COMIRB Protocol

PROTOCOL #: 14-0948

COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
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Project Title: **Ipilimumab and All-Trans Retinoic Acid Combination Treatment of Advanced Melanoma**

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I. Hypotheses and Specific Aims

The successful treatment of melanoma with immune checkpoint inhibitors, such as anti-CTLA-4 and PD-1 antibodies, has altered our thinking and approach to immunotherapy for solid tumors. Despite these advances, only a portion of patients experience a durable response suggesting that there is room for improvement via enhanced immunomodulatory approaches. Anti-CTLA-4 (ipilimumab) significantly improves overall survival and achieves long-lasting complete responses in some melanoma patients, the number of patients that achieve durable clinical benefit is limited and could be improved by a combined immunomodulatory approach. The objectives of our study are to assess the safety and efficacy of combined treatment with ipilimumab and all-trans retinoic acid (ATRA), brand name VESANOID, in melanoma patients. We hypothesize that combined treatment with ipilimumab and VESANOID will improve patient responses, increase tumor antigen-specific T cell responses, and decrease immunosuppressive myeloid-derived suppressor cells (MDSCs) in melanoma patients compared to patients treated with ipilimumab alone.

Specific Aims:

1. To establish the **safety and tolerability** of ipilimumab and VESANOID combination therapy in advanced melanoma patients

2. To demonstrate the reduction in **MDSC frequency and suppressive function** in peripheral blood of Advanced melanoma patients undergoing ipilimumab and VESANOID combination therapy

3. To determine if the addition of VESANOID to standard ipilimumab treatment increases the **frequency of tumor-specific T cell responses**

II. Background and Significance

Melanoma uniquely elicits profound immune responses and is one of the few tumors for which immunotherapies have been successful (1). However, melanoma counters current immunotherapeutic approaches by inducing immune escape mechanisms, a critical barrier that may contribute to low response rates and treatment failure (1). Melanoma continues to be a leading cause of death among adult cancer patients (2, 3). Furthermore, incidents of melanoma continue to increase across all age groups (4).

The antibody therapy specific for CTLA-4, an inhibitory molecule expressed on activated T cells and constitutively expressed on T_{regs}, has recently been shown to significantly improve overall survival and induce durable, long-lasting responses of greater than 5 years in approximately 20% of treated patients (5, 6). While the results of this monumental study highlight a crucial advantage of immunotherapies in achieving long-term immunity that is resistant to disease relapse, increased response rates to ipilimumab could greatly impact survival and clinical outcomes for patients with advanced-stage melanoma. One proven mechanism of action for this drug is enhancing the frequency and function of tumor-specific T cells (7). However, immunosuppression may limit the development of effective immune responses in some patients treated with ipilimumab. Improving clinical responses to ipilimumab by enhancing other immune-mediated mechanisms in a combinatorial approach is essential for the future success of immunotherapeutic strategies.

MDSCs are a mixed population of immunosuppressive myeloid cells that are key contributors to tumor-induced immunosuppression. Increased numbers of MDSCs are associated with larger tumor burdens, increased tumor progression, and shorter overall survival in several cancers, including melanoma (8-11). These data suggest that targeting MDSCs in a combinatorial approach may improve tumor-specific immune responses, increase clinical responses to immunotherapy, and ultimately increase survival in melanoma patients. In this proposal, we aim to decrease the frequency and/or function of MDSCs in melanoma patients treated with all-trans retinoic acid (ATRA).

Investigational product: All-trans retinoic acid (ATRA) is a vitamin A derivative that binds the retinoic acid receptor on MDSCs and differentiates immature monocytes into more mature dendritic cells (12). VESANOID is a standard treatment for patients with acute promyelocytic leukemia (APL).

VESANOID (tretinoin) is an FDA-approved commercially available retinoid that induces maturation of APL cells in culture (Appendix B). It is not approved in combination with Ipilimumab or for the treatment of patients with melanoma. It is available in a 10 mg soft gelatin capsule for oral administration. The recommended dose (for APL) is 45 mg/m2/day administered as two evenly divided doses until complete remission is documented. Therapy should be discontinued 30 days after achievement of complete remission or after 90 days of treatment, whichever occurs first.

VESANOID has been investigated in 114 previously treated APL patients and in 67 previously untreated (“de novo”) patients in one open-label, uncontrolled single investigator clinical study (Memorial Sloan-Kettering Cancer Center [MSKCC]) and in two cohorts of compassionate cases treated by multiple investigators under the auspices of the National Cancer Institute (NCI).

Virtually all patients with APL experience some drug-related toxicity, especially headache, fever, weakness, and fatigue. These adverse effects are seldom permanent or irreversible nor do they usually require interruption of therapy. Some of the adverse events are common in patients with APL, including hemorrhage, infections, gastrointestinal hemorrhage, disseminated intravascular coagulation, pneumonia, septicemia, and cerebral hemorrhage. There are no reported side effects in patients with solid tumors treated with a short course of 150 mg/m^2 VESANOID combined with high dose IL-2 or a dendritic cell vaccine (13, 14).

Pre-clinical findings: Pre-clinical data suggest that VESANOID abrogates the suppressive effects of MDSCs isolated from both human cancer patients and mouse cancer models in vitro (15-17). Furthermore, mice treated with VESANOID have decreased frequencies of MDSCs and increased frequencies of dendritic cells that improve T cell responses against tumors (18-20).

