Protocol Title:	A prospective, randomized, blinded, placebo-controlled, phase IIb trial of an autologous tumor lysate (TL) + yeast cell wall particles (YCWP) + dendritic cells (DC) vaccine vs unloaded YCWP + DC and embedded phase I/IIa trial with tumor lysate particle only (TLPO) vaccine in stage III and stage IV (resected) melanoma to prevent recurrence.
Protocol Number:	20141932
Study Drugs:	Autologous TLPLDC vaccine (tumor lysate, particle-loaded, dendritic cells) and Autologous TLPO (tumor lysate, particle only)
Sponsor:	Elios Therapeutics, LLC
Director:	George E. Peoples, MD, FACS Cancer Insight, LLC 1305 East Houston Street San Antonio, Texas 78205
IND:	TLPLDC IND#16101 & TLPO IND#17274
Principal Investigator:	Mark B. Faries, MD, FACS Head, Division of Surgical Oncology Co-Director, Melanoma Program Surgical Director, Experimental Therapeutics The Angeles Clinic and Research Institute, a Cedars-Sinai Affiliate 11818 Wilshire Blvd., Suite 220 Los Angeles, CA 90025
Medical Monitor:	Vance Sohn, MD
Protocol Version/Date:	2.4/ 15 February 2021

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1.0 PROTOCOL SYNOPSIS

Brief Title	A prospective, randomized, blinded, placebo-controlled, phase IIb trial of an autologous tumor lysate (TL) + yeast cell wall particles (YCWP) + dendritic cells (DC) vaccine vs unloaded YCWP + DC and embedded phase I/IIa trial in tumor lysate particle only (TLPO) vaccine in stage III and stage IV (resected) melanoma to prevent recurrence.	
Test Article	Name	Autologous TLPLDC vaccine (tumor lysate, particle loaded, dendritic cells) Autologous TLPO vaccine (tumor lysate, particle only)
	Activity	Induction of anti-cancer cell-mediated immune response
	Dosage form	Intradermal injection of 1-1.5x10^6 autologous TLPLDC or 1.0x10^8 TLPO monthly x 3 followed by boosters at 6, 12, and 18 months
Development Phase	Phase IIb (with embedded phase I/IIa)	
Indication	To prevent recurrences in disease-free stage III and stage IV (resected) melanoma patients	
Rationale	**Setting:** Melanoma is a potentially lethal skin malignancy. Patients with stage III and IV (resected) melanoma have a significant risk of recurrence in the 50-90% range. Once recurrent, these patients will likely die of disease due to the paucity of effective systemic therapies. Melanoma is an immunogenic cancer often eliciting an endogenous immune response; however, if this protective mechanism fails then the disease progresses. Multiple immunotherapies have been tested in melanoma attempting to reinstate this immune protection. Some of the immunotherapies which have shown promise have been cytokine therapies (IFN-α, IL-2) as melanoma is an immunogenic cancer often eliciting an endogenous immune response; however, if this protective mechanism fails then the disease progresses. Multiple immunotherapies have been tested in melanoma-PD-L1 antibodies have shown significant promise with the FDA approval of the former in the metastatic setting. While these FDA-approved immunotherapies are effective in a small percentage of melanoma patients, they are non-specific, often toxic, and poorly tolerated. Vaccines are meant to raise a more specific, tumor-targeted immune response with minimal toxicity. Unfortunately, the majority of melanoma vaccines have been tested in late-stage patients with substantial tumor burden and have shown minimal benefit. Current data suggests that the best time to intervene with a vaccine strategy is in the adjuvant setting after patients have been rendered disease-free by standard of care (SoC) therapies. In this setting, the vaccine-induced immune response can attack any minimal residual disease and hopefully provide long-term protection through immunologic memory with minimal toxicity.	

Rationale **(continued)**	**Vaccine:** The majority of melanoma vaccines tested to date have been antigen-specific vaccines targeting melanoma-specific or associated antigens and utilizing a variety of delivery systems and immune-adjuvants. As opposed to testing an "off the shelf" vaccine that might be able to treat a subset of patients, our approach has been personalized to the patient and applicable to all patients. Our vaccine approach consists of harnessing the most potent antigen presenting cell in the body – the dendritic cell (DC) – together with the full repertoire of tumor antigens from an individual's cancer. We have conducted phase I and II studies using an autologous DC-tumor cell fusion technique that has now been simplified into a DC-tumor cell lysate vaccine. The autologous tumor lysate (TL) is loaded into yeast cell wall particles (YCWP) that are naturally and efficiently taken up into the patient's DC. These autologous tumor lysate, particle-loaded, DC (TLPLDC) are injected intradermally (ID) monthly x 3 followed by boosters at 6, 12, and 18 months. The embedded phase I/IIa will introduce the next version of the vaccine, which simplifies the production process. It utilizes the autologous tumor to create the tumor lysate, which is then loaded into the yeast cell wall particles and then capped with silicate for stability. This tumor lysate, particle only (TLPO) vaccine, is then injected intradermally and taken up *in vivo* by dendritic cells and stimulates the patient's immune system to recognize the tumor. These autologous TLPO vaccines are injected in the same manner as the TLPLDC, monthly x3 followed by booster at 6, 12, and 18 months. **Prior Results:** To date, we have vaccinated a total of 36 patients with varying malignancies, and 25 of these have been late-stage melanoma patients. These personalized vaccines have a very favorable toxicity profile with only flu-like symptoms immediately after inoculations, and no related serious adverse events have been observed in any patient vaccinated to date. In the completed phase I/IIa melanoma trial, 25 stage IV patients were vaccinated. The median overall survival (OS) was 14.5 months (compared to a historic rate of 8.5 months), and there was a 27% OS at 3 yrs. There was a dose response with an OS improvement in patients receiving ≥3 inoculations compared to patients receiving less (log rank p=0.025). Additionally, among the small group of stage IV (resected) patients (n=4), the OS was 100% at 20 months median follow-up.
Primary Endpoint	For the TLPLDC phase IIb: To determine 24-month disease-free survival (DFS) in vaccinated (TLPLDC) vs control patients. For the TLPO phase I/IIa: To assess the safety of the vaccine per CTCAEv4.03
Secondary Endpoints	For the TLPLDC phase IIb: 1) To determine DFS and OS at 36 months in vaccinated (TLPLDC) vs control patients. 2) To document the safety of the vaccine.

Exploratory Endpoints	For both trials: 1) To document immunologic response to the vaccines through T-cell assays recognizing known melanoma-specific antigens. 2) To correlate vaccine immune response to clinical outcome. 3) To assess safety and tumor response of continued vaccination in combination with SoC therapies in patients after recurrence.
Patient Population	Stage IIIA-C, Stage IV (resected) melanoma patients identified prior to definitive surgery and anticipated to be clinically disease-free after surgery.
Treatment Arms	For the TLPLDC phase IIb: 1) Vaccinated group = autologous tumor cell lysate + yeast cell wall particles + autologous DC (TLPLDC) 2) Control group = empty yeast cell wall particles + autologous DC For the TLPO phase I/IIa: 1) Vaccinated group = autologous tumor cell lysate + yeast cell wall particles (TLPO)
Stratification, Randomization, and Crossover	Patients will be stratified based on stage III vs stage IV. Randomization will be 2:1 (vaccine:control). All patients will be offered open label active vaccine at first sign of recurrence. Non-recurrent control patients will be given the option of receiving active vaccine upon their completion of the trial. Upon randomization of the 120th patient in the TLPLDC phase IIb, the TLPO phase I/IIa trial will be initiated and 60 additional patients will be randomized 2:1 (TLPO phase I/IIa:TLPLDC).
Number of Patients	At least N= 180
Sample Size Justification	Assuming a baseline recurrence rate of 60% (corresponding to a DFS of 40%) at 2 years in this mixed group of stage III and stage IV (resected) melanoma patients, a sample size of approximately 120 will have 80% power to detect a statistical difference between treatment proportions controlling the type I error at alpha = 0.05 (two-sided). A blinded evaluation of the first 75 patients enrolled discovered an early recurrence rate of 12% prior to completion of primary vaccine series. To compensate for this early recurrence rate and preserve the power of the trial, enrollment will be extended by at least an additional 20 patients, now totaling at least 140. The TLPO phase I/IIa will be assessed primarily for the tolerability of the TLPO vaccine as determined by CTCAEv4.03. Additionally, immunologic assays will be assessed for vaccine induced T cell recognition of common melanoma-related antigens. Patients will be followed clinically per standard of care for disease status.
Number of Sites	10-12 sites will be established.
Duration of Trial	With 10-12 sites enrolling at 0.8 pts/site/month, we anticipate enrollment to be complete in 12-18 months. With a primary endpoint of 2 year DFS and secondary endpoints of 3 year DFS/OS, the trial duration is expected to be 4-4.5 years.

