A Phase II Clinical Trial of Vemurafenib with Lymphodepletion Plus Adoptive Cell Transfer and High Dose IL-2 and an Expansion Cohort for the BRAF Inhibitor and PD-1 Antibody Refractory Setting in Patients with Metastatic Melanoma

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Study Treatment Schema for first cohort
(accrual was completed as of 2/10/17 and is no longer accruing)

Assessment	Screening[1]	Days -28 to 0	Day 0	Week 6	Week 12, then every 3 months for 2 yrs[22]
Informed Consent[1]	X				
History and Physical Exam[2]	X	X	X	X	X
Vital Signs[2]	X	X	X	X	X
ECOG Performance Status	X	X			
HLA typing[3]	X				
CBC w/diff, CMP, LDH, PT/PTT[2]	X	X	X	X	X
EBV titre	X				
EKG[4]	X	X	X	X	X
Cardiac Stress test and PFTs[5]	X				
Dermatologic Examination[6]	X		X		X
Serum Pregnancy Test[7]	X	X			
Tumor V600 Mutation Analysis	X				
CT Thorax-Abdomen-Pelvis, MRI Scan of Brain[8,9]	X	X[8]		X	X
Hepatitis B and C, HTLV-1 and 2 titres	X				
HIV titre, free T4, TSH[10], RPR/FTA[11]	X				
Tumor harvest for TIL[12]	X				
Baseline Symptom Assessment	X				
Adverse Event Assessment[13]		X[13]	X[13]	X[13]	X[13]
Concomitant Medications	X				
Vemurafenib[14], tumor biopsies[15]		X[14]		X[14]	X[14]
Chest X-Ray[16]		X			
Urinalysis, Cyclophosphamide and Fludarabine[17]		X			
Adoptive Transfer of TIL[19]			X		
Single Cycle of high dose IL-2 after TIL			X		
Apheresis + immune deficiency panel[20]		X		X	
Correlative endpoints[21]		X[21]	X[20]	X[20]	X[20]

Footnotes of Study Schema for first cohort (Accrual Complete as of 2/10/17)
All visits and tests may be done +/- 7 days from scheduled date unless otherwise specified.
1. All laboratory and imaging studies must be complete and satisfactory within 30 days of signing the consent document with the exceptions of: HLA-typing which will not be repeated if performed previously, and PFTs/cardiac stress tests whose results are valid for 6 months if performed previously.
2. History & Physical Examination with vital signs will be performed at screening, prior to lymphodepletion, prior to adoptive transfer of the TIL, and at each subsequent clinic visit. Complete blood count with differential (CBC w/diff) and complete metabolic panel (CMP) will be obtained 1-4 weeks after initiation of vemurafenib and within 24 hours prior to apheresis. Only

one blood draw is needed if done within a time frame to satisfy both requirements prior to the first apheresis.
3. HLA typing will be sent at screening if not done previously.
4. EKGs will be obtained within 30 days prior to first dose of vemurafenib (and will not be repeated if screening EKG is completed within this window) 15 days after initiation of vemurafenib (+/- 7 days), every 4 weeks for the next 3 months, and every 12 weeks thereafter while on vemurafenib. EKG abnormalities will be acted upon per section 4.1.4a.
5. PFTs are required at screening only if there is a significant history of pulmonary disease that necessitates the use of supplemental oxygen, is associated with dyspnea on walking one block or less, or requires inhaler therapy more than once per week. A cardiac stress test will be done at screening in all pts greater than age 50 or who have a history of coronary artery disease. If PFTs and/or a cardiac stress test have been done within 6 months of screening, they will not be repeated unless there is an interval change in the patient's clinical status.
6. Full dermatologic history, skin, lymphatic, head, neck and oral mucosal examination will be performed by a dermatologist or a provider within the Cutaneous program at Moffitt at screening, four weeks after the start of vemurafenib and every 3 months while on vemurafenib. Any lesion suspected to represent a squamous or basal cell carcinoma, keratoacanthoma, or primary melanoma will be treated per local standard of care and per section 4.1.5a. Patients who stop vemurafenib for any reason will be followed with skin examinations for 6 months after study drug discontinuation or until death, withdrawal of consent, start of subsequent anti-cancer treatment, or loss to followup, whichever occurs first.
7. Serum pregnancy test will be performed on women of child-bearing potential (see 4.1.1f) within 7 days prior to starting vemurafenib and within 14 days of pre-chemotherapy apheresis.
8. Scans to evaluate disease will be done pre-treatment, within 30 days of initiation of chemotherapy, at weeks 6 and 12, then every 3 months for 2 years, every six months for 2 years, then yearly thereafter or until disease progression, withdrawal of consent, start of subsequent anti-cancer treatment, loss to followup, or death, whichever occurs first. Note CT of the head without and with IV contrast (if not allergic) will be substituted for brain MRI in cases of existing MRI contraindication.
9. Once patients are no longer followed by scheduled radiologic assessments for tumor burden and vemurafenib was been discontinued, a chest CT scan will be performed 6 months after the last dose of vemurafenib to evaluate for metastatic squamous cell carcinoma for all patients who have not withdrawn consent or have not been lost to followup.
10. If T4 and TSH are abnormal, thyroid or autoimmune workup will be done.
11. FTA only if RPR is positive per sections 4.1.2b and 4.1.3.
12. Tumor fragments from the harvest will be plated in the adoptive cell therapy lab for TIL growth. This will be followed by vemurafenib treatment initiation the next day.
13. Adverse events will be collected throughout the study protocol until 6 months after the last dose of vemurafenib, loss to followup, start of subsequent anti-cancer treatment, withdrawal of consent or death, whichever occurs first.
14. Vemurafenib begins the day after the TIL harvest. A CBC w/diff and CMP will be obtained after 1-4 weeks of treatment. Vemurafenib will continue until day -8 unless discontinued earlier by treating physician. Vemurafenib will be resumed the day after the patient has clinically recovered from the single cycle of high dose IL-2 and has an ANC of 500 per mcL or greater. Patients exhibiting a response on this trial will continue vemurafenib for a duration of up to 2 years or until progression, start of subsequent anti-cancer treatment, withdrawal of consent, loss to followup or death.
15. Per section 6.0, where feasible, tumor samples will be obtained by FNA or core biopsy before and seven days after Vemurafenib treatment, and again on Days 21 and 42 after T cell transfer and within 30 days after disease progression.
16. Chest X-Ray will be done within 14 days of apheresis per section 4.2.1.

17. Urinalysis will be done per section 4.2.2.
18. Cyclophosphamide will be administered on Days -7 and -6, Fludarabine will be administered on Days -5 to -1. A CBC w/ diff, CMP, magnesium, and phosphorus will be performed every 1-2 days of treatment during preparative cyclophosphamide/fludarabine and high dose IL-2 therapy per section 6.0. The CBC differential will not be performed if the subject's white blood cell count is not sufficiently high for automated differential.
19. Adoptive transfer will take place at day 0 prepared per Moffitt cell therapy SOP.
20. Immunodeficiency panels and apheresis will be performed within 14 days before initiation of lymphodepletion and 6 weeks after T cell transfer. The samples derived from the apheresis will be used for immune monitoring purposes and will not be used for therapy. CBC w/diff, CMP, LDH, PT/PTT, serum pregnancy test, EKG and Chest X-ray will be performed within 14 days prior to apheresis.
21. 8 large Green tops (~60 mL) will be drawn before and 7 days after initiation of vemurafenib and on Days 7, 14, 21, 28, 35, 70 (+/- 7 days) for T cell recovery/immune cell reconstitution before and after lymphodepletion, and in vitro assay for anti-tumor function by interferon-γ release as previously described (1, 3). On day 0, one large green top (~10 mL) will be drawn. This will minimize blood loss during the time of bone marrow suppression from the chemotherapy. Blood draws will be omitted if Hb < 8 or deemed not clinically feasible by the PI or treating co-investigator.
22. Follow-up visits with disease evaluation will be conducted at weeks 6 and 12 (+/- 7 days), then every 3 months (+/- 14 days) for two years, every six months (+/- 30 days) for 2 years, then yearly thereafter (+/- 90 days) or until disease progression, withdrawal of consent, loss to followup, or death, whichever occurs first. Adverse events will be collected throughout the study protocol until 6 months after the last dose of vemurafenib, loss to followup, start of subsequent anti-cancer treatment, withdrawal of consent or death, whichever occurs first.

Study Treatment Schema 2nd Cohort*		TIL Harvest	Start Vemurafenib	While on Vemurafenib		Hospital Stay			Hospital Stay						Stop Vemu			
Assessment	Screen[1]	D -35	D -21	week 2,4, q 1 mox3 then q 3 mos [3]	D -14	D -7	D -6	D -5	D 0	D+1	~D+7	~D+21	D+42 [23]	D+84 [23]	Month 6 [15]	Q 3 mos for 24 mos [23]	Q 6 mos for next 24 mos [23]	Q 1 yr [23]
Informed consent [1]	●																	
Medical history and Physical exam [2]	●	●	●	●	●				●			●	●	●		●	●	●
Vital Signs [2]	●		●	●	●	●	●	●	●	●		●	●	●		●	●	●
Performance Status	●		●		●				●							●	●	●
Baseline Symptom Assessment	●																	
Current Medications	●		●	●	●	●							●	●		●	●	●
EKG [3]	●			● [3]								●				●		
Cardiac Stress test and PFT [4]	●																	
Dermatologic Evaluation [5]	●			●												●		
Tumor V600 Mutational Analysis/Confirmation of Progression on Previous BRAF inhibitor	●																	
Blood Tests																		
CBC with diff, CMP	●			●	●	●	●	●	●	●			●	●		●	●	●
LDH	●				●								●	●		●	●	●
Blood clotting tests (PT/PTT)	●				●													
HLA typing (if not done before) [6]	●																	
EBV, Hepatitis B and C, HTLV-1 & 2 ab titers [7]	●																	
HIV, RPR (FTA if necessary) [7]	●																	
Thyroid Tests (Free T4, TSH) [8]	●				●				●							●		
Serum Pregnancy Test [9]	●				●													
PBMC Correlative Endpoint Collections [10]		●			●				● [10]		●[10]	●[10]	●[10]	●[10]				
CT Chest [11]-Abdomen-Pelvis, Brain MRI [12]	●				●								●	●		●	●	●
Tumor harvest for TIL [13]		●																
Adverse Event Assessment [14]			●	●	●	●	●		●	●		●	●	●		●		
Start/resume Vemurafenib [15]			●								●							
Stop Vemurafenib [15]	●					●									●			
Tumor biopsies (FNA/Core biopsy) [16]					●							●	●	●				● [16]
Urinalysis [17]	●				●			●	●									
Leukapheresis + Immunodeficiency panel [18]					●								●					
Cell Therapy Facility Start of Rapid Expansion [19]					●													
Lymphodepletion:																		
Cyclophosphamide (hospital x 2 days) [20]						●	●											
Fludarabine (outpatient x 5 days) [20]								●										
Cell Therapy Facility Rapid Expansion 2nd week [21]						●												
Adoptive Transfer of TIL [22]									●									
High dose IL-2 (hospitalization 7-10 days)										●								

Footnotes of Study Schema for <u>second cohort</u>

*All visits and tests may be done +/- 7 days from scheduled date unless otherwise specified.

1. All laboratory and imaging studies must be complete and satisfactory within 30 days of signing the consent document with the exceptions of: HLA-typing which will not be repeated if performed previously, BRAF V600 tumor mutational analysis, and PFTs/cardiac stress tests whose results are valid for 6 months if performed previously.
2. History & Physical Examination with vital signs will be performed at screening, prior to lymphodepletion, prior to adoptive transfer of the TIL, and at each subsequent clinic visit while on study.
3. EKGs will be obtained within 30 days prior to first dose of vemurafenib (and will not be repeated if screening EKG is completed within this window), 2 and 4 weeks after initiation of vemurafenib (+/- 7 days), then monthly for the next 3 months, and every 3 months thereafter while on vemurafenib. EKG abnormalities will be acted upon per section 4.1.4a.
4. PFTs are required at screening only if there is a significant history of pulmonary disease that necessitates the use of supplemental oxygen, is associated with dyspnea on walking one block or less, or requires inhaler therapy more than once per week. A cardiac stress test will be done at screening in all patients greater than age 50 or who have a history of coronary artery disease. If PFTs and/or a cardiac stress test have been done within 6 months of screening, they will not be repeated unless there is an interval change in the patient's clinical status.
5. Full dermatologic history, skin, lymphatic, head, neck and oral mucosal examination will be performed by a dermatologist or a provider within the Cutaneous program at Moffitt at screening, four weeks after the start of vemurafenib and every 3 months while on vemurafenib. Any lesion suspected to represent a squamous or basal cell carcinoma, keratoacanthoma, or primary melanoma will be treated per local standard of care and/or per section 4.1.5a. Patients who stop vemurafenib for any reason will be followed with skin examinations for 6 months after study drug(s) discontinuation for reporting of serious adverse events that are definitely related to vemurafenib until start of a subsequent anti-cancer therapy, death, withdrawal of consent or loss to followup, whichever occurs first.
6. HLA typing will be sent at screening if not done previously.
7. EBV IgG antibody to viral capsid antigen (needs to be +), Hepatitis B, C, HTLV I/II, HIV/HTLV ab, RPR (and FTA if RPR is positive) will be obtained at screening.
8. If T4 and TSH are abnormal, thyroid and/or autoimmune workup will be done.
9. Serum pregnancy test will be performed on women of child-bearing potential (see 4.1.1f) within 7 days prior to starting vemurafenib and within 14 days of pre-chemotherapy apheresis.
10. 8 large green tops (~60 mL) will be drawn at TIL harvest (day -35) and on Days -14, 0, +7, +14, +21, +42, +84 (+/- 7 days) for T cell recovery/immune cell reconstitution before and after lymphodepletion, and in vitro assay for anti-tumor function by interferon-γ release as previously described (1). On day 0, one large green top (~10 mL) will be drawn (shaded cell in table). This will minimize blood loss during the time of bone marrow suppression from the chemotherapy. Blood draws will be omitted if Hb < 8 or deemed not clinically feasible by the PI or treating co-investigator.
11. Once patients are no longer followed by scheduled radiologic assessments for tumor burden and vemurafenib has been discontinued, a chest CT scan will be performed 6 months (+/- 90 days) after the last dose of vemurafenib to evaluate for metastatic squamous cell carcinoma for all patients who have not withdrawn consent, have not started a subsequent anti-cancer treatment, and/or have not been lost to followup.
12. CT Scans (chest, abdomen, pelvis and other sites as determined by the treating physician) to evaluate disease will be done pre-treatment, within 30 days of initiation of chemotherapy, at weeks 6 and 12, then every 3 months for 2 years, every six months for 2 years, then yearly thereafter or until disease progression, withdrawal of consent, loss to followup, or death, whichever occurs first. Note that past day 84, brain MRIs will be done as clinically indicated by

the treating physician and in keeping with the standard of care. CT of the head without and with IV contrast (if not allergic) will be substituted for brain MRI in cases of existing MRI contraindication.
13. Tumor fragments from the harvest will be plated in the adoptive cell therapy lab for TIL growth and propagated in accordance with the current Moffitt Cell Therapy Facility SOP.
14. Adverse events will be collected throughout the study protocol beginning at initiation of treatment and continuing until any one of the following: progression, loss to follow-up, withdrawal of consent, death, or 30 days after the last dose of study treatment. Beyond 100 days from study treatment, subjects will continued to be followed for ongoing drug-related adverse events until resolved, return to baseline, deemed irreversible by the Moffitt treating physician, or until the subject is lost to follow-up, there is withdrawal of study consent, removal of the subject from the trial by the Moffitt treating physician, or start of a subsequent anti-cancer therapy.
15. Vemurafenib begins day -21 or approximately 2 weeks after the TIL harvest. Vemurafenib will be managed by the treating physician in accordance with the standard of care and/or label for this FDA approved drug. Vemurafenib will continue up until day -7 unless discontinued earlier by treating physician. Vemurafenib will be resumed the day after the patient has clinically recovered from the single cycle of high dose IL-2 and has an ANC of 500 per mcL or greater (around day +7) if determined to be appropriate at the discretion of the treating physician. Patients exhibiting a response on this trial will continue vemurafenib for 6 months unless dose-limiting side effects are experienced by the patient. This will be managed by the treating physician.
16. Per section 6.0, where feasible, tumor samples will be obtained by FNA or core biopsy approximately seven days after vemurafenib treatment (Day -14), and again on Days +21, +42, +84 and within 30 days after disease progression.
17. Urinalysis will be done per section 4.2.2.
18. Immunodeficiency panels and apheresis will be performed within 14 days before initiation of lymphodepletion and 6 weeks after T cell transfer. The samples derived from the apheresis will be used for immune monitoring purposes and will not be used for therapy. CBC w/diff, CMP, LDH, PT/PTT, serum pregnancy test, and EKG will be performed within 14 days prior to apheresis or per current Moffitt apheresis guidelines.
19. Rapid expansion of TIL commences on Day -14 per Cell Therapy Facility SOP.
20. Cyclophosphamide will be administered on Days -7 and -6. Fludarabine will be administered on Days -5 to -1. A CBC w/ differential, CMP, magnesium, and phosphorus will be performed every 1-2 days of treatment during preparative cyclophosphamide/fludarabine and high dose IL-2 therapy per section 6.0. The WBC differential will not be performed if the subject's white blood cell count is not sufficiently high for automated differential per the policy of the institution.
21. The second week of Rapid Expansion starts on Day -7 per Cell Therapy SOP.
22. Adoptive transfer will take place at day 0 prepared per Moffitt cell therapy SOP.
23. Follow-up visits with disease evaluation will be conducted at weeks 6 and 12 (+/- 7 days), then every 3 months (+/- 14 days) for two years, every six months (+/- 30 days) for 2 years, then yearly thereafter (+/- 90 days) or until disease progression, withdrawal of consent, loss to followup, or death, whichever occurs first. Serious adverse events definitely related to vemurafenib will be collected throughout the study protocol starting on the day of initiation of the vemurafenib until 100 days after the last dose of vemurafenib, start of a subsequent anticancer therapy, loss to followup, withdrawal of consent or death, whichever occurs first. Patients will undergo further followup after disease progression every 3 months for overall survival and report of any subsequent anti-cancer treatments that can be accomplished by visit, phone or email contact.

Abstract

Melanoma is particularly appealing to treat with immunotherapy, since a number of melanoma-specific antigens recognized by T cells have been identified over the past two decades. Although strategies to actively vaccinate patients with melanoma against tumor-specific antigens can induce increases in circulating antigen-specific T cells, tumor regressions have not been observed consistently in vaccinated patients. Lack of clinical responses despite the presence of tumor-reactive circulating T cells may be due to immune regulation or tolerance mechanisms.

One promising approach to circumvent suppressive or tolerogenic influences *in vivo* is to activate and expand tumor infiltrating lymphocytes (TIL) *ex vivo*, followed by adoptive cell transfer (ACT). Recently, it was demonstrated that melanoma patients receiving *ex vivo*-expanded tumor-reactive TIL in combination with high dose IL-2 showed relatively high rates of clinical responses and a significant proportion of one and two year survivors. Patients were treated with lymphodepleting chemotherapy prior to cell transfer in order to reset natural homeostatic mechanisms. In some patients, this resulted in high levels of long-term persisting circulating, tumor-reactive T cells, and encouraging responses in the range of 40-70% (1).