Treatment justification: Although VESANOID is a standard treatment for patients with acute promyelocytic leukemia, it has also been used to treat patients with solid tumors. Renal cell carcinoma patients treated with VESANOID followed by high dose IL-2 had reduced frequencies of circulating MDSCs with reduced immunosuppressive function during the VESANOID phase of the treatment regimen (14). However, the subsequent high-dose IL-2 treatment abrogated these effects and resulted in a lack of clinical benefit overall (14). In another recent trial, VESANOID was used in combination with a dendritic cell vaccine targeting p53 mutations in small cell lung cancer patients (13). The survival outcomes have not yet been reported; however, the study demonstrated that the frequency and function of MDSCs was reduced following VESANOID treatment and that p53-specific T cell responses were increased in more patients receiving the combinatorial therapy compared to patients receiving the dendritic cell vaccine alone (13).

Description of population to be studied: Advanced melanoma patients undergoing standard ipilimumab treatment.

Study rationale: Reducing immunosuppression by MDSCs during ipilimumab treatment may maximize T cell activation and expansion, increasing the overall effectiveness and success of ipilimumab treatment. This pilot clinical trial will demonstrate whether combinatorial immunotherapeutic approaches that augment tumor-specific T cells and simultaneously block immunosuppressive mechanisms are beneficial in melanoma patients.

Preliminary Studies

Our preliminary research has identified a population of immunosuppressive MDSCs (Lineage negative, HLA-DR negative, CD11b positive, and CD33 positive) that are increased in Stage IV melanoma patients relative to healthy donors. To establish MDSCs as a potentially important contributor to immunosuppression in melanoma patients, we compared the frequency and function of immunosuppressive MDSCs in the peripheral blood of healthy donors, Stage I, and Stage IV melanoma patients (11). We found significant increases in the frequency of MDSCs in the peripheral blood of Stage IV melanoma patients compared to healthy donors. We showed that the frequency of these cells correlates with an increase in regulatory T cells and an increased level of IL-6 and IL-8 cytokines in the peripheral blood. Furthermore, patients with clinical disease progression had an increased frequency of these cells. We also found that patients with a high frequency of MDSCs had decreased overall survival and an increased risk of death (hazard ratio 4.83, $p = 0.016$). This increased risk of death was independent of other clinical factors including age, gender, and treatment regimens. Finally, we demonstrated that MDSCs isolated from Stage IV melanoma patients were significantly more immunosuppressive than those isolated from healthy donors. Overall, these data suggest that MDSCs may be a significant contributor to immunosuppression in melanoma patients and that targeting these cells in a combinatorial approach may improve immune responses against melanoma.

VESANOID induces the differentiation of immature monocytes into more mature cells of the dendritic cell lineage (16, 17, 21). In agreement with these results, our preliminary data suggest that VESANOID induces maturation of in vitro-derived MDSCs. We cultured monocytes from a healthy donor with GM-CSF/IL-4 or GM-CSF/IL-6 to generate mature monocytes of the dendritic cell lineage (a control population) or MDSCs, respectively (22, 23). In contrast to the control population, MDSCs express low levels of HLA-DR and maintain high expression of CD14, consistent with the phenotype of an immature monocyte. Furthermore, MDSCs express increased levels of molecules that inhibit T cell responses such as Arginase I, NOX1, iNOS, and PD-L1. Conversely, MDSCs cultured with VESANOID are phenotypically more similar to the control dendritic cell population, expressing decreased levels of inhibitory molecules and CD14 and increased levels of HLA-DR. Most importantly, MDSCs derived in vitro with GM-CSF and IL-6 suppress T cell proliferation. However, treatment of these MDSCs with VESANOID eliminates their suppressive effects and restores T cell proliferation. Finally, MDSCs express higher levels of retinoic acid receptors RAR and RXR, suggesting that VESANOID treatment may selectively target these inhibitory cells. These preliminary data and our previous publications demonstrate that our research team has established the protocols necessary to accomplish the experiments outlined in this protocol.

III. Study Design and Research Methods

This is a Phase 2 investigator-initiated randomized open label clinical investigation aimed at evaluating the safety of combined VESANOID and ipilimumab treatment versus standard treatment with ipilimumab alone, and the effect of this combined treatment on the frequency and function of peripheral MDSCs in advanced melanoma patients being treated at the University of Colorado Cancer Center. This trial will be conducted in compliance with this protocol, Good Clinical Practice, and local and federal regulations.

Anticipated total number of enrolled research subjects: 60
Anticipated number to complete study: 48

Outcome Measures

- **Safety and tolerability** of ipilimumab and VESANOID combination therapy in advanced melanoma patients will be established using the Bayesian approach.

- **MDSC frequency** in peripheral blood of advanced melanoma patients undergoing ipilimumab and VESANOID combination therapy will be measured by flow cytometry.

- **MDSC suppressive function** in peripheral blood of advanced melanoma patients will be measured through the activation and proliferation of T cells in the presence of isolated MDSCs.

- Changes in the **frequency of tumor-specific T cell responses** attributable to the addition of VESANOID to standard ipilimumab therapy will be determined by the frequency of IFN-gamma producing cells after stimulation with melanoma antigens.

- Subjects with unresectable Stage III and Stage IV disease will be followed for RECIST 1.1

Description of Population to be Enrolled

We will identify patients undergoing treatment for melanoma in the cutaneous oncology clinic at the University of Colorado Cancer Center who are eligible for treatment with ipilimumab. Up to 60 patients will be enrolled with 48 patient anticipated to complete the study.

