A Randomized Phase 2 Pilot Study of Type I-Polarized Autologous Dendritic Cell Vaccines Incorporating Tumor Blood Vessel Antigen (TBVA)-Derived Peptides in Combination with Dasatinib in Patients with Metastatic Melanoma

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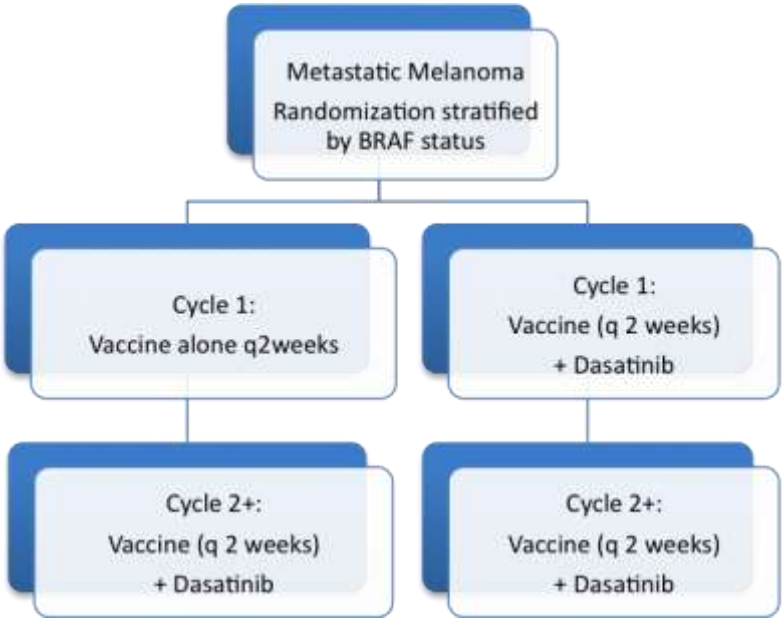
Metastatic Melanoma
Randomization stratified by BRAF status
Cycle 1:
Vaccine alone q2weeks
Cycle 1:
Vaccine (q 2 weeks)
+ Dasatinib
Cycle 2+:
Vaccine (q 2 weeks)
+ Dasatinib
Cycle 2+:
Vaccine (q 2 weeks)
+ Dasatinib

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LIST OF ABBREVIATIONS

AE	Adverse event
ANC	Absolute Neutrophil Count
BID	Twice a Day
BMS	Bristol-Myers Squibb Company
CBC	Complete Blood Count
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
EKG	Electrocardiogram
ESR	Expedited Safety Report
FDA	Food and Drug Administration
FSH	Follicle-Stimulating Hormone
GCP	Good Clinical Practice
HCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
HRT	Hormone Replacement Therapy
IB	Investigators' Brochure
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IND	Investigational New Drug (Application)
IRB	Institutional Review Board
NCI	National Cancer Institute
NSAE	Non-Serious Adverse Event
PFS	Progression Free Survival
PO	By Mouth
PR	Partial Response
QD	Once Daily
QoL	Quality Of Life
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper Limit of Normal
WBC	White Blood Count
WOCBP	Women of Child-Bearing Potential

1. OBJECTIVES

1.1 Primary Objectives

To determine the effect of the addition of dasatinib on the proportion of HLA-A2$^+$ melanoma patients that exhibit improved peripheral blood CD8$^+$ T cell responses against 3 or more peptide epitopes after active vaccination with Type I-polarized autologous dendritic cell (αDC1) vaccine incorporating 6 tumor blood vessel-associated antigen (TBVA)-derived peptides.

1.2 Secondary Objectives

- To determine the safety and feasibility of a regimen consisting of the combination of dasatinib and vaccine.
- To determine the objective response rate by RECIST 1.1 of melanoma patients treated with the combination of dasatinib and vaccine.
- To determine the progression-free and overall survival of patients treated with the combination of dasatinib and vaccine.
- To determine the quantitative effect of treatment on the following immunological endpoints; a) number of CD8$^+$ T cells infiltrating into melanoma lesions; b) number of suppressor cell populations and blood vessels in melanoma tumor biopsies; c) number of suppressor cell populations in patients peripheral blood, d) level of EphA2 protein expression in tumor biopsies, and e) serum concentration of the T cell-recruiting chemokine CXCL10/IP-10.

2. BACKGROUND

2.1 Melanoma

The incidence of melanoma continues to rise at an alarming pace. The American Cancer Society estimates that over 70,000 patients will be diagnosed with melanoma in 2011 with an estimated death toll of over 8,700 deaths (1). Early stages of the disease are surgically curable and adjuvant therapy of high risk disease is modestly effective in preventing recurrent metastatic disease. Once metastatic, melanoma is incurable with very high disease-fatality rates (2). Few agents have shown clinical benefit in this setting and none has shown a survival advantage, until ipilimumab, most recently with the first ever metastatic melanoma trial to show improvement of overall survival leading to FDA approval in March 2011. Hydroxyurea was the first agent to get FDA approval in 1967 followed by dacarbazine in 1971 which quickly became the "standard of care" as no other agent, singly or in combination, achieved a survival advantage over dacarbazine (3-5). Immunotherapy has achieved limited success with high-dose bolus IL-2 leading to durable responses in a small subset of patients (5-15%; ref. 6). The approval of ipilimumab is likely to alter the therapeutic landscape of metastatic melanoma but it should be noted that only 10-15% of patients respond and not all achieve durable responses (7). Even more promise is associated with the advent of highly selective BRAF-targeted agents that have led to

the highest response rates seen in melanoma in patients that harbor the BRAF mutation (8). While this is clearly encouraging, to date no durable responses have been described with BRAF inhibitors and those are only available for the 40-50% of patients that have the mutation. Therefore there continues to be an urgent need to develop therapeutic strategies that offer long term control of the disease.

Mutation-directed therapy has expanded the therapeutic opportunities for c-KIT-mutated melanomas. Unlike melanomas that occur in chronic sun-damaged areas, melanomas arising in non-sun exposed areas such as the mucosal surfaces, palms, soles, and nail beds are thought to result from different risk factors and perhaps different oncogenes. Indeed, melanomas with oncogenic mutations in c-KIT have been reported in several studies. Recently, c-KIT-activating mutations were reported in 21% of mucosal melanomas, 11% of acral melanomas, and 16.7% of melanomas arising in chronically sun-damaged skin (9-13).

2.2 Dasatinib

Dasatinib (BMS-354825; NSC-732517) is a potent, broad spectrum ATP-competitive inhibitor of 5 critical oncogenic tyrosine kinases/kinase families: BCR-ABL, SRC, c-KIT, PDGFR, and ephrin (Eph) receptor kinases. Overexpression or activation of these kinases plays a critical role in the etiology of various cancer types, as well as the malignant behavior associated with these diseases, such as unregulated proliferation and metastasis. The Philadelphia chromosome and resultant constitutively expressed BCR-ABL protein tyrosine kinase (PTK) is present in > 90-95% of patients with CML and 20% to 30% of adult patients with ALL. BCR-ABL activity is required for the cancer-causing ability of this protein. Dasatinib is ~500-fold more potent than imatinib in inhibiting BCR-ABL by binding to both active and inactive conformations of c-ABL, whereas imatinib only binds to the inactive state. This difference in binding may be responsible for the increased potency of dasatinib over imatinib (14).

Dasatinib is indicated for the treatment of newly diagnosed adults with CML in chronic phase, the treatment of adults with chronic, accelerated, myeloid or lymphoid blast phase CML with resistance or intolerance to prior therapy including imatinib, and the treatment of adults with Ph+ ALL with resistance or intolerance to prior therapy (14).

2.2.1 Preclinical Anti-tumor Activity

2.2.1.1 In Vitro Molecular Studies

Dasatinib competes with ATP for the ATP-binding site in the kinase domain of selected protein tyrosine kinases (PTKs) and has been shown to inhibit at least five protein tyrosine kinases/kinase families: SRC family kinases, BCR-ABL, c-KIT, EphA2 and the PDGFRβ receptor. Dasatinib is much more potent than imatinib mesylate. Dasatinib was 260-, 8-, 60-, and >1000-fold more potent than imatinib versus BCR-ABL, c-KIT, PDGFRβ, and SRC kinases, respectively (14).

2.2.1.2 Cellular Studies

Dasatinib inhibits the BCR-ABL kinase with an *in vitro* IC50 of 3 nM, a potency that was 260-fold greater than that of imatinib mesylate (IC50 = 790 nM). In cellular assays, dasatinib killed or inhibited the proliferation of all BCR-ABL dependent leukemic cell lines tested to date. Dasatinib also demonstrated undiminished antitumor activity against several preclinically- and clinically-derived models of imatinib mesylate resistance. These results demonstrate that dasatinib is effective in reducing the proliferation or survival of both imatinib mesylate-sensitive and resistant cells, and its inhibitory activity is not solely dependent on BCR-ABL.

2.2.1.3 In Vivo Studies

The activity of dasatinib against CML cells *in vitro* was reproduced *in vivo* against several human CML xenograft models grown subcutaneously in SCID mice. Against the K562/imatinib mesylate/R CML model, dasatinib was curative in 100% of the treated animals. In contrast, at its optimal dose and schedule, imatinib mesylate was inactive.

Dasatinib exhibited *in vivo* antitumor activity in a broad spectrum of solid tumor types including the rhabdomyosarcoma line RD1 implanted in nude mice. The activity appeared to be from a cytostatic rather than cytotoxic effect.

2.2.1.4 Preclinical Toxicology

Single or repeated oral administration of dasatinib principally affected the gastrointestinal (GI) tract, including the liver, the hematopoietic and lymphoid systems in rats and monkeys. Other prominent effects after single oral administration of dasatinib included renal and cardiac toxicity in rats at lethal doses, and cutaneous hemorrhage in monkeys. Dasatinib can also affect the immune system and bone turnover.

Dasatinib *in vitro* activity in the HERG/IKr and Purkinje-fiber assays indicated a moderate liability for prolongation of cardiac ventricular repolarization (QT interval) in the clinic. However, there were no dasatinib-related changes observed in electrocardiograms, nervous system function, respirations and heart rate, blood pressure, or arterial oxygen saturation in single-dose, 10-day, or 1-month oral toxicity studies in monkeys.

Dasatinib was found to exhibit a profile of broad-spectrum platelet inhibition best typified by anti-platelet agents such as the GPIIb/IIIa antagonists, integrelin and abciximab.

Finally, modulation of SRC kinase activity could also affect osteoclast morphology and function and bone remodeling. This effect could potentially result in an increase in bone mineral density and a phenotype analogous to osteopetrosis (14).

2.2.2 Clinical Pharmacokinetics

The pharmacokinetics of dasatinib has been evaluated in 229 healthy patients and in 137 patients with leukemia.

2.2.2.1 Absorption

Maximum plasma concentrations (C_{max}) of dasatinib are observed between 0.5 and 6 hours (T_{max}) following oral administration. Dasatinib exhibits dose proportional increases in AUC and linear elimination characteristics over the dose range of 15 mg to 240 mg/day. The overall mean terminal half-life of dasatinib is 3-5 hours (14).

Data from a study of 54 healthy patients administered a single, 100-mg dose of dasatinib 30 minutes following consumption of a high-fat meal resulted in a 14% increase in the mean AUC of dasatinib. The observed food effects were not clinically relevant.

2.2.2.2 Distribution

In patients, dasatinib has an apparent volume of distribution of 2505 L, suggesting that the drug is extensively distributed in the extravascular space. Binding of dasatinib and its active metabolite to human plasma proteins *in vitro* was approximately 96% and 93%, respectively, with no concentration dependence over the range of 100-500 ng/mL (14).

2.2.2.3 Metabolism

Dasatinib is extensively metabolized in humans, primarily by the cytochrome P450 enzyme 3A4. CYP3A4 was the primary enzyme responsible for the formation of the active metabolite. Flavin-containing monooxygenase 3 (FMO-3) and uridine diphosphate-glucuronosyl transferase (UGT) enzymes are also involved in the formation of dasatinib metabolites. In human liver microsomes, dasatinib was a weak time-dependent inhibitor of CYP3A4.

The exposure of the active metabolite, which is equipotent to dasatinib, represents approximately 5% of the dasatinib AUC. This indicates that the active metabolite of dasatinib is unlikely to play a major role in the observed pharmacology of the drug. Dasatinib also had several other inactive oxidative metabolites.

2.2.2.4 Elimination

Elimination is primarily via the feces. Following a single oral dose of [^{14}C]-labeled dasatinib, approximately 4% and 85% of the administered radioactivity was recovered in the urine and feces, respectively, within 10 days. Unchanged dasatinib accounted for 0.1% and 19% of the administered dose in urine and feces, respectively, with the remainder of the dose being metabolites.

2.2.3 Clinical Experience with Dasatinib in CML and Ph+ ALL

Four single-arm multicenter studies were conducted to determine the efficacy and safety of dasatinib in patients with CML or Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) resistant to or intolerant of treatment with imatinib. Resistance to imatinib included failure to achieve a complete hematologic response (within 3–6 months) or major cytogenetic response (by month 12) or progression of disease after a previous cytogenetic or

hematologic response. Imatinib intolerance included inability to tolerate 400 mg or more of imatinib per day or discontinuation of imatinib because of toxicity. The studies are ongoing. The results are based on a minimum of 6 months follow-up after the start of dasatinib therapy. Most patients had long disease histories with extensive prior treatment, including imatinib, cytotoxic chemotherapy, interferon, and stem cell transplant. The maximum imatinib dose had been 400–600 mg/day in about one-half of the patients and > 600 mg/day in the other half (14).

All patients were treated with dasatinib 70 mg, twice a day, on a continuous basis. The median durations of treatment were between 2.8 - 5.6 months (14).