Trial Design and Conduct	Stage III and Stage IV (resected) melanoma patients will be identified prior to definitive surgery and screened for inclusion/exclusion criteria. Eligible patients will be counseled and consented for tissue procurement. Enrolled patients will have their disease surgically resected and a portion (1mg minimum) of their melanoma sterilely frozen in provided freezing vials and storage tubes. This tissue will be shipped in liquid nitrogen shippers through FedEx to our central facility in Greenville, SC and stored frozen until vaccine preparation. If patients cannot be rendered disease-free, they will be considered screen failures for this study. If melanoma is being resected from multiple locations (primary and nodes, two different metastatic sites), then samples of each would be preferred but not mandatory. As indicated by SoC per the National Comprehensive Cancer Network (NCCN) guidelines and determined by the treating team, if a patient is to receive systemic therapy (chemotherapy or IFNα and/or radiation therapy), then the vaccinations will not begin until SoC therapy is completed. Although, patients on adjuvant check point inhibitors (CPI) may start the vaccine trial after showing tolerance of the CPI after 3 doses, given that there may a synergistic benefit and no added toxicity when adding the vaccine to CPI. Once SoC therapies are complete (or demonstrating tolerance on CPI after 3 doses) and the patient deemed clinically disease-free, they will be consented for treatment and randomized. Once consented, patients will receive a single injection of Neupogen (G-CSF) 300 µg (or its equivalent) SQ 24-48 hrs prior to having 70 mL of blood collected and sent to our central facility for DC isolation and preparation. Patients who cannot tolerate Neupogen, or its equivalent or refuse it, will have 120 mL of blood drawn and sent. Additional blood may be drawn if additional vaccine doses need to be made or re-made for any reason. Vaccines will be prepared by producing TL through freeze/thaw cycling and then loaded into pre-prepared YCWP. The TL-loaded YCWP will be introduced to the DC for phagocytosis thus creating the TLPLDC vaccine, which will be frozen in single dose vials. Each vial will contain 1-1.5 x 10^6 TLPLDC and will be labeled with the patient's unique study number. The TLPO is created in a similar manner with the freeze/thaw cycling, loaded into pre-prepared YCWP, and then capped with silicate for stability. The TLPO vaccine is then frozen in single dose vials of 1.0 x 10^8 TLPO and will be labeled with the patient's unique study number. Based on their randomization, autologous TLPLDC (active vaccine) or unloaded YCWP + autologous DC (control) or autologous TLPO will be sent back to the site in a blinded fashion. Upon randomization of the 120th patient the randomization will transition to 2:1 (TLPO phase I/IIa:TLPLDC). Regardless of assigned group, the site will receive 6 single dose vials to be injected intradermal monthly x 3 followed by boosters at 6, 12, and 18 months in the same lymph node draining area (preferably the anterior thigh). Patients must begin vaccinations between 3 weeks and 3 months from completion of SoC (or demonstrating tolerance on CPI after 3 doses). Frozen tumor will be maintained for active vaccines for all patients to include the control patients. The latter will be offered their active vaccine at time of recurrence in a crossover fashion. Additionally, control patients who do not recur will be offered active vaccine at the completion of the trial. Safety data will be collected on local and systemic toxicities and graded and reported per the Common Terminology Criteria for Adverse Events (CTCAE) v4.03. Disease-free status will be monitored per SoC as outlined by NCCN. Suspected recurrences will be documented with biopsy and pathologic confirmation. Time to recurrence will be based on date of randomization to time of confirmed recurrence. Recurrent patients will be offered participation in the open label portion of the study. New active vaccine will be made for all patients, and they will be inoculated at 0, 1, 2, 3, 6, and 9 mos. Patients will be treated per SoC for their recurrence. Safety and tumor response will be assessed per RECIST and iRECIST on their SoC follow-up scans. Blood (50 mL) will be collected from all patients prior to each inoculation and at 24 months from enrollment for a total of 7 time points or a total of 350 mL of blood over 2 years. The collected blood will be sent to our central facility for immunologic testing of the T-cell response.

Inclusion Criteria	1) Stage IIIA-C patients identified prior to their definitive surgery 2) Stage IV patients rendered clinically disease-free with surgery 3) Approximately 1 cm^3 is preferred (but 1 mg minimum) of accessible and dispensable tumor that will not interfere with pathologic staging 4) Clinically disease-free after SoC therapies OR clinically disease-free after 3 doses of CPI therapy, demonstrating tolerance of CPI, and completion of other SoC therapies 5) ECOG 0-1 performance 6) Not involved in other clinical trials 7) Capable of giving informed consent
Exclusion Criteria	1) Evidence of residual disease after surgery and SoC therapies 2) Insufficient tumor available to produce vaccine 3) Immune deficiency disease or HIV, active HBV, or active HCV 4) Steroids or other immunosuppressants 5) ECOG $\geq$2
Statistical Analysis	The primary endpoint for analysis of clinical efficacy is 24 months DFS when analyzed as a proportion using Pearson chi-square test. The intention-to-treat (ITT) population analyses and the per protocol (PP) population analyses will be co-primary given the high early recurrence rate. In the time -to-event analysis, Kaplan-Meier curves will be generated to display time to events for each treatment group. The null and alternative hypotheses are: H0: hV / hC = 1 Ha: hV / hC ≠ 1 where hV is the constant autologous TLPLDC vaccine hazard rate and hC is the constant unloaded YCWP + autologous DC control hazard rate. The active vaccine efficacy is demonstrated when hV /hC < 1; the alternative hypothesis is a 0.50 hazard ratio. The overall type 1 error rate of 5% will be used for the rejection of the null hypothesis in favor of the alternative hypothesis that autologous TLPLDC or TLPO vaccine will prolong DFS over that of unloaded YCWP + autologous DC. The stratification factors are the following: Stage (III vs IV) The time-to-event analysis efficacy analysis will be based on a proportional hazard model including the stratification factors to estimate the hazard ratio. Hazard ratios and 95% 2-sided confidence intervals will be presented. The model will also examine 2-way treatment interactions with the baseline stratification factors. Confirmatory analyses will be performed using a stratified log rank test for each of the stratification factors. Subgroup analyses will be displayed; any p-values for individual strata will be regarded as descriptive statistics. To explore the possibility of a treatment by investigative site interaction, the primary analysis will also be conducted by site for the ITT and PP populations. Sites that have enrolled large numbers of subjects relative to other trial centers will be identified to see if the results obtained at those investigative sites are influential. Investigative sites that individually represent fewer than 10 subjects will be combined for this exploratory analysis; geographic region will be substituted if the average number of subjects per site is fewer than 10.

Planned Survival Analyses	1) The primary analysis will be conducted at the completion of two years of follow-up on the last enrolled patient and will compare 2-year DFS between the two treatment arms. 2) An interim analysis is planned six months after enrollment of the 120th patient. This analysis will compare median DFS between treatment groups. 3) The secondary analysis will be conducted at the completion of 3 yrs. of follow-up on the final enrolled patient and will compare 3-year DFS and OS between treatment groups.