Patient Screening

All patients being evaluated for melanoma in the cutaneous oncology clinic for advanced disease are eligible for the trial based on the following criteria:

Inclusion Criteria

- Patients over the age of 18 year.
- Patients diagnosed with advanced melanoma.
- Patients that are considered candidates for ipilimumab therapy.
- Patients able to understand and willing to sign a written informed consent documents.
- Patients willing to have regular blood draws, one before treatment and four during or after treatment.

Exclusion Criteria

- Patients under the age of 18.
- Patients with Stage I or II, melanoma who are not candidates for Ipilimumab.
- Patients that have received systemic treatments within four weeks prior to the beginning of treatment.
- Women that are pregnant or nursing.
- Patients taking immunosuppressive medications.
- Patients with active autoimmune disease.
- Patients with known sensitivity to retinoic acid derivatives.
- Patients with aspartate aminotransferase (AST), alanine aminotransferase (ALT), or bilirubin > 2.5 × ULN.

Informed Consent Process

Following patient identification, patients may be approached by the Principal Investigator, Study Coordinator, or Sub-Investigator to discuss study participation. Treatment options and research-specific procedures will be carefully reviewed with the patient prior to enrollment in the study.

Allocation of Treatment

Treatment allocation will be determined by the breaking of a randomization envelope. Subjects will be randomized into two groups with 30 patients in each group. Arm A (No VESANOID Therapy) will receive the standard of care treatment with ipilimumab only, receiving the standard doses of 3 or 10mg/kg ipilimumab approximately every 3 weeks or longer according to standard of care protocols. Arm B (VESANOID Therapy) will receive

the standard 4 doses of 3 or 10mg/kg ipilimumab approximately every three weeks or longer according to standard of care protocols plus the supplemental treatment of 150 mg/m^2 VESANOID orally for 3 days surrounding each of the first four infusions of ipilimumab (day -1, day 0, day +1) for a total of 12 days of VESANOID treatment (Figure 1). The total daily dose of VESANOID may be taken anytime on the day before the ipilimumab infusion (day -1) according to the patient's preferences, prior to the ipilimumab infusion on the day of the infusion (day 0), and anytime on the day after the ipilimumab infusion (day 1) according to the patient's preferences.

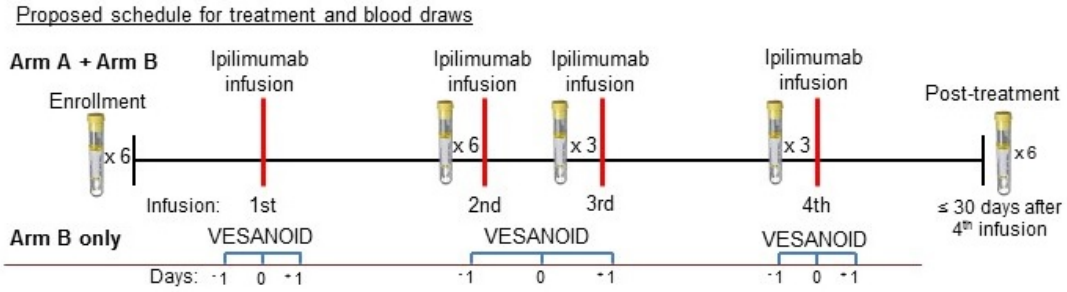

Figure 1. Diagram of the treatment and blood draw schedule for this study. Patients in Arm A and B will receive an infusion of ipilimumab approximately every three weeks for 12 weeks (a total of 4 infusions). Patients in Arm B will also receive 3 days of VESANOID treatment surrounding each infusion of ipilimumab, beginning one day prior to the infusion (a total of 12 days of VESANOID). Three tubes of peripheral blood (approximately 20 ml) will be drawn at enrollment (pre-treatment), prior to each infusion of ipilimumab, and three weeks after the final ipilimumab infusion, for a total of 5 time points. Three additional tubes of peripheral blood (for a total of approximately 40 ml) will be drawn at enrollment, at the 3rd infusion, and within 30 days of the final infusion for additional functional experiments. *(Note: prior to dosing is ≤72 hours)

Drug Distribution

Ipilimumab is standard of care for this patient population. VESANOID will be supplied by the Investigator through the University of Colorado Cancer Center pharmacy. VERSANOID is supplied as 10 mg capsules, two-tone (lengthwise), orange-yellow and reddish-brown and imprinted VERSANOID 10 ROCHE. It is packaged in high-density polyethylene opaque bottles of 100 capsule with child-resistance closure.

Patient Compliance

Patients in Arm B will self-administer VERSANOID capsules in the outpatient setting. At subsequent follow-up visits, patients will be asked about any noticeable side effects or intolerance to the VERSANOID. If patients were not able to take all the prescribed capsules, they will be instructed to bring any remaining pills to the clinic during each visit. Pharmacy personnel will count and record the number of used and unused drug at each visit on the Drug Compliance Assessment for Oral Investigational Product Document.

Study Procedures

Study assessments and sample collection time points are summarized in Table 1. Following the standard protocols for ipilimumab treatment, peripheral blood will be drawn at baseline and prior to each dose of ipilimumab to monitor electrolytes, liver function, thyroid function, and complete blood counts (CBCs). These assays will be performed in the clinical laboratories at the University of Colorado Hospital and monitored by the treating medical oncologists and study team, led by our collaborator and Director of the Melanoma Research Clinics at the University of Colorado Denver, Dr. Rene Gonzalez.