It should be noted that the results of ACT for metastatic melanoma have not been reported on an intention to treat basis, and despite this, there is still a significant fraction of patients treated with this regimen that will eventually progress and die of their disease. In our own experience with an active ACT trial for metastatic melanoma, an additional significant limiting factor is that approximately 40% of patients cannot be treated due to interval disease progression prior to the sufficient expansion of their TIL, which can take up to 8 weeks. While not explicitly reported in the literature to date, when factoring in this “drop-out rate” based upon our experience with ACT, the complete response (CR) rate would be estimated to be only 10% based upon intention to treat (unpublished observation). Thus, it would be attractive to treat patients during the interval when the TIL are being generated with an agent that will potentially limit disease progression during TIL growth and thus improve the drop-out rate. Furthermore, it would be attractive to include a potentially synergistic treatment after ACT, and thus augment the complete response rate.

Vemurafenib, a targeted inhibitor of the V600 mutated BRAF molecule, has emerged as an agent with significant anti-melanoma activity that could be used both before and after ACT in patients whose tumor harbors the mutation. Dr. Dmitry Gabrilovich has shown that BRAF inhibition sensitizes BRAF V600 mutated tumors to T cell-induced cytolysis *in vitro*, shown in section 1.5 below. As a putative mechanism, he has also demonstrated that BRAF inhibition upregulates the mannose-6-phosphate receptor (MPR) and increases tumor autophagy and T cell mediated destruction (see section 1.5 below). We therefore hypothesize that *in vivo* exposure of melanoma cells to a targeted inhibitor of BRAF will increase their expression of MPR and result in enhanced tumor destruction by transferred T cells. In addition Vemurafenib is associated with a 90% initial disease control rate that rarely, if ever, results in durable responses. Thus, we

further hypothesize that vemurafenib treatment while the TIL are generated *ex vivo* will reduce the proportion of patients who drop out due to progression between the time of tumor harvest and TIL transfer and lead to a higher rate of durable response in those patients.

To test these hypotheses, we originally proposed to accrue up to 53 patients with stage III or IV metastatic melanoma who were unresectable for curative intent and have a BRAF V600E, D or K mutation. Of note, these patients were BRAF inhibitor naïve (now designated cohort 1). Patients had tumor harvested, and tumor fragments were explanted *in vitro* for TIL growth. Patients then began treatment with vemurafenib, one day following the tumor harvest. Vemurafenib was then continued during the time required for TIL generation and preparation of the patient for ACT. Vemurafenib was stopped prior to the anticipated lymphodepletion and then was resumed after recovery from high-dose IL-2 administration. The co-primary endpoints of the trial were: 1. the proportion of sustained responses at 12 months on an intention-to-treat basis, and 2. the proportion of patients who drop out due to progression between the time of harvest and TIL transfer (i.e., the “drop-out rate”). The secondary endpoint was progression-free survival. In addition, important exploratory laboratory correlative endpoints included:

1. the determination of the change in MPR expression, Granzyme B expression and markers of autophagy (LC3 expression) in tumor biopsy samples obtained before and after vemurafenib and after TIL ACT and the correlation of changes to outcome.
2. the exploration of whether the change in the tumor reactivity of circulating T cells pre and post vemurafenib and after TIL ACT correlates with clinical outcome.

As of April 5, 2017, we have accrued 17 patients to this first cohort of this trial and observed a 41% objective response rate (Wilson’s 95% confidence interval 17.3-58.7%) based upon intention to treat and a 0% dropout rate due to disease progression between the time of harvest and TIL transfer (after a median followup of 21 months). The median progression-free and projected median overall survival calculated by exponential analysis was 12.4 and 49.4 months respectively. All responses that were sustained at 6 months are currently ongoing, with durations ranging from 11+ to 48+ months. However, due to changes to referral patterns, we have observed a dramatic drop off in accrual, as most patients with BRAF V600 mutated melanoma screened at our center have already progressed on BRAF inhibitor containing treatment in the past. Because most patients with metastatic melanoma we evaluate are ineligible due to prior progression on BRAF inhibitor containing treatment, we are very unlikely to complete the original trial design successfully. However, we have discovered that treatment with vemurafenib has activity against tumors that are resistant to BRAF-inhibitor containing therapy (see section 1.6 below). Of note, treatment after progression on BRAF-inhibitor containing therapy is an area of unmet clinical need (2). Therefore, we propose a second pilot cohort of 12 patients who will be accrued to the study after having progressed on BRAF-inhibitor containing treatment. Patients will also be required to have progressed on or have been intolerant to PD-1 antibody containing therapy as well to ensure patients have had optimal prior therapy and hence more likely to be recruited.

1.0 Background and Drug Information

1.1 Melanoma as an immune target and melanoma differentiation antigens

Patients with stage IV metastatic melanoma have a five-year survival rate of less than 5% associated with systemic chemotherapy according to most published clinical series. While chemotherapy regimens have been shown to induce objective regressions in 10-40% of melanoma patients, anti-tumor responses are generally short-lived and rarely result in complete clinical responses. Over the past fifteen years, immunotherapy has become a viable alternative to chemotherapy for the treatment of metastatic melanoma, largely because melanoma is one of the most immunogenic of all known cancers (1,3, 4).

The first indication that immune responses could alter the clinical course of established, invasive human cancers came from studies of the administration of interleukin-2 (IL-2) to patients with metastatic melanoma (5). While IL-2 has no direct impact on the growth of cancer cells, it does have a number of immune regulatory effects, including the expansion of T lymphocytes following activation by specific antigen and NK cells (6, 7). Approximately 15% of melanoma patients treated with IL-2 experience objective regressions, a portion of which are long-lasting and potentially curative (8, 9). This result suggested that activation of T lymphocytes with anti-tumor activity was responsible for the induction of tumor regressions (10).

Subsequent studies in both humans and in animal models have borne out these predictions and have allowed for the elucidation of the mechanisms behind the immune recognition of tumor cells at the molecular level. T cells specifically recognize antigens presented as small peptides in association with surface human leukocyte antigen (HLA) molecules (11, 12). Peptide antigens expressed on the surface of cancer cells have been demonstrated in multiple studies to induce T cell recognition leading to tumor cell killing and/or the release of helper and other cytokines. In the case of melanoma, a number of antigens have now been identified that can be recognized by both CD8+ cytotoxic T cells and CD4+ T-helper cells, including MART-1, gp100, MAGE-1, tyrosinase, TRP-1, TRP-2 and NY-ESO-1 (12, 13).

The MART-1/Melan-A gene encodes a 118 amino acid protein of 13 kD. With the exception of melanocytes and retina, no normal tissues express this antigen and no expression of the MART-1 gene product has been seen in cancers other than melanoma. MART-1, therefore, appears to represent a melanocyte lineage-specific antigen. The MART-1 antigen was expressed on a majority of fresh melanomas assayed by the Surgery Branch, National Cancer Institute (NCI) (14). The immunodominant nine amino acid peptide, AAGIGILTV, has been identified as the peptide in the MART-1 sequence that binds to the HLA-A2 molecule (15). A majority of melanoma specific TIL from HLA-A2+ individuals that recognized MART-1, reacted with this peptide as evidenced by the ability of these TIL to specifically recognize HLA-A2+ T2 cells or EBV cells pulsed with this peptide.

1.2 Initial studies of adoptive T cell transfer for the treatment of human cancer

The identification of T cells with the ability to specifically recognize melanoma antigens, along with the technological capability to expand these tumor-reactive T cells to large numbers in the laboratory, has led to the development of adoptive transfer protocols for patients with metastatic melanoma. Tumor-infiltrating lymphocytes (TIL) derived from resected tumors that were expanded *in vitro* were shown to be capable of specifically recognizing tumor antigens, particularly MART-1, in over two-thirds of melanoma patients (15, 16). Such TILs, when expanded to large numbers (greater than 20 billion) and adoptively transferred intravenously to patients along with IL-2, resulted in an objective response rate of 35% (17). This initial response rate was nearly twice that observed with IL-2 alone and was also seen in patients that were refractory to IL-2 treatment. However, most of the responses were transient and limited persistence of the transferred cells was observed (18).

1.3 Addition of non-myeloablative lymphodepletion to adoptive cell transfer

Mounting evidence suggests that the host immune environment can significantly impact the efficacy of adoptive cell transfer therapy. Data from mouse tumor models have demonstrated that sublethal doses of irradiation prior to adoptive transfer of tumor antigen-specific lymphocytes substantially increases the persistence and anti-tumor activity of the transferred cells (19). While the mechanisms leading to this enhanced T cell activity have not been precisely delineated, two non-mutually exclusive hypotheses may provide an explanation. A subset of $CD4^+$ T cells, expressing high levels of CD25 and the molecule FoxP3 and known as regulatory or suppressor T cells, is thought to have a negative impact on the activity of cytotoxic T cells *in vivo* (20, 21). It has been hypothesized that increased numbers of suppressor T cells in cancer patients may correlate with an unfavorable prognosis and that elimination of these cells may result in an improved efficacy of adoptive immunotherapy (22-25). Alternatively, prior depletion of lymphocytes may create ‘space’ for the adoptively transferred cells within the lymphocyte compartment (26). Under this model, homeostatic lymphocyte survival may result in increased proliferation and enhanced survival of transferred T cells, perhaps through a mechanism involving increased access to endogenous cytokines like IL-7 and IL-15 (27).

In a prior published protocol, patients with metastatic melanoma received a single dose of cyclophosphamide (Cytoxan) at 25 mg/kg prior to the administration of autologous TIL cells. Traffic of TIL to tumor sites was evaluated by labeling TIL with Indium-111 and performing sequential radionuclide scans. Of 26 patients that received cyclophosphamide, TIL trafficking to tumor was seen in 21 patients (81%) compared to TIL trafficking to tumor in 42% of patients that did not receive cyclophosphamide ($p=0.026$). No difference was seen in tumor regression rates. Thus, even with a mild and very transient leukopenia (about 5 days), evidence of increased lymphocyte trafficking to tumor was observed (28).

The animal and clinical studies cited above strongly suggest that the clinical effectiveness of these cells and their ability to survive and repopulate the host would be enhanced if patients were significantly immunosuppressed by the depletion of lymphocytes prior to the adoptive transfer of lymphocytes.

A recent clinical trial investigated the addition of a lymphodepleting conditioning regimen to adoptive cell transfer therapy in patients with metastatic melanoma. Patients received a lymphodepleting chemotherapy regimen consisting of high dose cyclophosphamide and standard doses of fludarabine before administration of highly selected, expanded, tumor-reactive TIL and IL-2 (29, 30). The lymphodepletion step resulted in a transient myelosuppression and the elimination of all circulating lymphocytes for approximately one week, after which time patients recovered endogenous marrow function and reconstituted their lymphocyte compartments towards normal levels within two to three weeks (31).

Because of the immunosuppression of fludarabine, one of the patients who had clonal repopulation from infused TIL cells and a complete response of metastatic melanoma, developed Epstein-Barr virus (EBV) - associated B cell lymphoma. This patient was EBV-naïve prior to the treatments. The potential source of EBV was thought to be multiple blood product transfusions after chemotherapy. The patient later died of complications from the treatment of his lymphoma. Another patient developed polyneuropathy manifested by vision blindness, motor and sensory defects, approximately 2 months after chemotherapy. The etiology of this complication is unknown, but was possibly related to the fludarabine (29).

In a recent publication in *Science*, six patients of thirteen demonstrated objective tumor regression and four additional patients showed mixed responses with substantial shrinkage of some lesions after lymphoid depletion and adoptive transfer of highly selected, expanded, tumor-reactive TIL and IL-2. Significant levels of tumor regression were observed in metastatic deposits in the liver, lungs, cutaneous and subcutaneous tissues, and lymph nodes. One patient had dramatic regression of axillary, pelvic and intraabdominal metastases, on-going at 17 months and was rendered free of disease by a surgical removal of one residual intraperitoneal lesion. Two other patients had marked, persistent lymphocytosis up to 3 weeks after the TIL infusion. Molecular and immunological analyses confirmed that these lymphocytes from their peripheral blood were the progeny of the infused TIL (17). In particular, in one patient, it was shown that one specific clone repopulated this patient's peripheral blood lymphocytes (PBL) up to 2 months after infusion. Immunohistochemistry studies revealed that specific clones from infused bulk oligoclonal TIL cells infiltrated the regressing tumor nodules. Although the mechanism for the continued proliferation *in vivo* of these rapidly expanded (REPed) bulk TIL cells and their anti-tumor effects remain to be determined, it appears that some cells in the REPed bulk TIL might have provided necessary cytokines (such as IL-2) for cytotoxic T lymphocytes to persist and survive *in vivo* and eventually kill the tumor. Alternatively, the chemotherapy regimen used in this protocol might have depleted endogenous suppressive lymphocytes. Seven of these 13 patients received a second cycle of treatment with the same chemotherapy regimen plus bulk TIL and high dose IL-2. All seven patients recovered from a second cycle (29). Four out of the 13 patients

developed vitiligo and one patient developed uveitis, which did not interfere with vision and reversed after steroid therapy (29).

More recent experience with lymphoid depletion, TIL and IL-2 at the Moffitt Cancer Center, MD Anderson Cancer Center, Sheba Medical Center in Israel and the National Cancer Institute suggest that so-called “young” TIL that are not selected for tumor reactivity but are grown from tumor fragments in IL-2 and pooled without an intermediate step to assay their tumor specific release of cytokine can be successfully used to treat patients with stage IV melanoma. In a presentation at the American Society for Clinical Oncology annual meeting in June 2011, Shapira-Frommer et al described their experience with 42 patients treated with “young” TIL at the Sheba Medical Center, demonstrating a 40% ORR and significant regression of tumor in patients with extensive metastatic disease. At the MD Anderson Cancer Center, 35 patients have been treated with cyclophosphamide-fludarabine followed by TIL and high-dose IL-2, with a 50% ORR. Tumor regression was associated with larger number of cells administered (30, 31). However, the results were not reported on an intention-to-treat basis and would be significantly lower if the drop-out rate was taken into consideration. In our experience, 25% of patient samples fail to grow TIL, and 32% of patients drop out prior to treatment.

Finally, the National Cancer Institute, which has pioneered TIL therapy for cancer, has described their long-term experience with up to 5 year follow-up in 93 patients treated with TIL that have had lymphoid depletion using the current cyclophosphamide-fludarabine regimen, or using that regimen with 200 or 1200 RAD of total lymphoid irradiation. The documented that 22% of their patients had complete responses, and that 93% of those patients were alive at 5 years, free of disease (1). Again, the results were not reported on an intention-to-treat basis. At Moffitt, we have failed to successfully grow TIL from harvested tumor samples in 25% of cases in our initial preclinical trial efforts to validate TIL growth (unpublished observation). In our first adoptive cell therapy trial, we have attempted to treat 19 patients and have successfully treated 13 patients (68%) with cyclophosphamide-fludarabine followed by TIL with IL-2. Of these, we have observed 5 partial responses and two patients with stable disease (32). Thus our observed response rate based upon intention to treat is 26%.

The collective results suggest that the non-myeloablative lymphodepleting chemo-preparative regimen identical to the above regimen proposed in this current study can be tolerated and is potentially efficacious for the treatment of advanced metastatic disease. This may be due to the homeostatic pressure created by the chemotherapy regimen. After ablation of the endogenous lymphocyte compartment, the infused TIL cells may expand better *in vivo* without competition from endogenous lymphocytes. At the NIH, marked proliferation of the transferred TIL cells was not observed without this course of chemotherapy. Thus, in the proposed study, the plan is to administer this preparative lymphodepleting chemotherapy regimen to patients prior to TIL cell infusion. The justification for the addition of vemurafenib, the selective mutated B-RAF inhibitor, to TIL therapy for two cohorts of patients who are either BRAF inhibitor treatment naïve and refractory is described in more detail below.

1.4 BRAF inhibition and Vemurafenib

Discovery of oncogenic v-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutations elucidated the central role of BRAF kinase in signaling pathways that control cellular proliferation. In normal cells, BRAF is recruited to the plasma membrane following activation of growth factor receptors that enable binding of guanosine triphosphate (GTP) to the rat sarcoma virus oncogene homolog (RAS) proteins. GTP-bound RAS binds directly to BRAF and this interaction results in activation of BRAF kinase activity. The best-characterized substrate of BRAF is mitogen-activated protein kinase kinase 1, also known as MEK1. Phosphorylation of MEK1 phosphorylates extracellular signal-regulated kinase 1 (ERK1) and ERK2, and phospho-ERK translocates to the nucleus where a variety of ERK substrates reside. Oncogenic mutations in BRAF result in gain-of-kinase- function, rendering the RAF-MEK1-ERK pathway constitutively active in the absence of its growth factors (331). Davies et al. (34) highlighted the prevalence of BRAF mutations in a variety of cancers and showed that the majority of mutations occur in codon 600, most frequently resulting in a valine to glutamate substitution (V600E). BRAF mutations have been identified in 50–68% of melanomas, 63% of low-grade serous ovarian carcinomas, and 4–14% of colorectal cancers, among many others (35). Generally, RAS and BRAF mutations are mutually exclusive (36, 37). Oncogenic BRAF mutations generally correlate with poor prognosis in a variety of different tumor types. Published data and the COSMIC database (38) indicate that over 90% of the BRAF mutations seen in melanoma occur in codon V600, and that over 90% of the V600 mutations are V600E (39). However, a variety of other uncommon variants, such as V600K, V600R and V600D have been observed, which can also result in constitutive activation of the BRAF kinase, and patients whose tumors harbor any known activating mutations in BRAF will be included in this trial (40).

Clinical Studies with Vemurafenib: Vemurafenib (also known as PLX4032, RO5185426 or RG7204) is a low molecular weight, orally available inhibitor of the activated form of the BRAF serine-threonine kinase enzyme. Vemurafenib selectively inhibits oncogenic BRAF kinase (41). The high level of selectivity of vemurafenib has been demonstrated in biochemical, cell-based and in vivo assays. The dose of vemurafenib was established in a multicenter, Phase I, dose- escalation study with a total of 55 patients (49 of whom with a diagnosis of melanoma). Once the recommended Phase II dose of 960 mg BID was demonstrated, 32 additional patients with metastatic melanoma and BRAF V600 mutations were enrolled in the extension phase of this study (42) to determine the ORR. The ORR for the 32 patients was 81% assessed by investigators, and median OS was 14.9 months (42).

In an open-label, multicenter Phase II study called BRIM2, presented at ASCO 2011, 132 patients were treated with oral vemurafenib 960mg BID, without scheduled dose interruption, until progression of disease or unacceptable toxicity. The primary objective of this study was to evaluate the best overall response rate (BORR) of vemurafenib as assessed by an independent review committee using the Response Evaluation Criteria in Solid Tumors (RECIST, v1.1) guidelines. Those results were presented at ASCO 2011, showing a 53% ORR and 5.7 month median PFS (43).