2.0 INTRODUCTION

2.1 Immunotherapy in Melanoma

Although melanoma is capable of eliciting an endogenous immunogenic response, the disease progresses when such protective mechanism fails. After standard of care (SoC) therapy of stage III and IV melanoma, clinically disease-free patients have a 50-90% risk of recurrence. Once recurrent these patients will likely die from their disease. Currently, chemotherapy, radiation therapy and immunotherapy are the main choices for metastatic disease with the hope of prolonging survival[1]. The majority of chemotherapy trials have been disappointing with no consensus standard therapy for care. Overall, immunotherapy appears to be the most promising. Even though immunotherapy has been studied for more than half a century as a treatment for cancer, recent advances in molecular immunology now make this strategy a viable option for the treatment of patients with advanced cancers[2].

Several immunotherapeutic strategies have been studied as methods to re-instate the innate and adaptive immune responses in melanoma. IFN-α and IL-2 immunotherapies are promising cytokine therapies having gained U.S. Food and Drug Administration (FDA) approval for treatment of metastatic melanoma. More recently, ipilimumab, a monoclonal antibody (mAb) targeting CTLA-4, was approved by the FDA for use in the metastatic setting in 2011.

IL-2 therapy is a common agent utilized in community practice despite its association with significant toxicities[3-5]. Multiple phase II trials have evaluated IL-2 therapy in conjunction with chemotherapy (cisplatin, vinblastine, dacarbazine, interferon-alpha, and IL-2), a treatment strategy with complete response rates of 15-21% and overall response rates as high as 64%[6-8] prompting FDA approval in 1998. A phase III trial evaluating biochemotherapy (cisplatin, vinblastine, dacarbazine, IL-2 and interferon alpha-2b) showed an increased response rate and progression-free survival in comparison to chemotherapy alone, but this was not associated with increased overall survival (OS) or improved quality of response[9]. Subsequent meta-analysis also showed no survival benefit of biochemotherapy in metastatic melanoma despite an improved overall response rate[10]. With toxicities of biochemotherapy substantially higher than chemotherapy alone, attempts to decrease this toxicity by administration of lower doses of IL-2 in the outpatient setting failed to show benefit of this approach vs chemotherapy alone[11-13]. Additional synergistic toxicities between IL-2 and ipilimumab (discussed below) therapies are also being brought into question[14].

Interferon therapy has been shown to increase DFS but the impact on OS remains unclear[14]. The World Health Organization conducted the initial major randomized trial examining low dose adjuvant interferon in resected stage III melanoma without an improvement in OS[15].

Two subsequent randomized studies in resected stage IIb/III melanoma also failed to show improvement in OS or recurrence-free survival[16].

However, in another prospective trial in stage II melanoma patients, DFS was increased by 41 months in the adjuvant interferon group[17]. In an adjuvant trial by the French Cooperative Group, a significant relapse-free survival benefit and trend towards increased OS was noted in clinically node-negative patients with melanoma > 1.5 mm[18]. A randomized trial by the Eastern Cooperative Oncology Group (ECOG 1684) examining high-dose interferon-α -2b has also shown significant improvement in relapse-free and OS at 6.9 months median follow-up in stage IIb and stage III melanoma; although, this effect later waned at 12.6 months median follow-up[19] with a larger follow-up trial confirming the lack of OS survival advantage[20]. A pooled analysis of the three randomized trials examining high dose interferon-α (E1684, E1690, and E1694) confirmed an improvement in relapse-fee survival in the high-risk melanoma population without improvement in OS[21]. The FDA has since approved the adjuvant use of pegylated interferon-α in node-positive melanoma patients based upon a significant 4-year relapse-free survival benefit (45.6% vs 38.9%) seen in a randomized trial of 1,256 patients with completely resected stage III melanoma[22]. Yet, despite FDA approval the high toxicity of adjuvant high-dose interferon is causing decreased use in most institutions although most National Comprehensive Cancer Network (NCCN) panelists agree on its role in specialized situations[14].

Ipilimumab gained FDA approval in the metastatic setting after a randomized phase III trial revealed a significant OS advantage from gp100 and ipilimumab combination therapy or ipilimumab monotherapy compared to gp100 monotherapy[23]. A significant OS benefit has been shown in a phase III trial of ipilimumab plus dacarbazine as well[24]. Unfortunately, ipilimumab's stimulation of T-cells may be associated with significant toxicity with immune-related events occurring in 60% of patients from the gp100 and ipilimumab combination trial. In this trial, 10-15% of the ipilimumab-associated immune-related adverse events were severe (grade 3 or 4) with immune-related toxicity attributed as the cause of seven deaths. As a T-cell stimulator, ipilimumab is specifically associated with significant risk of autoimmune reactions, and with the overall response rate occurring in less than 20% of patients, clinical effects often take months to become apparent. However, when therapeutic responses occur, they are often sustainable raising questions of application in the adjuvant setting. ECOG is currently conducting a prospective randomized trial of ipilimumab vs IFN-α in the adjuvant setting to prevent recurrence in stage III melanoma[25].

Although ipilimumab represents the only FDA approved immunostimulatory mAb, promising results are being obtained from clinical trials examining the safety and efficacy of the new programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway blocking antibodies. The PD-1/PD-L1 pathway has been increasingly utilized after being found to play an integral role in tumor-induced immunosuppression[26, 27]. Anti-PD-1 and anti-PD-L1 agents are currently being tested in early clinical trials[28].

A recent phase I trial boasts rapid response with 31% of advanced melanoma patients reported to have an objective response at 8 weeks with a median OS of 16.8 months for the total study population[29]. Noting the possible complementary role between CTLA-4 and PD-1, a phase I clinical trial cohort study examined this combination therapy with evidence of tumor regression in a substantial number of patients[30]. Given these results, a phase 3 trial comparing CTLA-4/PD-1 to either agent alone is currently underway (NCT01844505)[31].

Although promising, setbacks of current therapies include the small percentage of melanoma patients in which efficacy is shown, lack of specificity, and high toxicities. Although efficacious against some tumors, the potency of the above approaches is limited by their non-specific engagement of the host immune system. An ideal strategy will utilize the full potential of the host immune system but in a specific/targeted manner. As such, a tumor-targeted vaccine strategy is an appealing adjuvant strategy.

2.2 Dendritic Cell Vaccines

Sipuleucel-T (Provenge), an autologous dendritic cell-based vaccine, is the only FDA-approved cancer vaccine. The phase III trial examining Provenge's three-shot inoculation series improved OS by 4.1 months over placebo[32] without toxicity leading to regulatory approval for use in patients with metastatic castration-resistant prostate cancer in 2010.

Alone or in combination with cytokines, vaccines hold promise of eliciting a sustainable, more specific, tumor-targeted immune response with minimal toxicity. Several laboratories have demonstrated that tumor cells persist in part because they have one or more mutations that allow escape of immune surveillance[33-36]. As exemplified by sipuleucel-T, dendritic cells can be used as professional antigen-presenting cells (APC) to present tumor antigens to the immune system in an attempt to overcome this escape.

In order to stimulate robust anti-tumor immunity with activation of $CD8^+$ T-cells, tumor antigens must be presented by APC in the context of MHC Class I molecules. However, endocytosis of tumor antigens exposed to dendritic cells directs presentation by MHC Class II eliciting helper T-cells only, thus failing to engage the full power of the immune system with activation of a cytotoxic T-lymphocyte (CTL) response.

Laboratories have attempted to use gene transfer methods to introduce specific tumor antigens into dendritic cells from which they will be presented via MHC Class I[37-41]. Unfortunately, numerous challenges include: 1) the inability to identify and map all integral tumor specific antigens; 2) the limited number of known tumor antigen genes that can be introduced into the dendritic cell; and 3) the complicated, time-consuming process required of this strategy.