Tests and procedures	***Baseline***	***Active Treatment***			***Discontinuation of Treatment***[2]
	≤4 wks prior to start	Prior to each infusion[1]	Midway (prior to second infusion)	≤ 30 days after final infusion	
Medical History, exam, weight, physical signs, & adverse events assessment	X	X		X	X
Hematology group (WBC, ANC, Hgb, PLT)[7]	X	X		X	
Chemistry group and thyroid function [3,7]	X	X		X	
Research correlates					
Phenotypic characterization (3 yellow tops)	X	X[5]			
Functional characterization (+3 yellow tops)	X		X	X	
Tumor Measurement (CT, MRI, etc.) [4]	X[6]			X[8]	X

[1]Infusions of ipilimumab will occur approximately every 3 weeks or longer as per hospital standard of care.
[2]See "Protections against risk" below for conditions necessitating discontinuation of treatment. If treatment is discontinued, it is not necessary to repeat the specified evaluations.
[3]Creatinine, total bilirubin, AST, ALT, alkaline phosphatase, Ca, K, Na, albumin, total protein, TSH (with additional thyroid testing as clinically indicated)
[4]Baseline assessment prior to enrollment. Measurements will be taken using RECIST criteria.
[5]Except for the first infusion where the baseline blood draw will be used.
[6]Baseline tumor measurement window ≤90 days
[7]Labs drawn within 72 hours prior to infusion
[8]timeframe for scans to be consistent with standard of care practice

Table 1: Data and Sample Collection Schedule

Lab draws will be performed at regularly scheduled clinic visits as outlined in Figure 1. Peripheral blood mononuclear cells (PBMCs) will be isolated and flow cytometry will be used to quantify MDSCs and T cells.

- **Baseline immune response determination.** Participants in each arm of the study will be asked to provide 40 mls of blood prior to initiating treatment to evaluate baseline immune responses and immune cell frequencies.

- **Immune response monitoring.** Repeated blood samples of 20 mls will be drawn prior to the administration of the 2nd and 4th doses of ipilimumab for continued measurement of immune cell frequencies.

- **Post-treatment immune response determination.** Participants will also be asked to provide 40 mls of blood prior to the administration of the 2nd dose and 3 weeks after the 4th dose of ipilimumab for evaluation of post-treatment immune responses.

- **Cytokine analysis.** Up to 20 ml of plasma will be stored for five years following study completion for subsequent cytokine analysis.

Following the standard of care for ipilimumab treatment, will use standard imaging techniques (RECIST criteria version 1.1) every 3 months to monitor the appearance of new metastatic lesions and the size of existing lesions.

Specimen Analysis

Functional assays will be performed to assess the ability of isolated MDSCs to suppress T cell responses.

- **Determination of tumor-specific T cell response frequency.** Peripheral blood mononuclear cells (PBMCs) will be isolated over a ficoll gradient and five million cells will be banked to analyze the frequency of MDSCs and T cells using flow cytometry as previously published. Approximately five million PBMCs will be used to determine the frequency of tumor-specific T cell responses by stimulating the cells with a mixture of peptides derived from known melanoma tumor antigens and measuring IFN-gamma production by flow cytometry.

- **T cell suppression assay.** The remaining PBMC will be used to perform T cell suppression assays using magnetically separated MDSC populations, comparing the suppressive function of MDSCs throughout treatment. Any remaining MDSCs will be flash frozen and banked for gene expression studies analyzing the expression of known suppressive molecules by real-time quantitative PCR.

- **Cytokine analysis.** Multiplex cytokine array will be used for subsequent cytokine analysis. Concentration of cytokines related to the function of MDSCs (GM-CSF, VEGF, MIP-1 alpha, MIP-1 beta, IL-10, IL-6, and IL-8) and T cells (IFN-gamma, TNF-alpha, IL-1-beta, IL-2, IL-4, IL-5, IL-12p70, IL-17) will be analyzed.

Study Completion and Follow-up

Following the standard of care for ipilimumab treatment, standard imaging techniques will be used according to RECIST criteria version 1.1 approximately every 3 months to monitor the appearance of new metastatic lesions and the size of existing lesions.

Patients who complete or discontinue the study will have a final clinic visit and will be followed by office visit or phone contact every 2 months for two years.

IV. Anticipated Risks

The characterization of potential side effects and toxicities associated with combined ipilimumab and VESANOID treatment is an important objective of this protocol. As VESANOID has not been administered in combination with ipilimumab, there are no clinical or safety data concerning this drug combination. No adverse events related to VESANOID treatment in patients with solid tumors have been reported. However, patients with acute promyelocytic leukemia treated with VESANOID may develop Retinoic Acid Syndrome, otherwise known as Differentiation Syndrome, characterized by fever, dyspnea, weight gain, hypotension, and pulmonary infiltrates.

Expected adverse events known to occur in a minority of patients treated with ipilimumab include immune-mediated enterocolitis, hepatitis, dermatitis, neuropathies, and endocrinopathies. A recent study showed that of the patients treated with ipilimumab monotherapy, 14.5% reported grade 3 or higher immune-related adverse events (irAEs) (24). The same study showed that of the patients treated with ipilimumab combined with the gp100 vaccine, 10.3% reported grade 3 or higher irAEs (24). Most of these events occurred within the 12 week treatment schedule, with only 1 patient (0.15%) reporting a grade 3 irAE >70 days after the final dose of ipilimumab (24). Most severe adverse events were successfully managed using published protocol-defined guidelines (administration of corticosteroids, delays in treatment schedule, or discontinuation of treatment); 1.1% of deaths were attributable to irAEs.