Finally, a randomized, open-label, multicenter Phase III study called BRIM3 enrolled approximately 675 patients with treatment-naive metastatic melanoma and a BRAF V600E mutation. Patients were randomly assigned to be treated with either Vemurafenib 960 mg PO BID every day or intravenous dacarbazine 1000 mg/m2 on day 1 every 3 weeks. Those data were presented at ASCO 2011 and were also recently published, demonstrating a significant benefit for the vemurafenib-treated patients in overall survival (HR = 0.63, p=0.001) and PFS (HR = 0.74, p=0.001, 44). Response rates were 48% for the Vemurafenib arm, and 5% for Dacarbazine (44). Median PFS on Vemurafenib was 6.8 mos (95% CI: 5.6-7.6; 44). The results of BRIM3 and BRIM2 have been submitted to the FDA and has resulted in successful registration of vemurafenib for patients with BRAF V600 mutated metastatic melanoma.

1.5 Justification for combining ACT and Vemurafenib for the treatment of unresectable metastatic melanoma (cohort 1)

As discussed above, limitations to ACT include a significant drop out rate during the time required to prepare TIL for infusion, as well as a lack of response in 30-60% of patients who undergo successful treatment. It is our hypothesis that including BRAF inhibition both before and after TIL transfer will improve upon these limitations. Work by Dr. Dmitri Gabrilovich at the Moffitt Cancer Center, detailed below, supports the rationale for including a BRAF inhibitor after T cell transfer for BRAF inhibitor naïve tumors in the first cohort of this clinical trial. We have shown that treatment of sensitive tumor cells with a BRAF inhibitor can induce increased tumor recognition by T cells and can increase sensitivity to lysis of those cells by tumor-specific T cells via increased sensitivity of those target cells to granzyme B. The increased sensitivity was shown to be associated with up-regulation of the Mannose 6 Phosphate Receptor (MPR) on the tumor cells, which led to increased autophagy after exposure to granzyme B. The existence of this phenomenon, which has been demonstrated in animal models and using human tumor cells in vitro for melanoma, lung cancer and breast cancer, suggests that combining BRAF inhibitor therapy with immunotherapy, particularly adoptive cell therapy with antigen-specific T cells, might lead to additive if not synergistic anti-tumor effects if the BRAF inhibitor could be administered to patients with BRAF mutated tumors following ACT with TIL.

Effect of BRAF inhibitor on MPR expression. The effect of BRAF inhibitor PLX4720 on MPR expression was evaluated in 4 human melanoma cell lines with wild-type (w/t) BRAF (control) (SBCL22 and 2032p) or BRAF V600E mutation (SKMEL-28 and WM164p). At all tested doses (1.25-10 μM) PLX4720 did not affect MPR expression in BRAF wild-type SBCL22 or 2032p cells (Fig. 1A). In contrast, at relatively low doses (2 μM for SKMEL and 0.5 μM for WM164p) PLX4720 caused substantial up-regulation of MPR in BRAF mutant cells (Fig. 1A). In a xenograft model, human BRAF mutant melanoma cell line 1205Lu was established in SCID-NOD mice. When tumors reached 1 cm in diameter mice were treated by oral gavage with 100 mg/kg PLX4720 twice a day for 5 days. Tumors were collected at different times after that, and MPR expression was evaluated by IHC. PLX4720 caused substantial up-regulation of MPR 3 days after

start of the treatment (Fig. 1B). Thus, in BRAF mutant melanoma cells, targeted therapy resulted in up-regulation of MPR providing the rationale for further studies.

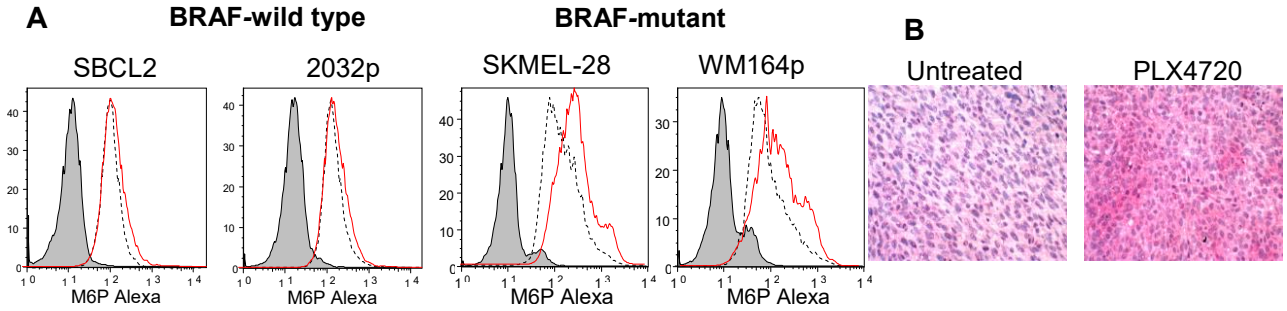


Figure 1. Effect of BRAF inhibitor on MPR expression.
A. Melanoma cell lines were treated overnight with the BRAF inhibitor PLX4720. Expression of surface MPR was measured by flow cytometry. Shaded area –isotype IgG, dotted gray line – untreated cells, red-line – treated cells. For BRAF w/t cells (SBCL2, 2032p) maximal dose of 10 μM is shown. For BRAF mutant cells: SKMEL cells – 2 μM and for WM164p cells – 0.5 μM doses are shown.

B. Treatment of SCID-beige mice bearing BRAF mutant melanoma (1205Lu) with PLX 4720 (100 mg/kg) twice a day for 3 days. Tumors were excised and MPR expression was detected by immunohistochemistry. Mag x 400

BRAF inhibitor potentiates anti-melanoma effect of CTLs *in vitro.* To test the ability of a BRAF inhibitor to sensitize melanoma cells to CTLs we used the 624 cell line established from a patient with melanoma. These cells carry the BRAF V600E mutation and are positive for HLA-A2. Tumor infiltrating lymphocytes (TIL) were isolated and expanded from a HLA-A2$^+$ melanoma patient. These TILs demonstrated HLA-A2 restricted cytotoxicity against tumor cells (data not shown). In our experiments pre-treatment of tumor cells with BRAF inhibitor PLX-4720 substantially enhanced CTL-mediated killing of melanoma cells (Fig. 2). These preliminary data suggest that BRAF inhibition may potentiate the effect of CTLs against sensitive melanoma cells and provide additional rationale for the addition of BRAF inhibition following ACT.

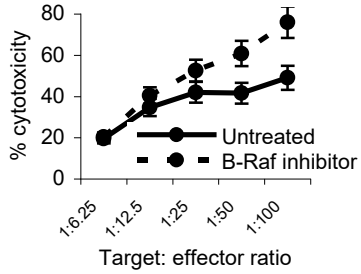


Figure 2. BRAF inhibitor sensitizes melanoma cells CTL killing. TILs were obtained from a HLA-A2$^+$ patient with metastatic melanoma. Cells were expanded with anti-CD3/CD28 antibodies and IL-2. HLA-A2$^+$ 624 melanoma cell line was used as target. Target cells were treated with 20μM of the BRAF inhibitor PLX-4720 for 24 hr and then used in a standard ^{51}Cr release assay.

Autophagy as a possible mechanism of MPR up-regulation. Next, we studied the possible mechanism of MPR up-regulation. Treatment with the BRAF inhibitor PLX-4720 induced both MPR and evidence of autophagy, shown in Figure 3**,** in which treated cells were stained for LC3 (which is a known marker of autophagy) (red fluorescence) and there was a clear dose dependence of autophagy induction with higher doses of PLX-4720.

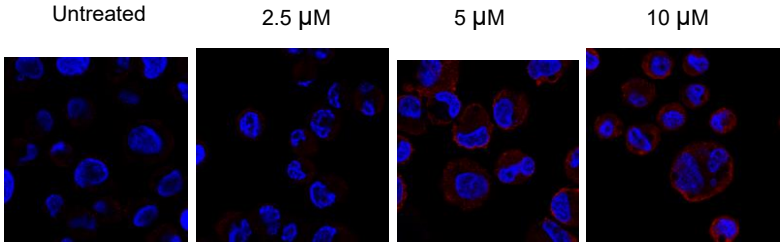


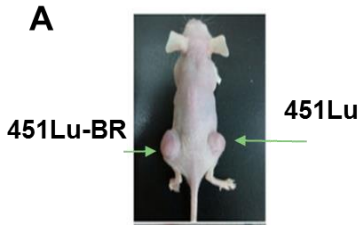
Figure 3. BRAF inhibitor causes autophagy of human melanoma cells with BRAF V600E mutation. 1205Lu melanoma cells was treated with indicated concentrations of PLX-4720 overnight and cells were stained with DAPI (blue) and LC3 antibody (red fluorescence) (Mag. 400).

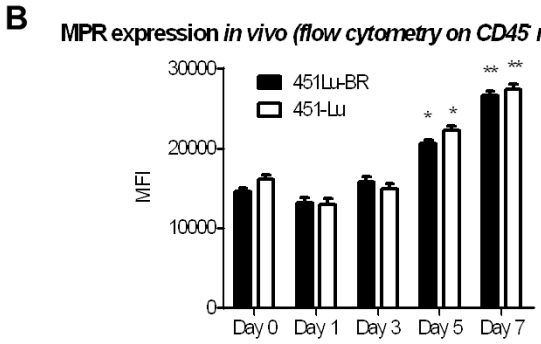
In both cohorts of this trial, the BRAF inhibitor will be stopped prior to the administration of chemotherapy and high dose IL-2 due to the uncertain toxicity that may result in the combination of treatments. Nonetheless, upon clinical recovery from high dose IL-2, the BRAF inhibitor may then be continued as such treatment may enhance the anti-tumor efficacy of the transferred T cells as discussed above. An important advantage of the addition of the BRAF inhibitor vemurafenib prior to ACT in BRAF inhibitor treatment naïve patients is that drop-out of patients during the interval between TIL harvest and cell administration will be minimized since patients will receive treatment during the period of TIL expansion with a drug that in 90% of cases will prevent short-term disease progression. In our experience, the drop out rate prior to ACT is approximately 40% (unpublished data). Since the median PFS of vemurafenib is 6.8 months, with very few durable responses noted in any trials, it should be practical to assess the contribution of TIL if the median PFS is prolonged to 12 months or more, and if a significant proportion of responses are sustained at 6 and 12 months. We will monitor the number of drop-outs of patients who are harvested for TIL and are unable to receive their cells due to rapid progression, and we will also assess the proportion and durability of PRs and CRs at 6 and 12 months.

1.6 Justification for combining ACT and Vemurafenib for the treatment of unresectable metastatic melanoma after progression on BRAF inhibitor-containing therapy and progression or intolerance to PD-1 antibody (cohort 2)

Despite the advances in the use of targeted inhibitor therapy for melanomas with BRAF V600-containing activating mutations, treatment after progression remains a significant unmet clinical need. This is illustrated by results of the BRIM7 study reported at ASCO 2016 that included a subset of patients who progressed on vemurafenib then treated with the combination of vemurafenib and the MEK inhibitor cobimetinib. Objective response rate was 15% with a median progression-free survival of 2.8 months (45). To meet the need for more effective treatment of patients who have progressed on BRAF inhibitor containing therapy, the laboratory of Dr. Gabrilovich has generated data that support the use of vemurafenib to sensitize tumors resistant to BRAF inhibitor

containing therapy, as resistant tumors still upregulate MPR in hypoxic conditions and are more sensitive to granzyme B-mediated cell death, which is a prominent mechanism by which TIL mediate the regression of tumors as discussed above. These data below support the incorporation of a second cohort of patients for this trial who have progressed on BRAF-inhibitor therapy in the past, with vemurafenib, not used as a direct anti-tumor cytotoxic agent, but rather a sensitizing agent for TIL-mediated anti-tumor immunity (see below).





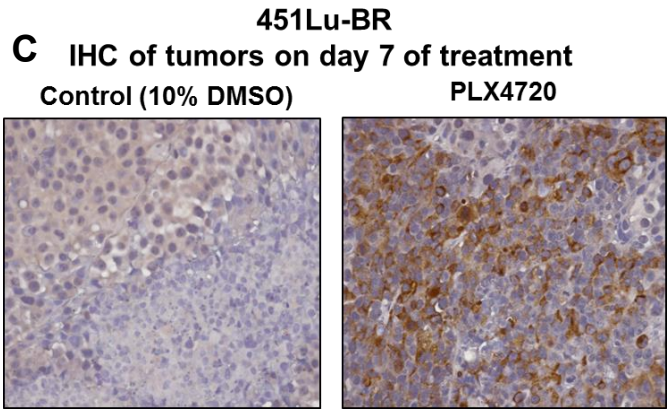


Figure 4. BRAF Inhibition upregulates MPR in resistant tumors in a mouse model of metastatic melanoma

A. Demonstration of in vivo growth of BRAF inhibitor sensitive (451Lu) and resistant (451Lu-BR) cell line in a mouse model.

B. Demonstration that MPR upregulation occurs in vivo at days 5 and 7 after BRAF inhibitor treatment in both BRAF inhibitor sensitive (451Lu) and resistant (451Lu-BR) cell line in a mouse model. Results were confirmed by flow cytometry quantitation of MPR mean fluorescence intensity.

C. Demonstration that MPR upregulation occurs in vivo at day 7 after PLX-4720 treatment in BRAF inhibitor resistant (451Lu-BR) cell line in a mouse model, confirmed by immunohistochemistry.

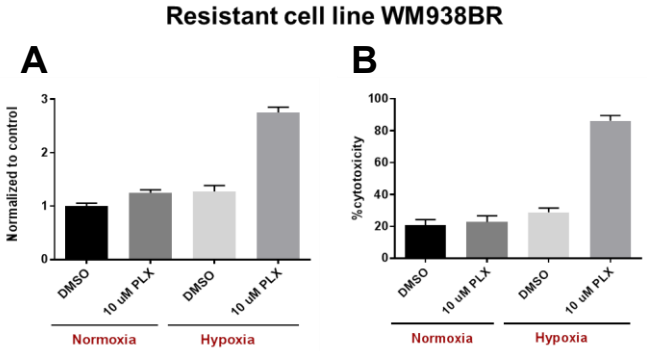


Figure 5. In resistant human cell lines, BRAF Inhibition upregulates MPR and induces sensitivity to Granzyme B mediated cell death under hypoxic conditions

A. Demonstration of MPR upregulation mediated by BRAF inhibition in a human cell line under hypoxic conditions

B. Demonstration that under hypoxic conditions, BRAF inhibition induces sensitivity to Granzyme B mediated cell death under hypoxic conditions.

Given that the purpose of vemurafenib for patients in cohort 2 is to upregulate MPR rather than to exert a direct antitumor effect, prospective patients for cohort 2 will be required to have progressed on PD-1 antibody based therapy per section 4.1.1s. This measure is to ensure that the patients in cohort 2 have been treated with and progressed on the most effective treatment available prior to enrolling in the second cohort where there will not be planned anti-tumor directed systemic therapy during the time of TIL growth (4 to 6 weeks), and prior anti-PD1 treatment will not interfere with the outcome assessment for this trial since the time for eligibility screening and time to grow TIL will result in a >8 week washout from prior anti-PD1 treatment.

2.0 Objectives

The co-primary objectives for the **first cohort** of this study (BRAF inhibitor and PD1 antibody naïve) will be to: 1. improve the 12 month PR + CR rate based upon RECIST 1.1 criteria on an intention-to-treat basis, and 2. to improve the drop-out rate in patients undergoing ACT. A secondary objective is an evaluation of progression-free survival.

The primary objective for the **second cohort** of the study (BRAF-inhibitor treatment resistant and PD-1 antibody treatment resistant or intolerant) will be to determine whether patients who have progressed on BRAF-inhibitor containing therapy in the past who receive vemurafenib added to adoptive cell therapy with tumor-infiltrating lymphocytes exhibit a relatively higher proportion of responses (CR+PR) at 12 months by RECIST 1.1 criteria when compared to prior experience with patients who have progressed after BRAF-inhibitor and PD-1 antibody containing treatment. A secondary objective is an evaluation of progression-free survival.

Additional exploratory laboratory correlative endpoints for both cohorts will include:
1. The determination of the change in MPR expression and markers of autophagy in tumor biopsy samples obtained before and after vemurafenib and after TIL ACT in patients whose tumors have or have not previously progressed on BRAF-inhibitor containing treatment.
2. The exploration of whether the change in tumor reactivity of circulating T cells pre and post ACT correlates with clinical outcome.

3.0 Pharmacology

3.1 Interleukin-2 (Aldesleukin, Proleukin)

Interleukin-2 (IL-2) will be manufactured and supplied by Prometheus, San Diego, CA. Prometheus, Inc will supply the IL-2 for the in vitro portion of the trial, and the Moffitt Cancer Center pharmacy will purchase IL-2 from commercial sources for the treatment portion of the trial. IL-2 is a 133 amino acid protein primarily secreted by T-cells in

response to various antigenic stimuli. The cytokine acts through a specific IL-2 receptor consisting of α, β, γ subunits. In addition to T-cell proliferation, IL-2 leads to activation and proliferation of natural killer (NK) cells, increasing their tumoricidal activity. Other actions of IL-2 include augmentation of B-cell growth and immunoglobulin production, enhancement of interferon (IFN)-γ and tumor necrosis factor (TNF)-β production from T-cells, IL-6 production by monocytes, modulation of histamine release by basophils, and upregulation of IL-2 receptors. This triggers the release of various other cytokines leading to the total immune/ inflammatory reaction and resultant toxicity.

IL-2 will be administered as an inpatient treatment. Grade III toxicities common to IL-2 include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, constitutional symptoms, and laboratory changes. Additional Grade IV and V toxicities have been seen with IL-2 (See Appendix).

3.2 Fludarabine

Fludarabine phosphate is a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-β-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination. Fludarabine is a purine antagonist antimetabolite. Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

It will be purchased by the Moffitt Cancer Center pharmacy from commercial sources. Fludarabine is supplied as a fludarabine phosphate powder in the form of a white, lyophilized solid cake. The fludarabine powder is stable for at least 18 months at 2 – 8 degrees C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribonucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

Fludarabine is administered as an IV infusion in 100 mL 0.9% sodium chloride, USP over approximately 15 to 30 minutes. At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression. However, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fatigue, anorexia, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy,

blindness, and coma is rare at the currently administered doses. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, and allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of chronic lymphocytic leukemia.

3.3 Cyclophosphamide (Cytoxan)

Cyclophosphamide is a synthetic anti-neoplastic drug chemically related to the nitrogen mustards. It is biotransformed principally in the liver to active alkylating metabolites by a mixed function microsomal oxidase system. These metabolites interfere with the growth of susceptible rapidly proliferating malignant cells. The mechanism of action is thought to involve cross-linking of tumor cell DNA.