Given the potential disadvantages of specific antigen identification, an ideal strategy involves inclusion of the entire content of tumor cells within the dendritic cells. As a result, the entire portfolio of potential tumor antigens will be included within the APC. Formation of a dendritic and tumor cell hybrid cell is one method to harness the DC with the full repertoire of tumor antigens from an individual's cancer[37, 42]. As such, it is an approach personalized to the patient while potentially available to all patients. Both peptide and tumor lysate-loaded dendritic cells have been previously utilized to vaccinate patients[43-49]. A preferred method to manufacture a tumor cell/dendritic cell hybrid uses un-cultured, post-irradiation tumor cells to produce a non-proliferative hybrid cell or dendritoma that retains both the character of the tumor cells in addition to the ability to act as an effective APC. Animal studies have shown these hybrid cells capable of producing an anti-tumor specific immune response[50-58]. Results from in vitro studies [37, 59, 60] and human clinical trials[42, 60] are also encouraging. Scientists have observed that DC pulsed with tumor proteins can be administered repeatedly without side effects, suggesting the safety of DC-based vaccines.

Drawbacks of this approach include the time consuming production process that makes application of a dendritoma vaccine within the clinical setting difficult. Therefore, we developed a novel technique by which hybrid cells can be easily purified from a fusion mixture, instantly and without culture[61]. Hybrid cells are instantly purified from these fusions between DC and tumor cells. This process allows these dendritomas to retain the characteristics of the tumor cell as well as the ability of the DC to act as an effective APC. Animal studies have confirmed that the purified dendritomas are better activators than fusion mixtures in stimulating specific anti-tumor immunity[62]. With the hypothesis that the use of highly purified hybrid cells from DC-tumor cell fusion would be more effective than the entire fusion mixture in stimulating tumor cell-specific antitumor immunity, we examined this instant dendritoma purification technique in a pilot study of ten stage IV renal cell carcinoma patients and phase I and IIa trials in stage IV melanoma patients. In combination with IL-2 therapy, dendritomas prepared from autologous DC and tumor cells were administered by subcutaneous injection. With toxicities related to IL-2 therapy, these studies showed both clinical and immunologic disease-specific responses[63].

2.3 New Vaccine Technology

Unfortunately, although injection of these dendritomas allows for effective presentation of tumor epitopes to patients' immune systems to stimulate a $CD8^+$ CTL anti-tumor response[61, 62] sufficient tumor tissue is often unavailable to create a dendritoma vaccine for most solid tumor patients.

New technology whereby patients' tumor cell lysate is loaded into micro particles to be phagocytized by DC offers an alternative when sufficient tumor tissue in not available.

Reproducing the strong $CD8^+$ immune response observed previously with the dendritoma vaccines, this technology takes advantage of an immature DC's avid phagocytosis of small particles in the range of 1-5 microns. Using a minimum 250-500 microgram sample of tumor tissue, it is repeatedly subjected to freeze-thaw cycling to create a tumor lysate suspension that is then loaded within the inner space of denatured yeast cell wall particles (YCWP). These YCWP are then incubated with and are engulfed by immature DC. These DC now contain tumor lysate within their cytoplasm, and tumor antigens are processed through the endogenous pathway. These tumor antigen-loaded professional APC are the vaccine. More specifically, the YCWP allow for the tumor proteins to escape the lysosome and thus digestion, so epitope products are not shuttled to the MHC Class II pathway. Instead, these particles deliver protein tumor antigens into the cytoplasm where they are digested by the proteasome into epitopes that are then processed by TAP (transporter associated with antigen processing) and bound to MHC class I molecules in the endoplasmic reticulum. In short, tumor lysate from the patient is loaded into YCWP, which are then phagocytized by patient dendritic cells. These tumor lysate, particle-loaded DC (TLPLDC) are then administered as the vaccine.

Theoretically, injection of this solid tumor vaccine intradermally near a lymph node allows the TLPLDC to effectively "present" the tumor epitopes to the patient's T-cells and to stimulate the generation of antitumor immune responses.

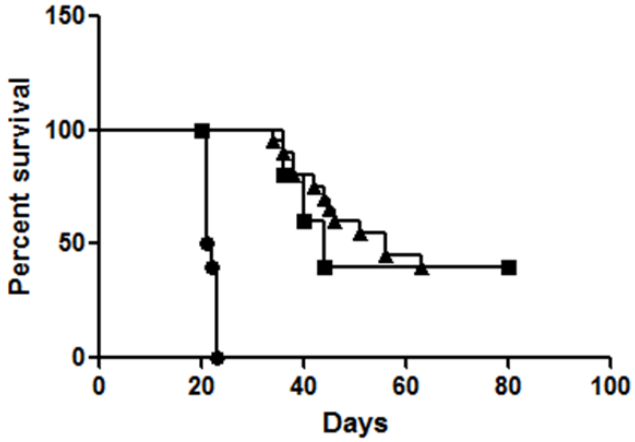
The next iteration of the vaccine production, TLPO, has removed the DC loading ex vivo and allows the DC to phagocytize the tumor lysate in vivo. The tumor lysate is produced in the same manner as TLPLDC with the autologous tumor tissue undergoing the freeze/thaw cycles and then loaded into the yeast cell wall particles. The TL-loaded YCWP are then capped with silicate to generate the TLPO vaccine. The TLPO vaccine removes the steps of harvesting the DC, maturing them, and loading the DC ex vivo. The TLPO method allows the DC to be loaded in vivo and present the tumor epitopes.

The concept of using TLPLDC as tumor-antigen-presenting cells in a therapeutic vaccine was initially tested in a murine metastatic melanoma model. In these studies, dendritic cells were prepared from mouse bone marrow and an established murine metastatic melanoma cell line was used as the source of the tumor cells. The dendritic cells were loaded with YCWP containing B16F0 tumor lysate. Three days prior to the TLPLDC preparation, female C57BL/6J mice were challenged with B16F0 melanoma cells by intravenous injection. Once the TLPLDC were prepared, each mouse in the treatment group was injected subcutaneously with TLPLDCs and this vaccination repeated for three weekly doses. The mice were monitored up to four weeks for pulmonary metastasis. At the end of four weeks, the mice were sacrificed and the metastases were counted. All four control animals that were not treated with TLPLDC had more than 50 tumors. On the other hand, none of the treated animals had measurable metastases. This experiment has been replicated multiple times, and these data

indicate that TLPLDC are effective in treating cancer in a proven animal model system.

The TLPO Investigator's Brochure details the specifics of the preclinical testing, but in summary, to test the TLPO concept, first YCWP were loaded with fluorescence labeled albumin and then half capped with silicate and half left uncapped. These particles were shaken in solution and tested for fluorescence leak at 1 and 2 hours. The fluorescence leak experiments demonstrated decreased leak from capped YCWP at 1 and 2 hours (15.8% vs 24.7% and 6.7% vs 16.6%, respectively). Next, the capped and uncapped YCWP were added to cultured mouse macrophage cells to evaluate DC uptake at 20 minutes, 1 hour and 2 hours; cells were then lysed and centrifuged to evaluate the amount of fluorescent material in the cytoplasm versus organelles. There was increased uptake of capped YCWP in DC cells compared to uncapped, particularly at 2 hours after introduction (fluorescence readings of 11065 vs. 3928). Lysed DC loaded with capped YCWP demonstrated delivery of 68.9% of contents to the cytoplasm vs. 48.8% in uncapped YCWP. Next, C57B mice were injected with 100ul saline (group #1), 10^6 empty capped YCWP (group #2) or 1mcg murine GMCSF (group #3). Five hours post-injection, 100ul saline was injected into the same site, then withdrawn, placed on a slide and examined under the microscope at 160X to evaluate the number of monocytic cells. The in vivo murine model showed greatly increased recruitment of monocytic cells in the mice receiving empty capped YCWP vs. GMCSF or saline alone. Then the TLPLDC vs TLPO was compared in a B16 melanoma model with mice followed to 80 days. In this murine survival model, there was improved survival in those treated with TLPLDC or TLPO vs. controls.