Following standard protocols for ipilimumab treatment, patients will be closely monitored for expected signs and symptoms of diarrhea, pain, mucus or bloody stool, fever, rash, muscle weakness, sensory alterations, headache, fatigue, mental status changes, hypotension, and visual disturbances.

Potential VESANOID Side Effects
Virtually all patients experience some drug-related toxicity, especially headache, fever, weakness, and fatigue. These adverse effects are seldom permanent or irreversible nor do they usually require interruption of therapy. Some of the adverse events are common in patients with APL, including hemorrhage, infections, gastrointestinal hemorrhage, disseminated intravascular coagulation, pneumonia, septicemia, and cerebral hemorrhage. The following describes the adverse events, regardless of drug relationship, that were observed in patients treated with VESANOID (tretinoin).

Typical Retinoid Toxicity
The most frequently reported adverse events were similar to those described in patients taking high doses of vitamin A and included headache (86%), fever (83%), skin/mucous membrane dryness (77%), bone pain (77%), nausea/vomiting (57%), rash (54%),

mucositis (26%), pruritus (20%), increased sweating (20%), visual disturbances (17%), ocular disorders (17%), alopecia (14%), skin changes (14%), changed visual acuity (6%), bone inflammation (3%), visual field defects (3%).

Body as a Whole
General disorders related to VESANOID (tretinoin) administration and/or associated with APL included malaise (66%), shivering (63%), hemorrhage (60%), infections (58%), peripheral edema (52%), pain (37%), chest discomfort (32%), edema (29%), disseminated intravascular coagulation (26%), weight increase (23%), injection site reactions (17%), anorexia (17%), weight decrease (17%), myalgia (14%), flank pain (9%), cellulitis (8%), face edema (6%), fluid imbalance (6%), pallor (6%), lymph disorders (6%), acidosis (3%), hypothermia (3%), ascites (3%).

Respiratory System Disorders
Respiratory system disorders were commonly reported in APL patients administered VESANOID (tretinoin) . The majority of these events are symptoms of the RA-APL syndrome (see boxed WARNINGS). Respiratory system adverse events included upper respiratory tract disorders (63%), dyspnea (60%), respiratory insufficiency (26%), pleural effusion (20%), pneumonia (14%), rales (14%), expiratory wheezing (14%), lower respiratory tract disorders (9%), pulmonary infiltration (6%), bronchial asthma (3%), pulmonary edema (3%), larynx edema (3%), unspecified pulmonary disease (3%).

Ear Disorders
Ear disorders were consistently reported, with earache or feeling of fullness in the ears reported by 23% of the patients. Hearing loss and other unspecified auricular disorders were observed in 6% of patients, with infrequent (<1%) reports of irreversible hearing loss.

Gastrointestinal Disorders
GI disorders included GI hemorrhage (34%), abdominal pain (31%), other gastrointestinal disorders (26%), diarrhea (23%), constipation (17%), dyspepsia (14%), abdominal distention (11%), hepatosplenomegaly (9%), hepatitis (3%), ulcer (3%), and unspecified liver disorder (3%). Incidents of suspected treatment-related hepatic toxicity will be closely monitored until resolution. If LFT abnormalities do not resolve with cessation of treatment and/or steroid therapy, consideration will be given to hepatitis B and C testing.

Cardiovascular and Heart Rate and Rhythm Disorders
Arrhythmias (23%), flushing (23%), hypotension (14%), hypertension (11%), phlebitis (11%), cardiac failure (6%) and for 3% of patients: cardiac arrest, myocardial infarction, enlarged heart, heart murmur, ischemia, stroke, myocarditis, pericarditis, pulmonary hypertension, secondary cardiomyopathy.
Central and Peripheral Nervous System Disorders and Psychiatric
Dizziness (20%), paresthesias (17%), anxiety (17%), insomnia (14%), depression (14%), confusion (11%), cerebral hemorrhage (9%), intracranial hypertension (9%), agitation (9%), hallucination (6%) and for 3% of patients: abnormal gait, agnosia, aphasia, asterixis, cerebellar edema, cerebellar disorders, convulsions, coma, CNS depression, dysarthria, encephalopathy, facial paralysis, hemiplegia, hyporeflexia, hypotaxia, no light reflex, neurologic reaction, spinal cord disorder, tremor, leg weakness, unconsciousness, dementia, forgetfulness, somnolence, slow speech.
Urinary System Disorders

Renal insufficiency (11%), dysuria (9%), acute renal failure (3%), micturition frequency (3%), renal tubular necrosis (3%), enlarged prostate (3%).
Miscellaneous Adverse Events
Isolated cases of erythema nodosum, basophilia and hyperhistaminemia, Sweet's syndrome, organomegaly, hypercalcemia, pancreatitis and myositis have been reported.
Cardiovascular
Cases of thrombosis (both venous and arterial) involving various sites (eg, cerebrovascular accident, myocardial infarction, renal infarct) have been reported rarely.
Hematologic
Rare cases of thrombocytosis have been reported.
Skin
Genital ulceration
Miscellaneous Adverse Events
Rare cases of vasculitis, predominantly involving the skin, have been reported.

The toxicity profile of VESANOID and Ipilimumab may overlap causing difficulty in determining the causative agent. Our investigators have many years of prior experience treating patients with Ipilimumab and their clinical judgement will be used to assess the toxicity and any potential relationship.