The primary efficacy endpoint in chronic phase CML was major cytogenetic response (MCyR), defined as elimination (complete cytogenetic response, CCyR) or substantial diminution (by at least 65%, partial cytogenetic response) of Ph+ hematopoietic cells. The primary endpoint in accelerated phase, myeloid blast phase, and lymphoid blast phase CML, and Ph+ ALL was major hematologic response (MaHR), defined as either a complete hematologic response or no evidence of leukemia as defined in Table 1.

Most cytogenetic responses occurred after 12 weeks of treatment, when the first cytogenetic analyses were performed. Hematologic and cytogenetic responses were stable during the 6-month follow-up of patients with chronic phase, accelerated phase, and myeloid blast phase CML. The median durations of major hematologic response were 3.7 months in lymphoid blast CML and 4.8 months in Ph+ ALL.

There were no age- or gender-related response differences (14).

Table 1: Efficacy in Dasatinib Clinical Studies in CML and Ph+ ALL (All Treated Populations)[a]

	Chronic (n=186)	Accelerated (n=107)	Myeloid Blast (n=74)	Lymphoid Blast (n=42)	Ph+ ALL (n=36)
Hematologic Response Rate[b] (%)					
MaHR (95% CI)	n/a	**59 (49–68)**	**32 (22–44)**	**31 (18–47)**	**42 (26–59)**
CHR (95% CI)	**90 (85–94)**	33 (24–42)	24 (15–36)	26 (14-42)	31 (16–48)
NEL (95% CI)	n/a	26 (18–36)	8 (3–17)	5 (0.6–16)	11 (3.1–26)
Cytogenetic Response[c] (%)					
MCyR (95% CI)	45 (37–52)	31 (22–41)	30 (20–42)	50 (34–66)	58 (41–74)
CCyR (95% CI)	33 (26–40)	21 (14–30)	27 (17–39)	43 (28–59)	58 (41–74)

a Numbers in bold font are the results of primary endpoint.

b Hematologic response criteria (all responses confirmed after 4 weeks):
Major hematologic response: (MaHR) = complete hematologic response (CHR) + no evidence of leukemia (NEL).
CHR (chronic CML): WBC ≤ institutional ULN, platelets <450,000/mm^3, no blasts or promyelocytes in peripheral blood, <5% myelocytes plus metamyelocytes in peripheral blood, basophils in peripheral blood ≤ institutional ULN, and no extramedullary involvement.

CHR (advanced CML/Ph+ ALL): WBC ≤ institutional ULN, ANC ≥1000/mm^3, platelets ≥100,000/mm^3, no blasts or promyelocytes in peripheral blood, bone marrow blasts ≤5%, <5% myelocytes plus metamyelocytes in peripheral blood, basophils in peripheral blood ≤ institutional ULN, and no extramedullary involvement.

NEL: same criteria as for CHR but ANC ≥500/mm^3 and <1000/mm^3, and/or platelets ≥20,000/mm^3 and ≤100,000/mm^3.

c Cytogenetic response criteria: complete (0% Ph+ metaphases) or partial (>0%–35%). MCyR (0%–35%) combines both complete and partial responses.

n/a = not applicable.

2.2.4 Phase 1 Experience of Dasatinib in Solid Tumors other than Melanoma

In a Phase 1 study (CA180003) conducted by Bristol-Myers Squibb (BMS), dasatinib was administered on a twice a day schedule to 42 patients with refractory solid tumor. To date, doses up to 160 mg, twice a day, on a 5-day on/2-day off schedule have been administered. A dose of 120 mg, twice a day, continuous daily schedule is currently under investigation.

No severe clinical toxicity has been encountered. Gastrointestinal symptoms were reported in most patients, fatigue was reported in 17 patients (40%) and rash in 10 patients (24%). Edema, lethargy and headache were uncommon and appear to be dose-related. Grade 3 asymptomatic hypocalcemia was considered dose-limiting in one subject, Grade 2 rash was considered dose-limiting in two other patients, and Grade 2 nausea and vomiting (with dysarthria, lightheadedness and lethargy in a 49 kg subject taking concurrent diazepam) was considered dose-limiting in one subject.

In another Phase 1 study (CA180021), dasatinib was administered on a once daily schedule to 24 patients at doses up to 180 mg. Pleural effusions were observed in three patients at the 180 mg dose level (one with pneumonia and two with malignant effusion). A dose of 250 mg, once daily, is currently under consideration. Hypocalcemia, GI symptoms, and skin rash have been mild and infrequent.

To date, the safety profile in solid tumor patients has been similar to that in chronic phase CML patients with the exception of severe myelosuppression, which has not been observed in solid tumor patients and is considered related to efficacy against the leukemia as noted above, and severe bleeding which is secondary to thrombocytopenia in most instances (14).

2.2.5 Phase 2 Experience of Dasatinib in Melanoma Patients

Kluger *et al.* (15) have recently reported results of their Phase 2 study in which 39 patients with advanced-stage, chemotherapy-naïve, unresectable melanoma received dasatinib twice daily in order to assess treatment impact on objective response rates and progression-free survival (PFS), as well as, any associated toxicities. The initial dosing of 100 mg, twice daily, was reduced to 70 mg, twice daily, after treating 17 patients due to dose-limiting toxicities including fatigue, dypsea, and pleural effusion. Two patients exhibited partial response to therapy, while an additional 3 patients displayed minor responses, with a median PFS of 8 weeks and 13% of patients with a PFS of at least 6 months. Although the study failed to meet its clinical endpoints of a 30% response rate or 6-month PFS, and it displayed minimal efficacy as a single agent in unselected patients, the Kluger study suggested potential biased activity of dasatinib in patients

without c-KIT mutations. This suggestion will be further inspected in an ongoing Eastern Cooperative Oncology Group (ECOG) Phase 2 study investigating the activity of dasatinib in patients with c-KIT mutated melanoma. Given the possibility of reduced clinical benefit for dasatinib in patients with c-KIT mutant melanomas, and in the absence of additional insights from the open ECOG trial, we have chosen to exclude such patients in this protocol.

Regardless of the melanoma patient cohort selected for inclusion, however, Hersey *et al.* (16) suggest that receptor tyrosine kinases like dasatinib will exert greatest clinical benefit when integrated in combination therapy designs, such as the current dasatinib + vaccination schema.

2.3 Potential for Vaccine-Induced $CD8^+$ T cell Targeting of Tumor Antigens versus Tumor Blood Vessel Antigens

The finding of T lymphocyte infiltrates in melanomas and other solid tumor types (17-19) resulted in a series of studies to evaluate the adoptive transfer of patient-derived T cells in customized adoptive immunotherapy (AIT) approaches. Tumor-infiltrating lymphocytes (TIL) isolated from cancer patients were expanded to large numbers *ex vivo* using either rIL-2 or rIL-2/rIL-4 culture supplementation (20). These lymphocytes were then administered intravenously (IV) in combination with systemic rIL-2 therapy. However, the adoptive transfer of TIL appeared no more effective than rIL-2 alone in promoting clinical benefit (20, 21).

Studies performed using such T lymphocytes provided one of the first demonstrations of a T cell response to human melanoma through the development of a series of T cell lines. $CD8^+$ T cell lines showed specificity for autologous tumor cells in terms of mediating lytic activity and in producing IFN-γ (22, 23). More recent studies have gone on to define a vast array of melanoma-associated antigens and their corresponding MHC restricting elements that allow for tumor cell recognition by specific $CD8^+$ (i.e. HLA-A2-restricted among others; ref. 24) Melanoma cells (over)express a broad range of antigens that are capable of being recognized by T cells (25), each of which may be considered for integration into clinical vaccine formulations for the treatment of patients diagnosed with this disease.

Cancer vaccines based on tumor-associated antigens (TAA) have been extensively evaluated in both translational models and in the clinic (26). Although by most accounts TAA-based vaccines have been found to be immunogenic in promoting increased frequencies of Ag-specific T cell responses in a large proportion of treated patients, they have only rarely proven curative (27). This limitation in efficacy may relate, at least in part, to the heterogeneity of cancer cells found within a given tumor lesion, particularly with regard to sub-population immunophenotypes. Hence, tumor cells expressing lower levels of MHC molecules, molecules associated with the antigen-presentation machinery and/or therapeutically targeted tumor antigens have clear survival advantages in the face of specific vaccination (28-30). Indeed, many times patients that have exhibited objective clinical responses to immunomodulatory therapies ultimately progress

with tumors characterized by defects in their antigen-processing machinery and immunophenotype (31, 32).

A theoretical means by which to promote anti-tumor immunity, while coordinately circumventing the immunophenotypic "instability" of cancer cells themselves, involves the development of vaccines targeting tumor-associated stromal cells, such as (myo)fibroblasts, vascular pericytes, and VEC (33-35). Conditioned by the cancer microenvironment, tumor-associated blood vessel cells are believed to express phenotypes that distinguish them from comparable cell populations found in normal tissues, hypothetically allowing for their biased immune targeting by specific T effector cell populations (36-38). Interestingly, the prior vaccination of mice with fibroblast or endothelial cell lines has been reported to provide some degree of protection against subsequent tumor growth (39, 40). Furthermore, prophylactic peptide- and/or recombinant vaccines based on tumor blood vessel-associated antigens (TBVA) such as endoglin (CD105), NG2, PDGFRβ, VEGFR1, or VEGFR2 have been reported to provide partial protection against challenge with tumor cell lines that fail to express these antigens, presumably based on T cell-mediated anti-neovascular activity in the TME (41-47). In a very limited number of studies employing therapeutic models, VEGFR1 and VEGFR2 peptide-based vaccines were shown capable of modestly slowing the progressive growth of subcutaneous (SQ) tumors in mice (44, 47).

We have recently reported that IL-12 cytokine gene-therapy of established tumors growing in HLA-A2 transgenic mice (i.e. these mice exhibit a $CD8^+$ T cell repertoire that mimics that of $HLA\text{-}A2^+$ humans) results in $CD8^+$ T cell-mediated protective immunity directed at host blood vessel cells within the tumor microenvironment (48). $CD8^+$ T cells isolated from effectively-treated animals recognized a range of HLA-A2-presented peptides derived from TBVA. We have more recently shown (49) that vaccination of HHD mice with TBVA-derived peptides results in protective immunity against multiple types of solid cancers (including melanoma) under conditions that are non-permissive for direct T cell recognition of tumor cells. Indeed, when applied in the therapeutic setting, such vaccines can be curative, based on Type-1 $CD8^+$ T cell targeting of tumor- (but not normal tissue-) associated blood vessel cells (vascular pericytes and/or endothelial cells). Since the efficacious peptides derived from the TBVA DLK1 (delta-like kinase 1), EphA2, HBB (hemoglobin-β), NRP1 (neuropilin 1), RGS5 (regulator of G-signaling protein 5) and TEM-1 (tumor endothelial marker-1) that bear identical sequences in mouse and man, and can be recognized by $CD8^+$ T cells isolated from cancer patients (48), these translational data support the use of these specific peptides in vaccine formulations for $HLA\text{-}A2^+$ patients with solid cancers, including melanoma.

2.4 Immune Dysfunction in Patients with Advanced-Stage Solid Cancers

Type-1 (i.e, Th1 $CD4^+$ T cell and Tc1 $CD8^+$ T cell) responses play critical roles in the rejection

of tumors (50, 51). Th1 cells secrete IFN-γ and IL-2, both of which promote cellular immunity, in part by providing helper signals for the development of Tc1 (cytotoxic T) lymphocytes (50). Th2-type cells produce IL-4 and IL-5, and typically promote a humoral (i.e. antibody-mediated) immune response, while Th3 and regulatory T (T_{reg}) cells produce immunosuppressive cytokines (IL-10/TFGβ) that can dampen both Type-1 and Type-2 immune responses (51). The majority of studies have indicated that the most prevalent cytokine profile observed in patients with melanoma is consistent with a Type-2 functional bias *in situ* (52, 53). In prior studies we found little evidence for local Type-1 immune responses within the tumor; i.) there is a minimal expression of mRNA encoding for IL-2 and IFN-γ associated with TIL, and less than 5% of tumor infiltrating lymphocytes (TIL) expressed the activation marker, IL-2Rα (54). TIL were defective in their proliferative and cytotoxic capacities (54, 55), and there was also an impairment in Type-1 T cell responses against tumor-associated antigens in peripheral blood T cells harvested from RCC patients with active disease (52). An analysis of MAGE-6- and EphA2-specific $CD4^+$ T cells revealed the predominance of Type-2 responses (i.e. characterized by IL-5 but not IFN-γ production) after *in vitro* stimulation with autologous dendritic cells (DC) pulsed with MAGE-6 and EphA2 peptide epitopes (52).

Apoptosis may contribute to the immune dysfunction observed for T cells in cancer patients. In many solid cancers, a sizeable proportion of tumor-infiltrating lymphocytes (mainly T cells) exist in a pro-apoptotic state, and an even greater number of T cells undergo apoptosis following *in vitro* stimulation (56). Peripheral blood T cells isolated from cancer patients are more sensitive to activation induced cell death (AICD) versus comparable T cell populations harvested from healthy normal donors (57, 58). Most recently we observed that tumor antigen-specific T cells in the peripheral blood of melanoma patients with active disease are more sensitive to apoptosis when compared to influenza-specific T cells recovered from these same individuals (58).

In addition to these intrinsic defects associated with anti-tumor T cells in melanoma patients, there are also "extrinsic" mechanisms linked to the functional suppression of otherwise protective anti-tumor T cells. These include the increased numbers and function of T_{reg} and MDSC observed to be enriched in cancer patients (59-62).