Figure 1: Murine Survival Model TLPLDC vs TLPO vs Control



Squares=TLPLDC, n=10
Triangles=TLPO, n=20
Circles=controls, n=5

Finally, in a large animal equine model, 4 grey horses with melanoma were treated with autologous TLPO created from a biopsy of their individual lesions, injected every 2 weeks for a total of 4 vaccinations. The target lesions were assessed over 6 months by standard RECIST criteria. The equine model revealed one horse to be a complete responder (100% resolution of the target lesion) and the remaining 3 as partial responders (50%, 68%, and 45% reduction in tumor size).

Through this series of results, the silicate capped TLPO particles have proven to be effective at retaining loaded contents, have improved uptake by DC and, specifically, effective delivery to the cytoplasm of DC. In early animal studies, TLPO appears to have equivalent efficacy compared to the TLPLDC vaccine, but eliminates the need for ex vivo DC loading. The TLPO vaccine has not been used in humans previously, and as such, the TLPO portion of this study is considered a first in man study and will be conducted as a phase I/IIa trial with safety, immune response and early clinical efficacy endpoints.

2.4 Prior/Ongoing Clinical Trials with Autologous Tumor/DC Vaccine

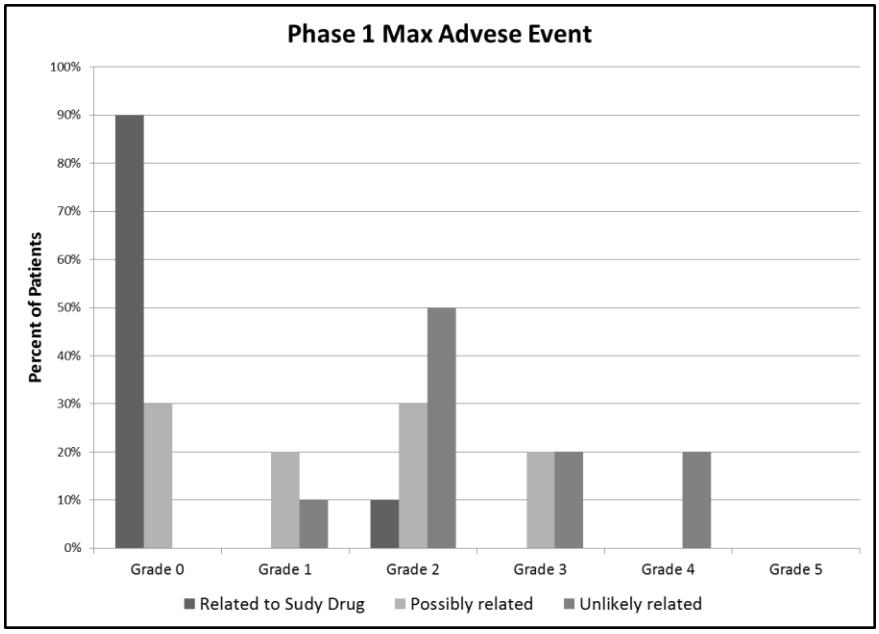
We have conducted phase I/IIa clinical trials examining DC + autologous tumor vaccination of solid tumor patients in combination with IL-2. To date, we have vaccinated 35 late-stage melanoma and renal cell carcinoma patients.

In a phase I trial (IND# 8851), ten patients with stage IV melanoma received a vaccination every three months at a dose ranging from 500,000-1,000,000 or more dendritomas. IL-2 dose was increased from 3 MIU/m^2/day if tolerated to a maximum dose of 9 MIU/m^2/day for five days (administered one day after the first

dendritoma vaccine but not after subsequent vaccinations). There were two unrelated serious adverse events: one patient died due to progressive disease 19 days after the first vaccination, and another patient experienced thrombocytopenia that led to a splenectomy 73 days after the last vaccination.

The maximum level of toxicity experienced by the patients in the phase I trial is depicted in Figure 1. The most common adverse events experienced by patients included fever (100%), chills (50%), hypotension (40%), nausea (40%), anemia (40%), arthralgia/myalgia (30%), weight gain (30%), stomatitis (30%), and edema (30%). The average survival of nine evaluable patients was 198 days, with one patient alive and disease-free 12 years after initial vaccination[64].

Figure 2: Adverse Events by Grade



The phase IIa trial (IND# 8851) vaccinated 15 patients with stage IV melanoma. Each patient received a vaccination every six weeks at a dose ranging from 250,000-1,000,000 or more dendritomas up to six times depending on the availability of dendritomas and tumor cells. IL-2 dose was administered from 3 MIU/m^2/day on days 1, 3, and 5 after the first vaccination. There were two unrelated serious adverse events: one patient (on Coumadin) experienced gastric bleeding and a second experience hypoxia after IL-2 administration and required blood transfusions for treatment of anemia. Erythema (33%), fever (33%), headache (27%), and arthralgia/myalgia (27%) were the most common adverse events experienced by patients.

Other commonly experienced adverse events included rash (20%), pain (20%), chills (20%), fatigue (20%), and nausea (20%). The average survival of the ten deceased patients was 590 days. Five patients remain alive.

With 23 evaluable patients out of the 25 enrolled in the completed phase I/IIa melanoma trials, the median overall survival (OS) was 12.5 months in comparison to the median OS of 8.5 months (4.7 to 11.5) [65] seen in historical controls (Table 1 and Figure 2). One patient with stage IV melanoma had a partial response 12 weeks after initial vaccination and received five vaccinations with a complete response (Figure 3).

At interim follow-up assessment, 13 patients had died, three subjects had progressive disease, two subjects had stable disease, one subject had progressive disease, and four subjects had complete response (Figure 4). Of note, OS of all four stage IV patients with completely resected disease was 100% at 20 months. Although difficult to draw a dose response conclusion in a limited number of patients, the four subjects who had a complete response received three to six vaccinations and the one patient who had a partial response had five vaccinations.

Table 1: Evaluable Patients

	All	Evaluable*
n	25	23
Age	59	59.35
Female	56% (14)	57% (13)
Male	44% (11)	43% (10)
Initial Stage*		
* I	24% (6)	26% (6)
* II	24% (6)	26% (6)
* III	24% (5)	17% (4)
*IV	28% (7)	30% (7)
Stage at Inoculation		
* IV (NED)	16% (4)	17% (4)
*IV	80% (20)	82% (19)

Figure 3: Overall Survival

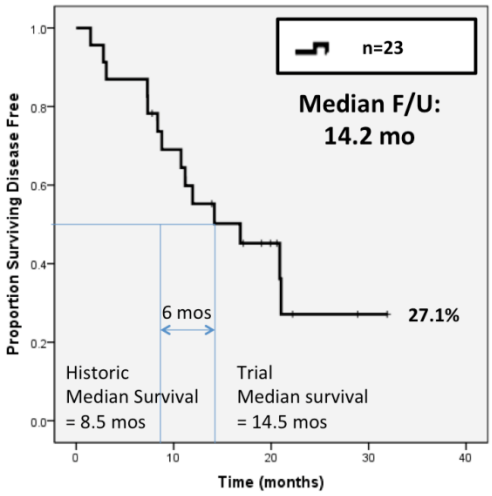


Figure 4: Number of Inoculations

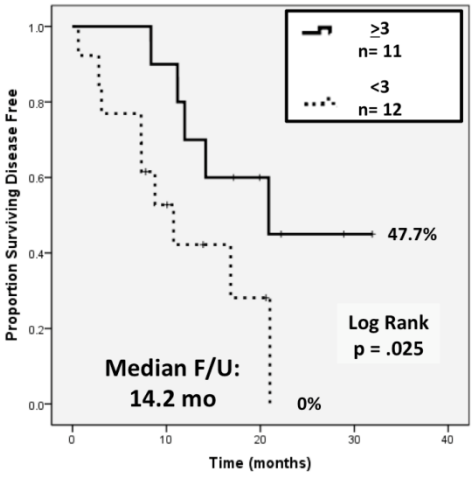
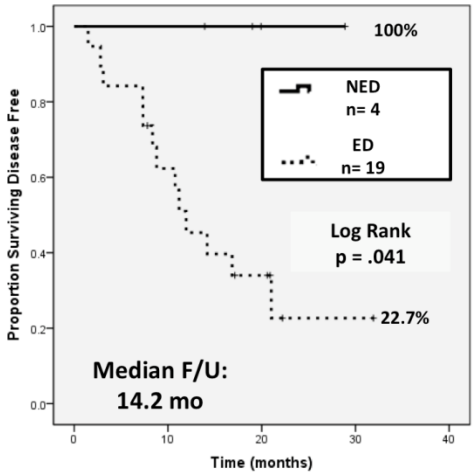


Figure 5: No Evidence of Disease



Overall, data from the above trials in late-stage melanoma patients showed the safety of a dendritoma vaccine itself as well as increased survival when compared to conventional treatments.