Cyclophosphamide is well absorbed after oral administration with a bioavailability greater than 75%. The unchanged drug has an elimination half-life of 3 to 12 hours. It is eliminated primarily in the form of metabolites, but from 5% to 25% of the dose is excreted in urine as unchanged drug. Several cytotoxic and non-cytotoxic metabolites have been identified in urine and in plasma. Concentrations of metabolites reach a maximum in plasma 2 to 3 hours after an IV dose. Plasma protein binding of unchanged drug is low but some metabolites are bound to an extent greater than 60%. It has not been demonstrated than any single metabolite is responsible for either the therapeutic or toxic effects of cyclophosphamide. Although elevated levels of metabolites of cyclophosphamide have been observed in patients with renal failure, increased clinical toxicity in such patients has not been demonstrated.

Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkylating agent and possesses potent immunosuppressive activity. The serum half-life after intravenous administration ranges from 3 to 12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

Cyclophosphamide will be obtained from commercially available sources by the Moffitt pharmacy. It will be diluted in 250 mL NS and infused over approximately two hours. The dose will be based on the patient's body weight, but to prevent undue toxicity, it will not exceed a dose greater than 140% of the maximum ideal body weight per Metropolitan Life Insurance Company, Height and Weight Tables. Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea, and vomiting may occur, especially after high-doses. Diarrhea, hemorrhagic colitis, and mucosal and oral ulceration have been reported in patients. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as an uroprotective agent Mesna may not be effective in all patients.

Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity due to allopurinol induction of hepatic microsomal enzymes. At high doses, cyclophosphamide can also result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain occurs. Cardiotoxicity has been observed at high doses of cyclophophamide. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis.

3.4 Mesna (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891)

Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

Mesna was developed as a prophylactic agent to reduce the risk of hemorrhagic cystitis. Analogous to the physiological cysteine-cystine, mesna is rapidly oxidized to its major metabolite, mesna disulfide (dimesna). Mesna disulfide remains in the intravascular compartment and is rapidly eliminated by the kidneys. In the kidney, the mesna disulfide is reduced to the free thiol compound, mesna, which reacts chemically with the urotoxic metabolites, resulting in their detoxification.

Mesna will be obtained commercially and is supplied as a 100 mg/mL solution. Intact ampules are stored at room temperature. Diluted solutions (1 to 20 mg/dL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% normal saline, or 24 hours in normal saline. It will be diluted up to 20 mg Mesna/mL fluid in D5W or normal saline and will be administered intravenously as a continuous infusion. Toxicities include nausea, vomiting and diarrhea.

3.5 Vemurafenib (RG 7204, PLX_4032)

The dose of vemurafenib at 960 mg BID was identified in the Phase I dose-finding study PLX 06-02 and is established as the recommended dosage for Phase II and III trials for the treatment of melanoma. At the recommended Phase II vemurafenib dose of 960 mg BID, the Vemurafenib mean half-life was approximately 50 hours (range 30–80 hours,

ref 40). With the twice-daily dosing regimen, all patients were exposed to relatively constant daily levels of the drug at steady state.

The formulated vemurafenib drug product is provided as 240-mg film-coated tablets packed in bottles for oral administration. For additional batch-specific instructions and information, vemurafenib will be labeled in compliance with Good Manufacturing Procedures (GMP). The drug label will include the contents, protocol number, batch number, and storage conditions, as well as any required statements that the drug is: "For Clinical Trial Use Only." Patients will be requested to store the vemurafenib at the recommended storage conditions noted on the label out of the reach of children or other cohabitants. Vemurafenib should be stored at room temperature between 15^0C and 25^0C and should be protected from excessive exposure to sunlight. For further details, please see the Investigator's Brochure.

The following AEs can occur in patients treated with vemurafenib (See the full Prescribing Information on the serious risks of vemurafanib). Details of these events are provided in the current vemurafenib Investigator's Brochure (v13, January 2016).

- New Primary Cutaneous Malignancies
- New Non-Cutaneous Squamous Cell Carcinoma
- Progression of malignancies associated with rRAS mutation tumor Promotion in BRAF Wild-Type Melanoma
- Serious Hypersensitivity Reactions including anaphylaxis and Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS Syndrome)
- QT Prolongation: Monitor ECG and electrolytes before and during treatment
- Hepatotoxicity
- Severe Dermatologic Reactions, including Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis
- Photosensitivity
- Serious Ophthalmologic Reactions
- Embryo-Fetal Toxicity
- Radiation Sensitization and Radiation Recall: Severe cases have been reported
- Renal Failure
- Dupuytren's Contracture and Plantar Fascial Fibromatosis

AEs with vemurafenib have been predominantly mild in severity and transient, even with continuous dosing (over 15 months of treatment in 1 patient). At the recommended Phase II and Phase III dose of 960 mg BID, AEs have been consistent with the safety profile observed in the Phase I setting. Treatment-related Grade 3 AEs and dose-limiting toxicities (DLTs) have been successfully managed by a temporary discontinuation of vemurafenib and/or a reduction in dose. See Section 4.1.5 for details with respect to dose modification and management guidelines for vemurafebin-related toxicities.

3.5.1 Safety of Vemurafenib in the Post Marketing Setting

One case of progression of NRAS-mutated chronic myelomonocytic leukemia (CMML) occurred in a male patient with metastatic melanoma treated with vemurafenib for less than 2 weeks (46). After the first dose of vemurafenib, laboratory results showed a marked leucoytosis and monocytosis and vemurafenib treatment was subsequently stopped. There was a temporal relationship between vemurafenib treatment and increase in white blood cell (WBC) and absolute monocyte counts, through multiple cycles of dechallenge and rechallenge. In vitro studies demonstrated proliferation of the leukemic cell population, an effect that was reversed upon drug withdrawal. Further, the cells exhibited dose-dependent and reversible activation of ERK in the NRAS-mutated leukemic clone. On the basis of its mechanism of action, vemurafenib may cause progression of cancers associated with RAS mutations. Vemurafenib should be used with caution in patients with a prior or concurrent cancer associated with RAS mutation. Detailed accounts of these events are in the current vemurafenib Investigator's Brochure.

3.5.2 Expanded Access and Post-Approval Safety Studies

In Study ML25597, the safety profile observed was consistent with that reported in previous studies. The number of patients with Grade 3 or 4 treatment-related adverse events was higher in patients with an ECOG PS of 2 or 3 than in those with PS 0 or 1 (10% vs. 5%, respectively). Adverse events requiring a dose reduction (at least 1 level) occurred in 11% of patients, and 9 patients (2%) experienced events leading to vemurafenib withdrawal, including 2 with repeated QT interval prolongation (47).

In Study MO25515, the safety profile observed was consistent with that reported in previous studies. Overall 3052/3222 (94.7%) patients experienced an AE of any grade and 30.6% had an SAE. Grade 3-4 AES were reported in 45.9% of the patients, 6.1% discontinued treatment due to an AE, and 85 patients (27%) died due to an AE.

3.6 G-CSF (Granulocyte Colony-Stimulating Factor)

G-CSF will be obtained commercially and is supplied in 300 mcg/ml and 480 mcg/ml vials. G-CSF should be refrigerated and not allowed to freeze. The product bears the expiration date. It is generally stable for at least 10 months when refrigerated. The appropriate dose is drawn up into a syringe. G-CSF will be given daily subcutaneously as a blood product support if needed. The side effects of G-CSF are skin rash, myalgias and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

3.7 Levofloxacin (Levaquin) and Trimethoprim and Sulfamethoxazole double strength (TMP / SMX DS, Bactrim)

Levofloxacin is used to prevent infections caused by bacteria. It is a synthetic broad spectrum antibacterial agent. The mechanism of action of levofloxacin involves inhibition of bacterial topoisomerase IV and DNA gyrase, enzymes required for DNA replication, transcription, repair, and recombination. An alternative antibiotic by mouth for patients who are allergic to levofloxacin will be cephalexin.

TMP/SMX DS will be obtained by the Moffitt Cancer Center pharmacy from commercial sources. It will be used for the prevention of Pneumocystis jiroveci pneumonia/Pneumocystis carinii Pneumonia. The oral dose is 1 tablet PO bid twice a week until absolute lymphocyte count is ≥1000/mcL. Like other sulfa drugs, TMP/SMX DS can cause allergies, fever, nausea, and vomiting. Allergies typically develop as a widespread itchy red rash with fever eight to fourteen days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur. Patients with pre-existing sulfa drug allergies may be prescribed pentamadine inhaler treatment, atovaquone, or equivalent drug that covers Pneumocytis, or may undergo sulfa allergy desensitization treatment.

3.8 Acyclovir (Zovirax) and Valacyclovir Hydrochloride (Valtrex)

Acyclovir and valacyclovir will be obtained by the Moffitt Cancer Center pharmacy from commercial sources.

Valacyclovir is the hydrochloride salt of *L*-valyl ester of acyclovir. It is rapidly converted to acyclovir, which has demonstrated antiviral activity against herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and varicella-zoster virus (VZV). The inhibitory activity of acyclovir is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV. This viral enzyme converts acyclovir into acyclovir monophosphate, a nucleotide analogue. The monophosphate is further converted into diphosphate by cellular guanylate kinase and into triphosphate by a number of cellular enzymes. Acyclovir triphosphate stops replication of herpes viral DNA.

Acyclovir will be used to prevent the occurrence of herpes virus infections. It is supplied as powder for injection in 500 mg vials. Reconstitute in 10 mL of sterile water for injection for bacteriostatic water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7 mg/ml or less and infused over 1 hour to avoid renal damage. Oral tablets of 200 and 800 mg are available, if the patient is able to tolerate medication by mouth. Reversible renal insufficiency has been reported with intravenous but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs has been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made, or the drug should be discontinued. Stomach upset, headache, nausea, rash, hives, diaphoresis, hematuria;

hypotension, and thrombocytosis have been reported. Hair loss from prolonged use has also been documented. Acyclovir will not be used concomitantly with other nucleoside analogs that interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

3.9 Fluconazole (Diflucan)

Fluconazole will be obtained by the Moffitt Cancer Center pharmacy from commercial sources. It will be used for prophylaxis against fungal infections. It is available in 200 mg tablets. It can cause headache, nausea, vomiting, diarrhea or abdominal pain, and liver damage that may be irreversible. It can cause rashes and itching, which in rare cases has caused Stevens Johnson Syndrome. It has several significant drug interactions. The package insert should be consulted prior to prescribing. For IV administration in patients who cannot tolerate the oral preparation, Fluconazole comes in 2 mg/mL solution for injection, and prepared according to Moffitt Cancer Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hr.

3.10 Ondansetron hydrochloride (Zofran)

Ondansetron hydrochloride will be obtained by the Moffitt Cancer Center pharmacy from commercial sources. It will be used to control nausea and vomiting during the chemotherapy preparative regimen. It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritis, constipation and urinary retention. Consult the package insert for a complete list of side effects and specific dose instructions.

3.11 Furosemide (Lasix)

Furosemide, a loop diuretic, will be obtained by the Moffitt Cancer Center pharmacy from commercial sources. It will be used to enhance urine output during the chemotherapy preparative regimen with cyclophosphamide. Adverse effects include dizziness, vertigo, paresthesias, weakness, orthostatic hypotension, photosensitivity, rash and pruritis. Consult the package insert for a complete list of side effects and specific dose instructions.

3.12 Cell Preparation

The procedures and reagents for expanding the human TIL cells will be followed according to the Moffitt Cell Therapy Core SOP.

4.0 Eligibility Assessment and Enrollment

Patient evaluation for eligibility and registration will occur utilizing a two-step design.

4.1 Screening, Initiation of Vemurafenib, and TIL Expansion

Patients must fulfill all of the following screening criteria to be eligible for the study.

4.1.1 Screening Inclusion Criteria

a. Patients must have unresectable metastatic stage IV melanoma or stage III in-transit or regional nodal disease and in the opinion of the PI or treating Co-investigator is an acceptable candidate for ACT.
b. Residual measurable disease after resection of target lesion(s) for TIL growth
c. Tumor must have a B-RAF V600E, D, R or K mutation by pyrosequencing, Cobas assay, or equivalent (40, 48)
d. Age greater than or equal to 18 years.
e. Clinical performance status of ECOG 0 - 1. ECOG performance status of 0-1 will be inferred if the patient's level of energy is ≥ 50% of baseline.
f. Women of childbearing potential (defined as having a menstrual cycle within the past 12 months and not having had a surgical procedure to accomplish sterilization) must have a negative serum pregnancy test within seven days of starting vemurafenib.
g. Adequate renal, hepatic, coagulation and hematologic function, including creatinine of less than or equal to 1.7 gm/dL, total bilirubin less than or equal to 2.0 mg/dL, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dL, AST and ALT of less than 3X institutional upper limit of normal, PT and PTT within 1.5 times the institutional upper limit of normal, hemoglobin of 8 gm/dL or more, WBC of 3000 per mcL and total granulocytes of 1000 per mcL or more, and platelets of 100,000 per mcL or more.
h. Patients must have a positive screening EBV antibody titre on screening test noted in 4.1.3.
i. Patients with antibiotic allergies per se are not excluded; although the production of TIL for adoptive transfer includes antibiotics, extensive washing after harvest will minimize systemic exposure to antibiotics.
j. At screening, patients with ≤ 3 untreated CNS metastases may be included provided none of the untreated lesions are > 1 cm in greatest dimension, and there is no peri-tumoral edema present on brain imaging (MRI or CT if MRI is contraindicated).
k. At screening, patients with ≤ 3 treated CNS metastases treated with either surgical resection and/or radiation therapy may be included. Patients may be included if the largest lesion is ≤ 1 cm, and there is no evidence of progressive CNS disease on brain imaging at least 28 days after treatment.
l. At screening, patients may be included if the largest lesion is > 1 cm or > 3 in number, if the patient **has underdone treatment** with surgery and/or radiation

therapy and there is no evidence of progressive CNS disease on brain imaging at least 90 days after treatment.

m. At screening, patients must have no known history of congenital long QT syndrome and must have a corrected mean QTcF interval ≤ 450 msec at baseline.
n. No evidence of ongoing cardiac dysrhythmia ≥ grade 2 (NCI CTCAE, v4.0)
o. A cardiac stress test (stress thallium, stress Multiple Gated Acquisition study, dobutamine echocardiogram or other equivalent stress test) is required to rule out reversible cardiac ischemia in those over 50 years of age or with a known history of coronary artery disease.
p. Pulmonary function tests (FEV1>65% or FVC>65% of predicted are required) in those who have a history of pulmonary disease that necessitates the use of supplemental oxygen, or is associated with dyspnea on walking one block or less, or requires inhaler therapy more than once per week.
q. All laboratory and imaging studies must be completed and satisfactory within 30 days of signing the consent document, with the exceptions of: negative serum pregnancy test for women of child-bearing potential (defined in section 4.1.1f) must be negative within 7 days of starting vemurafenib, HLA-typing which will not be repeated if performed previously, and PFTs/cardiac stress tests whose results are valid for 6 months if performed previously.
r. For cohort 2, patients must have had progressive disease while on a BRAF-inhibitor containing therapy per RECIST 1.1 criteria at the time of screening. Of note this is not an inclusion criterion for patients accrued to cohort 1.
s. For cohort 2, patients must have had progressive disease (per RECIST 1.1 criteria) or intolerance to treatment while on a PD-1 antibody containing therapy at the time of screening. Of note this is not an inclusion criterion for patients accrued to cohort 1.

4.1.2 Screening Exclusion Criteria

a. Patients with active systemic infections requiring intravenous antibiotics, coagulation disorders or other major medical illness of the cardiovascular, respiratory or immune system, which in the opinion of the PI or treating Co-investigator is not acceptable risk for ACT, are excluded.
b. Patients testing positive for HIV titre, Hepatitis B surface antigen, Hepatitis B core antibody, Hepatitis C antibody, HTLV I or II antibody, or both RPR and FTA positive are excluded.
c. Patients who are pregnant or nursing are excluded.
d. Patients needing chronic, immunosuppressive systemic steroids are excluded (i.e. greater than 10 mg prednisone or equivalent steroid daily).
e. Patients with autoimmune diseases that require immunosuppressive medications are excluded.
f. Presence of a significant psychiatric disease, which in the opinion of the principal investigator or his designee, would prevent adequate informed consent or render immunotherapy unsafe or contraindicated.

g. Patients with > 3 untreated CNS metastases or evidence of peri-tumoral edema will be excluded.
h. Patients with ≤ 3 untreated CNS metastases but with at least one lesion >1 cm or peri-tumoral edema will be excluded.
i. Patients with congenital long QT syndrome are excluded.
j. Patients with invasive malignancy other than melanoma at the time of enrollment and within 2 years prior to the first vemurafenib administration are excluded, except for adequately treated (to the satisfaction of the treating physician) basal or squamous cell carcinoma of the skin, in situ carcinoma of the cervix, in situ ductal adenocarcinoma of the breast, in situ prostate cancer, or limited stage bladder cancer or other cancers from which the patient has been disease-free for at least 2 years.
k. Patients who are unable to swallow pills are excluded.
l. Patients with treated CNS metastases > 1 cm or > 3 in number will be excluded if there is evidence of progressive CNS disease on brain imaging at least 90 days after treatment with surgery and/or radiation therapy (see section 4.1.1 l).
m. Patients unable to comprehend and give informed consent are excluded.
n. Patients screening for cohort 1 who have had previous BRAF inhibitor treatment are excluded (this applied for cohort 1 only; this is not an exclusion for patients accrued to cohort 2; n/a should be selected on the eligibility form for this exclusion criterion for cohort 2 patients).
o. Male patients with female partners of childbearing potential (defined in section 4.1.1f) who do not agree to use two FDA-accepted forms of contraception during sexual intercourse with women of child-bearing potential from the start of vemurafenib and up to at least 6 months after discontinuing vemurafenib are excluded.
p. Female patients of childbearing potential (defined in section 4.1.1f) who do not agree to use two FDA forms of contraception during sexual intercourse from the start of vemurafenib and up to at least 6 months after discontinuing vemurafenib are excluded.
q. Subjects who are unwilling or unable to comply with the following: The following foods/supplements are prohibited at least 7 days prior to initiation of and during study treatment involving vemurafenib:
   - St. John's wort or hyperforin (potent cytochrome P450 CYP3A4 enzyme inducer)
   - Grapefruit juice (potent cytochrome P450 CYP3A4 enzyme inhibitor)
r. Patients who decline possible transfusion of blood products will be excluded.

4.1.3 Screening Procedures

All visits and tests may be done +/- 7 days from scheduled date unless otherwise specified.