There is growing evidence that $CD4^+CD25^{hi+}$regulatory T cells (T_{reg}) may play an important role in suppressing the development of anti-tumor immunity in cancer patients (63). The frequency of T_{reg} is elevated in tumor sites and/or the peripheral blood of patients with advanced tumors (59-62). T_{reg} cells can impair induction of both antigen-specific and nonspecific T cells in cancer patients (63) and predict reduced survival in multiple cancer cell types (64, 65). Relevant to therapy, experimental models have shown that removal of T_{reg} cells facilitates the therapeutic efficacy of protective immunity. Depletion of T_{reg} cells in mouse models using anti-CD25 antibody enhances anti-tumor activity (66). Furthermore, reducing peripheral blood T_{reg} cell

numbers in melanoma patients enhances immunocompetence and responsiveness to active immunization (67-69).

MDSC act to suppress T cell effector function, and represent a heterogeneous population of cells derived from the myeloid lineage that accumulate in tumor bearing hosts as a result of tumor induced alterations in myelopoiesis (70). MDSC have mostly been studied in mice where they are identified by the co-expression of CD11b and Gr-1, accumulate in lymphoid organs and tumors of tumor bearing mice, and mediate T cell impairment that is reversed with tumor removal or with $CD11b^+$ or $Gr\text{-}1^+$ cell depletion (71). MDSC have been described in human patients with many cancers including kidney cancer (72, 73), where they are defined as cells that express the common myeloid marker CD33, but lack markers of mature myeloid cells such as the MHC class II molecule HLA-DR. The accumulation of these cells in the peripheral blood of advanced-stage cancer patients has been associated with a decrease in the number and effectiveness of peripheral blood DC, as well as a decrease in T cell expression of TCR-ζ (signaling) chain and IFN-γ production (70-73). Studies performed in metastatic patients suggest these inhibitory effects are mediated via MDSC-derived reactive oxygen species (ROS) and MDSC-induced depletion of key biosynthetic amino acids (i.e. arginine, cystine, cysteine and tryptophan; refs. (70-74).

2.5 Dendritic Cells

Pre-clinical studies using Dendritic Cells (DC) to stimulate specific anti-tumor T cell responses have been encouraging (75-79). DC are the most potent antigen presenting cells (APCs), capable of efficiently internalizing and presenting antigen in the context of co-stimulatory signals and cytokines that are essential to the induction of effective long-lasting T cell-mediated immunity. Animal models have demonstrated that DC, pulsed *ex vivo* with defined tumor antigens (peptide epitopes) or material derived from tumor cells, are capable of eliciting protective, antigen-specific immune responses (75, 80). Importantly, clinical trials have confirmed that DC pulsed with defined (tumor) peptide epitopes or tumor cell lysates induce anti-tumor T cell responses, and that occasionally, such immunity coincides with complete tumor regression in late stage patients (81-83).

2.6 Type-1 Polarized DC

Two functions of dendritic cells are believed to be important for the ability of DC to induce Type-1 Th1 cells and Tc1; i.e. high co-stimulatory activity and high secreted protein levels of anti-cancer cytokines, especially IL-12 (79-83). Many previous clinical trials have relied on the use of either fully-matured DC exhibiting high stimulatory function but low IL-12 secretion, or immature DC that display low stimulatory/high IL-12 secretion functions. Using a novel culture method, it is now possible to generate mature DC that exhibit high levels of co-stimulatory activity and IL-12 production (84). Such DC are referred to as Type-1 polarized DC or αDC1. *In*

vitro observations show that αDC1 induce up to 50-fold higher frequencies of tumor antigen-specific $CD8^+$ T cells after *in vitro* sensitization (the equivalent of vaccination) when compared to conventionally matured DCs that have been previously employed in clinical trials (84).

In addition to their potent immunostimulatory function and high-level production capacity for Type-1 polarizing cytokines, αDC1 exhibit a stable phenotype that is resistant to tumor-associated immunosuppressive factors, including IL-10 and PGE_2 (84-87). This suggests the possibility of using αDC1 as a biologic adjuvant to enhance the clinical efficacy of cancer vaccines, despite the immune suppressive environment known to exist in many patients with melanoma.

Recently the original αDC1 protocol based on fetal bovine serum-supplemented cultures has been modified to allow αDC1 generation in serum-free media (84). This has facilitated the translation of αDC1 into ongoing Phase 1/2 clinical trials being performed at the University of Pittsburgh Medical Center (UPMC)/University of Pittsburgh Cancer Institute (UPCI) in the setting of melanoma (UPCI 03-118, BB-IND 11,754), colorectal cancer (UPCI 05-063; BB-IND 13,234), and glioma (UPCI 05-115; BB-IND 12,415). Thus far clinical activity for vaccination has been observed in 2 of the initial 4 melanoma patients who completed treatment (1PR 12M+ in a patient with Stage IIIb disease; 1 SD 11M+ in a patient with stage IV lung disease). The two first patients with resected metastatic CRC are undergoing follow-up for time to recurrence and immunologic evaluations. We also recently completed a Phase 1/2 αDC1 (1-3 x 10^7 cells) + glioma peptide (derived from the EphA2 [specifically $EphA2_{883-891}$ to be used in the current vaccine formulation], IL-13Rα2, TKL-40 and gp100 antigens) vaccine trial of 22 HLA-$A2^+$ patients with malignant glioma (88). More than half of the patients exhibited vaccine-associated increases against at least one of the 4 treatment peptides, with increased levels of serum IFN-α and CXCL10/IP-10 post-vaccination. Nine patients achieved a PFS exceeding 12 months, with one patient developing a complete response to treatment. To date, these regimen have proven to be well-tolerated, with no untoward side effects observed in any of these settings.

2.7 Improved Anti-Tumor Efficacy for Combined Dasatinib + Vaccine Therapy

Given our own and others' recent reports supporting the ability of tyrosine kinase inhibitors (such as sunitinib and dasatinib) to mitigate at least a portion of the immunosuppressive circuits (i.e. regulatory T cells and myeloid-derived suppressor cells) present in cancer patients, we hypothesize that the co-application of dasatinib will increase the immunogenicity of specific vaccination protocols.

We recently performed and published proof-of-concept, pre-clinical studies in which we observed superior anti-tumor efficacy for therapies combining TKI (sunitinib) + vaccination (DC1/peptide) approaches (89). This was particularly evident when sunitinib was applied at the time of the initial vaccination or the vaccine booster. Treatment effectiveness was associated

with the acute loss of (and/or failure to recruit) cells bearing MDSC or T_{reg} phenotypes within the melanoma microenvironment and the corollary, prolonged enhancement of specific Type-1 $CD8^+$ T cell responses in the melanoma-draining lymph node (TDLN) and the melanoma lesion itself. Enhanced Type-1 T cell infiltration of tumors was associated with treatment-induced expression of VCAM-1 and CXCR3 ligand chemokines (CXCL9/Mig, CXCL10/IP-10 and CXCL11/ITAC) in vascular/peri-vascular cells within the tumor microenvironment (TME), with the combined therapy benefits negated by administration of blocking antibodies against CXCR3 or vascular cell adhesion molecule (VCAM)-1. These data support the ability of sunitinib to enhance the capacity of vaccines to elicit Type-1 $CD8^+$ T cell responses in tumor-bearing hosts and to concomitantly (re)condition the TME to become more receptive to the recruitment and prolonged therapeutic action of such vaccine-induced $CD8^+$ T effector cells. More recently we have observed similar tendencies when combining dasatinib and DC1/peptide vaccines in murine melanoma models (**Fig. 1**).

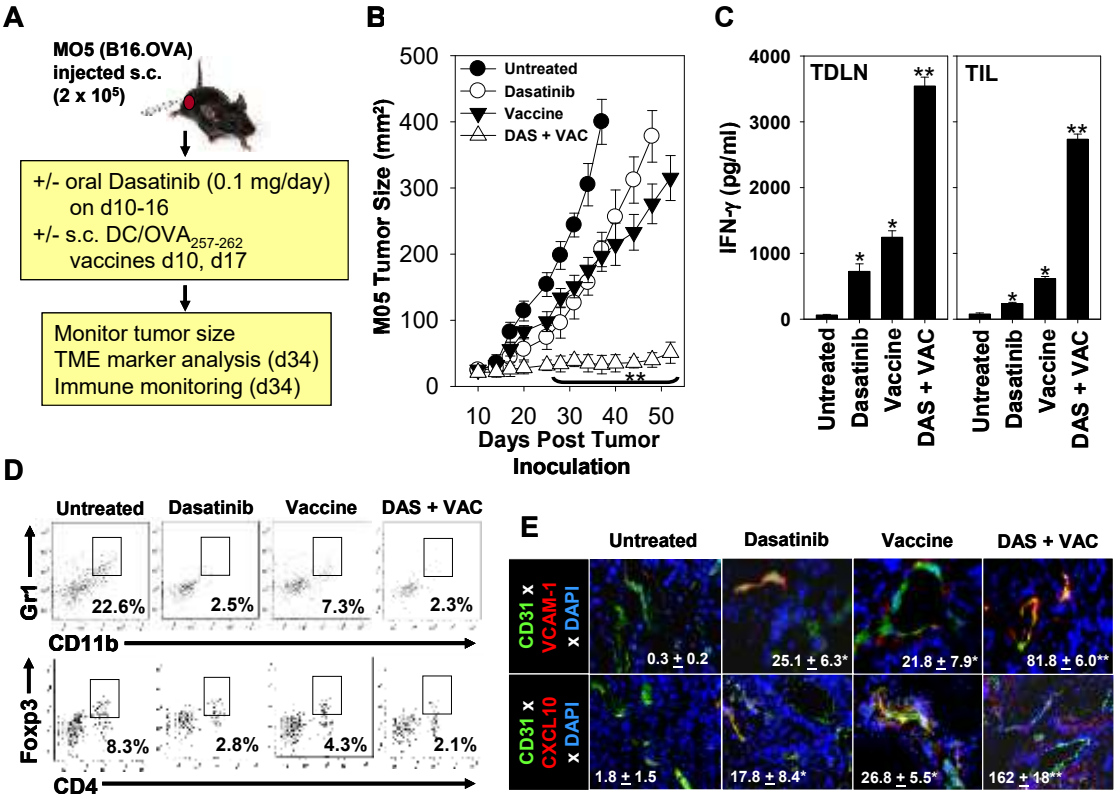

Fig. 1. Combination dasatinib + DC1/peptide-based vaccination results in superior activation of specific CD8+ T cells and anti-tumor efficacy in a murine melanoma model. (A) M05 (B16.OVA) melanoma cells were injected s.c. into syngenic C57BL/6 mice and allowed to establish for 10 days, at which time animals were randomized for tumor size and cohorts of 7 mice/group were left untreated, or they were treated with orally administered dasatinib (DAS; 0.1 mg/day for 7 consecutive days), a contralateral s.c. vaccine (VAC) consisting of 10^6 OVA peptide-pulsed dendritic cells (DC) or a combination of DAS + VAC. Animals were then monitored for tumor growth (***B***) and at day 34, 2 mice/group were sacrificed for analysis of specific T cell (isolated from the tumor-draining lymph node (TDLN) of tumor (TIL)) recognition of the OVA peptide based on IFN-γ secretion as monitored by ELISA (***C***). Day 34 tumors were also isolated and analyzed for their content of myeloid-derived suppressor cells (MDSC; bearing a $CD11b^+Gr1^+$ phenotype) and regulatory T cells (Treg; bearing a $CD4^+Foxp3^+$ phenotype) by flow cytometry (***D***);

percentage of cells bearing the specified phenotype is reported in panel insets) and for expression of VCAM-1 and CXCL10 proximal to $CD31^+$ vascular endothelial cells using immunofluorescence microscopy (***E***); mean +/- SD number of cells over 10 high-power fields are reported in panel insets). Three experiments were performed independently, each yielding similar data, *p < 0.05 versus untreated; **p < 0.05 versus all other groups.

