

6. Paulos CM, Kaiser A, Wrzesinski C, Hinrichs CS, Cassard L, **Andrea Boni**, Muranski P, Sanchez-Perez L, Palmer DC, Yu Z, Antony PA, Gattinoni L, Rosenberg SA and Restifo NP (2007). "Toll-like receptors in tumor immunotherapy" Clinical Cancer Research. 2007 Sep; 13(18 Pt 1):5280-9. PMCID: PMC2131730.
7. Paulos CM, Wrzesinski C, Kaiser A, Hinrichs CS, Chieppa M, Cassard L, Palmer DC, **Andrea Boni**, Muranski P, Yu Z, Gattinoni L, Antony PA, Rosenberg SA and Restifo NP. (2007) "Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells via TLR4 signaling" J Clin Invest. 2007 Aug; 117(8):2197-204. PMCID: PMC1924500.
8. Muranski P, **Andrea Boni**, Wrzesinski C, Citrin DE, Rosenberg SA, Childs R and Restifo NP (2006) "Increased intensity lymphodepletion and adoptive immunotherapy: How far can we go?" Nature Clinical Practice Oncology. 2006 Dec; 3(12):668-81. PMCID: PMC1773008.
9. **Andrea Boni**, Iezzi G, Degl'Innocenti E, Jacchetti E, Camporeale A, Grioni M, Camporeale A and Bellone M(2006) "Prolonged exposure of dendritic cells to maturation stimuli favors the induction of type-2 cytotoxic T lymphocytes" Eur J Immunol. 2006 Dec; 36(12):3157-66
10. **Andrea Boni**, Iezzi G, Degl'Innocenti E, Grioni M, Bertilaccio MTS and Bellone M (2006). "Type-2 Cytotoxic T Lymphocytes Modulate the Activity of Dendritic Cells toward Type-2 Immune Response" Journal of Immunology. 2006 Aug; 177(4):2131-7.
11. Degl'Innocenti E, Grioni M, **Andrea Boni**, Camporeale A, Bertilaccio S, Freschi M, Monno A, Arcelloni C, Greenberg NM and Bellone M (2005). "Impact of Dendritic Cell-Based Vaccine on Peripheral Tolerance to a Prostate Cancer Associated Antigen" Eur J Immunol. 2005 Jan; 35(1):66-75.
12. Camporeale A, **Andrea Boni**, Iezzi G, Degl'Innocenti E, Grioni M, Mondino A and Bellone M (2003) "Critical Impact of the Kinetics of Dendritic Cells Activation on the in Vivo Induction of Tumor-Specific T Lymphocytes." Cancer Res. 2003 Jul 1; 63:3688-3694

D. Research Support

Ongoing Research Support: None

Completed Research Support: None

OTHER AGENCIES to whom this application has been submitted:

The proposed studies represent approximately one half of Specific Aim 3 in the following grant application:

NIH R01 CA120777-01 10/1/11 – 9/30/16
"Mechanisms of Concomitant Tumor Immunity and Autoimmunity"
Role: PI
Submission date: March 5, 2011, Competitive Renewal, first submission
Score: 28 (23%tile)

CURRENT SUPPORT

Pending Research Support

RSG-214269 01/01/12 – 12/31/15
American Cancer Society
"Tumor-specific T cell Memory and the Role of Autoimmunity"
Role: PI
Submission date: April 6, 2011 (A2)
Score: Outstanding

R21 03/01/12 – 05/31/14
NIH
"CD8 T cell Memory in Autoimmune Vitiligo"
Role: PI
Submission date: June 5, 2011

Planned Submissions

NIH R01 CA120777-01 08/01/12 – 07/30/17
"Mechanisms of Concomitant Tumor Immunity and Autoimmunity"
Role: PI
Submission date: November 5, 2011 (Competitive Renewal, second submission)
Previous Score: 28 (23%tile)

Ongoing Research Support

R01 CA120777-01 Turk (PI) 07/01/06 – 05/30/12 (no cost extension)
NIH $70,000 no cost extension
"Mechanisms of Concomitant Tumor Immunity"
Goal: characterize mechanisms whereby progressive, poorly-immunogenic tumors prime and suppress protective T cell responses.
Role: Principle Investigator

1R43AI088948-01(SBIR) Turk (sub-award PI) 04/01/10 – 09/30/11
NIH $118,000
"Angiogenesis antagonist plus CD40-TLR agonist adjuvant combination vaccine"
Goal: to develop a combination therapy using proprietary anti-CD40/TLR adjuvant vaccine combined with a blocking Ab to the angiogenesis factor VEGFR2 as well as the cytoreductive chemotherapeutic agent cyclophosphamide.
Role: Principle Investigator of Dartmouth Sub-award, 5% effort

R01 CA123079-01A1 Noelle (PI) 05/01/07-04/30/12
NIH
"Synergy of the innate and acquired immune responses in tumor immunology"
Goal: Evaluate the impact of combined CD40 and TLR signaling on the induction of cell -mediated immune responses,and the induction of protective anti-tumor immunity.
Role: Co-investigator, 10% effort

R01 AI067405-01A2 Berwin (PI) 03/15/07-02/29/12
NIH
"Mechanisms of Chaperone-Mediated Immune Responses"
Goal: Determine the basis of chaperone-mediated tumor rejection.
Role: Co-investigator, 5% effort

1R AI -01(SBIR Phase II) Turk (sub-award PI) 01/15/11 – 01/14/14
NIH $30,000/year
"Preclinical Development of a Novel and Powerful Immunotherapeutic"
Goal: To optimize CD40-TLR adjuvant treatment for clinical vaccine studies in melanoma.
Role: Principle Investigator of Dartmouth Sub-award, 5% effort

RSG-10-229-01-LIB Berwin (PI)
American Cancer Society
"Ovarian Cancer Inhibition by Depletion of Myeloid-Derived Suppressor Cells."
Role: Co-investigator, 5% effort

1 U54 CA151662-01 Baker (PI) and Fiering (Project PI)
NIH
Center of Cancer Nanotechnology, Project 4 "Magnetic Nanoparticle Therapy for Ovarian Cancer."
Role: Co-investigator, 10% effort