Dose Modifications for VESANOID

If patients experience grade 3 or 4 toxicity that is believed to be related to the VESANOID, then the VESANOID will be held until the toxicity resolves to grade 0 or 1. If the toxicity appears to be related to a combination of Ipilimumab and VESANOID, the VESANOID will be held. After the toxicity has resolved, the VESANOID will be restarted at the next scheduled time at a reduced does of 100mg/m2. If the grade 3 or 4 toxicity returns, the VEASOIND will be held indefinitely.

The severity of the adverse events will be rated on a scale of Grade 1-4 and reported according to the Data and Safety Monitoring Plan using CTCAE version 4.0. Medical oncologists at the University of Colorado Hospital Cutaneous Oncology Clinic specialize in melanoma and have extensive experience treating patients with immune-based therapies that cause immune-related adverse events. The primary investigator will be notified of all adverse events that occur during this trial and will work with Dr. Gonzalez and the treating oncology team to determine the likelihood of VESANOID’s contribution to the adverse event.

Risks to the patient include potential disclosure of protected health information, pain and bruising due to standard risks with venipuncture, and adverse events associated with ipilimumab and/or ipilimumab combined with VESANOID. The risks associated with participation in this trial will be discussed in full detail during the consent process and will be clearly presented to the patient in advance. All potential subjects have will have the option of receiving standard of care or supportive care/hospice care.

Protections Against Risk

Patients will be screened prior to study enrollment and monitored for adverse events during the study. Participants will be instructed to notify the clinical provider immediately for any adverse events.

- **Subject eligibility determination:** Study applicants will undergo complete laboratory tests and physical exams to determine their eligibility and safety of their participation in this study. Study applicants will be excluded if they do not meet the eligibility criteria. Subjects will also be excluded if other clinical problems that may contraindicate their participation in this study exist.

- **Adverse event assessment:** During the treatment phase of the study, participants will be asked about adverse events at each clinic visits and vital signs will be obtained before administering the study medication. Patients will not receive the medication if they have any signs of symptoms that may contraindicate its administration. All adverse events occurring during the course of the study will be collected, documented, and reported to the principal investigator. Each clinic visit, a study investigator will review the adverse events since the previous visit. The study investigators will follow all adverse events to the point of a satisfactory resolution. A study participant may have their medication discontinued or may be withdrawn from the study if the medically responsible investigator determines it is the best decision in order to protect the safety of a participant. All adverse events will be assessed to determine if they meet criteria for a severe adverse event. Adverse event collection will conclude at the final study visit, within 30 days of their final ipilimumab infusion.

- **Serious adverse event assessment:** Severe adverse events, as defined by the FDA, will be systemically evaluated at each clinic visit. Any severe adverse event, whether or not related to the study medication, will be reported to the principal investigator, the COMIRB, and the UCCC DSMC. Initial severe adverse event reports will be followed by submission of a completed severe adverse event reports to the above parties. In the event that a patient withdraws from the study or the investigator discontinues a patient due to severe adverse events, the patient will have the appropriate follow-up medical monitoring. Monitoring will continue until the problem has resolved or stabilized with no further change expected, is clearly unrelated to the study medication, or results in death. A summary of the severe adverse events that occurred during the previous 6 months will be included in the data safety monitoring report to the DSMC.

VESANOID Related Toxicity

Significant VESANOID related toxicity is defined as any >= grade 3 non-hematologic toxicity related to VESANOID, or other grade VESANOID related adverse events that lead to treatment discontinuation. An exception is VESANOID related headache not optimally managed with supportive care therapies (acetaminophen or non-steroidal anti-inflammatory medication).

Toxicity and Study Discontinuation Criteria

The trial stopping criteria outlined in Tables 2 and 3 below will be followed to avoid posing undue risk to additional patients. 24 patients will potentially be recruited in each arm of our study as necessary to fulfill the efficacy endpoint. Toxicity in Arm B will be monitored using the Bayesian approach (25, 26) as extended by Thall and Sung (27). Toxicity is defined as AEs of grade 3 or higher, any >= grade 3 non-hematologic toxicity related to VESANOID or combined Ipilimumab and VESANOID toxicity of grade 3 or higher.. Historical data of similar patients show an overall toxicity rate of 10-15% for ipilimumab treatment alone (Arm A). It is expected for the current trial that Arm A and B will have toxicity rates at a maximum of 15% and 25%, respectively.

The prior probabilities of toxicity for Arm A and B are modeled by minimally informative prior distributions (*Beta* (0.3, 1.7)). Denoting the historical probabilities of the rate of toxicity in Arm A by p(TOX, H), and the probabilities of the rate of toxicity in Arm B by p(Tox, Arm B), the following formula will be applied to calculate the trial stopping decision criteria: for Arm B, patient recruitment will stop if Prob{p(TOX, H) + δ_{TOX} < p(TOX, Arm B)| data} > 0.8, where δ_{TOX} = 0.1.
Patients in Arm B will be monitored according to the stopping boundaries for toxicity shown in Tables 2 and 3. If recruitment to Arm B is stopped due to toxicity, recruitment to Arm A will also be stopped. Patients with significant irAEs will be treated using protocol-defined guidelines.

Table 2: Calculated Trial Stopping Criteria for Ipilimumab 3mg/kg

# of patients in each arm	Stopping Criteria (x)[1]
6	3
12	5
18	7
24	End of trial
[1]Recruitment to both arms of the trial will be discontinued if the number of patients with grade 3 or higher irAEs in arm B is greater than or equal to x.	