All patients must sign an informed consent form and a negative pregnancy test (serum) must be documented for women of childbearing potential at screening and within 7 days of starting vemurafenib. Once enrolled, patients will have the following standard laboratory procedures performed: CBC w/diff, CMP, LDH, Magnesium, Phosphorus, and urinalysis with micro. In addition the patient will be screened for Hepatitis B Surface Antigen, Hepatitis B Core Antibody, Hepatitis C Virus Antibody, HIV 1/HIV 2

Antibody, HTLV I/II Antibody, RPR Qualitative (and FTA only if RPR is positive), and EBV IgG antibody to viral capsid antigen, within 30 days of signing the informed consent. TSH and free T4 will be done, and if abnormal, a workup for thyroid and/or autoimmune condition will ensue. If a condition listed in 4.1.2.e is found, patients will be excluded. On their own, abnormal TSH or free T4 results are not exclusionary. If not previously done, HLA typing will also be done at screening for potential future laboratory assays involving TIL function. However, HLA type is not a specific inclusion or exclusion criterion. Patients will have appropriate diagnostic imaging performed including a CT or MRI of the brain and CTs of the thorax, abdomen, pelvis. CT of the neck and imaging of the extremities may be indicated depending on the clinical presentation at the discretion of the treating physician. If patients have had redundant screening studies (laboratory or imaging tests) performed for another reason within 30 days of enrollment, they will not be repeated, with the exception of pregnancy testing. If HLA testing has been done previously, it will not be repeated. If patients are being resected for other reasons, tumor may be harvested and cultures initiated for TIL prior to results of the screening studies. However, treatment may not be initiated until after all screening criteria have been met. If the patient is later found to be ineligible, the TIL will be destroyed or will be de-identified and used for in vitro research purposes in the manner exactly described by Moffitt protocol 15375 and/or 50148.

4.1.4 Vemurafenib Treatment

All visits and tests may be done +/- 7 days from scheduled date unless otherwise specified.

While T-cells are being grown, patients will be treated with vemurafenib at 960 mg PO BID (starting on the first business day following tumor harvest for patients on cohort 1, and starting two week following tumor harvest for patients on cohort 2, until one day prior to the planned initiation of chemotherapy. If patients are required to stop vemurafenib treatment due to intolerance, they may still proceed with the adoptive cell therapy phase of the protocol at the discretion of the treating physician.

4.1.5 Dose Modifications and Management Guidelines for Vemurafenib-Related Toxicity

The ACT portion of the protocol may be continued at the discretion of the treating investigator if vemurafenib is discontinued due to toxicity that is definitely related to vemurafenib.

For management of specific toxicities and dose modification guidelines see below:

For New Primary Cutaneous Malignancies

No dose modifications are recommended.

For Other Adverse Reactions

Permanently discontinue vemurafenib for any of the following:

- Grade 4 adverse reaction, first appearance (if clinically appropriate) or second appearance

  QTcF prolongation $>$500 msec and increased by $>$60 msec from pre-treatment values. In addition, electrolytes (K, Mg, and Ca) should be monitored and any electrolyte

abnormalities should be corrected prior to reinstitution of therapy. ECG should be monitored weekly until the QTcF interval decreases to <500 msec or returns to baseline before reinstituting therapy at a reduced dose (see Table 1).

Table 1 Vemurafenib Dose Modification Schedules for QTcF Prolongation

Dose Modification Schedule Based on Prolongation of the QT Interval - QTcF Value	Recommended Dose Modification
QTcF >500 msec at baseline	Treatment not recommended.
QTcF increase meets values of both >500 msec and >60 msec change from pre-treatment values	• Rule out other risk factors for arrhythmia (e.g., myocardial ischemia); check for electrolyte disturbances (particularly potassium and magnesium levels) in all cases. • Evaluate concomitant medications to determine if there is co-administration of drugs that prolongs QTcF interval in all cases (e.g., 5-HT_3 receptor antagonist anti-emetics) • Interrupt dosing of vemurafenib and continue ECG monitoring until QTcF interval decreases below 500 ms. Electrolytes abnormalities should be corrected in all cases. • Plan to seek a cardiologist consultation or advice.
1st occurrence of QTcF >500 msec during treatment and change from pre-treatment value remains <60 msec	• Temporarily interrupt treatment until QTcF decreases below 500 msec. • See monitoring measures in the Investigator's Brochure (Section 4.4). • Resume dosing at 720 mg twice daily (or 480 mg twice daily if the dose has already been lowered).
2nd occurrence of QTcF >500 msec during treatment and change from pre-treatment value remains <60 msec	• Temporarily interrupt treatment until QTcF decreases below 500 msec. • See monitoring measures in the Investigator's Brochure (Section 4.4). Resume dosing at 480 mg twice daily (or discontinue permanently if the dose has already been lowered to 480 mg twice daily).
3rd occurrence of QTcF >500 msec during treatment and change from pre-treatment value remains <60 msec	Discontinue permanently.

Table 2 Dose Modification for Other Adverse Reactions

Recommended Vemurafenib Dose Modification		
Toxicity Grade (CTC-AE)*	**Vemurafenib dose changes during current treatment period**	**Dose modification at resumption of treatment**

Recommended Vemurafenib Dose Modification		
Toxicity Grade (CTC-AE)*	**Vemurafenib dose changes during current treatment period**	**Dose modification at resumption of treatment**
Grade 1 or tolerable Grade 2	No change	N/A
Intolerable Grade 2 or Grade 3		
1st Appearance^	Interrupt until resolved: grade 0 – 1	Reduce dose by 240 mg twice daily
2nd Appearance^	Interrupt until resolved: grade 0 – 1	Reduce dose by 240 mg twice daily
3rd Appearance^	Discontinue permanently	N/A
Grade 4		
1st Appearance^	Discontinue permanently or interrupt until resolved: grade 0 – 1	Reduce dose to 480 mg twice daily
2nd Appearance^	Discontinue permanently	N/A

4.1.6 Concomitant and Excluded Therapy while Subjects are on Vemurafenib

- Effects of Vemurafenib on Drug Metabolizing Enzymes

Results from an *in vivo* drug-drug interaction study in patients with cancer demonstrated that vemurafenib is a moderate CYP1A2 inhibitor, a weak CYP2D6 inhibitor and a CYP3A4 inducer. Co-administration of vemurafenib increased the AUC of caffeine (CYP1A2 substrate) 2.6-fold and increased the AUC of dextromethorphan (CYP2D6 substrate) by 47%, while it decreased the AUC of midazolam (CYP3A4 substrate) by 39%. Concomitant use of vemurafenib with agents with narrow therapeutic windows that are metabolized by CYP1A2, CYP2D6, and CYP3A4 is not recommended as vemurafenib may alter their concentrations. If co-administration cannot be avoided, exercise caution and consider a dose reduction of the concomitant CYP1A2 and CYP2D6 substrate drug.

Co-administration of vemurafenib resulted in an 18% increase in the area under the curve of S-warfarin (CYP2C9 substrate). Patients who require systemic anticoagulation are excluded from this protocol (see 4.2.2k).

- Drugs that Inhibit or Induce CYP3A4

Based on *in vitro* data, vemurafenib is a substrate of CYP3A4, and therefore, concomitant administration of strong CYP3A4 inhibitors or inducers may alter vemurafenib concentrations. Strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, indinavir, nelfinavir, voriconazole) and inducers (e.g., phenytoin, carbamazepine,

rifampin, rifabutin, rifapentine, and phenobarbital) should be used with caution when co-administered with vemurafenib.

- Drugs that May Cause QTc Prolongation or Cardiac Arrhythmia

Exposure-dependent QT prolongation was observed in an uncontrolled, open-label Phase 2 QT sub-study in previously treated patients with $BRAF^{V600E}$ mutation-positive metastatic melanoma. QT prolongation may lead to an increased risk of ventricular arrhythmias, including Torsade de Pointes. Treatment with vemurafenib is not recommended in patients with uncorrectable electrolyte abnormalities, long QT syndrome, or who are taking medicinal products known to prolong the QT interval.

Please reference the vemurafenib package insert for additional information.

4.1.7 Pregnancy and Vemurafenib

If a patient is suspected to be pregnant, vemurafenib must be immediately discontinued. If it is subsequently confirmed that the patient is not pregnant, dosing may be resumed.

4.2 Adoptive Cell Treatment – Chemotherapy/TIL Infusion

Patients must fulfill all of the following criteria to be eligible for the adoptive cell treatment phase. Laboratory testing CBC w/diff, CMP, PT/PTT, serum β-HCG pregnancy test (for women of child-bearing potential), EKG, and CXR must be complete and satisfactory within 14 days of the pre-chemotherapy apheresis. Appropriate diagnostic imaging performed including a CT or MRI of the brain and CTs of the thorax, abdomen, pelvis. CT of the neck and imaging of the extremities may be indicated depending on the clinical presentation must be complete and satisfactory within 30 days of initiation of chemotherapy, and the identical criteria and management will be followed as discussed in sections 4.1.1 j, k and l. Any measurable disease found on physical examination will be documented within 30 days prior to initiation of chemotherapy. However, if there are no measurable metastatic lesions present on physical examination, then no physical measurements will be documented.

4.2.1 Apheresis

Apheresis will be performed on all patients prior to chemotherapy and following TIL transfer.

a. Patients will be evaluated by a physician or Advanced Registered Nurse Practitioner/Physician Assistant for clearance and certification within 24 hours prior to the initiation of the apheresis regimen
b. CBC w/diff and CMP (with Magnesium and Phosphorus) will be completed within 24 hours prior to the apheresis regimen.

4.2.2 Chemotherapy/Adoptive Cell Transfer Inclusion Criteria

a. Patients must have adequate TIL available as described in the Moffitt Cell Therapy Core SOP.
b. Male patients with female partners of childbearing potential and female patients of childbearing potential (defined in section 4.1.1f) must agree to use contraception for four months after receiving the preparative regimen or while on vemurafenib and for 6 months after the last dose of vemurafenib.
c. For women of child bearing potential (defined in section 4.1.1f), a negative serum pregnancy testing will be verified within 14 days prior to pre chemotherapy apheresis.
d. Clinical performance status equivalent to ECOG 0-1 at the clinical visit prior to apheresis.
e. Absolute neutrophil count greater than or equal to 1000/mcL.
f. Platelet count greater than or equal to 100,000/mcL.
g. Hemoglobin greater than or equal to 8.0 g/dL.
h. Serum ALT and AST less than three times the institutional upper limit of normal.
i. Serum creatinine less than or equal to 1.7 mg/dL.
j. Total bilirubin less than or equal to 2.0 mg/dL, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dL.
k. PT and PTT within 1.5 times the institutional upper limit of normal
l. Patients with EKG within 14 days of initiation of chemotherapy demonstrating no new rhythm, axis or ST segment changes will be included. If clinically significant, new EKG changes are present, patients may be included if cardiac stress test indicates no cardiac ischemia or clearance is provided by a cardiologist (see section n below).
m. Urinalysis within 14 days of initiation of chemotherapy demonstrating no evidence of a urinary tract infection.
n. A stress cardiac test (stress thallium, stress multiple gated acquisition study, dobutamine echocardiogram or other equivalent stress test that will rule out cardiac ischemia) within 6 months of lymphodepletion in those over 50 years of age or with a known history coronary artery disease.
o. Pulmonary function tests (FEV1>65% or FVC>65% of predicted are required) within 6 months of lymphodepletion in those who have a history of pulmonary disease that necessitates the use of supplemental oxygen, or is associated with dyspnea on walking one block or less, or requires inhaler therapy more than once per week.
p. Complete history and physical examination noting in detail the exact size and location of any lesions that are detected on examination will be performed within 30 days prior to initiation of chemotherapy.
q. Appropriate diagnostic imaging performed including a CT or MRI of the brain and CTs of the thorax, abdomen, pelvis. CT of the neck and imaging of the extremities may be indicated depending on the clinical presentation must be complete and satisfactory within 30 days of initiation of chemotherapy.

5.0 Treatment Plan

5.1 Outline

T cells will be expanded in a state-of-the-art GMP cell growth facility at Moffitt that will allow compliance with all FDA regulations regarding investigational cell transfer products. Our adoptive cell therapy core laboratory has demonstrated that we can consistently obtain T cell expansions of greater than 400 fold (Figure 6), which is required to treat patients with cell numbers consistent with previous clinical experiences at the National Cancer Institute (NCI) (goal $\geq$ ~20 x 10^9 cells per patient).

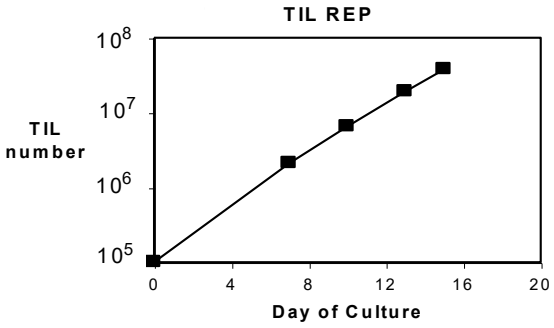


Figure 6: Rapid Expansion (REP) of tumor Infiltrating Lymphocytes. T cells were cultured in the presence of irradiated HPCA feeders, anti-CD3, and IL-2, as previously described. Total cell counts were measured at serial time points to assess proliferation. Total expansion was consistently over 400 fold.

Apheresis will be performed via a 2-armed approach or via a temporary central venous catheter. Approximately a 7 liter exchange lasting 2 to 3 hours using a Gambro Spectra machine will be performed to generate cells for immune monitoring and research related testing. ~60 mL of blood (8 x 10 mL greentop tubes) will be collected if the apheresis cannot be completed.

All patients will receive vemurafenib at a dose of 960 mg PO BID starting 1 day after their tumor harvest for TIL growth (cohort 1) or approximately 14 days after their tumor harvest for TIL growth (cohort 2). The patient will utilize a drug diary to record vemurafenib dosing details such as time, date and number of pills taken. The pill diary will be reviewed at the end of each vemurafenib cycle (approximately 30 days). Unless there is intolerance, this treatment will continue up until 1 day prior to initiation of cyclophosphamide (occurring on day -7). Cyclophosphamide will be administered at 60 mg/kg/day I.V. in 250 mL NS over approximately 2 hours on Days –7 and –6. The dose will be based on the patient's body weight, but to prevent undue toxicity, it will not exceed a dose greater than 140% of the maximum ideal body weight per Metropolitan Life Insurance Company, Height and Weight Table. Of note, duration of cyclophosphamide infusion is a guideline and will not be tracked for monitoring purposes in keeping with the current clinical practice at Moffitt Cancer Center. Fludarabine will then be infused at 25 mg/m^2 IVPB daily over approximately 30 minutes

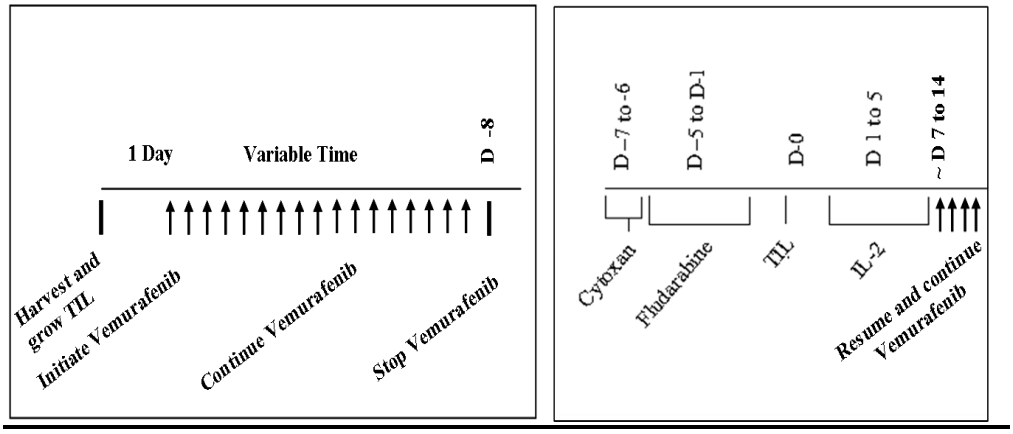
on Days –5 to –1. To prevent undue toxicity with fludarabine, the dose will be based on body surface area (BSA), but will not exceed a dose calculated on surface areas based on body weights greater than 140% of the maximum ideal body weight per Metropolitan Life Insurance Company Height and Weight Tables. Of note, duration of fludarabine infusion is a guideline and will not be tracked for monitoring purposes in keeping with the current clinical practice at Moffitt Cancer Center. On day 0, all patients will receive up to $1x10^{12}$ T cells administered according to the Moffitt Cell Therapy Core TIL SOP. Of note, the precise duration of TIL infusion will not be tracked for monitoring purposes. Eight (8) to sixteen (16) hours after completing the T cell infusion, all patients will receive high dose interleukin-2 (IL-2) on an inpatient basis at the standard dose of 720,000 IU/kg as an intravenous bolus over an approximate 15 minute period every 8-16 hours for up to 6 doses on Days 1 to 3 (allowing for doses to be skipped), as tolerated. Doses will be skipped if patients reach Grade III or IV toxicity due to high dose IL-2, except for the reversible Grade III toxicities common to high dose IL-2 such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes (i.e. platelets, creatinine, total bilirubin) as detailed in the appendix. If the toxicity is easily reversed by supportive measures, then additional doses may be continued up to a maximum total of 6 doses. Of note, duration of IL-2 infusion is a guideline and will not be tracked for monitoring purposes in keeping with the current clinical practice at Moffitt Cancer Center. There will be no dose modification for IL-2, except for morbid obesity, per standard practice for the use of high dose IL-2. As above, doses may be skipped for hypotension or low urine output of grade III or IV that is not controlled with dopamine and/or neosynephrine, but IL-2 may continue if that toxicity is at least partially reversed with supportive measures according to the judgment of the treating attending physician. Patients will discontinue high dose IL-2 treatment for altered mental status, supra-ventricular arrhythmias that require medication, evidence of myocarditis, uncontrolled hypotension, urine output less than 600 mL per 24 hours and/or creatinine of ≥3.5 gm/dL in spite of maximal supportive measures, bilirubin of ≥8 gm/dL, positive blood culture, or evidence of infection. IL-2 will be permanently discontinued in patients who develop a life-threatening ventricular arrhythmia, life-threatening infection, myocardial infarction, or permanently altered mental status. Unless there was previous intolerance, vemurafenib will be re-started at the same dose as previously administered upon clinical recovery from IL-2 related toxicities and with ANC of 500 per mcL or greater. Treatment with vemurafenib will be for a duration of up to 2 years (for cohort 1) or 6 months (for cohort 2), or until progression of disease, dose-limiting toxicity, loss to followup, or withdrawal of consent by the patient. The patient will utilize a drug diary to record vemurafenib dosing details such as time, date and number of pills taken.

For the evaluation of peripheral blood cells, blood samples in 8 large green top tubes (~60 mL) collected in CPT or heparin tubes will be on Days -35, 0, +7, +14, +21, +28, +42, and +84, (+/- 7 days for each time point), where feasible. On day 0, prior to TIL infusion, 10 mL of blood will be collected to verify lymphodepletion. This reduced amount will minimize the blood loss during the time of bone marrow suppression from the chemotherapy. These blood draws are in addition to apheresis that will be performed prior to lymphodepletion and at week 6 (+/- 7 days). These time points were

selected based on previous studies at the National Cancer Institute. During high dose IL-2 administration and recovery from subsequent toxicity (Days 1-6), peripheral blood mononuclear cell (PBMC) yields are low, possibly due to adhesion of immune cells to vasculature. Therefore, day 7 has been selected as the earliest feasible time point to obtain peripheral blood samples post initiation of IL-2, for evaluation of the immediate effects of ACT. The other days have been selected as later time points to evaluate T cell persistence, function, and immune cell subset reconstitution. Recovered circulating T cells will be assessed for autologous or HLA-matched anti-tumor function by interferon-γ release as previously described (32), and persistence of reactivity will be correlated to clinical outcome. In addition, when clinically feasible, tumor samples may be obtained by fine needle aspiration (FNA) or core biopsy after vemurafenib treatment on day -14 (excess tumor from TIL harvest will be used for a pre-treatment baseline), and again on Days 21, 42 and 84 (+/- 7 days) after T cell transfer and at time of progression (within 30 days). The tumor biopsy time points have been limited to 4 samples because of the invasiveness of the procedure. Tumor biopsies will only be performed percutaneously on those patients with clinically accessible tumors, and is a strictly optional procedure for research purposes after the biopsy on day 21 (if tumor is accessible on day 21 by percutaneous biopsy). The tumor biopsies will not be required and are not an eligibility criterion per se. Pre and post vemurafenib and post ACT tumor samples will be assayed for MPR expression and markers of autophagy. Based upon our ACT experience, we anticipate that approximately one third of patients will have tumor accessible to tumor biopsy before and after treatment.