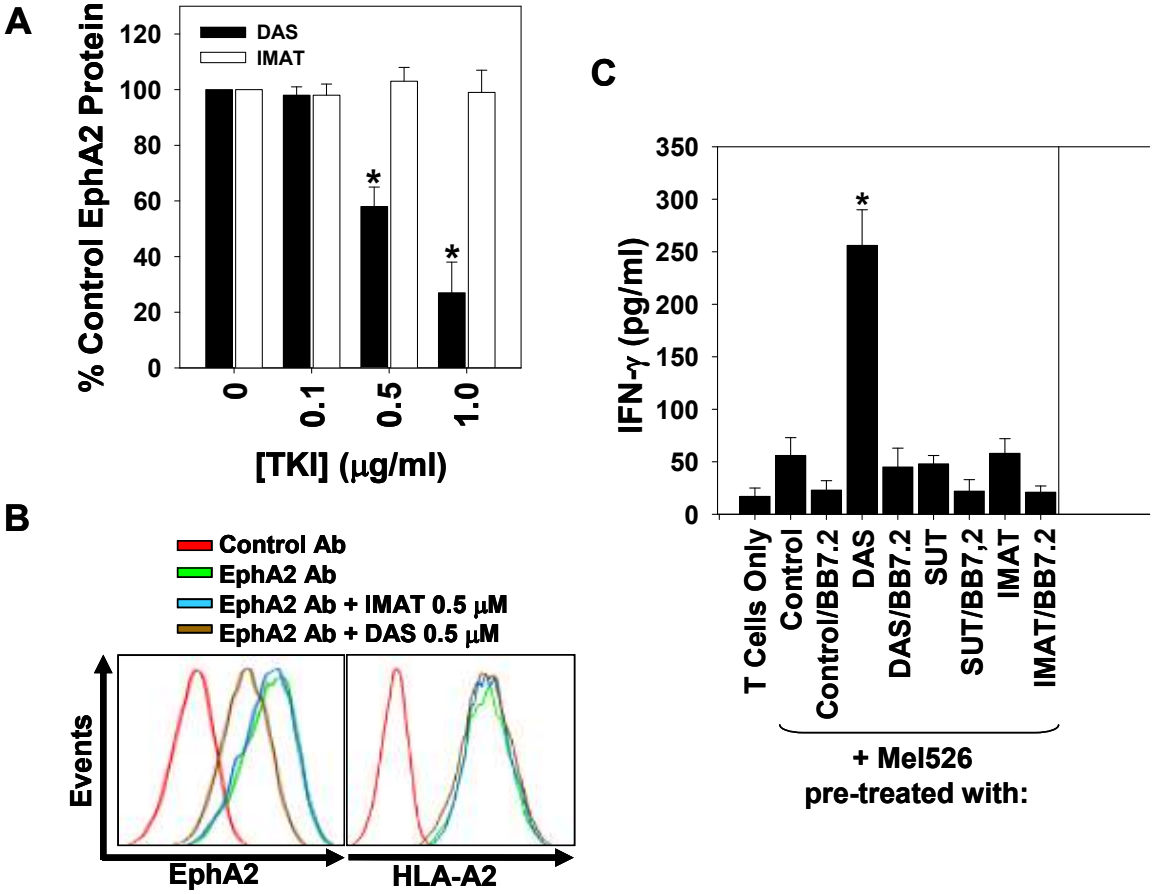

Fig. 2. Dasatinib (but not imatinib) pre-treatment of HLA-$A2^+$ human melanoma cell-line Mel526 increases degradation of EphA2 and improves tumor cell recognition by EphA2-specific $CD8^+$ T cells. In addition to its inhibitory action on src kinases, dasatinib has been reported to inhibit signaling through the receptor tyrosine kinase EphA2 (90) which is commonly over-expressed by human melanomas in situ (91) and shown to represent a critical oncogene in melanoma tumorigenic potential (92). We treated the human HLA-$A2^+$, $EphA2^+$ with various doses of dasatinib or imatinib for 24h and analyzed EphA2 protein by western blotting. Panel ***A*** reports the percentage of control EphA2 expression in the TKI-treated melanoma cells. This loss of EphA2 protein is due to the proteasome-dependent degradation of the protein based on the ability of MG-132 and lactacystin to block protein loss (data not shown). Using flow cytometry (***B***), we also showed that cell surface expression of EphA2, but not HLA-A2 class I molecules was reduced selectively after treatment with dasatinib. Finally, since proteasomal processing is required for the generation of many MHC class I-presented peptides to $CD8^+$ T cells, we next analyzed whether TKI (0.5 mg/ml) pre-treatment enhanced tumor cell recognition by anti-EphA2 $CD8^+$ T cells based on their secretion of IFN-γ after stimulation with these melanoma cells (***C***). To validate the HLA-A2-restricted nature of T cell recognition, we showed that addition of the anti-HLA-A2 blocking antibody BB7.2 to T cell/melanoma cultures ablated specific IFN-γ production by the $CD8^+$ T cells. *p < 0.05 versus control untreated tumor cells (Control) and dasatinib-treated tumor cells + BB7.2 antibody (DAS/BB7.2).

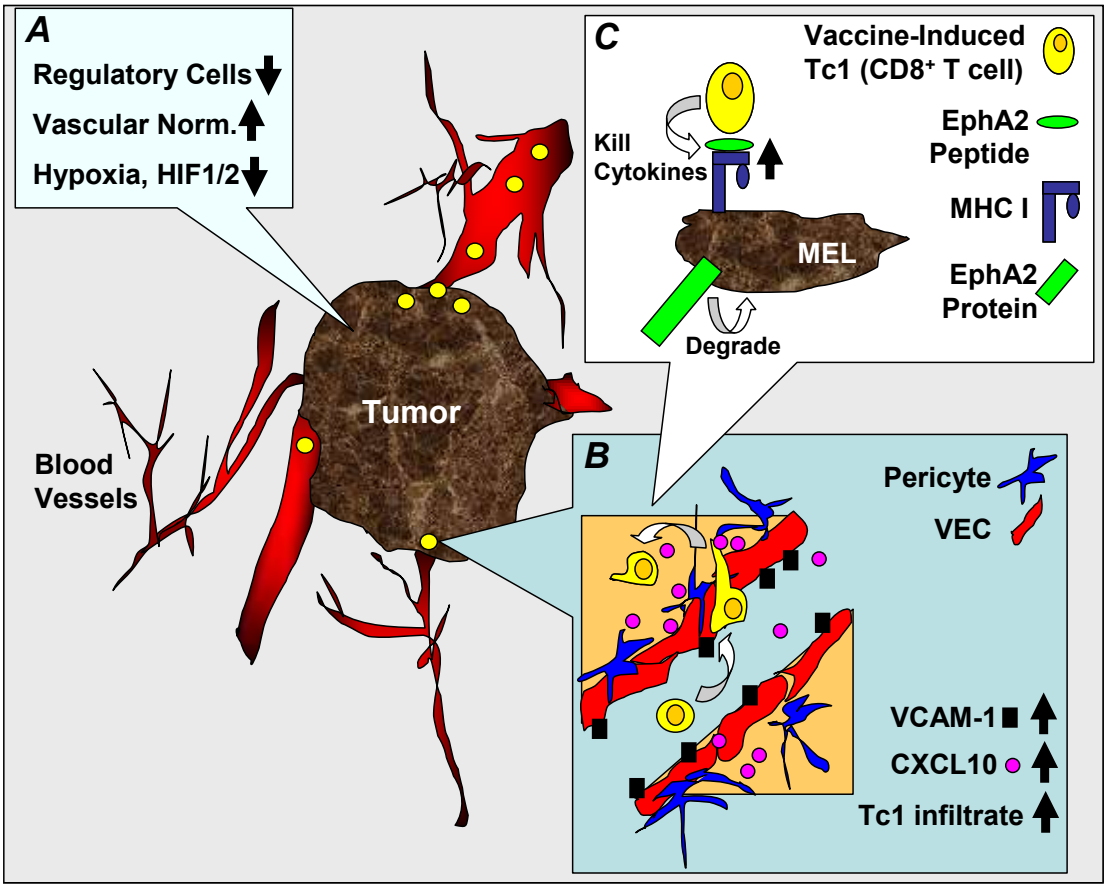

Fig. 3. Proposed mechanism of action for dastinib + vaccine co-therapy. Dasatinib is proposed to mediate beneficial anti-tumor impact based on a series of effects including its acute inhibition of immunoregulatory cells (MDSC and Treg) and normalization of blood vasculature within the tumor microenvironment (TME), leading to reduced hypoxia and interstitial fluid pressure (**A**). Stromal cells within the TME alter their expression of chemokines and adhesion molecules, such as CXCL10 and VCAM-1 that are required for recruitment and infiltration of protective $CXCR3^{+}VLA4^{+}$ Type-1 $CD8^{+}$ T cells (**B**). Dasatinib also mediates direct effects on $EphA2^{+}$ melanoma cells, by increasing the proteolytic processing of this protein into T cell recognized peptide epitopes, leading to enhanced anti-tumor activity mediated by anti-EphA2 TIL *in vivo* (**C**).

3. STUDY RATIONALE

3.1 Importance and Rationale for the Trial

Current therapeutic approaches available for patients with advanced-stage melanoma remain inadequate, and existing approaches including those involving immunotherapy with cytokines and/or targeted strategies have resulted in disappointingly low rates of durable and complete responses. Correcting immune dysfunction in advanced-stage melanoma patients using TKI such as dasatinib is proposed to relicense the patient's immune system to respond optimally to specific immunization. The integration of antigens expressed by tumor-associated blood vessel cells provides a means to selectively target the genetically-/antigenically-heterogeneous population of tumor cells in the advanced-stage melanoma patient.

The current trial represents a pilot, randomized, Phase 2 study to determine the activity and safety of intradermal administration of αDC1s loaded with a mixture of six TBVA-derived

peptides at the time of, or a cycle prior to, starting study treatment with the TKI dasatinib.

3.2 Rationale for Vaccine Dose, Route of Administration and Schedule

We propose to analyze $CD8^+$ T cell responses against the TBVA DLK1, EphA2, HBB, NRP1, RGS5, and TEM1 in peripheral blood of HLA-$A2^+$ melanoma patients prior to, during the course of, and one month after the last dose of dasatinib. Based on the strong Type-1 polarizing potential of αDC1 *in vitro*, we hypothesize that these vaccines will enhance Type-1 $CD8^+$ T cell responses against at least 3 of the 6 peptides included in the vaccine (particularly when patients receive concurrent dasatinib administration, as this removes the regulatory action of MDSC/T_{reg} suppressor cells).

We chose to evaluate a mixture of six TBVA-derived epitopes to be applied to αDC1s as an intradermal vaccine injection into 28 HLA-$A2^+$ patients with advanced-stage melanoma. This choice is based on the finding of superior anti-tumor efficacy in HLA-A2 transgenic tumor models for the pooled peptide vaccine approach and the relevance of the previously identified TBVA-derived peptides (which share sequence identity in both human and mouse TBVA) in the HLA-$A2^+$ human patient setting (48, 49). Our combinational vaccine + dasatinib modeling suggest that optimal therapeutic benefit against established M05 melanoma occurred when sunitinib administration was initiated at the time of initial vaccination or at the time of boosting.

Systemic review and meta-analysis of previous DC-based vaccine trials in cancer patients suggests that: i.) vaccine-induced T cell responses are associated with beneficial clinical outcome; ii.) mature DC (such as αDC1) were superior activators of specific immunity and a better clinical prognosis when compared to immature DC; iii.) while a threshold dose of DC is required in the vaccine in order to promote specific immunity, a vast increase in DC number over that threshold did not generally yield superior efficacy (93-98). A recent study by Verdijk *et al.* suggests that intradermal delivery of DC-based vaccines in patients with advanced stage melanoma was clinically equitable to the delivery of these cells directly into lymph nodes (95), while a report from Lesterhuis and colleagues argues that intradermal delivered DC-based vaccines were superior to intranodal delivered vaccines in promoting melanoma-specific T cell activation *in vivo* (96). *In vivo* tracking of intradermal injected DC in melanoma patients suggests that approximately 4% of the administered DC actually migrate to tissue-draining lymph nodes and that the delivery of approximately 5×10^5 (vaccine) DC are needed to promote clinically-meaningful levels of antigen-specific T cells (95). By extrapolation, these figures indicate that intradermal injection of a vaccine containing approximately 10^7 mature antigen-loaded αDC1 would be anticipated to provide a quasi-optimal degree of immune stimulation that may be associated with clinical benefit. Pre-clinical, clinical, and mathematical modeling all suggest that optimal vaccine-induced immunity and benefit to the tumor-bearing host can be best achieved through repeated immunization (3-5 vaccines) provided over a regular-interval

schedule (93-98). Since there is no consensus in the literature for an optimal time interval between the individual vaccinations, we have adopted a protocol involving 4 intradermal vaccines every 2 weeks, which is a commonly employed schedule for DC-based vaccines (96).

4. STUDY DESIGN

This is a single-center, prospective randomized, pilot, Phase 2 trial evaluating the activity, safety and immune effects of dasatinib given in combination with an autologous type-1 polarized DC vaccine. Dasatinib will be administered at the standard dose and schedule recommended by the FDA (70 mg BID). The autologous type-I DC vaccine will be administered either prior to, or concomitant with, the inititation of dasatinib administration. Patients will be vaccinated intradermally with the αDC1/peptide mixture on days 1 and 15 of every cycle on an outpatient basis in the University of Pittsburgh Clinical and Translational Research Center (UPCI-CTRC). For those patients starting therapy with vaccine alone, dasatinib will be initiated on day 29 after receiving the first immunization. Unless patients are removed from study, they will be treated for at least 6 cycles or disease progression. In cases where there is continued clinical benefit and no additional vaccine product is available, patients can continue to be treated on single agent dasatinib. Additional leukapheresis procedures may be performed at the discretion of the Investigator/sub-investigator for patients that derive benefit but require the production of additional vaccine.

4.1 Leukapheresis

Leukapheresis (3 hours, 180 minutes) is a minimal risk procedure. Prior to the procedure each subject's venous access will be evaluated. If a subject does not have acceptable venous access a pheresis catheter will need to be put in place. All selected patients will undergo a single limited leukapheresis once they have been deemed eligible and prior to the first course of vaccination. If the patients receive benefit from the vaccine, but require additional cells for vaccine production, the leukapheresis may be repeated at the discretion of the investigator. The additional leukapheresis will take about two hours (120 minutes) to complete. One time of the subject's blood volume will be processed per procedure. The product is delivered immediately to the UPCI Immunologic Monitoring and Cellular Products Laboratory (IMCPL) from the UPCI-CTRC. Additional leukapheresis procedures may be performed at the discretion of the Investigator/sub-investigator for patients that derive benefit but require production of additional vaccine.

Leukapheresed product will be immediately processed as described in the Chemistry, Manufacturing, and Control section of BB-IND 11754, and a part of it will be used for the first vaccination course (Week 1). The remainder of the product will be cryopreserved as described. If cytopenia (WBC < 2000/mm^3 or platelets < 40,000/mm^3) develops during, or as a result of, leukapheresis, the procedure will be postponed until recovery. This will not be considered an adverse event. Samples from each cell product will be obtained for hemoglobin, hematocrit, total

WBC, and differential and platelet count.

4.2 Vaccine

4.2.1 Formulation

Dendritic cells (DC) are derived from autologous (the subject's own) adherent mononuclear cells (monocytes) in the peripheral blood obtained from leukapheresis. In this case, "biologic product" and "biologic substance" are the same. We prefer the term "biologic product."

The vaccine will be manufactured in the UPCI-IMPCL, under cGMP conditions.