Table 3: Calculated Trial Stopping Criteria for Ipilimumab 10mg/kg

Safety stopping boundary based on 95% exact lower confidence interval >=true grade 3 or 4 AEs rate

Number of enrollment	Stop for the number of AEs assuming the true AEs rate is 54% (single drug arm A)	Stop for the number of AEs assuming the true AEs rate is 60% (combo drug arm B)
7	7	No stop
8	8	8
9	9	9
10	>=9	10
11	>=10	11

12	>=11	>=11
13	>=11	>=12
14	>=12	>=13
15	>=13	>=14
16	>=13	>=14
17	>=14	>=15
18	>=15	>=16
19	>=15	>=16
20	>=16	>=17
21	>=17	>=17
22	>=18	>=19
23	>=18	>=19
24	>=19	>=20

V. Data Handling and Record Maintenance

Specimen labeling: All collected specimens will be labeled with the study number, patient number, and date. Specimens will be collected by the research staff, processed according to protocol-specific procedures, and stored in locked freezers until assays are performed. To maintain confidentiality patient names and hospital numbers will not be used. Only the clinical provider, the study coordinator, and data manager will have access to the subject identities.

Clinical data collection: Clinical data will be collected during each visit by the clinical provider and stored in a locked office or cabinet with access limited to the study coordinator and/or data manager. Age, gender, pathologic features of the melanoma, co-morbid conditions, and concomitant medications will be recorded.

Paper forms (Appendix 1) will be used for the initial recording of clinical data. Data will be recorded legibly in black ballpoint. Corrections will be made legibly, initialed, and dated. Correction fluid and covering labels will not be used. Data quality will be monitored by random inspection of the completed forms by a research assistant and any problems will be discussed with the PI.

Database: Study data will be collected and managed using REDCap (Research Electronic Data Capture). REDCap is a secure web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails and a de-identified data export mechanism to common statistical packages (28). The database is hosted at the University of Colorado–Denver Development and Informatics Service Center (DISC), which will be used as a central location for data processing and management. REDCap data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the DISC. The research team can create and design surveys in a web browser and engage potential respondents using a variety of notification methods.

The database will be designed and maintained by the study coordinator and the data manager. The study coordinator will enter the following data at the conclusion of each clinic visit:

- Subject information form (name, hospital identification number, study identification number, date of birth, gender, ethnicity, and Stage of disease)
- Subject visit form (date of visit, treatment received, and pill counts)
- Adverse event form (signs and symptoms, onset, severity, toxicity grade, action taken, outcome, likeliness of being study related)

The data manager will enter the following data at the completion of the study:

- Disease history (primary diagnosis, disease progression)
- Treatment history (previous treatments)
- Sample Information (blood volume, cell counts, storage location, assays performed)

Data and Safety Monitoring

The Principal Investigator in collaboration with Dr. Rene Gonzalez, a medical oncologist and director of the Melanoma Research Clinics at the University of Colorado Denver, will be responsible for monitoring the trial per the trial monitoring plan, monitoring the safety and efficacy of the trial, executing the DSM plan and for complying with all reporting requirements to local and federal authorities. This oversight will be accomplished through additional oversight from the Data and Safety Monitoring Committee (DSMC) at the University of Colorado Cancer Center (CU Cancer Center). The DSMC is responsible for ensuring data quality and patient safety for all clinical studies at the CU Cancer Center.

The team of treating medical oncologists and the PI will meet weekly to discuss the progress of the clinical trial and any adverse events. The principal investigator will provide a Data Safety Monitoring report to the DSMC every 6 months that will include a protocol summary; current enrollment numbers; summary of toxicity data to include specific SAEs, UAPs and AEs; any dose modifications; all protocol deviations; and protocol amendments. The DSM report to the DSMC will also include, if applicable, the results of any efficacy data analysis conducted. Results and recommendations from the review of this six month report by the DSMC will then need to be submitted by the site to the IRB of record at the time of continuing review. A summary of the DSMC activities follows:

- Conduct of internal audits
- Ongoing review of all reportable adverse events and all serious/unanticipated adverse events
- Has the authority to close and/or suspend trials for safety or trial conduct issues
- May submit recommendations for corrective actions to the CU Cancer Center's Executive Committee

Per the CU Cancer Center Institutional DSM Plan, SAEs, UAPs, and reportable AEs are reported to the DSMC and the IRB. All SAEs, UAPs, and reportable AEs are to be reported to the DSMC within 5 business days of receiving notification of the occurrence.

The principal investigator will be notified of any recommendation in order that he may alert all investigators involved in the trial with regard to the action plan.

Each subject's treatment outcomes will be monitored at weekly team meetings by the Investigators and Clinical Research Coordinators (CRCs). Toxicity will be addressed in real time and communicated to the team, then submitted to DSMC as indicated. Data regarding number of subjects, significant toxicities, dose modifications, and responses will be discussed and documented in the minutes.
The PI will provide a DSM report to the UCCC DSMC on a six month basis after the first patient is enrolled. DSM reports will contain data from all participating sites. The DSM report will include summaries of minutes taken at monthly meetings, the participants' demographic characteristics, expected versus actual recruitment rates, treatment retention rates, any quality assurance or regulatory issues (including a summary of any protocol deviations), summary of AEs and SAEs, summary of dose modifications, and any actions or changes with respect to the protocol. The DSM report to the DSMC will also include, if applicable, the results of any efficacy data analysis conducted. Results from these reviews will be provided to all participating investigators to submit to their IRBs at the time of continuing review.