The current study's first cohort was a single arm phase II trial that employed the Simon two-stage Minimax design to prospectively evaluate the complete and partial response rate at 12 months, and the "drop-out" rate. Progression-free survival was a secondary endpoint. Survival of infused TIL will be monitored by peripheral blood samples obtained as described above. Up to 53 patients were planned to be treated on the first cohort. **As discussed above, accrual to the first cohort has been closed as of February 10, 2017 and the results are presented in the abstract section above.**

Treatment schema - first cohort:



Treatment (first cohort)

One day after TIL harvest: Start vemurafenib as described above until day -8

Day -7 and Day -6
Cyclophosphamide 60 mg/kg/day IV in 250 mL NS over approximately 2 hrs.
Mesna 20 mg/kg with D5W or NS at 125 mL/hr infused intravenously over approximately 24 hours.

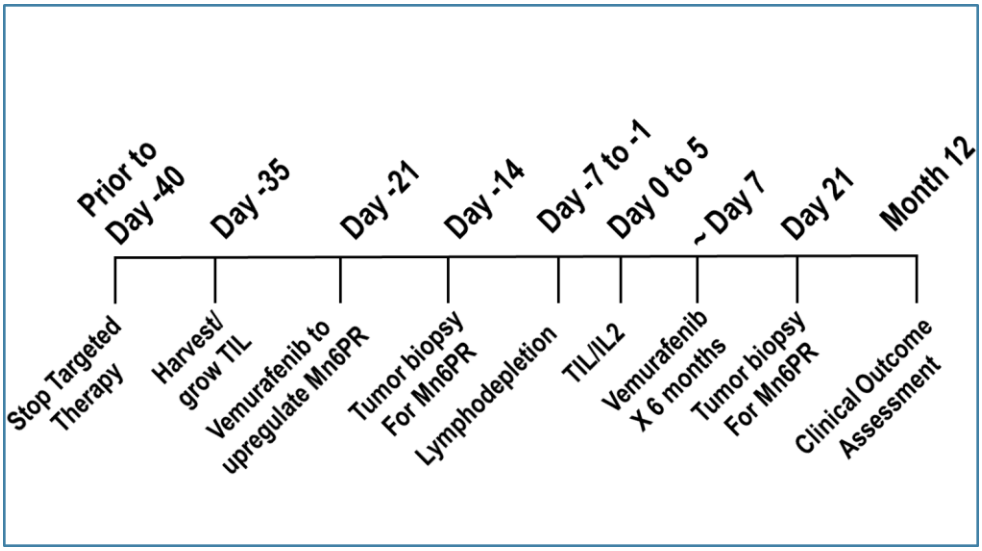
Day -5 to Day -1:
Fludarabine 25 mg/m^2 IVPB daily over approximately 30 minutes for 5 days.

Day 0:
T cell infusion will be administered according to the Moffitt Cell therapy core SOP. Vital signs (temperature, blood pressure, pulse and respiratory rate) and pulse oximetry will be monitored every 15 minutes during the TIL infusion, every 30 minutes for 1 hour after TIL infusion, and hourly thereafter for a total of 4 hours after completion of the infusion.

Days 1-3:
High dose IL-2, 720,000 IU/kg IV bolus (about 15 minutes) every 8-16 hours for up to 6 doses or tolerance, beginning approximately 12-16 hours after T cell infusion.
On the day after clinical recovery from high dose IL-2 (approximately day +7 to 14), Vemurafenib will be resumed if the ANC is 500 or greater.

The current study's second cohort is a single arm pilot expansion to prospectively evaluate the complete and partial response rate at 12 months for patients who have had disease progression after prior BRAF inhibitor containing therapy and progression after or intolerance to PD-1 antibody containing therapy.

Treatment schema - second cohort:



Treatment - second cohort -- Please note that the days are approximate and may change based upon rate of TIL propagation and the clinical status of the patient.

At screening (prior to Day -40): stop BRAF inhibitor containing therapy also known as targeted therapy (if active) at least 5 days prior to anticipated TIL harvest.

Day -35: TIL harvest and blood draw for PBMC

Day -21: Vemurafenib treatment to upregulate MPR as described above

Day -14: Tumor biopsy for MPR immunohistochemistry and blood draw for PBMC approximately 1 week post vemurafenib

Day -7 to -1: Lymphodepletion with cyclophosphamide and fludarabine

Day 0 to +7: TIL and high dose IL2/recovery as described above

Day +7: Resume vemurafenib and continue for up to 6 months in order to maintain upregulation of MPR for enhanced TIL efficacy. Of note, the vast majority of responders to ACT exhibit a response by 6 months (unpublished observation). Blood draws on days 0, +7, +14, +21, +28, +42, +84 for PBMC.

Day +21, +42, +84: Tumor biopsy to assess MPR post TIL transfer

Month 12: Clinical outcome assessment

5.2 Prophylaxis

5.2.1 Infection Prevention and Pneumocystis jiroveci Pneumonia/Pneumocystis carinii Pneumonia Prophylaxis

Patients will receive levofloxacin at 500 mg daily (or cephalexin at 500 mg TID if allergic to levofloxacin) until ANC recovers to greater than 500/mcL and the fixed combination of trimethoprim (TMP) and sulfamethoxazole (SMX) as double strength (DS) tablet [DS tabs = TMP 160 mg/tab and SMX 800 mg/tab] po BID twice a week. TMP/SMX-DS will be taken by patients beginning on Day -7 and continuing until $CD4^+$ lymphocyte counts are >200 cells/mcL. At the discretion of the treating physician, patients with sulfa allergies will receive either desensitization protocol or aerosolized pentamidine 300 mg per nebulizer, atovaquone or equivalent medication that will be started within one week of cyclophosphamide and continued monthly until $CD4^+$ lymphocyte count is greater than 200 cells/mcL tested 6 weeks after cell therapy. If $CD4^+$ counts are below 200 cells/mcL, then continued prophylaxis as above will continue, and subsequent re-testing will occur in accordance with the standard of care as determined by the treating physician.

Patients will be given antibiotics (levofloxacin, or ceftazidine if allergic to levofloxacin) intravenously during high dose IL-2 therapy if unable to tolerate po intake.

5.2.2 Herpes Virus Prophylaxis

At the time of the T cell infusion, patients with positive HSV serology will be administered valacyclovir 500 mg po daily (or equivalent formulary medications) if patient is able to take oral medications or acyclovir 5 mg/kg IVPB every 8 hours if patient needs intravenous medications or equivalent, which is continued until absolute neutrophil count is greater than 500/mcL. Reversible renal insufficiency has been reported with IV administered acyclovir but not with oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs has been reported with higher doses of acyclovir. If symptoms occur, a dosage adjustment will be made or the drug be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs (e.g. ganciclovir), which interfere with DNA synthesis. In patients with renal disease, the dose is adjusted as per product labeling.

5.2.3 Fungal Prophylaxis

Patients will begin fluconazole 200 mg po daily with the T cell infusion (Day 0) and continue until the absolute neutrophil count is >500/mcL.

5.2.4 Empiric Antibiotics

Patients will be switched from levofloxacin to more broad spectrum intravenous antibiotics (cefepime, piperacillin/tazobactam, meropenem, or equivalent) if there is neutropenic fever per existing Moffitt protocol, i.e. for fevers $\geq$38 C x 1 hour or $\geq$38.5 C x any duration, with an ANC less than 500/mcL. Aminoglycosides should be avoided unless there is clear evidence of sepsis. Infectious disease consultation will be obtained for patients when deemed appropriate by the treating attending physician.

5.2.5 Blood Product Support

In order to reduce duration of neutropenia following chemotherapy and T cell infusion, G-CSF will be given at 5 mcg/kg/day daily subcutaneously until neutrophil counts reach >1.5 K/mcL Using daily CBC's as a guide, the patient will also receive platelets and packed red blood cells (PRBC's) as needed. In general, attempts will be made to keep Hb >7.5 gm/dL, and platelets >10,000/mcL (>20,000/mcL if there is history of brain metastasis), per the judgment of the treating provider. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection. Irradiated blood and blood products should be used.

6.0 Evaluation During Study

All visits and tests may be done +/- 7 days from scheduled date unless otherwise specified.

Please refer to section 4.1.5 above for evaluation and management while on vemurafenib. During the preparative cyclophosphamide-fludarabine regimen and high dose IL-2 therapy, patients will have a CBC w/diff and CMP drawn every 1 to 2 days of treatment. On the day of TIL infusion, patients will be monitored with vital signs at baseline and after cell infusion as described in section 5.1. Blood samples will be obtained on days -35, -14, 0, +7, +14, +21, +28, +42, and +84 +/- 7 days, where

feasible. This is in addition to apheresis that will be performed within 14 days of lymphodepletion and at week 6 (+/- 7 days). These samples will be used to evaluate T cell persistence and function by co-culture with autologous or HLA-matched tumor targets and subsequent interferon-γ ELISA as previously described (3). Where feasible, tumor samples will be obtained by FNA or core biopsy on day -14 (after vemurafenib treatment; please note that excess sample from TIL harvest will be used as pretreatment baseline), and again on Days 21, 42 and 84 (+/- 7 days) after T cell transfer and within 30 days of progression. Tumor samples obtained before and after treatment will be compared for MPR expression and markers of autophagy. Secondary laboratory assays will be correlated to clinical outcome.

Clinical evaluation for tumor response with physical examinations and appropriate CT scans (routine chest, abdomen and pelvis, as well as any additional areas deemed appropriate by the study physician) will be performed approximately at 6 weeks (± 7 days) and at 12 weeks (± 7 days) after the cell infusion, at which time patients will be clinically restaged. Then, after the 12 week disease evaluation, patients will have follow-up visits at 3 month intervals +/- 14 days for the next 2 years, every 6 months +/- 30 days for the next 2 years, then yearly thereafter +/- 90 days. Past the week 12 evaluation, brain imaging will be done as clinically indicated by the treating physician and in keeping with the existing standard of care. The follow-up assessments will be performed for all patients on the study until disease progression, withdrawal of consent, loss to follow-up or death. After confirmed disease progression subjects may be followed every 3 months for overall survival and report of any subsequent anti-cancer treatments. This can be accomplished by visit, phone or email contact.

Excess blood and tissue specimens collected in the course of this research project may be de-identified, banked and provided to investigators involved with other melanoma research pre-clinical projects.

6.1 Correlative endpoint studies

All visits and tests may be done +/- 7 days from scheduled date unless otherwise specified.

Where feasible, tumor samples obtained by fine needle aspirate or core biopsy on day -14 (post vemurafenib treatment; please note that excess sample from TIL harvest will be used as pretreatment baseline), and 21, 42 and 84 days (+/- 7 days) post ACT and within 30 days of disease progression. Tumor samples will be analyzed for the following biomarkers by immunohistochemistry: autophagy using the LC-3 monoclonal antibody, granzyme B using the 1F11 monoclonal antibody, and MPR expression using the AB32815 antibody (Abcam) with a 0, 1 (mild), 2 (moderate) or 3 (high) staining scale. Induction of the above parameters at each time point will be determined if at least 75% of all samples show an increase of at least 1 level in the IHC staining, which will be evaluated in a blinded fashion by a Moffitt pathologist. We will correlate changes in the above biomarkers to objective response at 12 months. We expect objective responders

to exhibit enhanced intensity of biomarker expression after treatment compared to non-responders.
The generation of effector T cell activity in peripheral blood capable of recognizing tumor cells will be assessed by the use of a PBMC-target co-culture assay for 24 hours followed by an ELISA for gamma interferon as has been described previously (1). PBMC will be obtained by pheresis pre and post adoptive transfer. Effectors at a 2:1 ratio to targets will be used for a 24 hour incubation, and supernatants will be removed. Aliquots will be used for a gamma interferon ELISA assay, and/or multiplex cytokine bead array or equivalent assay. Controls will include no effector and no target samples, as well as an effector-irrelevant target control. All assays will be performed in triplicate. Observation of more than 200 picograms per mL of gamma interferon or a statistically significant increase in pro-inflammatory cytokine release over the controls is required for an assay to be declared positive at a given time point. It is our hypothesis that PBMC responders will exhibit an enhanced and more durable change in tumor-induced interferon-gamma release and other pro-inflammatory cytokine responses compared to non-responders.

6.2 Additional Descriptive Correlative Analyses

Up to 72 de-identified samples labeled with a 5 digit ID (pre- and post-treatment pheresis products and expanded TIL derived from the first stage of the minimax accrual) will be sent to MD Anderson over a 3-year period for a study entitled “SNP array analysis on PBMC and TIL as predictors of T-cell infiltration into melanoma and clinical responses” led by Dr. Laszlo Radvanyi or his replacement. If results are promising, additional specimens derived from the second stage of the minimax accrual will be sent.

This project will entail small nucleotide polymorphism (SNP) array assays to probe TIL specimens from patients successfully treated with TIL therapy. TIL specimens will be used to generate SNP panels. Immunologically-relevant genes will be identified and expression patterns will be descriptively compared between responders and non-responders. The aim will be identify potentially interesting immunologically-relevant SNPs associated with improved probability to respond to TIL therapy and longer progression-free survival (PFS) and overall survival (OS) following TIL therapy. Data will be used to design correlative immunologic assays for future adoptive cell therapy trials.

Furthermore, PBMC from patients enrolled on the TIL trial will be analyzed using the same SNP array assay platform. PBMC from both treated and untreated patients will be included. The aim is to identify immunologically relevant SNPs that separate patients with successful or unsuccessful TIL outgrowth. While exploratory, this particular SNP analysis may therefore turn out to be a predictor of who will successfully grow TIL and may lead to a biomarker fingerprint panel for patient selection.

In addition, TIL and pre and post pheresis specimens will be subjected to TCR clonotyping using a high-throughput TCR CDR3 region sequencing system (ImmunoSEQ™). The data will be used to track single persistent T-cell clones in specific TIL subsets in vivo. TIL persistence will be correlated to progression-free survival.

Moffitt will provide MIT/Broad Research institute up to the 17 melanoma specimens from patients that were accrued to the first cohort and up to 12 melanoma specimens from the patients to be accrued to the second cohort to determine if there are SNVs that are non-synonymous compared to PBMC that are associated with tumor response, and also to find out if there are mutations that are clearly associated with resistance to response.

Of note, deidentified samples of patients treated with TIL from other centers (including MD Anderson and the NCI) will be included in a merged analysis. Analyses will be descriptive and exploratory since the total sample size as well as the number of potentially interesting analytes are unknown at this time. The merged results may be used to inform the design of future adoptive cell therapy trials as well as preclinical research projects.

7.0 Evaluation of Toxicity

Please refer to sections 7.4 for evaluation and reporting of toxicity related to vemurafenib.

7.1 Adverse Event Definition

An Adverse event (AE) is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with metastatic melanoma that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as tumor biopsies).
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

7.2 Serious Adverse Event Definition

An AE should be classified as a serious adverse event (SAE) if the following criteria are met:

- It results in death (i.e., the AE actually causes or leads to death).
- It is immediately life-threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).

- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the IMP.

It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

7.3 Reporting of Adverse Events to IRB and FDA

This study will utilize the National Cancer Institute Common Terminology Criteria (CTC) for Adverse Events version 4.0 for toxicity and Adverse Event reporting. A copy of the CTC version 4.0 can be found online at the NCI website. All appropriate treatment areas should have access to a copy of the CTC version 4.0.

The principal investigator will monitor the data and toxicities to identify trends and will be responsible for revising the protocol as needed to maintain safety. Serious adverse events will be reported to the IRB in accordance with IRB and Moffitt institutional policies and to the FDA as described below.

Reporting Serious Adverse Events (SAEs) to the FDA

Any event that is both serious and unexpected must be reported to the FDA as soon as possible and, in no event, later than 7 days (death or life-threatening event) or 15 days (all other SAEs) after the investigator's or institution's initial receipt of the information. SAEs may be reportable to the FDA for both FDA-approved (post-market) drugs as well as investigational agents.

For an event to be reportable to the FDA, it must meet ***both*** of the following requirements as determined by the principal investigator of the study:

1. The event is possibly, probably or definitely ***related*** to the agent
2. The event is ***unexpected*** (i.e., not listed in the protocol, investigational brochure, or informed consent)

See additional reporting guidelines and requirements from vemurafenib study-drug supplier, Genentech, in section 7.4)

Expedited reporting to the FDA on a MEDWatch 3500A form will be used for all unexpected serious adverse events as defined in 21 CFR 312.32. Additionally, all of the following events will be reported in the same manner unless judged to be attributable to disease or other pre-existing condition:

- all non-hematologic Grade 4-5 adverse events
- all grades 3-5 infusion reactions attributed to TIL
- all grades 3-5 autoimmune events

TIL infusion reactions will be readily identified as there is an 8-16 hour window between TIL infusion and initiation of high dose IL-2.

In the annual report to the FDA, all grade 3-5 toxicities not felt due to the melanoma or preexisting disorders will be added to the summary reports.

Reporting events for an APPROVED, POST-MARKET AGENT:

The current MedWatch mandatory reporting form FDA 3500A can be found on the FDA website:

http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

Use: Form FDA 3500A - Mandatory Reporting

Complete the ***MedWatch*** report and mail it to the appropriate address below (please also see **FDA reporting fax number** under section 7.4.2):

For Drugs:

Central Document Room
Center for Drug Evaluation and Research
Food and Drug Administration
5901-B Ammendale Road
Beltsville, MD 20705-1266

For Biologics, including Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps):

Center for Biologics Evaluation and Research
OBE Division of Epidemiology, (HFM-220)
Food and Drug Administration
1401 Rockville Pike
Rockville, MD 20852-1448

Reporting of events determined to be related to the approved, post-market agent should be completed and mailed to the FDA by the study coordinator. A copy of the report should be sent to the H. Lee Moffitt Cancer Center Regulatory Affairs IND Office for inclusion in the annual report.

To report an event for an INVESTIGATIONAL AGENT under investigational new drug applications:

Send a narrative of the event with circumstances, IND number, and why it is related and unexpected to the H. Lee Moffitt Cancer Center Regulatory Affairs IND Office. A MedWatch report for the event would suffice. These events should not be reported or sent directly to the FDA by the study coordinator. The Regulatory Affairs IND Office at Moffitt Cancer Center will submit the reportable event as an IND safety report to the FDA on behalf of the principal investigator.