4.2.2 Storage and preparation

The final product is placed in vials with labels identifying each unique vaccine lot and cryopreserved. DCs used in the vaccine will be suspended in 5% human serum albumin (HSA) and delivered to the clinic for administration. For preparation of the vaccines, the labeled vials of cryopreserved αDC1 are removed from storage in liquid nitrogen and quickly thawed in a 37^{o}C water bath. After 3 washes in sterile medium, thawed αDC1 will be suspended in saline with 5% human serum albumin (HSA), placed in sterile syringe for administration to the subject and delivered to the clinic for administration, depending on time of notification, it can be as early as 10 am. Each syringe will be labeled with a custom-designed label, identifying the subject and the vaccine. Both saline and HSA are clinical grade (NDC 0944-0490-01 and NDC 0338-0049-31) and available from UPMC hospital pharmacy as injection products for humans.

4.2.3 Administration

The autologous type-I DC vaccine will be administered intradermally either prior to, or concomitant with, the initiation of dasatinib administration. The injections will be performed on an outpatient basis in the UPCI-CTRC.

4.2.4 Anticipated Adverse Events (Vaccine)

Dendritic cell-based vaccines have been extensively evaluated in thousands of cancer patients over the past 15 years (25, 27, 79, 81, 82, 95, 96) and found to be safe and extremely well-tolerated. The experience of the UPMC/UPCI in trials involving vaccination with αDC1 has similarly shown that this cellular therapy is safe (88). Since the toxicities observed for the TKI are not of an immunologic nature, we do not anticipate any increase in adverse event frequency or severity for the dasatinib + vaccine cohort (Arm B) to exceed the level previously observed for dasatinib alone.

Dendritic cell vaccines carry a potential risk of development of autoimmune disease (such as lupus or vitiligo). Whereas development of vitiligo has been reported with similar vaccines administered to melanoma subjects, and correlated with positive response to vaccination, other side-effects have not been reported. Based on the previous trials with DC-based vaccines, the chances of developing an allergic reaction to DC-based vaccines are rare. There is a chance that

subjects could have an allergic reaction to the vaccine.

4.3 Dasatinib

Dasatinib is provided by BMS in 3 different strengths:

Strength	Description
5 mg	white, round, film coated tablet
20 mg	white to off-white, biconvex, round, film coated tablet with either "20" or "BMS" debossed on one side and "527" on the other side
50 mg	white to off-white, biconvex, oval, film coated tablet with either "50" or "BMS" debossed on one side and "528" on the other side

4.3.1 Packaging and Labeling

Dasatinib is supplied as 5 mg, 20 mg, and 50 mg film-coated tablets containing dasatinib with lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, hydroxypropyl cellulose, and magnesium stearate. The tablet coating contains hydroxypropyl methylcellulose, titanium dioxide, and polyethylene glycol (triacetin in the 5 mg film-coated tablet). Tablets for clinical studies are supplied in high-density polyethylene bottles containing a desiccant and cotton. The bottles are heat-induction sealed with child resistant caps.

Each bottle is labeled in an open label manner. Labels contain, at a minimum, the following information: product name, tablet strength, batch number, directions for use, storage conditions, and appropriate caution statements.

4.3.2 Storage

Bottles containing dasatinib tablets should be stored at 15° - 25°C.

The investigational product should be stored in a secure area according to local regulations. The investigator is responsible for ensuring that it is dispensed only to study patients and only from official study sites by authorized personnel, as dictated by local regulations.

The investigator is responsible for ensuring that the investigational product is stored under the appropriate environmental conditions (temperature, light, and humidity).

If concerns regarding the quality or appearance of the investigational product arise, do not dispense the investigational product, and contact BMS immediately.

4.3.3 Handling and Dispensing

Procedures for proper handling and disposal of anticancer drugs should be considered.

Dasatinib tablets consist of a core tablet (containing the active drug substance), surrounded by a film coating to prevent exposure of pharmacy and clinical personnel to the active drug substance.

If tablets are crushed or broken, pharmacy and clinical personnel should wear disposable chemotherapy gloves. Personnel who are pregnant should avoid exposure to crushed and/or broken tablets.

The Investigator (or assigned designee, i.e., study pharmacist) will dispense the proper number of each strength tablet to the subject to satisfy dosing requirements for the study. The containers provided to the subject should be labeled with proper instructions for use. The lot numbers, dosing start dates and the number of tablets for each dosage strength must be recorded on the drug accountability pages of record for the site. The subject must be instructed to return all unused dasatinib in the provided packaging at each subsequent visit.

4.3.4 Drug Ordering and Accountability

Initial Orders of dasatinib should be requested by completing the Dasatinib (Sprycel®) Drug Supply Form for Investigator Sponsored Studies and submitting the request form electronically via e-mail at least 5-7 business days before the expected delivery date. Deliveries will be made Tuesday through Friday.

Initial drug supply will be provided for a 12-week treatment period per subject.

Please send drug supply form to: srcsupply@bms.com.

Re-supply requests should be sent to the appropriate email address (see Section 4.3.4). Please check "Re-supply" on the drug supply form. Re-supply requests should be submitted at least 5-7 business days before the expected delivery date. Deliveries will be made Tuesday through Friday.

4.3.5 Safety of Dasatinib in Clinical Studies in CML and Ph+ ALL

The data described below reflect exposure to dasatinib in 911 patients with leukemia from 1 Phase 1 and 5 Phase 2 clinical studies. The median duration of therapy was 6 months (range 0–19 months).

The majority of dasatinib-treated patients experienced adverse drug reactions at some time. Drug was discontinued for adverse drug reactions in 6% of patients in chronic phase CML, 5% in accelerated phase CML, 11% in myeloid blast phase CML, and 6% in lymphoid blast phase CML or Ph+ ALL.

The most frequently reported serious adverse events (SAEs) included pyrexia (9%), pleural effusion (8%), febrile neutropenia (7%), gastrointestinal bleeding (6%), pneumonia (6%), thrombocytopenia (5%), dyspnea (4%), anemia (3%), cardiac failure (3%), and diarrhea (2%).

All treatment-emergent adverse events (excluding laboratory abnormalities), regardless of relationship to study drug, that were reported in at least 20% of the patients in dasatinib clinical studies are shown in **Table 2**.

Table 2. Adverse Events Reported ≥20% in Clinical Studies in CML and Ph+ ALL

	All Patients (n=911)		Chronic Phase (n=488)	Accelerated Phase (n=186)	Myeloid Blast Phase (n=132)	Lymphoid Blast Phase and Ph+ ALL (n=105)
	All Grades	Grades 3/4	Grades 3/4			
Preferred Term	Percent (%) of Patients					
Fluid Retention	50	9	6	6	23	9
Superficial Edema	36	1	0	2	3	2
Pleural Effusion	22	5	3	3	14	8
Diarrhea	49	5	3	10	8	6
Headache	40	2	2	2	4	6
Hemorrhage	40	10	3	18	23	17
Musculoskeletal Pain	39	4	2	3	6	13
Pyrexia	39	5	1	5	13	9
Fatigue	39	3	2	4	4	8
Skin Rash	35	1	1	1	1	4
Nausea	34	1	<1	0	5	2
Dyspnea	32	6	5	7	11	9
Cough	28	<1	<1	1	1	0
Infection (including bacterial, viral, fungal, non-specified)	34	7	4	8	15	13
Infection/Inflammation	26	1	1	1	5	1
Abdominal Pain	25	2	1	2	4	6
Pain	26	2	<1	1	5	4
Vomiting	22	1	1	2	2	2
Febrile Neutropenia	9	8	2	11	17	20

Table 3. CTC Grades 3/4 Laboratory Abnormalities in Clinical Studies in CML and Ph+ ALL

	Chronic Phase (n=488)	Accelerated Phase (n=186)	Myeloid Blast Phase (n=132)	Lymphoid Blast Phase and Ph+ ALL (n=105)
	Percent (%) of Patients			
Hematology Parameters				
Neutropenia	49	74	83	81
Thrombocytopenia	48	83	82	83
Anemia	18	70	70	51
Biochemistry Parameters				
Hypophosphatemia	11	13	23	21
Hypocalcemia	2	9	20	15

	Chronic Phase (n=488)	Accelerated Phase (n=186)	Myeloid Blast Phase (n=132)	Lymphoid Blast Phase and Ph+ ALL (n=105)
Elevated SGPT (ALT)	1	4	7	11
Elevated SGOT (AST)	1	2	5	8
Elevated Bilirubin	<1	1	5	8
Elevated Creatinine	0	2	1	1

CTC grades: neutropenia (Grade 3 ≥0.5–1.0 × 10^9/L, Grade 4 <0.5 × 10^9/L); thrombocytopenia (Grade 3 ≥10–50 × 10^9/L, Grade 4 <10 × 10^9/L); anemia (hemoglobin ≥65–80 g/L, Grade 4 <65 g/L); elevated creatinine (Grade 3 >3–6 × upper limit normal range (ULN), Grade 4 >6 × ULN); elevated bilirubin (Grade 3 >3–10 × ULN, Grade 4 >10 × ULN); elevated SGOT or SGPT (Grade 3 >5–20 × ULN, Grade 4 >20 × ULN); hypocalcemia (Grade 3 <7.0–6.0 mg/dL, Grade 4 <6.0 mg/dL); hypophosphatemia (Grade 3 <2.0–1.0 mg/dL, Grade 4 <1.0 mg/dL).

4.3.6 Laboratory Abnormalities

Myelosuppression was commonly reported in all patient populations. The frequency of Grade 3 or 4 neutropenia, thrombocytopenia, and anemia was higher in patients with advanced CML or Ph+ ALL than in chronic phase CML. Myelosuppression was reported in patients with normal baseline laboratory values as well as in patients with pre-existing laboratory abnormalities. (**Table 3**)

In patients who experienced severe myelosuppression, recovery generally occurred following dose interruption and/or reduction; permanent discontinuation of treatment occurred in 1% of patients.

Grade 3 or 4 elevations of transaminases or bilirubin and Grade 3 or 4 hypocalcemia and hypophosphatemia were reported in patients with all phases of CML but were reported with an increased frequency in patients with myeloid or lymphoid blast CML and Ph+ ALL. Elevations in transaminases or bilirubin were usually managed with dose reduction or interruption. Patients developing Grade 3 or 4 hypocalcemia during the course of dasatinib therapy often had recovery with oral calcium supplementation (**Table 3**).

4.3.7 Anticipated Adverse Events (Dasatinib)

4.3.7.1 Myelosuppression

Treatment with dasatinib is associated with severe (NCI CTC Grade 3 or 4) thrombocytopenia, neutropenia, and anemia. Their occurrence is more frequent in patients with advanced CML or Ph+ ALL than in chronic phase CML. Complete blood counts should be performed weekly for the first month and then monthly thereafter, or as clinically indicated. Myelosuppression was generally reversible and usually managed by withholding dasatinib temporarily or dose reduction (14).

4.3.7.2 Bleeding Related Events

In addition to causing thrombocytopenia in human patients, dasatinib caused platelet dysfunction

in vitro. Severe CNS hemorrhages, including fatalities, occurred in 1% of patients receiving dasatinib. Severe gastrointestinal hemorrhage occurred in 7% of patients and generally required treatment interruptions and transfusions. Other cases of severe hemorrhage occurred in 4% of patients. Most bleeding events were associated with severe thrombocytopenia.

Patients were excluded from participation in dasatinib clinical studies if they took medications that inhibit platelet function or anticoagulants. Caution should be exercised if patients are required to take medications that inhibit platelet function or anticoagulants.

4.3.7.3 Fluid Retention

Dasatinib is associated with fluid retention, which was severe in 9% of patients, including pleural and pericardial effusion reported in 5% and 1% of patients, respectively. Severe ascites and generalized edema were each reported in 1%. Severe pulmonary edema was reported in 1% of patients. Patients who develop symptoms suggestive of pleural effusion such as dyspnea or dry cough should be evaluated by chest X-ray. Severe pleural effusion may require thoracentesis and oxygen therapy. Fluid retention events were typically managed by supportive care measures that include diuretics or short courses of steroids (14).

4.3.7.4 QT Prolongation

In vitro data suggest that dasatinib has the potential to prolong cardiac ventricular repolarization (QT interval). In single-arm clinical studies in patients with leukemia treated with dasatinib, the mean QTc interval changes from baseline using Fridericia's method (QTcF) were 3–6 msec; the upper 95% confidence intervals for all mean changes from baseline were <8 msec. Nine patients had QTc prolongation reported as an adverse event. Three patients (<1%) experienced a QTcF >500 msec.

Dasatinib should be administered with caution to patients who have or may develop prolongation of QTc. These include patients with hypokalemia or hypomagnesemia, patients with congenital long QT syndrome, patients taking anti-arrhythmic medicines or other medicinal products that lead to QT prolongation, and cumulative high-dose anthracycline therapy. Hypokalemia or hypomagnesemia should be corrected prior to dasatinib administration (14).

5. STUDY TREATMENT PLAN

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

5.1 Dasatinib Administration

All patients will receive dasatinib at a starting dose of 70 mg twice daily by mouth in the outpatient setting. Dasatinib will be supplied as 50 mg and 20 mg tablets. Patients will take 1 of the 50 mg tablets and 1 of the 20 mg tablets twice daily, approximately every 12 hours, at the same time each day. Dasatinib may be taken with or without food. Patients are to swallow the

tablets whole. The tablets should not be cut, crushed or dissolved. Study medication will be self-administered and patients will be asked to keep a daily diary of the time and dose of drug ingested.

Patients on Arm A will start dasatinib administration on cycle 2, day 1 (week 5), while those patients in Arm B will start dasatinib administration on cycle 1, day 1 (week 1). Study treatment will continue for at least 6 cycles or disease progression. In case of vaccine depletion patients may continue on dasatinib alone and there is evidence of clinical benefit.