The adverse events listed above were felt to be related the administration of IL-2 and not the vaccine itself. In addition, better survival rates were seen when all visible tumor was removed at the time of surgery.

A similar vaccination strategy was also utilized in a phase I, open label, study of ten patients with stage IV renal cell carcinoma (IND# 9519). Each patient received a vaccination every six weeks at a dose ranging from 500,000-1,000,000 or more dendritomas. IL-2 dose was increased from 3 MIU/m2/day if tolerated to a maximum dose of 9 MIU/m2/day for five days (administered one day after first dendritoma vaccine but not after subsequent vaccinations). Twelve weeks after initial vaccination, one patient died, four patients had progressive disease, three had stable disease, and one had a partial response. At final follow-up, nine patients are deceased. The remaining patient was withdrawn due to medical problems requiring steroid treatment, a prohibited concomitant medication. This patient is being followed for survival. The cumulative summary of adverse events from the clinical study showed that fever (90%), nausea (70%), vomiting (60%), anemia (60%) and chills (60%) were the most common AEs experienced by the patients. Other commonly experienced AEs included unspecified pain (50%), stomatitis (40%) and weight loss (40%). Average survival of the first six deceased patients in the phase I trial was 274 days with three patients living greater than 18 months and one patient still alive with no evidence of disease at two years. These results suggest a possible clinical benefit although a larger, controlled trial is needed to draw meaningful conclusions.

As discussed above, the concept of an autologous tumor/DC vaccine has been made simpler with the use of the YCWP loading system, which allows the efficient and effective delivery of autologous tumor lysate to the patient's DC. In addition to being simpler, the TLPLDC vaccine has been shown to be more effective in animal models compared to the dendritoma approach although both systems utilize autologous tumor and DC. We are currently utilizing the new technology of TLPLDC in an ongoing treatment registry study of multiple solid tumors in Cayman. This non-IND foreign clinical trial is being conducted by GCP and meets the criteria of 21 CFR 312.120. Thus far, 10 patients have completed the vaccination series of $1x10^6$ TLPLDC intradermally per monthly inoculation x 4 with very limited toxicity (no grade >2). Clinical activity has been documented in 60% of the wide variety of patients vaccinated to date (2 complete responses, 2 partial responses, and 2 with stable disease). Thus far, 40% have shown progressive disease, but none of these received all four inoculations. One partial responder subsequently developed progressive disease. Initial clinical response in stage IV melanoma patients has been impressive but in small numbers. One of three vaccinated stage IV melanoma patients has achieved a complete pathologic response (see detailed information in the Investigator's Brochure). Based on this initial safety data using the new technology and our prior promising clinical responses with autologous tumor/DC vaccination in stage IV melanoma patients, especially resected stage IV, we are moving forward with a phase IIb trial in the adjuvant setting.

3.0 OBJECTIVES

In this combined study, we intend to assess the ability of the tumor lysate, particle-loaded, dendritic cell (TLPLDC) vaccine given in the adjuvant setting to prevent recurrences in patients with stage III and IV (resected) melanoma. Also, the tumor lysate, particle only (TLPO) vaccine is being studied in an embedded phase I/IIa trial with safety, immune response and early efficacy endpoints as described below.

3.1 Primary Objective

For the TLPLDC phase IIb, the primary objective is to compare DFS at 24 months between the vaccinated (TLPLDC and TLPO) and control groups analyzed as a proportion.

For the TLPO phase I/IIa, the primary objective is to assess the tolerability of the TLPO vaccine per CTCAEv4.03.

3.2 Secondary Objectives

For the TLPLDC phase IIb:

- Compare DFS and overall survival (OS) at 36 months between the vaccinated (TLPLDC and TLPO) and control groups from time of randomization.

- Compare safety between vaccinated (TLPLDC and TLPO) and control groups by assessing local and systemic toxicities using standard National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03.

3.3 Exploratory Objectives

For both trials:

- Evaluate immune responses among the vaccinated patients and compare to the control patients.

- Correlate immunologic responses to clinical outcome.

- Assess ongoing vaccination after recurrence for safety and tumor response in combination with SoC therapies to include checkpoint inhibitors.

4.0 STUDY DESIGN

4.1 Description of Study

This will be a multi-center, prospective, randomized, blinded, placebo-controlled phase IIb trial of the autologous TLPLDC vaccine and a phase I/IIa trial of the TLPO vaccine (Appendixes A and B). The vaccines to be used in this study are investigational and will be used under Investigational New Drug (IND) applications (IND#16101 for TLPLDC and IND#17274 for TLPO). The Sponsor of these INDs is Elios Therapeutics, LLC.

The target study population is melanoma patients after complete surgical excision with high risk for recurrence. High-risk patients are defined as:

- Stage III resected patients. Stage IIIA, IIIB, and IIIC will be allowed; however, it is unlikely that many stage IIIA patients will be enrolled given the requirement for tumor to produce the vaccine (detailed later).

- Stage IV resected patients. All clinical/radiographic disease must be resected.

Stage III and Stage IV (resected) melanoma patients will be identified prior to definitive surgery and screened for inclusion/exclusion criteria.

Eligible patients will be counseled and consented for tissue procurement (consent #1). Enrolled patients will have their disease surgically resected per SoC (need not occur at the study site), and a portion (approx. 1 cm^3 preferred but 1 mg minimum of their melanoma sterilely frozen in provided freezing vials and storage tubes.

This tissue will be shipped in liquid nitrogen shippers through FedEx to our central facility in Greenville, SC and stored frozen until vaccine preparation. If patients cannot be rendered disease-free, they will be considered screen failures for this study. If melanoma is being resected from multiple locations (i.e., primary and nodes or two different metastatic sites), then samples from all locations would be preferred but is not mandatory.

As indicated by SoC per the NCCN guidelines and determined by the treating team, if a patient is to receive systemic therapy (chemotherapy or IFN-α) and/or radiation therapy, then the vaccinations will not begin until SoC therapy is completed. Although, patients on adjuvant check point inhibitors (CPI) may start the vaccine trial after showing tolerance of the CPI after 3 doses, given that there may be a synergistic benefit and no added toxicity when adding the vaccine to CPI. Once the patient is deemed clinically disease-free, they will be consented (consent #2) for treatment and randomized. Consenting should occur as close to completion of SoC therapies as possible (or after demonstrating tolerance to CPI after 3 doses), but may overlap completion of systemic and/or radiation therapies by no more than one month. Once consented, patients will receive a single

injection of Neupogen (G-CSF) 300 µg (or its equivalent) subcutaneously 24-48 hrs prior to having 70 mL of blood collected from the patient and sent to our central facility for DC isolation and preparation. Patients who cannot tolerate Neupogen (or its equivalent) or refuse it, will have 120 mL of blood drawn and sent to our central facility for DC isolation and preparation. Additional blood may be drawn if additional vaccine doses need to be made or remade for any reason.

Vaccines will be prepared by producing tumor lysate (TL) through freeze/thaw cycling of the autologous tumor and then loaded into pre-prepared yeast cell wall particles (YCWP). The TL-loaded YCWP will be introduced to the DC for phagocytosis; thus creating the TLPLDC vaccine which will be frozen in single dose vials. Each vial will contain 1-1.5 x 10^6 TLPLDC and will be labeled with the patient's unique study number. The TLPO will be created by taking the TL-loaded YCWP and capping them with silicate. The TLPO vaccine will be frozen in single dose vials containing 1 x 10^8 TLPO and will be labeled with the patient's unique study number. Vaccine production and QA testing for vaccine release and shipping will take approximately three weeks.