If the principal investigator determines that the event could be possibly related and unexpected to *both the post-market agent and the investigational agent*, then the following should be done:

- Submit the MedWatch report to the appropriate post-market address at the FDA as written above.
- Send the MedWatch report to regulatory affairs at Moffitt Cancer Center. The office will submit and IND safety report on the behalf of the principal investigator.

SAEs should be reported in accordance with all external sponsors' recommendations in addition to the above requirements (see section 7.4).

All adverse events will be reported to the FDA in the annual report with CTCAE grade and attribution (unrelated, unlikely, possible, probable, or definite) according to this table:

Attribution	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Unrelated			X	X	X
Unlikely			X	X	X
Possible	X	X	X	X	X
Probable	X	X	X	X	X
Definitive	X	X	X	X	X

Adverse events that are grade 1 or grade 2 that are unrelated or unlikely to be related to the protocol treatment will not be reported. These include but are not limited to:

i. Fatigue
ii. Weakness
iii. Bone, joint or muscle pain
iv. Alopecia
v. Loss of appetite, nausea, vomiting
vi. Chemistry abnormalities (phosphorus, calcium, glucose)

In addition to the above table, adverse events that are known to be commonly due to metastatic melanoma, chemotherapy, and/or other documented pre-existing conditions, as well as conditions that have no clinical significance will not be reported. <u>These events are not required to be documented in Oncore and are not required to be reported to the FDA in the annual report. These include but are not limited to:</u>

- Abnormalities in hematologic parameters that are definitely due to myelosuppressive therapeutic effect will not be reported such as:
  i. Anemia, neutropenia, lymphopenia, thrombocytopenia
  ii. Epistaxis or bleeding except for hemorrhage requiring inpatient hemodynamic monitoring or transfusion
- Laboratory abnormalities that are without significant clinical importance to the acute safety/condition of the patient:

i. LDH (increased or decreased)
ii. Alkaline phosphatase (increased or decreased)
iii. Low levels of the following: AST, ALT, creatinine, BUN, uric acid, bilirubin, albumin, total protein
iv. Electrolyte abnormalities Grade 2 or less (sodium, potassium, bicarbonate, CO_2, magnesium)
v. Coagulation abnormalities (eg shortened PT, PTT, increased fibrinogen)

- General therapy related events:
  i. Catheter related events
  ii. Grade 1 or Grade 2 adverse events related to FDA-approved non-immunotherapeutic medications (including but not limited to antibiotic and anti-emetic use)

7.4 Assessment of Safety For Vemurafenib

7.4.1 Safety plan for vemurafenib

Toxicities will be evaluated utilizing the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (NCI CTCAE v4.0). If toxicity occurs, the toxicity will be graded, and appropriate supportive care treatment will be administered to decrease the signs and symptoms thereof.

Safety Monitoring for Potential Risks Associated with Vemurafenib

(a) Cutaneous Squamous Cell Carcinoma due to vemurafenib

Cases of cutaneous squamous cell carcinoma (cuSCC), including both SCCs of the skin and keratoacanthomas (KAs), have been reported in patients treated with vemurafenib. The incidence of cuSCC in vemurafenib-treated patients in Trial 1 (NP22657) was 24%. CuSCC usually occurred early in the course of treatment with a median time to the first appearance of 7 to 8 weeks. Of the patients who experienced cuSCC, approximately 33% experienced >1 occurrence with median time between occurrences of 6 weeks. Potential risk factors associated with cuSCC in vemurafenib clinical studies included age (≥ 65 years), prior skin cancer, and chronic sun exposure. In the clinical trials, cases of cuSCC were managed with excision, and patients were able to continue treatment without dose adjustment. It is recommended that all patients receive a dermatologic examination prior to initiation of therapy and at every subsequent study visit. Dermatologic exam may be performed by a dermatologist or by the treating oncologist. Any suspicious skin lesions should be excised, sent for dermatopathologic evaluation, and treated as per standard of care. Monitoring should be considered for 6 months following discontinuation of vemurafenib.

Routine skin exams should be performed at every study visit. Suspicious lesions or rashes should be referred to a dermatologist. Any lesion at baseline or during treatment clinically suspected of representing a cutaneous squamous cell carcinoma, basal cell carcinoma, actinic keratosis, KA, or other skin conditions identified should be treated as per local standard of care. Skin biopsies should be performed by a dermatologist, as necessary with histopathologic

interpretation of suspected lesions. Biopsy-proven non-melanoma skin cancers should be excised.

(b) Other Malignancies due to vemurafenib

Based on mechanism of action, vemurafenib may promote malignancies associated with activation of RAS through mutation or other mechanisms. Monitor patients receiving vemurafenib closely for signs or symptoms of other malignancies.

Reports of non-cuSCC have been received involving patients receiving vemurafenib Patients should undergo a head and neck examination, consisting of at least a visual inspection of oral mucosa and lymph node palpation prior to initiation of treatment and every 3 months during treatment. In addition, patients should undergo a chest CT scan prior to initiation of treatment and every 6 months during treatment.

(c) Hypersensitivity Reactions due to vemurafenib

Serious hypersensitivity reactions, including anaphylaxis, have been reported in association with vemurafenib and upon re-initiation of treatment. Severe hypersensitivity reactions included Stevens-Johnson syndrome, toxic epidermal necrolysis, generalized rash, and erythema or hypotension. In patients who experience a severe hypersensitivity reaction, vemurafenib treatment should be permanently discontinued.

(d) QT Prolongation due to vemurafenib

Exposure-dependent QT prolongation was observed in an uncontrolled, open-label Phase II QT sub-study in previously treated patients with BRAFV600E mutation-positive metastatic melanoma. QT prolongation may lead to an increased risk of ventricular arrhythmias, including Torsade de Pointes.

Treatment with vemurafenib is not recommended in patients with uncorrectable electrolyte abnormalities, long QT syndrome, or who are taking medicinal products known to prolong the QT interval.

ECG and electrolytes, including potassium, magnesium, and calcium, should be monitored before treatment with vemurafenib and after dose modification. Monitoring of ECGs should occur monthly during the first 3 months of treatment, followed by every 3 months thereafter or more often as clinically indicated. Patients with baseline QTcF >450 ms are not eligible for this study. If during treatment the QTcF exceeds 500 ms (NCI CTCAE Grade 3), vemurafenib treatment should be temporarily interrupted, electrolyte abnormalities should be corrected, and cardiac risk factors for QT prolongation (e.g., congestive heart failure, bradyarrhythmias) should be controlled. Re-initiation of treatment should occur at a lower dose once the QTcF decreases below 500 ms (see table 1 above).

Permanent discontinuation of vemurafenib treatment is recommended if after correction of associated risk factors, the QTcF increase meets values of both >500 ms and >60 ms change from pre-treatment values (see table 1).

(e) Liver Laboratory Abnormalities due to vemurafenib

Liver laboratory abnormalities have occurred with vemurafenib. Liver enzymes (transaminases and alkaline phosphatase) and bilirubin should be monitored before initiation of treatment and monthly during treatment or as clinically indicated. Laboratory abnormalities should be managed with dose reduction, treatment interruption, or treatment discontinuation.

An analysis of liver-related adverse events (AEs) reported with vemurafenib use showed that 63 cases (out of estimated exposure of approximately 20,000 patients) of medically confirmed serious adverse events (SAEs) were consistent with drug-induced liver injury (DILI) based on clinical chemistry criteria from the DILI Expert Working Group (Aithal et al. 2011). Of the 63 cases, two were assessed as severe (both reported as hepatic failure). There were no reported deaths among the 63 cases of liver injury; the outcome of one case of severe liver injury was reported as completely resolved with vemurafenib discontinuation, while information on the outcome of the second case of severe liver injury is not available at this time. The median time to onset of the AEs was 44 days after initial dose. The median alanine aminotransferase (ALT) to alkaline phosphatase (ALP) ratio was calculated as 1.5, suggesting a trend towards cholestatic pattern of liver injury. The analysis did not reveal any risk factors or populations at risk.

(f) Neutrophil Laboratory Abnormalities due to vemurafenib

A review of the Roche safety database found neutropenia to be an uncommon (6 cases per 1000 person-years, 0.6%) adverse drug reaction associated with the use of vemurafenib, typically occurring during the first 6-12 weeks of treatment. It appeared to be reversible usually within 2 weeks, with either temporary interruption, dose reduction, or discontinuation of vemurafenib, and in some cases was managed with granulocyte colony-stimulating factor (G-CSF).

(g) Photosensitivity due to vemurafenib

Mild to severe photosensitivity was reported in patients treated with vemurafenib in clinical trials. All patients should be advised to avoid sun exposure while taking vemurafenib. While taking the drug, patients should be advised to wear protective clothing and use a broad spectrum UVA/UVB sunscreen and lip balm (SPF 30) when outdoors to help protect against sunburn.

For intolerable Grade 2 (tender erythema covering 10%–30% body surface area) or greater photosensitivity, dose modifications are recommended.

(h) Ophthalmologic Reactions due to vemurafenib

Serious ophthalmologic reactions, including uveitis, iritis, iridocyclitis, and retinal vein occlusion, have been reported. Monitor patients routinely for ophthalmologic reactions.

(i) New Primary Malignant Melanoma due to vemurafenib

New primary melanomas have been reported in clinical trials. Cases were managed with excision, and patients continued treatment without dose adjustment. Monitoring for skin lesions should occur as outlined

(j) Dupuytren's contracture and Plantar Fascial Fibromatosis due to vemurafenib Dupuytren's contracture and plantar fascial fibromatosis have been reported with vemurafenib. The majority of cases were grade 1 or 2, but severe, disabling cases of Dupuytren's contracture have also been reported.

7.4.2 Safety Parameters, Definitions and Guidelines while the Subject is on vemurafenib

Safety assessments will consist of monitoring and reporting AEs and SAEs that are considered related to vemurafenib, all events of death, AEs of Special Interest (AESIs), and any study specific issue of concern.

Adverse Events

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the patient that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with metastatic melanoma that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations).
- If applicable, AEs that occur prior to the first dose of vemurafenib associated with medication washout, no treatment run-in, or other protocol-mandated intervention.
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

Serious Adverse Events

An AE should be classified as an SAE if the any following criteria are met:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the patient at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the IMP.

- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above).

Methods and Timing for Assessing and Recording Safety Variables while the Subject is on vemurafenib

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study are collected and reported to the FDA, appropriate IRB(s), and Genentech, Inc. in accordance with CFR 312.32 (IND Safety Reports).

Adverse Event Reporting Period

During screening AEs are not recorded unless they are SAEs which are related to protocol-mandated procedures. ALL AEs (including SAEs) must be recorded from the time of first vemurafenib administration and end 30 days following the last administration of vemurafenib or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

Assessment of Adverse Events

All AEs and SAEs whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the vemurafenib (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes

There is a plausible temporal relationship between the onset of the AE and administration of the vemurafenib, and the AE cannot be readily explained by the patient's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the vemurafenib; and/or the AE abates or resolves upon discontinuation of the vemurafenib or dose reduction and, if applicable, reappears upon re-challenge.

No

Evidence exists that the AE has an etiology other than the vemurafenib (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to vemurafenib administration (e.g., cancer diagnosed 2 days after first dose of study treatments).

Expected AEs are those AEs that are listed or characterized in the Package Insert or current Investigator's Brochure.

Unexpected AEs are those not listed in the Package Insert or current Investigator's Brochure or not identified. This includes AEs for which the specificity or severity is not consistent with the description in the Package Insert or Investigator's Brochure. For example, under this definition,

hepatic necrosis would be unexpected if the Package Insert or Investigator's Brochure only referred to elevated hepatic enzymes or hepatitis.

7.4.3 Procedures for Eliciting, Recording, and Reporting Adverse Events while the Subject is on vemurafenib

Eliciting Adverse Events

A consistent methodology for eliciting AEs at all patient evaluation time points should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

(a) Guidelines for Diagnosis versus Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

(b) Deaths

All deaths that occur during the protocol-specified AE reporting period (see Section 7.4.2), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

(c) Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

(d) Hospitalizations for Medical or Surgical Procedures

Any AE that results in an inpatient hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a patient is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the

procedure itself, should be reported as the SAE. For example, if a patient is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study

(e) Pregnancy

If a female patient becomes pregnant while receiving investigational therapy or within 30 days after the last dose of vemurafenib, a report should be completed and expeditiously submitted to the Genentech, Inc. follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female patient exposed to the vemurafenib should be reported as an SAE.

(f) Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a patient has completed or discontinued study participation if attributed to prior vemurafenib exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female patient who participated in the study, this should be reported as an SAE.

(g) Reconciliation of vemurafenib

The Sponsor agrees to conduct reconciliation for the product. Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange quarterly line listings of cases received by the other party. If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

(h) Adverse events of Special Interest (AESI)

AESIs are defined as a potential safety problem, identified as a result of safety monitoring of vemurafenib.

AESIs are specific to the protocol and take into consideration the study design, study population, and safety issues that will be characterized further by the study. AESIs are to be reported within the same reporting timeframe as SAEs

AESIs for vemurafenib may include the following:

- Grade ≥ 3 rash and/or arthralgias
- Grade ≥ 3 photosensitivity
- Grade ≥3 elevations of AST, ALT, serum bilirubin, GGT, OR cases of elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice
- Grade ≥3 QT interval prolongation
- Any cutaneous and non-cutaneous primary malignancy, including SCC, KA, basal cell carcinoma (BCC), new primary melanoma.

(i) Adverse Event Reporting

Investigators must report all SAEs and AESIs to Genentech within the timelines described below. The completed MedWatch/case report should be faxed immediately upon completion to Genentech Drug Safety at:

(650) 225-4682

OR

(650) 225-5288

- Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available.
- Serious AE reports that are related to the vemurafenib and AEs of Special Interest (regardless of causality) will be transmitted to Genentech within fifteen (15) calendar days of the Awareness Date.
- Serious AE reports that are unrelated to the vemurafenib will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
- Additional Reporting Requirements to Genentech include the following:
- Any reports of pregnancy following the start of administration with the vemurafenib will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
- All Non-serious AEs originating from the Study will be forwarded in a quarterly report to Genentech.

Note: Investigators should also report events to their IRB as required.

<u>Medwatch 3500a Reporting Guidelines</u>

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (Section 5) of the MedWatch 3500A form:

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics

- Investigator's assessment of the relationship of the AE to each vemurafenib and suspect medication

(j) Follow-up Information

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e., D.O.B., initials, patient number), protocol description and number, if assigned, brief AE description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report).

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the patient for whom and AE was reported. For questions regarding SAE reporting, you may contact the Genentech Drug Safety representative noted above or the MSL assigned to the study. Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available and/or upon request.

MedWatch 3500A (Mandatory Reporting) form is available at
http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/default.htm

(k) Study Close-Out Requirements Specified by Genentech

Any study report submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. The Clinical Study Report (final study report) by the Sponsor-Investigator should be copied to Genentech. Additionally, any literature articles that are a result of the study should be sent to Genentech. Copies of such reports should be mailed to the assigned Clinical Operations contact for the study:

Email: Vemurafenib-gsur@gene.com

Fax: 866-959-4186

For this Investigator-Initiated IND Study, some additional reporting requirements for the FDA apply in accordance with the guidance set forth in 21 CFR § 600.80 (see section 7.4.1 to 7.4.3).

Events meeting the following criteria need to be submitted to the Food and Drug Administration (FDA) as expedited IND Safety Reports according to the following guidance and timelines:

7-Calendar Day Telephone or Fax Report

The Investigator is required to notify the FDA of any fatal or life-threatening AE that is unexpected and assessed by the investigator to be possibly related to the use of vemurafenib.

An unexpected AE is one that is not already described in the vemurafenib Investigator's Brochure. Such reports are to be telephoned or faxed to the FDA and Genentech within 7 calendar days of first learning of the event.

15-Calendar Day Written Report

The Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of vemurafenib. An unexpected AE is one that is not already described in the vemurafenib investigator brochure.

Written IND Safety reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the investigator with the IND concerning similar events should be analyzed and the significance of the new report in light of the previous, similar reports commented on.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA, Genentech, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a Medwatch 3500 form, but alternative formats are acceptable (e.g., summary letter):

FDA fax number for IND Safety Reports: 1 (800) FDA 0178

All written IND Safety Reports submitted to the FDA by the Investigator must also be faxed to Genentech Drug Safety:

Fax: (650) 225-4682 or (650) 225-4630

All written IND Safety Reports submitted to the FDA by the Investigator must also be faxed to the site IRB:

Chesapeake IRB

6940 Columbia Gateway Drive, Suite 110

Columbia, MD 21046

Tel: (877)-992-4724

Email: adviser@chesapeakeirb.com; reference # Pro00014483

Questions Related to Safety Reporting

For questions related to safety reporting, please contact Genentech Drug Safety:

Tel: (888) 835-2555

Fax: (650) 225-4682 or (650) 225-4630

IND Annual Reports

Copies to Genentech:

All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. Copies of such reports should be faxed to Genentech Drug Safety:

Fax: (650) 225-4682 or (650) 225-4630

Post-Marketing 15-Day "Alert Report":

The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening AE that is unexpected and assessed by the investigator to be possibly related to the use of vemurafenib. An unexpected AE is one that is not already described in the Investigator's Brochure. Such reports are to be submitted to the FDA (2 copies) at the following address: Central Document Room, 12229 Wilkins Avenue, Rockville, MD 20852.

All post-marketing 15-Day "Alert Reports" submitted to the FDA by the Sponsor-Investigator must also be faxed to:

Genentech Drug Safety

Fax: (650) 225-4682 or (650) 225-4630

Please use the safety reporting fax cover sheet in the appendices of this protocol for your fax transmission.

For questions related to safety reporting, contact:

Genentech Drug Safety

Tel: 1-888-835-2555

OR

Fax: (650) 225-4682 or (650) 225-4630

(l) Special situation reports

In addition to all AEs, pregnancy reports and AESIs, the following Special Situations Reports should be collected and transmitted to Genentech even in the absence of an Adverse Event within thirty (30) calendar days:

- Data related to the Product usage during pregnancy or breastfeeding
- Data related to overdose, abuse, off-label use, misuse, inadvertent/erroneous administration, medication error or occupational exposure, with or without association with an AE/SAE unless otherwise specified in the protocol
- Data related to a suspected transmission of an infectious agent via a medicinal product (STIAMP)
- Lack of therapeutic efficacy

In addition, reasonable attempts should made to obtain and submit the age or age group of the patient, in order to be able to identify potential safety signals specific to a particular population

(m) Aggregate Reports

Moffitt Cancer Center will forward a copy of the Publication to Genentech upon completion of the Study.

7.5 Vemurafenib Treatment Considerations

All patients will be advised to avoid prolonged sun exposure while taking vemurafenib and for at least 10 days after study drug discontinuation. Patients should also be advised to use a broad-spectrum sun-screen and lip balm of at least SPF30 to help protect against sunburn (see section 4.1.4b). For acneiform rash, investigators should consider treatment with minocycline.