The dosing time may be adjusted as required for subject convenience. If doses are missed for toxicity, they should not be replaced. If a dose is not taken due to an error, it may be taken up to 12 hours later. If vomiting occurs within 30 minutes of intake, that dose may be repeated. Patients will be provided with a diary in which to record any changes in dosing of study drug. Dasatinib should not be taken with grapefruit containing food or beverages.

REGIMEN DESCRIPTION					
Agent	**Premedications/ Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
αDC1 Vaccine	None	10^7 cells	Intradermal injection	Arms A and B: every 2 weeks starting on Cycle 1, day1	28 days (4 weeks)
Dasatinib	Take with or without food	70 mg	Orally, twice a day	Arm A: Daily starting on Cycle 2, day 1 Arm B: daily starting on Cycle 1, day 1	

5.2 Vaccine administration

The DC vaccine will be administered by a single intradermal injection of approximately 10^7 cells (a minimum of 5 x 10^6 cells is allowable due to manufacturing limitations), with all the DCs being administered on days 1and 15 of each cycle. The intradermal administration will be in the vicinity of the four nodal drainage groups of the four extremities and performed on an outpatient basis in the UPCI-CTRC. Study treatment will continue for at least 6 cycles or disease progression.

5.3 Duration of Study Treatment

In the absence of treatment delays due to adverse events, treatment may continue for at least 6 cycles until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),

- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator/sub-investigator.
- Study is terminated
- Loss of ability to freely provide consent

Patients may continue on dasatinib alone in case the vaccine product is no longer available and there is evidence of clinical benefit.

5.4 Duration of Follow Up

Patients will be followed for 1 year (±2 months) per standard of care visits or through medical records review after removal from study treatment or until death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

6. STUDY CALENDAR

Schedules shown in the Study Calendar below are provided as an example and should be modified as appropriate.

Screening evaluations are to be conducted within 1 week of leukapheresis. Scans and x-rays must be done ≤ 4 weeks prior to baseline. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Week	Up to -1[d] from baseline	Baseline	1	2	3	4	5[e]	6[e]	7[e]	8[e]	Off Study Treatment[f]
Informed consent	X										
HLA-A2 Screening[a]	X										
BRAF, c-KIT mutation[a]	X										
Demographics	X										
Medical history	X										
Physical exam	X		X		X		X		X		X
Vital signs	X		X		X		X		X		X
Height	X										
Weight	X		X		X		X		X		X
Performance status	X		X		X		X		X		X
CBC w/diff, platelets	X		X	X	X	X	X	X	X	X	X
Serum chemistry[b]	X		X	X	X	X	X	X	X	X	X
B-HCG[c]	X										
EKG (as indicated)	X										
Leukapheresis		X[i]									

Week	Up to -1[d] from baseline	Baseline	1	2	3	4	5[e]	6[e]	7[e]	8[e]	Off Study Treatment[f]
Vaccine production		X									
Dasatinib Arm A Arm B			 X	 X	 X	 X	 X X	 X X	 X X	 X X	
DC Vaccine[e]			X		X		X		X		
Immune monitoring-PBMC		X[g]			X		X		X		X
Tumor Biopsy		X[g]					X				
AE evaluation			◄ X ►								X
Tumor measurements	X		Tumor measurements are repeated every 8 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.								X
Radiologic evaluation	X		Radiologic measurements should be performed every 8 weeks.								X[h]

a: Not necessary if already known.
b: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, magnesium.
c: Serum pregnancy test (women of childbearing potential).
d: Screening is to be performed in the UPCI-CTRC.
e: Treatment with DC vaccine and dasatinib will continue for at least 6 cycles (or until disease progression), the weeks of those cycles will follow the tests and procedures listed for weeks 5 through 8 above for each subsequent cycle.
f: Four weeks after the last dasatinib administration.
g: Can happen at any time prior to start of study treatment.
h: If removed from the study for reasons other than DP.
i: Additional leukapheresis procedures may be performed at the discretion of the Investigator/sub-investigator for patients that derive benefit but require production of additional vaccine.

There is a window of ±7 calendar days available for scheduling/rescheduling treatment and/or procedures at the discretion of the Sub-investigator, and as discussed with the Investigator if a course is missed or a subject's treatment and/or testing day(s) need to be rescheduled due to the subject's inability to comply with the study calendar (this includes but is not limited to: hospitalizations, business, vacation plans, travel from long distances for study treatment, in advance of the scheduled date to allow ready access to the result(s), reduce financial burden on the subject [i.e. non-UPMC insurance coverage] or reduce travel inconvenience, illness, transportation issues, holidays, family emergencies, etc.).

7. DOSE-LIMITING TOXICITIES

7.1 Definition of Dose-Limiting Toxicity

Toxicities will be scored according to the NCI Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.0.

Dose-limiting toxicity (DLT) is defined as the following regimen-related events experienced during Cycle 1 and 2 for Arm A and during Cycle 1 for Arm B:

- Grade 4 neutropenia or thrombocytopenia which lasts more than 7 days;
- Grade 3 or 4 febrile neutropenia; or
- Grade 3 or greater non-hematological toxicities; this includes grade 3 or greater diarrhea, nausea or vomiting which last more than 7 days despite adequate treatment (with loperamide for diarrhea, 5HT3 antagonists, steroids and dopamine antagonist for N/V).

7.2 Dasatinib Dosing delays/modifications

Dose Level	Dasatinib Dose
0	70 mg twice a day
-1	50 mg twice a day
-2	100 mg once a day

This study will use the CTCAE (Common Terminology Criteria for Adverse Events) version 4.0 for toxicity and serious adverse event reporting. A copy can be downloaded from the CTEP homepage http://ctep.cancer.gov.

7.2.1 Grades 1 or 2 hematologic or non-hematologic toxicities

No routine dose interruptions or reductions will occur for Grades 1 or 2 toxicities. If the subject experiences an intolerable Grade 2 toxicity, dasatinib may be reduced 1 dose level without temporarily withholding study drug. If the toxicity resolves to baseline, dasatinib may be escalated to the previous dose level. If the intolerable toxicity recurs after re-escalation, dasatinib should be reduced 1 dose level and re-escalation of dose should not occur.

7.2.2 Grades 3 or 4 hematologic or non-hematologic toxicities

If the subject experiences a clinically significant Grade 3 non-hematologic toxicity or any Grade 4 non-hematologic toxicity or Grade 3 or 4 neutropenia or thrombocytopenia, dasatinib must be withheld until the toxicity resolves to Grade 1 or less or returns to baseline at the start of therapy, and the dose must be reduced 1 dose level when drug is restarted. If the clinically significant Grade 3, or Grade 4 non-hematologic toxicity, or Grade 3 or 4 neutropenia or thrombocytopenia recurs on the lower dose of dasatinib, drug must be withheld until the toxicity resolves to Grade 1 or less or returns to baseline, and the dose must be reduced to level -2 when restarted. If the clinically significant Grade 3, or Grade 4 non-hematologic toxicity, or Grade 3 or 4 neutropenia or thrombocytopenia recurs on dose level -2 of dasatinib, the subject's treatment with dasatinib will be discontinued but follow-up for tumor progression and survival will continue.

7.3 Vaccine Dosing delays/modifications

Although past experience at UPCI and other clinical centers performing DC-based vaccinations suggests that immunization with DC1-based vaccines will be well-tolerated and have little if any serious adverse side effects, to ensure patient safety we will closely monitor for any potential autoimmunity. No dose modifications will be allowed.

8. PATIENT SELECTION

8.1 Inclusion Criteria

- Patients must be HLA-$A2^{+}$ and have histologically confirmed melanoma that is metastatic (Stage IV) or unresectable Stage IIIB/C and for which standard curative or palliative measures do not exist or are no longer effective.

- Patients must have measurable disease by RECIST 1.1, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as $\geq$ 20 mm with conventional techniques or as $\geq$ 10 mm with spiral CT scan, MRI, or calipers by clinical exam. See Section 11 for the evaluation of measurable disease.
- Patients should have at least 2 subcutaneous, intracutaneous, and accessible tumor deposits, lymph node or other site available for biopsy purposes. Patients that have one biopsiable site that can be amenable to 2 biopsies (pre- and post-) will be considered eligible.
- Prior chemotherapy, immunotherapy, or targeted therapy is allowed as long as it did not include dasatinib.
- Age $\geq$ 18 years. Because no dosing or adverse event data are currently available on the use of dasatinib in patients < 18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.
- ECOG performance status $\leq$ 2 (Karnofsky $\geq$ 60%, see **Appendix A**).
- Life expectancy of greater than 12 weeks.
- Patients must have normal organ and marrow function as defined below:
  - Leukocytes $\geq$ 3,000/μL
  - absolute neutrophil count $\geq$ 1,500/μL
  - absolute lymphocyte count $\geq$ 500/μL
  - platelets $\geq$ 100,000/μL
  - total bilirubin within normal institutional limits
  - AST(SGOT)/ALT(SGPT) $\leq$ 2.5 X institutional upper limit of normal
  - Creatinine $\leq$ 2.0 X institutional upper limit of normal
- Serum magnesium, potassium and adjusted (or ionized) calcium $\geq$ the institutional lower limit of normal. (Supplementation of electrolytes prior to screening is allowed).
- Sexually active women and men of childbearing potential must agree to use an effective method of birth control during the course of the study and for up to 3 months following the last dose of the study drug, in a manner such that risk of pregnancy is minimized. Surgical sterilization, intrauterine device, birth control pills, or barrier method (e.g. condom and/or diaphragm with spermicidal agents) are acceptable forms of birth control. Women of childbearing potential must have a negative pregnancy test (serum) within 7 days prior to treatment. A pregnancy test is not required for registration. Women who have not menstruated for more than 2 years will be considered postmenopausal, thus not of childbearing potential.
- Ability to understand and the willingness to sign a written informed consent document.

8.2 Exclusion Criteria

- Patients who have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier.
- Patients with documented c-KIT mutations.
- Patients who are receiving any other investigational agents.
- Patients with known active brain metastases should be excluded. Patients with treated brain metastases with documented stability for 4 weeks are eligible.

- History of allergic reactions attributed to compounds of similar chemical or biologic composition to dasatinib or any of the components of the vaccine being administered as part of this study.
- Women who are pregnant or nursing/breastfeeding.
- History of significant bleeding disorder unrelated to cancer, including:
  - Diagnosed congenital bleeding disorders (e.g., von Willebrand's disease)
  - Diagnosed acquired bleeding disorder within one year (e.g., acquired anti-factor VIII antibodies)
- Patients currently taking medications that inhibit platelet function (i.e., aspirin, dipyridamole, epoprostenol, eptifibatide, clopidogrel, cilostazol, abciximab, ticlopidine, and any non-steroidal anti-inflammatory drug) because of a potential increased risk of bleeding from dasatinib.
- Patients currently taking anticoagulants (warfarin, heparin/low molecular weight heparin [e.g., danaparoid, dalteparin, tinzaparin, enoxaparin]) because of a potential increased risk of bleeding from dasatinib.
- Diagnosis of unstable angina or myocardial infarction within 6 months of study entry.
- Patients currently taking one or more of the following drugs that are generally accepted to have a risk of causing Torsades de Pointes:
  - quinidine, procainamide, disopyramide
  - amiodarone, sotalol, ibutilide, dofetilide
  - erythromycins, clarithromycin
  - chlorpromazine, haloperidol, mesoridazine, thioridazine, pimozide
  - cisapride, bepridil, droperidol, methadone, arsenic, chloroquine, domperidone, halofantrine, levomethadyl, pentamidine, sparfloxacin, lidoflazine.
- Diagnosed or suspected congenital long QT syndrome.
- Prolonged QTc interval on pre-entry electrocardiogram (> 450 msec) within 30 days prior to study registration.
- Any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or Torsades de pointes)
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with dasatinib. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

8.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

8.4 General Concomitant Medication and Supportive Care Guidelines

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are allowed. The administration of growth factors (e.g. procrit, neupogen) is

allowed. ASCO guidelines for providing growth factor support are recommended. The administration of any other anticancer agents including chemotherapy and biologic agents is not permitted. The administration of interferons is not allowed. The use of other concurrent investigational drugs is not allowed.

8.4.1 Bisphosphonates

Intravenous bisphophonates will be withheld for the first 8 weeks of treatment due to the risk of hypocalcemia. After the need for Ca^{2+} supplementation has been assessed and levels documented to be > lower limit of normal, patients on prior bisphosphonate may be restarted with caution at the investigator's discretion.

8.4.2 Antacids

Short-acting antacid agents may be taken, but it is recommended that these not be taken from 2 hours before to 2 hours after dosing of dasatinib. *In vitro* solubility data indicate that dasatinib may have decreased solubility and absorption at pH > 4. Until further data are available, patients should try to avoid taking proton pump inhibitors and H2 antagonists.

8.4.3 Medications that prolong the QT interval

Ideally, patients should not take medications known to prolong the QT interval. However, should the Investigator believe that beginning therapy with a potentially QT prolonging medication (other than the ones explicitly prohibited) is vital to an individual subject's care, the Investigator must check that the subject's prior on-therapy ECG has not shown a QTcF ≥ 480 msec or an increase in QTc ≥ 60 msec over the baseline value. Additional ECG(s) will be done one week later or more at the Investigator's discretion to ensure the subject's safety.

8.4.4 Medications that affect CYP3A4

Caution is warranted when administering dasatinib to patients taking drugs that are highly dependent on CYP3A4 for metabolism and have a narrow therapeutic index. Systemic exposures to these medications could be increased while receiving dasatinib. In *in vitro* studies, dasatinib is a strong inhibitor of the human CYP3A4 enzyme and a weak inhibitor of CYP1A2, CYP2D6 and CYP2C19. Dasatinib shows time-dependent inhibition of CYP3A4; however, there appears to be a low probability for drug-drug interactions due to metabolism-dependent CYP3A4 inactivation. Results from an *in vitro* hPXR trans-activation study suggest that dasatinib has little potential to induce CYP3A4 through the activation of hPXR.