Based on their randomization, either autologous TLPLDC or TLPO (active vaccine) or unloaded YCWP + autologous DC (control) will be sent back to the site in a blinded fashion. The patients, referring physicians, site PIs, and site pharmacists will be blinded to group assignment. Regardless of assigned group, the site will receive six single dose vials to be injected intradermal monthly x 3 followed by boosters at 6, 12, and 18 months (Appendix C) from initial inoculation in the same lymph node draining area (preferably the anterior thigh). Patients must begin vaccinations between 3 weeks and 3 months from completion of Standard of Care (SoC) or patients on adjuvant check point inhibitors (CPI) may start the vaccine trial after showing tolerance of the CPI after 3 doses.

Frozen tumor will be maintained for all patients. Patients who recur will have the option to participate in the open label portion of the trial. At the time of confirmed recurrence, the study site can request to have the patient unblinded, and the patient will sign the open label consent form (consent #3). In the open label study, all patients will have new vaccine doses produced from stored tumor and a new blood draw. If the patient is having surgery, additional tumor tissue may be collected or may be required if insufficient tumor is available in the freezer.

Once open label consent has been signed, patients will receive a single injection of Neupogen (G-CSF) 300 µg (or its equivalent) subcutaneously 24-48 hrs. prior to having 70 mL of blood collected from the patient and sent to our central facility for DC isolation and preparation. Patients who cannot tolerate Neupogen (or its equivalent) or refuse it, will have 120 mL of blood drawn and sent to our central facility for DC isolation and preparation. Additional blood may be drawn if additional vaccine doses need to be made or remade for any reason. After vaccine is produced and shipped to the study site, patients will begin receiving the inoculations on the following schedule: 0, 1, 2 and 3 months ± 1 week followed by booster inoculations at 6 and 9 months ± 2 weeks from initial inoculation for a total

of six doses (Appendix C). These vaccinations will be occurring in the background and not interfere with any SoC therapies indicated and initiated to treat the patient's recurrence per NCCN guidelines. For the open label study, patients will be followed for a total of 12 months with tumor assessments by iRECIST and RECIST (Appendix F) on SoC follow-up imaging

Control patients who do not recur will be offered active vaccine at the completion of the trial. It will be their choice whether to proceed with active vaccination.

Safety data will be collected on local and systemic toxicities, graded and reported per the NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (Appendix D).

Disease-free status will be monitored per SoC as outlined by NCCN for three years. Suspected recurrences will be documented with biopsy and pathologic confirmation whenever possible. Time to recurrence will be based on date of randomization to time of confirmed recurrence.

In addition to the initial blood collection for vaccine preparation, 50 mL of blood will be collected from all patients prior to each of the six inoculations and at 24 months from enrollment for a total of seven time points or a total of 350 mL of blood over two years. The collected blood will be sent to our central facility for immunologic testing of the T-cell response. The immunologic responses will be correlated with the clinical outcomes. Patients will follow a similar pattern if they recur and participate in the open label portion of the study.

We plan to enroll at least 180 patients (randomized 2:1, vaccine:control) up to 120 patients; after enrollment of the 120th patient the randomization will transition to 2:1 (TLPO phase I/IIa:TLPLDC) for the remaining 60 patients. The TLPO portion of the study is being conducted as an embedded phase I/IIa trial. With 10-12 sites enrolling a 0.8 pts/site/month, we anticipate enrollment to be complete in 12-18 months. With a 2-year DFS primary endpoint and 3-year DFS/OS secondary endpoints, the trial duration is expected to be 4-4.5 years. Open label patients will be followed for a year after recurrence.

4.2 Rationale for Study Design

Based on our prior phase I/IIa clinical trials in stage IV melanoma, clinically disease-free patients realized the greatest benefit from vaccination. As such, stage III and IV (resected) patients are the target population for this adjuvant trial. Prevention of recurrence is the primary objective.

Assuming a baseline recurrence rate of 60% (corresponding to a DFS of 40%) at two years based on published data in this mixed group of stage III (predominantly IIIB and IIIC) and stage IV (resected) melanoma patients, a sample of size of 120 will have 80% power to detect a statistical difference in proportions between treatment arms controlling the type I error at alpha = 0.05 (two-sided).

A blinded evaluation of the first 75 patients enrolled discovered an early recurrence rate of 12% prior to completion of primary vaccine series. To compensate for this early recurrence rate and to preserve the power of the trial, enrollment will be extended by at least an additional 20 patients, now totaling at least 140. After the randomization of the 120th patient, then randomization will transition to a 2:1 (TLPO phase I/IIa:TLPLDC) for an additional 60 patients resulting in an additional 20 patients receiving the TLPLDC vaccine (therefore 140 as stated above). The total expected enrollment after the initiation of the TLPO phase I/IIa trial is at least 60 patients (40 TLPO phase I/IIa and 20 additional TLPLDC; Appendix B). In the TLPO phase I/IIa, the tolerability of the TLPO vaccine will be assessed primarily. Given the embedded nature of the trial and the randomization of the patients to the TLPO phase I/IIa vs TLPLDC, there will be an opportunity to compare the toxicity profiles of the two vaccines.

In an attempt to allow all patients to benefit from receiving an autologous TLPLDC or TLPO vaccine and to assess the potential for synergistic benefit of active vaccination added to SoC therapies, all patients regardless of randomization will be offered the opportunity to participate in the open label portion of the study if they should recur. These open label secondarily treated patients will be followed for documentation of treatment response. Additionally, control patients who do not recur can request active vaccination after they complete the trial.

4.3 Outcome Measures

4.3.1 Primary outcome measures

Disease state will be determined by the patients' own physicians at the individual study sites during their routine follow-up screening. This will occur for all enrolled patients, regardless of randomization, approximately every three to six months for three years from randomization with clinical exam, laboratory and radiographic surveillance as indicated. This follow-up strategy is SoC for these patients per NCCN guidelines. If records are not available, patients, or their referring physicians, will be contacted to discern their disease status. Recurrences will be confirmed pathologically, whenever possible, per standard of care.

For the TLPLDC phase IIb, the primary outcome measure of the trial is DFS at 24 months compared between the vaccinated (TLPLDC) and control groups after the final enrolled patient completes two years of follow-up.

An interim analysis will be performed six months after the 120th patient is enrolled. This analysis will compare median DFS between vaccinated (TLPLDC) and control groups.

For the TLPO phase I/IIa, the primary outcome measure is safety as assessed by CTCAE v4.03 graded toxicity scale (Appendix D).

4.3.2 Secondary outcome measures

For the TLPLDC phase IIb, the secondary outcome measures include 36 months DFS and OS and assessing local and systemic toxicities.

DFS and OS at 36 months will be assessed and compared between vaccinated (TLPLDC) and control groups. This assessment will be conducted after the last enrolled patient has been followed for three years.

For both studies, standard local and systemic toxicities will be collected and graded per the NCI CTCAE v4.03 graded toxicity scale (Appendix D).

For both the primary and the booster inoculations, patients will be monitored for 30 minutes after the inoculation with vital signs taken as needed. Local or systemic toxicities will be collected and graded by the research staff at the patient's next visit. Serious AEs will be reported as described in Section 6.0.

4.3.3 Exploratory outcome measures

For both trials, immune responses will be primarily documented using the assessment of local reactions and future CTL assays on stored blood and assessment of stored serum pre- and post-vaccination. Each of these measurements will be performed regardless of randomization. Detailed descriptions of these assays/tests are described in Sections 5.4.4, 5.4.5, and 5.4.6.