Special consideration must be taken with any patient on study with an increased QTcF interval. Please refer to section 4.1.5 and table 1 for further details regarding QTcF interval.

During chronic vemurafenib therapy, please refer to section 4.1.5 for evaluation and dose modification. Temporary discontinuation of drug for up to 8 weeks is allowed. Temporary discontinuation longer than 8 weeks should be discussed with the principal investigator. If a treatment interruption occurs for reasons other than an AE, and it is determined that vemurafenib will be re-started, no dose reduction is required.

Once patients are no longer followed by scheduled radiologic assessments for tumor burden and vemurafenib was been discontinued, a chest CT scan will be performed 6 months (+/- 90 days) after the last dose of vemurafenib to evaluate for metastatic squamous cell carcinoma for all patients who have not withdrawn consent, have not died, not initiated a new cancer treatment, and have not been lost to followup.

7.6 Chemotherapy Treatment Considerations

The use of the non-myeloablative regimen in this protocol is a major procedure which entails serious discomforts and hazards for the patient. Although it is anticipated that this protocol is relatively safe because of the expected recovery of the patients' bone marrow within 2 to 4 weeks, fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life-threatening metastatic cancer. The major hazards are infection and disease progression. The major discomforts are nausea, mucositis, anorexia, diarrhea, fever and malaise. Side effects of common drugs used in this non-myeloablative regimen include:

Cyclophosphamide: Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, and SIADH.

Fludarabine: Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity, and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL patients treated with fludarabine.

Antimicrobials in general: Allergic reactions, renal impairment, nausea, vomiting, hepatic damage, marrow suppression.

7.7 Adoptive cell therapy with TIL Treatment Considerations

A variety of side effects that potentially overlap with high dose IL2 have been associated with adoptive cell therapy in our experience and at the NCI. Prominent long-term side effects include: vitiligo, high frequency hearing loss and uveitis. Careful evaluation to ascertain the toxicity, immunologic effects and anti-tumor efficacy of cell infusions will be performed. Toxicities will be monitored and documented on a daily basis beginning at day 0 (T-cell infusion) and continuing until discharge from the hospital following the initial IL-2 infusion. Patients with treatment-limiting toxicity will be followed until the event is resolved to grade I or less, or six months from the onset of the event.

7.8 High Dose IL-2 Treatment Considerations

A variety of side effects have been associated with high-dose IL-2 administration in our experience at the NCI and a listing of these side effects in 652 patients who received 1,039 treatment courses are listed in the Appendix.

8.0 Criteria for Stopping the Trial Due to Toxicity

The criteria for stopping the study and halting enrollment and the administration of study regimen will be as follows:
1. If 33% or greater (≥ 4) of the first 12 patients treated on this trial have non-hematologic grade IV organ toxicity related to the study treatment that is immediately life-threatening, and occurring upon or after the T cell transfer or definitely attributable to vemurafenib, or
2. If 17% or greater (≥ 2) of the first 12 patients treated on this trial have Grade V toxicity (death) related to the study treatment upon or after T cell transfer or definitely attributable to vemurafenib within 3 months of the conditioning regimen that is not due to disease progression or other pre-existing condition.

Of note, the first cohort has accrued 17 subjects as of 2/10/2017 and has successfully passed the criteria outlined in this section.

9.0 Criteria for Response

The initial disease assessment at 6 weeks will be subsequently confirmed at the 12 month assessment

9.1 Evaluation of Target Lesions[1]

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

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

Progression (PD): At least a 20% increase in the sum of LD of target lesions taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions if the sum demonstrates an absolute increase of at least 5 mm.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD.

[1] All measurable lesions up to a maximum of 5 lesions 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) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease. At least one lesion defined radiologically must be present. R.E.C.I.S.T. Version 1.1 will be referenced for determination of response.

9.2 Evaluation of non-target lesions[2]

Complete Response (CR): Disappearance of all non-target lesions any pathologically enlarged lymph nodes (whether target or non-target) must have a reduction in short axis to ≤10 mm, and normalization of tumor marker level.

Non-Complete Response: Persistence of one or more non-target lesions.

Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions.

[2] All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as “present” or “absent.”

9.3 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.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

10.0 Criteria for Removal from the Study

Patients will be taken off the study if: (a) the patient voluntarily withdraws, (b) there is significant noncompliance, (c) there is progression of disease by RECIST 1.1 criteria that is confirmed at the week 12 assessment or death, or (d) any immediately life-threatening grade IV non-hematologic organ-specific adverse event (neurologic, pulmonary, cardiac, gastrointestinal, genitourinary, hepatic or dermatologic) not felt due to melanoma, or a pre-existing condition. Any patient who develops an immediately life-threatening Grade IV non-hematologic organ-specific toxicity due to cell infusion will be taken off protocol.

11.0 Statistical Considerations and Data Analysis

11.1 Overview

This trial was originally designed as a single-arm phase II trial employing the Simon two-stage Minimax design to prospectively evaluate the response (CR+PR) rate at 12 months and the drop-out rate as co-primary endpoints, and as a secondary endpoint progression-free survival of the regimen of vemurafenib plus TIL administered after lymphodepletion and followed by a single cycle of high dose IL-2. Up to 53 patients were originally planned to be treated on this trial. Due to changes in treatment options and referral patterns, we are very unlikely to complete the original trial design successfully. However, we have discovered that the treatment has activity against tumors that are resistant to BRAF-inhibitor containing therapy. Of note this patient population represents a significant fraction of patients treated at Moffitt Cancer Center and is an area of unmet clinical need. Therefore, we propose a second cohort of 12 patients who will be accrued to the study after having progressed on BRAF-inhibitor containing treatment. Patients in the second cohort will be required to have progressed on prior PD-1 antibody containing therapy in order to ensure that effective treatment options have been exhausted.

The intention-to-treat approach will be used for data analysis for both cohorts of this trial. That means all patients who undergo surgical resection for the intention of growing TIL will be included in the data analysis. Patients who are lost to follow-up or drop out of the study prior to their scheduled response evaluation at any time after tumor harvest for TIL propagation due to any reason will be treated as non-responders for response determination and censored for time-to-event type of endpoints at the time of their last assessment/follow-up if no relevant event has occurred by then. Patients in the second cohort who undergo tumor harvest but do not undergo cell therapy due to disease progression will be counted as non-responders. A secondary analysis that includes only all eligible patients may be conducted in addition to the primary analysis described above at the discretion of the trial PI and study biostatistician. Other sub-analyses may also be considered on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., 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 co-primary objectives for the first cohort of this study (BRAF-inhibitor treatment naïve) was to determine whether patients receiving vemurafenib added to adoptive cell therapy with tumor-infiltrating lymphocytes exhibit: 1) a relatively higher proportion of responses (CR+PR) at 12 months by RECIST 1.1 criteria when compared to our prior experience with ACT on an intention-to-treat basis and 2) a relatively lower “drop-out rate”, i.e., the proportion of patients not able to actually receive harvested TIL. Secondary objectives will be to evaluate the overall progression-free survival as well as some exploratory laboratory endpoints of interest.

The primary objective for the second cohort of the study (BRAF-inhibitor treatment resistant and PD-1 antibody resistant or intolerant) will be to determine whether patients who have progressed after BRAF-inhibitor containing therapy and progressed after or are intolerant to PD-1 antibody in the past, who receive vemurafenib added to adoptive cell therapy with tumor-infiltrating lymphocytes exhibit a relatively higher proportion of responses (CR+PR) at 12 months by RECIST 1.1 criteria when compared to expected survival upon progression after PD-1 antibody and BRAF-inhibitor containing treatment.

11.2 Trial Design

The study was originally designed as a Simon two-stage Minimax design, with a desired complete and partial response (CPR) rate of ≥ 50% at 12 months compared to the expected CPR rate ≤ 30% from standard TIL therapy by an intention-to-treat approach. With that design, using an alpha error of 0.047 and beta error of 0.098, we needed to observe more than 7 patients successfully showing CPR at 12 months in the first 24 patients, and then proceed to add another 29 patients, and observe more than 21 patients showing CPR at 12 months from standard TIL therapy out of the total of 53 patients treated to consider this trial a success. Given the accrual to date, we are very unlikely to be able to complete that trial design successfully. However, we have discovered that the treatment has activity against tumors that are resistant to BRAF-

inhibitor containing therapy. Therefore we propose a second cohort that accrues 12 subjects who have previously progressed on previous PD-1 antibody and BRAF-inhibitor containing therapy. This cohort is expected to have a substantially enhanced rate of accrual since this is an area of unmet clinical need. The second cohort will enroll patients whose tumors have progressed on prior PD-1 antibody and BRAF inhibitor containing therapy in the past. Patients will undergo tumor harvest for TIL propagation on day -35 and start vemurafenib on day -21 to sensitize tumors to immune mediated cell death. Patients will receive intravenous (IV) chemotherapy consisting of cyclophosphamide on Days –7 and –6, fludarabine on Days –5 to -1, IV T cells on Day 0, and high dose IL-2 on Days 1 to 2. Vemurafenib will resume after the patient has clinically recovered from high-dose IL-2 based upon the judgment of the treating physician. The primary endpoint is CPR at 12 months, and the results from the second cohort will be successful if a CPR rate of 15% is observed, given an expected rate of ~5% associated with alternative therapies (49-51). Based upon long-term results reported with chemotherapy (49) and ipilimumab (50, 51), the null objective response rate at 12 months for patients in cohort 2 is 5%. The trial will be considered successful if the observed objective response rate is ≥15% (α=0.12). As a reference, if the true objective response rate is 20%, then the power associated with analysis of the second cohort would be 73%; and if the true objective response rate is 25%, then the power associated with analysis of the second cohort would be 84%.

11.3 Outcomes and Data Analysis

Overall response (OR) is defined as the patient being alive at month 12, and tumor size evaluated at screening and at month 12 using the RECIST 1.1 criteria to be a complete response (CR) or partial response (PR). Evaluations will be made by CT scan approximately 12 months from the date of tumor harvest, and by clinical evaluation during the first 12 months. The complete response rate, complete and partial response rate (CPR), and drop-out rate will be summarized using both a point estimate and its 95% exact confidence interval based on the binomial distribution.

Progression-free survival (PFS), defined as the time from study entry to disease progression, relapse or death due to any cause, whichever is earlier, will be summarized with the exponential survival estimator. Confidence intervals for the median and survival rates at different time points will be constructed if needed and appropriate. This secondary endpoint will be reported descriptively. Overall survival (OS) is defined as the time from initiation of the study protocol to date of last patient contact or death due to any cause, whichever is latest. OS data will be analyzed and reported in the manner described for PFS. Additional survival follow-up for subjects who have progressed may continue until the patient is lost to follow-up or withdraws consent. After confirmed disease progression subjects may be followed every 3 months for overall survival and report of any subsequent anti-cancer treatments. This can accomplished by visit, phone or email contact. The study will end once survival follow-up has concluded.

For the correlative endpoints, data on the Mannose-6 Phosphate Receptor (MPR), granzyme B and autophagy expression marker LC3 by immunohistochemistry will be collected at the time of tumor harvest (pre) and 7 days post start of vemurafenib treatment and at least 21 days post ACT, whenever feasible, on a 0 (negative), 1-3 (weak), 4-6(moderate) or 7-9 (high) staining scale. Successful induction of the above biomarkers at each time point will be declared if at least 75% of patients show an increase of at least 1 level in the IHC staining. We anticipate that responders will exhibit enhanced intensity of biomarker expression levels after treatment compared to non-responders. The changes in MPR, Granzyme B and autophagy expression levels in tumor biopsy samples obtained before and after vemurafenib and after TIL ACT will be summarized for each time point, and their correlation to complete response will be explored using a generalized linear model with an appropriate link function. A similar analysis will be performed for the change in the tumor reactivity of circulating T cells pre and post vemurafenib and after TIL ACT with clinical outcome.

11.4 Toxicity Monitoring and Stopping Rules

All patients will be evaluable for toxicity from the time of their first protocol treatment. Toxicity will be reported by type and severity according to the NCI CTC version 4. The criteria for stopping the study and halting enrollment and the administration of study regimen will be as follows:

1. If 33% or greater (≥ 4) of the first 12 patients treated on this trial have non-hematologic grade IV organ toxicity related to the study treatment that is immediately life-threatening, and occurring upon or after the T cell transfer or definitely attributable to vemurafenib, or
2. If 17% or greater (≥ 2) of the first 12 patients treated on this trial have Grade V toxicity (death) related to the study treatment upon or after T cell transfer or definitely attributable to vemurafenib within 3 months of the conditioning regimen that is not due to disease progression or other pre-existing condition.

Of note, the first cohort has accrued 17 subjects as of 2/10/2017 and has successfully passed the criteria outlined in this section.

11.5 Accrual

We have successfully accrued 17 subjects to the first cohort as of 2/10/17. We plan on accruing 12 patients for the second study cohort. Anticipated accrual rate is 1 patient every 6 weeks. The accrual of up to 12 patients for the entire study will take approximately 18 months.

11.6 Evaluation of Response

All patients treated on 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.

Patients in response categories 4-9 should be considered as failing to respond to treatment. Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

12.0 Data Entry and Protocol Management

For the purposes of this study at Moffitt Cancer Center, the Protocol Data Management System (OnCore) will be employed. All patients will be registered in OnCore before any study specific tests are performed. For patients who consent for the trial but are not eligible and do not undergo excision of tumor for TIL propagation, the only data that will be collected are the data that are collected via OnCore at the time that the patient is consented. No case report forms for screening procedures are required to be completed and will be marked as not applicable. The screening visit in OnCore will be certified according to Moffitt Cancer Center policy/procedure.

This phase II investigator-initiated trial will be monitored continuously by the PI and the assigned study clinical trial coordinator and discussed at the Cancer Center's phase II meetings, which typically occur every week. Compliance with the Moffitt Data Safety Monitoring Plan and Protocol Monitoring Committee guidelines will be followed.

To ensure adherence to the protocol, safety and monitoring reports will be submitted to the Protocol Monitoring Committee (PMC) once 30% of the total accrual has been met or once the early stopping rule threshold has been met (whichever comes first) or more frequently if requested by the PMC. A final safety and monitoring report will be submitted to the PMC within 3 months of the last subject having been enrolled. These reports will be assembled by the study trial coordinator and reviewed by the PI. The study trial coordinator and the PI will ensure accuracy, quality control and management of the data.

Internal audits will be conducted by the Compliance Office in accordance with applicable regulatory standards. The following elements will be reviewed:

1. Source documentation verification of eligibility and protocol compliance
2. Regulatory review of IRB compliance and external reporting requirements
3. Drug/device accountability and handling
4. Completeness and quality of data

The Compliance Office will conduct and report the findings of audits to the PMC.

13.0 Administrative Procedures

13.1 Changes to the Protocol

Any change or addition to this protocol requires a written protocol amendment that must be approved by the IRB. A copy of the written approval of the IRB must

be received before implementation of any changes. The IRB must review and approve all amendments to the protocol.

13.2 Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol and Good Clinical Practice, as described in:

13.2.1 International Conference on Harmonisation (ICH)

- Harmonized Tripartite Guidelines for Good Clinical Practice 1996

13.2.2 US 21 Code of Federal Regulations dealing with clinical studies

- Including parts 50 and 56 concerning informed consent and IRB regulations.

13.2.3 Declaration of Helsinki

- Concerning medical research in humans
  (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice.

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Appendix

Appendix Subtitle: High-dose Interleukin-2 Toxicities

15. Appendix

Toxicity of Treatment of 283 Patients With Melanoma or Renal Carcinoma With High-Dose Bolus Interleukin 2 *

Toxic Effect	No. (%) of Courses by Diagnosis		
	Melanoma	Renal Cell	Total
Chills	42 (20%)	40 (17%)	82 (18%)
Pruritis	20 (10%)	20 (8%)	40 (9%)
Mucositis	0 (0%)	2 (1%)	2 (0%)
Nausea	78 (37%)	92 (39%)	170 (38%)
Diarrhea	68 (33%)	67 (28%)	135 (30%)
Malaise	37 (18%)	28 (12%)	65 (15%)
Peak bilirubin, µmol/L (mg/dL)			
1.7-34.2 (0.1-2.0)	30 (14%)	40 (17%)	70 (16%)
34.9-102.6 (2.1-6.0)	126 (60%)	153 (64%)	279 (62%)
104.3-171.0 (6.1-10.0)	41 (20%)	34 (14%)	75 (17%)
>171.0 (>10.0)	12 (6%)	11 (5%)	23 (5%)
Oliguria <80 mL/8 h	39 (19%)	60 (25%)	99 (22%)
Anuria <240 mL/24 h	3 (1%)	15 (6%)	18 (4%)
Weight gain, % of body weight			
0.0-5.0	53 (25%)	77 (32%)	130 (29%)
5.1-10.0	88 (42%)	99 (42%)	187 (42%)
10.1-15.0	44 (21%)	46 (19%)	90 (20%)
15.1-20.0	16 (8%)	13 (5%)	29 (6%)
>20.0	8 (4%)	3 (1%)	11 (2%)
Peak creatinine, µmol/L (mg/dL)			
8.8-176.8 (0.1-2.0)	98 (47%)	44 (18%)	142 (32%)
185.6-530.4 (2.1-6.0)	105 (50%)	172 (72%)	277 (62%)
539.2-884.0 (6.1-10.0)	6 (3%)	18 (8%)	24 (5%)
>884.0 (>10.0)	0 (0%)	4 (2%)	4 (1%)
Edema	3 (1%)	1 (0%)	4 (1%)
Respiratory distress	4 (2%)	14 (6%)	18 (4%)
Respiratory failure requiring intubation	4 (2%)	9 (4%)	13 (3%)
Pleural effusion	3 (1%)	2 (1%)	5 (1%)
Somnolence	3 (1%)	19 (8%)	22 (5%)
Coma	1 (0%)	9 (4%)	10 (2%)
Disorientation	25 (12%)	37 (16%)	62 (14%)
Hypotension	97 (46%)	136 (57%)	233 (52%)
Angina	2 (1%)	3 (1%)	5 (1%)
Myocardial infarction	0 (0%)	2 (1%)	2 (0%)
Arrythmias	17 (8%)	12 (5%)	29 (6%)
Anemia requiring transfusion, no. of units transfused			
0	171 (82%)	182 (76%)	353 (79%)
1-5	35 (17%)	47 (20%)	82 (18%)
5-10	3 (1%)	6 (3%)	9 (2%)
11-15	0 (0%)	3 (1%)	3 (1%)
Platelet nadir, cells x 109/L			
0-20	7 (3%)	9 (4%)	16 (4%)

20-60	72 (34%)	59 (25%)	131 (29%)
60-100	64 (31%)	73 (31%)	137 (31%)
>100	66 (32%)	97 (41%)	163 (36%)
Infection	9 (4%)	9 (4%)	18 (4%)
Line sepsis	3 (1%)	6 (3%)	9 (2%)
Death	0 (0%)	3 (1%)	3 (1%)
TOTAL	209 (100%)	238 (100%)	447 (100%)

*Reference 1; includes only grade 3 and 4 toxicity except where detailed.