Until information regarding exposure-toxicity and exposure-response relationships is available with dasatinib, concomitant CYP3A4 inhibitors and inducers should be avoided since they could alter the systemic exposure to dasatinib. Incubations with recombinant human CYP450 isozymes suggest that dasatinib is primarily metabolized by the CYP3A4 enzyme. Many other enzymes appear capable of metabolizing dasatinib, including CYP1A1, 2C9, 2E1, FMO3, 1B1, 2B6, 2A6, 2C8, and 4A1; however, it is unknown at this time what contributions these enzymes may have

to the total metabolic clearance of dasatinib.

Based on pre-clinical data, dasatinib may increase the likelihood of bleeding. Hence, patients undergoing surgical procedures, including dental procedures, should be instructed to inform their doctors of this potential increased risk. Patients requiring surgery should stop dasatinib at least 1 day prior to surgery, when feasible. As dasatinib has the potential to cause hemorrhage and its effect on wound healing is unclear, a minimum 2 week wait time after surgery is recommended and further do not resume medication until recommended by the attending surgeon based on the post-operative course.

Palliative radiation is allowed provided that the irradiated lesion is not the sole target lesion for response assessment.

The following medications are prohibited:

- St. John's wort (Hypericum perforatum): May decrease dasatinib plasma concentrations unpredictably. Patients receiving dasatinib should not take St. John's wort.
- H2 Blockers/Proton Pump Inhibitors: Long-term suppression of gastric acid secretion by H2 blockers or proton pump inhibitors (eg, famotidine and omeprazole) is likely to reduce dasatinib exposure. The concomitant use of H2 blockers or proton pump inhibitors with dasatinib is not recommended. The use of antacids should be considered in place of H2 blockers or proton pump inhibitors in patients receiving dasatinib therapy (see Section 5.2.2, antacids).

9. RESEARCH SAMPLES

Patient peripheral blood and tumor biopsies will be obtained at various time points prior to, and after, the initiation of therapy.

If the patient is determined to express the HLA-A2 antigen on their peripheral blood cells, to express a wild-type phenotype, and they pass all additional inclusion/exclusion criteria, after written consent, they may be entered on trial.

Those patients determined to express c-KIT mutations will be excluded from study, while BRAF mutational status will be used to stratify patients during randomization to ensure a balanced proportion of patients with the mutation on both arms.

9.1 Biopsy tissue

Melanoma biopsies will be obtained prior to the first vaccination (baseline) and week 5 (date of the third vaccination). Patients should have at least 2 subcutaneous, intracutaneous, and accessible tumor deposits, lymph node or other site available for biopsy purposes or one biopsiable site that can be amenable to 2 biopsies (pre- and post-) will be considered eligible.

9.2 Blood samples

At least 3 weeks prior to study treatment, peripheral blood will be obtained for the screening of patient HLA-A2 expression status (if not already known) and for baseline testing. Peripheral blood will be obtained every 2 weeks on trial beginning Cycle 1 week 3.

10. CORRELATIVE STUDIES

We expect to observe that HLA-A2/TBVA peptide dextramer$^+$ CD8$^+$ T cells (i.e. CD8$^+$ T cells imaged by flow cytometry using a fluorescently-labeled, antigen-specific probe) will exhibit higher frequencies in the peripheral blood and a greater propensity to produce IFN-γ after the initiation of αDC1-based vaccines. Since dasatinib is expected to alter the recruiting capacity of the tumor microenvironment based on activation of VCAM-1 expression on the tumor-associated vascular endothelial cells and locoregional production of CXCR3 ligand chemokines, it is very possible (and likely clinically-preferred) that the frequency of TBVA-specific CD8$^+$ T cells will selectively decline in the patients peripheral blood if the combined therapy is performing as expected based on our preclinical modeling data (given recruitment away out of the blood and into the tumor lesion). Based on a recent publication by Ernstoff and colleagues (99), circulating levels of the CXCR3 ligand CXCL10 (aka IP-10), as well as, the Okada and colleagues Phase 1/2 trial results for αDC1/peptide vaccination of glioma patients (88) may become elevated under treatment conditions in patients that are more prone to exhibit objective clinical response to effective immunotherapy. As a consequence, we will analyze levels of serum CXCL10 before, during and after combined vaccine + dasatinib therapy to determine correlation with TBVA-specific CD8$^+$ T cells in the blood versus tumor over time post-treatment.

10.1 Immune Monitoring Analysis of TBVA-specific CD8$^+$ T cell responses (Primary Endpoint).

Rationale and Hypothesis: Translation and clinical vaccine trials have demonstrated that DC/peptide-based vaccines effectively activate specific CD8$^+$ T cells in tumor-bearing hosts that may be detected in peripheral blood, and that individuals that exhibit objective clinical response to such vaccine therapies tend to derive from the cohort of patients that display detectable increases in T cell responses post-vaccination (25-27). Our own pre-clinical studies support the effectiveness of DC1/peptide vaccination to elicit protective/therapeutic T cell-mediated immunity in melanoma models *in vivo* (49), supporting the hypothesis that αDC1/peptide vaccination of advanced stage melanoma patients will result in increased quantities of specific CD8$^+$ T cells in patient peripheral blood and that those individuals in which improved response to many peptides can be observed are those that are more likely to demonstrate clinical benefit.

Method: Using fluorescently-labeled HLA-A2/peptide dextramer probes and intracellular staining for the Type-1 cytokine IFN-γ, we can simultaneously determine how the frequency of CD8$^+$ T cells specific for TBVA peptides changes over time post-vaccination and how many of

these T cells are Type-1 effector T cells. By screening for *in vitro* reactivity of T cells against melanoma antigen-derived peptides, we will also be able to assess how vascular-targeting T cell responses may promote the corollary ability of the immune system to recognize melanoma-associated peptide epitopes (i.e. "epitope spreading") that is hypothesized by many to be a strong surrogate for the clinical efficacy of immunotherapeutic approaches (25-27).

10.2 Quantitation of $CD8^+$ T cells, Treg, MDSC and blood vessels in melanoma biopsy tissue

Rationale and Hypothesis: Tumor progression is believed to be linked to the accumulation of suppressor cell populations (both MDSC and Treg) and strong pro-angiogenic signals, as well as, "prevention" of Type-1 T cell recruitment within the tumor microenvironment (65-74). Our preliminary data in murine melanoma models support the ability of dasatinib (particularly when combined with DC1/peptide vaccines) to counteract these biologic endpoints *in vivo* (**Figs. 1, 3**). We hypothesize that such changes may also be evidenced in effectively treated melanoma patients by analyzing melanoma biopsies taken post- versus pre-treatment and that the greatest "normalization" of the tumor microenvironment will be observed after treatment with combined dasatinib + vaccine therapy.

Method: Immunofluorescence microscopy will be used to analyze tumor sections of melanoma biopsies for expression of the markers CD8α (T effector cells), CD11b + CD33 + lack of HLA-DR (lineage-negative MDSC), CD11b + CD15 + lack of CD14 (neutrophilic MDSC), CD11b + CD14 + lack of CD15 (myeloid MDSC), CD4 + Foxp3 (Treg cells) and CD31 + NG2 (blood vessels). After staining and washing, sections will be covered in Gelvatol (Monsanto, St. Louis, MO) and a coverslip applied. Positively-stained cells will be quantitated by analyzing the images at a final magnification of x20 using Metamorph Imaging software (Molecular Devices, Sunnyvale, CA).

10.3 T_{reg} analysis in PBMC

Rationale and Hypothesis: Cancer patients have commonly also been shown to have elevated populational frequencies of Treg (based on the $CD4^+Foxp3^+$ phenotype) circulating in the peripheral blood. Alternate TKI, such as sunitinib, have been shown capable of reducing peripheral blood Treg levels within the first 4 week cycle of drug administration, in concert with a rebound in Type-1 T cell numbers and function in PBMC (59). Based on our pre-clinical data (**Fig. 1**), we hypothesize that dasatinib with provide a similar effect in melanoma patients and that those patients exhibiting the greatest degree of Treg reduction post-therapy will be more likely to respond favorably against the peptide epitopes contained in the vaccine formulation.

Method: Peripheral blood cells will be analyzed by flow cytometry using specific antibodies against CD3 (all T cells), CD4 + Foxp3 (Treg), CD4 + $CD25^{hi}$ (Treg) Results will expressed as percentage of $CD25^{+hi}/Foxp3^+$ cells out of total $CD3^+/CD4^+$ viable cells.

10.4 MDSC Analysis in PBMC

Rationale and Hypothesis: Similar to Treg, levels of cells expressing an MDSC phenotype have been reported to be elevated in the peripheral blood of cancer patients, including patients with advanced-stage melanoma (60, 61). TKI, such as sunitinib and dasatinib can reduce the frequency of such suppressor cells to a variable degree when used as a therapy (ref. 60, **Fig. 1**). We hypothesize that melanoma patients treated with dasatinib will exhibit reduction in MDSC frequencies in PBMC, with the degree of loss correlating with the patient's ability to respond favorably against the peptide epitopes contained in the vaccine formulation.

Method: Analysis of MDSC percentages in patient PBMC will be performed using flow cytometry and anti-human antibodies against CD11b, CD11c, CD14, CD15, CD33 and HLA-DR as previously described (100).

10.5 EphA2 Protein Levels in tumor biopsies

Rationale and Hypothesis: We have previously shown that drug treatments (including dasatinib *in vitro*) that promote the proteasome-dependent degradation of the tumor (and tumor vascular endothelial) cell-associated protein EphA2 lead to improved recognition by specific $CD8^+$ T cells (refs. 101, 102 and **Fig. 2**). We hypothesize that administration of dasatinib to melanoma patients will promote the loss of EphA2 protein within the tumor lesion, leading to an enhancement in the sensitivity of $EphA2^+$ cells in the tumor microenvironment to EphA2-specific $CD8^+$ T cells that have been activated as a consequence of αDC1/peptide-based vaccination.

Method: Western blotting and immunofluorescence microscopy will be used to quantitate EphA2 protein expression in melanoma biopsies pre- versus post-vaccination.

10.6 CXCL10 Levels in Patient Serum

Rationale and Hypothesis: We and others have recently shown that therapeutic $CD8^+$ T cells require the production of CXCR3 ligand chemokines within the tumor microenvironment in order to effectively home to these disease sites. Two recent clinical trials, including our αDC1/glioma peptide vaccination trial in patients with brain tumors strongly support CXCL10 (aka IP-10) as a chemokine associated with superior clinical outcome to immune-based therapy (88, 99). We hypothesize that this will also be the case in our αDC1/TBVA peptide vaccinated patients with melanoma where Type-1 $CXCR3^+$ responder T cells require a gradient of CXCL10/IP-10 (as detected in serum) in order to traffick to tumor sites *in vivo*.

Method: Patient serum levels of CXCL10 will be monitored using Luminex fluorescent bead technology according to manufacturer's protocol.

11. MEASUREMENT OF EFFECT

Although objective tumor response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients

should be re-evaluated every 8 weeks. In addition to a baseline scan, confirmatory scans will also be obtained ≥ 4 weeks following initial documentation of an objective response.

11.1 Antitumor Effect

For the purposes of this study, patients should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained no less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1; ref. 104). Changes in the largest diameter (uni-dimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or as ≥ 10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured

and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

11.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be

imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥ 10 mm diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of

CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
- FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Investigator).

11.1.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/ not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.6 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

12. STATISTICAL CONSIDERATIONS

12.1 Study Design

The primary objective of this study is to evaluate the effects of combination therapy of dasatinib and vaccine on immune response rate. Here a patient who responded to at last 3 of the 6 peptides is considered to have a positive immune response. The secondary objectives of this trial include evaluation of clinical response rate, overall survival (OS), progression free survival (PFS), and immunological endpoints, which include number of $CD8^+$ T cells, MDSC/Treg regulatory cells and blood vessels in tumor lesions, level of EphA2 protein expressed within the tumor lesion and the level of the CXCL10/IP-10 chemokine in patient serum pre- versus post-treatment.

We expect that the proportion of patients that are not evaluable for immune endpoint will be 10% or less. To ensure a total of 28 evaluable patients, up to 31 evaluable patients will be randomized in 1:1 ratio to receive either:

A. Vaccine alone starting in the first 28-day cycle followed by vaccine combined with daily dasatinib starting on the first day of the second cycle (Arm A)
B. Vaccine combined with daily dasatinib starting on the first day of cycle 1 (Arm B)

The UPCI randomizer will be used for the randomization. The randomization will be stratified by BRAF mutation status.

12.2 Safety Monitoring

The dose limiting toxicity (DLT) is defined in section 7.2.2. Bayesian analysis will be used to continuously monitor the DLTs in both arms to ensure that the study will continue enrollment without interruption as long as the underlying true DLT rate is 20% or less. The posterior probability (PPr) will be calculated from the study's accumulated data on DLTs. A weak prior distribution of Beta (0.1, 1.9) was used. If PPr > 0.7, that is, there is a 70% or higher chance that more than 20% of the patients will experience DLTs, the enrollment of the study arm will stop pending a review of both the data and the study's implementation.

The following table listed the suspension rule based on the Bayesian analysis calculation.

Bayesian rule for suspending enrollment	
Suspend accrual if n patients experience a DLT	in N patients treated
2	2-4
3	5-9
4	10-14

The operating characteristics of this study design can be expressed in terms of probability of early suspending the enrollment for the combination arm under the assumptions of various the

true rates of SAEs. The following table calculated these probabilities based on the simulation of 10,000 hypothetical trials.