Serious Adverse Events, Unanticipated Problems, and Other Reportable Events

All SAEs will be submitted to the DSMC mailbox within 5 business days of Primary Investigator becoming aware of the event. All SAEs and UAPs will be submitted to COMIRB within 5 business days of Primary Investigator becoming aware of the event. A composite list of all reportable events will be provides to COMIRB with each annual review.

An adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- death
- life-threatening adverse event
- inpatient hospitalization or prolongation of existing hospitalization
- persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Unanticipated Problems (UAPs) include adverse events which in the opinion of the principal investigator comprise

- both unexpected and probably or definitely related to the drug
- any unforeseen development that potentially increases the likelihood of harm to participants or others in the future
- information that indicates a change to the risks or potential benefits of the research

- an actual unforeseen harmful or unfavorable occurrence to participants or others that relates to the research protocol (injuries, psychological events, drug errors).

Other Reportable Adverse Events include

- death unrelated to study treatment if occurring within 30 days of the last study treatment
- death at least possibly related to study treatment at any timepoint
- secondary malignancy
- pregnancy
- fetal death
- neonatal death

VI. Data Analysis Plan

We will compare the frequency of MDSC subsets, the suppressive function of MDSCs, the frequency of T cell subsets, the expression of activation markers, and the CTL response within each group using one-sided one-group t-tests with an alpha of 0.05. Two-group t-tests will be used to compare the changes before and after treatment between groups. P values and corresponding effect sizes will be reported. Kaplan-Maier overall survival curves and interval censored progression-free survival curves for the two arms will be generated. Median survival times and 95% confidence intervals will be reported. Correlations between the frequencies of MDSCs and T cell subsets or expression of activation markers will be evaluated using Pearson Correlation Coefficients.

The characterization of potential side effects and toxicities associated with combined ipilimumab and VESANOID treatment is an important objective of this protocol. Safety outcomes will be assessed using the Bayesian approach. Study discontinuation criteria will be followed as outlined in Table 2.

Power Analysis

We powered our study based on the second of our primary objectives, to show that VESANOID treatment reduces the frequency of MDSCs in melanoma patients. Our previous study showed that a small sample size was sufficient to detect two-fold increases in the frequency of MDSCs in Stage IV melanoma patients ($n = 34$, $p = 0.041$) (11). Furthermore, a recent clinical trial combining VESANOID and a dendritic cell vaccine found approximately two-fold decreases in the frequency of MDSCs with 12 patients treated with VESANOID ($p = 0.02$) (13). With a conservative assumption of a large variance for the change in MDSC frequency in each group (assume the measurement for pre- and post-treatment has a correlation coefficient = 0.1 and used the larger variance of the two to obtain a variance = 6 for the change), and a conservative estimate for the magnitude of change in the Arm B (1.8, or 10% less than was observed in the previous clinical trial using VESANOID), a sample size of 24 patients in each arm will provide 80% power to detect differences in the change of MDSC frequency between Arm A and Arm B using one-sided two-group t-tests with an alpha of 0.05. This sample size will also provide

over 85% power to detect the change of 1.5 in MDSC frequency before- and after-treatment in Arm B using one-sided one-group t-tests with an alpha of 0.05.

Potential Scientific Problems

Combined treatment with VESANOID and ipilimumab may result in excessive toxicity. To reduce this likelihood, we proposed a short course of VESANOID treatment at a dose with low reported toxicity. The number of agents approved to treatment advanced melanoma has changed significantly in the past few months making projections on enrollment more challenging. However, one recent change is the approval of ipilimumab for the adjuvant treatment of resected stage III melanoma. We believe this may allow for the recruitment of additional patients who would receive ipilimumab as standard of care. Our capable clinical research team will closely monitor the safety and recruitment of patients, feasibly recruiting 48 patients within the time frame of this study. Due to the expertise and proficiency of our laboratory research team, we do not anticipate technical difficulties that would limit our ability to complete this project. However, the effect of VESANOID may be weaker than the previous study on which we based our patient sample size. Although we believe this study will provide valuable information regardless of the outcome, we plan to compare the effects of VESANOID in patients with high versus low pre-treatment frequencies of MDSCs as a potential biomarker for targeting this combined treatment to subsets of melanoma patient's in future clinical trials.

Knowledge to be Gained

Approximately 20% of patients receiving ipilimumab monotherapy respond to treatment and have prolonged median survival of approximately 4 months. Patients in both arms of the study may potentially benefit from ipilimumab treatment. Our hypothesis is that adding VESANOID to ipilimumab will reduce immunosuppression and may potentially increase the efficacy of ipilimumab. Therefore, the possible benefits outweigh the risks associated with participation in this study. Furthermore, the exploratory research aims of the study may guide future therapy by improving our general knowledge about melanoma induced immunosuppression regardless of the clinical outcomes.

This trial is testing the general hypothesis that combining drugs that target immune cell activation with drugs that target blocking immunosuppression are more beneficial to the treatment of melanoma patients. We will also be collecting data on the response of the immune system to this treatment both in frequency and in function. The knowledge to be gained is how VESANOID affects the immune system, whether it augments the immune cell activation caused by ipilimumab, and whether it improves clinical responses to ipilimumab. Although we are testing these hypotheses in melanoma patients, this knowledge may be applicable to other cancer patients where ipilimumab is indicated. Furthermore, these data may help in the design of future combinatorial treatments as new drugs that target immune cell activation become available.

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Appendix I

Sample Data Collection Forms

Appendix II

Current FDA Approval/ VESANOID Package Insert