Frequentist properties of Bayesian rule for suspending enrollment		
Probability of DLTs	Probability of suspending the enrollment	Median sample size for the combination arm
0.05	0.02	14
0.1	0.09	14
0.15	0.22	14
0.2	0.36	14
0.25	0.53	13
0.3	0.67	8
0.35	0.79	7
0.4	0.88	4

12.3 Sample Size/Accrual Rate

We estimated an accrual rate of approximately 1-2 patients per month therefore, we the accrual will be completed within 20 months. We plan a 12 month follow up after the last patient entered the study. Therefore, the study will be completed within 32 months after the initiation of the study.

The power calculation for the proposed study is based on the comparison of the positive immune response rate between the two treatment arms. Based on a preliminary data of 15 patients, the immune response rate for the vaccine only arm is close to 0%. With 14 evaluable patients in each arm, we will have 80% power to distinguish a difference between 5% immune response rate (of the vaccine arm) and 50% immune response rate (of the combination arm) with 1-sided Fisher exact test ($\alpha = 0.025$).

12.4 Data Analysis

12.4.1 Analysis Sets

Evaluable Patients are patients who meet all of the protocol inclusion/exclusion criteria and begin treatment with the protocol assigned regimen. All evaluable patients will be used in the analysis of safety, immune response, clinical response, OS and PFS.

12.4.2 Baseline Characteristics

Baseline characteristics on all evaluable patients will be provided on demographic variables (age, sex, race/ethnicity), performance status, laboratory parameters, prior treatments, and disease characteristics, including tumor size, number of nodes involved, and metastatic sites.

12.4.3 Safety Profile

NCI CTCAE version 4.0 will be used to evaluate the serious adverse events (SAEs) in each cycle of the treatment, and for 30 days beyond the last protocol specified treatment. Sever AEs rate for each treatment arm will be calculated and the corresponding exact 95% confidence interval (CI) will be provided. All adverse events that are determined to be possibly, probably or definitely related to treatment will be tabulated according to grade and type (according to the NCI CTCAE,

Version 4.0). For each adverse event category, frequencies will be tabulated by treatment group according to the highest grade per patient within 30 days after any study treatment.

12.4.4 Efficacy Analysis

The immune response rate, defined as proportion of patients that responded to ≥ 3 out of the 6 peptides, for each study arm will be calculated with 95% exact CI. The clinical response rate for each study arm will be estimated by the percentage of patients achieving CR or PR by RECIST criteria, with corresponding exact 95% CI. Both immune response rate and clinical response rate of the two treatment groups will be compared using Fisher exact test. As an exploratory analysis, we will also evaluate and compare the immune response for the B-raf mutant carrier and non-carrier in similar manner as described above.

The Kaplan-Meier estimate of PFS and OS with corresponding 95% confidence band will be provided for each dose level. The corresponding median survival time (with 95% confidence limits) will be determined, along with OS and PFS estimates at selected time points. The exact log rank test will be used to compare the PFS and OS between the two study arms.

Exploratory analysis will be conducted to study the association between the positive immune response and:

a. Objective clinical response.
b. CD8+ T cell infiltration in tumor after cycle 1.
c. Reduction in suppressor cells in the tumor and blood.
d. Reduction in blood vessel density in the tumor after cycle 1.
e. Reduction in EphA2 protein expression in tumor after cycle 1.
f. Increased level of the CXCR3 ligand chemokine CXCL 10/IP-10 in patient serum after cycle 1.

Chi-square (or Fisher exact) test will be used to test the association between immune response and the categorical outcomes (e.g. objective clinical response). Wilcoxon test will be used to compare the continuous outcomes (e.g. $CD8^+$ T cell infiltration, suppressor cell populations, tumor blood vessel density, EphA2 protein expression, chemokine level) between the immune responders and non-responders.

12.5 Reporting and Exclusions

12.5.1 Evaluation of toxicity

All patients will be evaluated for toxicity from the time of their first treatment with either vaccine alone or dasatinib with vaccine.

12.5.2 Evaluation of response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4)

progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

13. DATA SAFETY MONITORING PLAN

Investigator/Sub-investigators, regulatory, CRS management, clinical research coordinators, clinical research associates, data managers, and clinic staff meet monthly in disease center Data Safety Monitoring Boards (DSMB) to review and discuss study data to include, but not limited to, the following:

- serious adverse events
- subject safety issues
- recruitment issues
- accrual
- protocol deviations
- unanticipated problems
- breaches of confidentiality

Minutes from the DSMB meetings are available to anyone unable to attend the center DSMB.

All toxicities encountered during the study will be evaluated on an ongoing basis according to the NCI Common Toxicity Criteria version 4.0. All study treatment associated adverse events that are serious, at least possibly related and unexpected will be reported to the IRB. Any modifications necessary to ensure subject safety and decisions to continue, or close the trial to accrual are also discussed during these meetings. If any literature becomes available which changes the risk/benefit ratio or suggests that conducting the trial is no longer ethical, the IRB will be notified in the form of an Unanticipated Problem submission and the study may be

terminated.

All study data reviewed and discussed during these meetings will be kept confidential. Any breach in subject confidentiality will be reported to the IRB in the form of an Unanticipated Problem submission. The summaries of these meetings are forwarded to the UPCI DSMC which also meets monthly following a designated format.

For all research protocols, there will be a commitment to comply with the IRB's policies for reporting unanticipated problems involving risk to subjects or others (including adverse events). DSMC progress reports, to include a summary of all serious adverse events and modifications, and approval will be submitted to the IRB at the time of renewal.

Protocols with subjects in long-term (survival) follow-up or protocols in data analysis only, will be reviewed twice a year rather than monthly by the disease center DSMB.

Both the UPCI DSMC as well as the individual disease center DSMB have the authority to suspend accrual or further investigate treatment on any trial based on information discussed at these meetings.

All records related to this research study will be stored in a locked environment. Only the researchers affiliated with the research study and their staff will have access to the research records.

14. ADVERSE EVENT REPORTING

14.1 Adverse event definitions

Adverse event (AE) means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

> *Reasonable possibility*, for the purpose of IND safety reporting, means there is evidence to suggest a causal relationship between the drug and the adverse event.

Life-threatening adverse event or life-threatening suspected adverse reaction is considered "life-threatening" if, in the view of the sponsor-investigator, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse event or serious suspected adverse reaction is considered "serious" if, in the view of the sponsor-investigator, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life

functions, or a congenital anomaly/birth defect.

> Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unexpected adverse event or unexpected suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

> For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. “Unexpected,” as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

14.2 Recording/Reporting requirements

14.2.1 Eliciting AE Information

Research subjects will be routinely questioned about AEs at study visits.

14.2.2 Recording Requirements

All observed or volunteered adverse events (serious or non-serious) and abnormal test findings, regardless of study group or suspected causal relationship to the study drug(s) will be recorded in the subjects’ case histories. For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a serious adverse event) and; 2) an assessment of the casual relationship between the adverse event and the study drug(s).

AEs or abnormal test findings felt to be associated with the investigational drug or study treatment(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the Sponsor-Investigator.

14.2.2.1 Abnormal Test Findings

An abnormal test finding will be classified as an adverse event if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms.
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy.
  Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an AE.
- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical study.
- The test finding is considered an AE by the Sponsor-Investigator of the IND application.

14.2.2.2 Causality and severity assessment

The Sponsor-Investigator of the IND application will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s); and 3) if the adverse event meets the criteria for a serious adverse event.

If the Sponsor-Investigator's final determination of causality is "unknown and of questionable relationship to the study drug(s)", the adverse event will be classified as associated with the use of the study drug(s) for reporting purposes. If the Sponsor-Investigator's final determination of causality is "unknown but not related to the study drug(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

14.3 Reporting of Suspected Adverse Reactions

All events meeting the definition of a serious adverse event should be recorded on a MedWatch 3500A Form (http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf) or departmental SAE form. Copies should be sent to:

1. Investigator-Sponsor
2. crssafetysubmissions@upmc.edu
3. Local Institutional Review Board per institutional reporting requirements

In addition to completing appropriate patient demographic and suspect medication information, the report should include as applicable the following information that is available at the time of report within the Event Description (section 5) of the MedWatch 3500A form:

- CTCAE term(s) and grade(s)
- Current status of study drug

- All interventions to address the AE (testing and result, treatment and response)
- Hospitalization and/or discharge dates
- Event relationship to study drug

Follow-up reports:
Additional information may be added to a previously submitted report by adding to the original MedWatch 3500A report and submitting it as follow-up or creating supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form.

14.4 Review of Safety Information: Sponsor Responsibilities[1]

The sponsor must promptly review all information relevant to the safety of the drug obtained or otherwise received by the sponsor from foreign or domestic sources, including information derived from any clinical or epidemiological investigations, animal or in vitro studies, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities and reports of foreign commercial marketing experience for drugs that are not marketed in the United States.

14.5 Reporting of Adverse Reactions to the FDA

14.5.1 Written IND safety reports

The Sponsor-Investigator will submit a written IND Safety Report (i.e., completed FDA Form 3500 A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be a *serious and unexpected, suspected adverse reaction*. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to all participating investigators (if applicable) and sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

For each written IND Safety Report, the Sponsor-Investigator will identify all previously submitted IND Safety Reports that addressed a similar suspected adverse reaction experience and will provide an analysis of the significance of newly reported, suspected adverse reaction in light of the previous, similar report(s) or any other relevant information.

Relevant follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the information is available and will be identified as such (i.e., "Follow-up IND Safety Report").

If the results of the Sponsor-Investigator's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the Sponsor-Investigator will submit a written IND Safety Report as

[1] 21 CFR Sec. 312.50

soon as possible, but in no event later than 15 calendar days, after the determination was made.

14.5.2 Telephoned IND safety reports – fatal or life-threatening suspected adverse reactions

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the Sponsor-Investigator will notify the responsible review division of the FDA by telephone or facsimile transmission of any *unexpected, fatal or life-threatening suspected adverse reaction.*

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

14.6 Reporting adverse events to the responsible IRB

In accordance with applicable policies of the University of Pittsburgh Institutional Review Board (IRB), the Sponsor-Investigator will report, to the IRB, any observed or volunteered adverse event that is determined to be 1) *associated with the investigational drug or study treatment(s)*; 2) *serious*; and 3) *unexpected*. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse events will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the sponsor-investigator's receipt of the respective information. Adverse events which are 1) *associated with the investigational drug or study treatment(s)*; 2) *fatal or life-threatening*; and 3) *unexpected* will be reported to the IRB within 24 hours of the Sponsor-Investigator's receipt of the respective information.

Follow-up information to a reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the Sponsor-Investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the Sponsor-Investigator will report the adverse event to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

15. WITHDRAWAL OF SUBJECTS DUE TO ADVERSE EVENTS

Therapy will be immediately discontinued for any grade 4 toxicity that becomes apparent or any toxicity outlined in the Regimen Limiting Toxicities Section 9.1.4. Study treatment will be reinitiated for any grade 3 (except toxicities outlined in the Regimen Limiting Toxicities) toxicity pending the reversal of such toxicity after withholding treatment. In the event of any adverse effect, appropriate medical treatment will be instituted and study treatment will be discontinued if the above toxicity remains.

16. QUALITY CONTROL AND QUALITY ASSURANCE

Independent monitoring of the clinical study for protocol and Guidelines on Good Clinical Practice compliance will be conducted periodically (i.e., at a minimum of annually) by qualified staff of the Education and Compliance Office – Human Subject Research, Research Conduct and Compliance Office, University of Pittsburgh.

The Investigator (i.e., the study site principal investigator) and the University of Pittsburgh and University of Pittsburgh Medical Center will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

17. DATA HANDLING AND RECORD-KEEPING

The Investigator (i.e., the study site principal investigator) will maintain records in accordance with Good Clinical Practice.

The investigator will retain the specified records and reports for up to 2 years after the marketing application is approved for the investigational drug; or, if a marketing application is not submitted or approved for the investigational drug, until 2 years after investigations under the IND have been discontinued and the FDA so notified.

18. ETHICS

18.1 Institutional Review Board (IRB) Approval

The investigator (i.e., the study site principal investigator) will obtain, from the University of Pittsburgh Institutional Review Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment, if applicable.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the investigator will promptly notify the University of Pittsburgh IRB of the deviation.

The University of Pittsburgh IRB operates in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice.

In the event that the University of Pittsburgh IRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of an sponsor's decision to modify the previously accepted clinical protocol, the sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change that significantly affects the safety of subjects, the scope of the

investigation, or the scientific quality of the study. Examples of protocol changes requiring the submission of a Protocol Amendment include:

- Any increase in drug dosage or duration of exposure of individual subjects to the investigational drug beyond that described in the current protocol, or any significant increase in the number of subjects under study.
- Any significant change in the design of the protocol (such as the addition or deletion of a control group).
- The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or AE; or the dropping of a test intended to monitor the safety of the investigational drug.

18.2 Ethical and Scientific Conduct of the Clinical Study

The clinical study will be conducted in accordance with the current IRB-approved clinical protocol; ICH Guidelines on Guidelines on Good Clinical Practice; and relevant policies, requirements, and regulations of the University of Pittsburgh IRB, University of Pittsburgh and University of Pittsburgh Medical Center, Commonwealth of Pennsylvania, and applicable federal agencies.

18.3 Subject Informed Consent

The investigator (i.e., the study site principal investigator) will make certain that an appropriate informed consent process is in place to ensure that potential research subjects, or their authorized representatives, are fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The investigator, or a sub-investigator(s) designated by the sponsor, will obtain the written, signed informed consent of each subject, or the subject's authorized representative, prior to performing any study-specific procedures on the subject. The date and time that the subject, or the subject's authorized representative, signs the informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the subject's case history. The investigator or sub-investigator will retain the original copy of the signed informed consent form, and a copy will be provided to the subject, or to the subject's authorized representative.

The investigator will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the investigator will obtain the informed consent of enrolled subjects for continued participation in the clinical study.

19. REFERENCES

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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.