Phenotypic assays (dimer/tetramer) for clonal CTL expansion against common melanoma-associated antigens such as gp-100 and MART-1 as well as functional assays like ELISPOT against the same antigens will be performed on banked/stored blood. Antibody and cytokine responses may be assessed on the stored serum. Blood/serum will be collected prior to each inoculation (0, 1, 2, 6, 12, 18 months) and at study completion at 24 months from randomization. Comparisons will be made between the immunologic responses in the vaccinated (TLPLDC and TLPO) patients vs the control patients.

In a series of exploratory analyses, the results of these immunologic measures will be correlated with clinical outcome in the vaccinated (TLPLDC and TLPO) patients.

Additional exploratory outcomes will compare clinical and immunological outcomes based on the dose of vaccine (TLPLDC and TLPO) and number of inoculations received.

If enrolled patients recur, they will be offered participation in the open label portion of the study regardless of their randomization. All patients will have new vaccine produced, and they will receive inoculations at 0, 1, 2, 3, 6, and 9 mos (Appendix C). They will receive the vaccinations in the background of their SoC therapies initiated to treat their recurrence. These patients will be followed for safety and tumor response in combination with their SoC therapies and assessed per iRECIST and RECIST (Appendix F) of their SoC follow-up scans.

4.4 Safety Considerations

In our previous trials, the safety of the autologous tumor/DC vaccines has been excellent. In the completed melanoma phase I where the autologous tumor/DC vaccine was given with IL-2, the most common adverse events experienced by patients included fever (100%), chills (50%), hypotension (40%), nausea (40%), anemia (40%), arthralgia/myalgia (30%), weight gain (30%), stomatitis (30%), and edema (30%). There were no SAE's related to the vaccine/IL-2.

In the completed melanoma phase IIa where the IL-2 dose and frequency was reduced, the most common adverse events were erythema (33%), fever (33%), headache (27%), and arthralgia/myalgia (27%). Other commonly experienced adverse events included rash (20%), pain (20%), chills (20%), fatigue (20%), and nausea (20%).

Many of these toxicities are attributable to IL-2, which will not be used in the current study. We have seen minor toxicities (grade 1 and 2) transient flu-like symptoms in the 12 patients vaccinated without IL-2 in the treatment registry with the new technology, TLPLDC vaccine. We anticipate a similar favorable safety profile in this trial, but all local and systemic toxicities will be documented, graded by the NCI CTCAE v4.03 and reported as indicated below in Section 6.0.

Additionally, there is a small risk associated with the protocol-dictated single dose of Neupogen, or its equivalent, prior to the initial blood draw for DC isolation. Neupogen is a FDA-approved drug for increasing the white blood count, is commonly used in oncology patients, and has a well-tolerated toxicity profile.

Thus far, in this study, we have seen no significant local reactions in any of the blinded patients vaccinated with the TLPLDC vaccine or placebo nor has there been any systemic safety signal noted by the DSMB. However, for the TLPO vaccine phase I/IIa component, this is a first in human study; therefore, the safety of this vaccine is unknown. As such, the primary endpoint of this embedded trial is safety. Since the silicate-capped, tumor lysate-loaded YCWP are being given alone (not contained within autologous DC), then the vaccinated patients may form a different reaction to this version of the vaccine. While the phase I/IIa TLPO trial is not a dose escalation trial, we will modify the dose based on local and systemic toxicities.

Dose Reductions of Vaccine. For patients experiencing robust local reactions (>100mm of erythema/induration and/or threatened skin disruption), or >/= grade 3 local and/or systemic reactions, or >/= grade 2 hypersensitivity reactions, the dose of the vaccine will be reduced by 50% for subsequent inoculations. If reactions/toxicities recur with subsequent inoculations, the vaccine dose will continue to be reduced serially by 50%. If dose reduction does not control toxicities or they worsen despite reductions, then all further inoculations will be halted.

4.5 Compliance with Laws and Regulations

This study will be conducted in accordance with current FDA regulations and Good Clinical Practices (GCPs), and local ethical and legal requirements.

5.0 MATERIALS AND METHODS

5.1 Subjects

Patients over the age of 18 years with a diagnosis of stage III or IV melanoma capable of being rendered clinically disease-free after surgery will be targeted. Patients will be recruited from medical and surgical oncology and/or hematology/oncology clinics at the individual study sites. All patients will be properly counseled and consented.

5.1.1 Subject selection

Potentially eligible patients, specifically patients with clinical stage III and stage IV resectable melanoma will be identified by staff in the medical and surgical and/or the hematology/oncology clinics at the individual study sites.

A research nurse and/or study coordinator will approach these patients about being in the trial and will introduce the trial to the prospective volunteer. If the volunteer is interested and appears eligible, the nurse will arrange to counsel the patient. The nurse will thoroughly screen the patient for inclusion and exclusion eligibility criteria. If the patient remains interested and eligible, informed consent will be obtained for tissue procurement (consent #1).

Written informed consent will be obtained from all study participants. Prospective participants will be provided with a copy of the consent form #1 to read.

The research nurse coordinator and/or study coordinator, or Principal Investigator (PI), will explain the study and review the consent form with the patient. Subjects will be given ample time to ask and have all questions answered prior to signing the consent form.

After SoC surgery (which need not occur at the study site), if the patient is rendered clinically disease-free, sufficient tumor is available for vaccine production (1 cm^3 preferred, 1mg minimum), and all other eligibility criteria are met, then the patient can be counseled and consented for randomization and treatment (consent #2). This consenting should occur as close to completion of indicated SoC therapies as possible (or after demonstrating tolerance to CPI after 3 doses), but may overlap completion of systemic and/or radiation therapy by no more than one month. Once the second written informed consent is obtained, the patient will receive a single injection of Neupogen (G-CSF) 300 µg (or its equivalent) subcutaneously 24-48 hours prior to having 70 mL of blood collected from the patient and sent to our central facility for DC isolation and preparation. Patients who cannot tolerate Neupogen (or its equivalent) or refuse it, will have 120 mL of blood drawn and sent.

Additional blood may be drawn if additional vaccine doses need to be made or remade for any reason. The full course (six doses) of the TLPLDC or TLPO vaccine vs placebo vaccine (empty YCWP + DC) will be produced at one time after randomization. The production and QA testing for vaccine release will take approximately three weeks. Vaccination cannot begin until after all SoC therapies are completed, but the inoculation series must begin within 3 weeks and 3 months from completion of SoC therapies.

Tumor will be maintained on all patients to include the control patients so that active vaccine can be reproduced if necessary, in the case of the vaccine patients or be made for the control patients in the case of recurrence or upon request at the end of the primary endpoint.

5.1.2 Inclusion criteria

Patients will be included in the study based on the following criteria:

- 18 years or older
- Eastern Cooperative Oncology Group (ECOG) performance status 0,1 (Appendix E)
- AJCC stage III or IV completely resectable melanoma identified before surgery
- Approximately 1 cm^3 preferred but 1 mg minimum of accessible and dispensable tumor that will not interfere with pathologic staging
- Clinically disease-free after surgery
- Completing SoC adjuvant therapy per NCCN guidelines to include chemotherapy, radiation therapy, and/or biologic therapy as clinically indicated. (Consent #2 should be signed as close to completion of SoC as possible but may overlap completion by up to one month.)

  ONLY for patients on CPI therapy: The patient has completed all other SoC therapy per NCCN and has demonstrated tolerance of treatment after 3 doses of CPI therapy without serious adverse events and remains clinically disease-free. (Consent #2 should be signed after the 3rd dose of CPI therapy.)
- Vaccinations initiated between 3 weeks and 3 months from completion of SoC multi-modality cancer care or after tolerance to CPI therapy has been determined.
- Adequate organ function as determined by the following laboratory values:

- ANC ≥ 1,000/μL
- Platelets ≥ 75,000/μL
- Hgb ≥ 9 g/dL
- Creatinine ≤ 1.5 x upper limit of normal (ULN) or Creatinine clearance ≥ 50% of lower limit of normal (LLN)
- Total bilirubin ≤ 1.5 ULN
- ALT and AST ≤ 1.5 ULN

- For women of child-bearing potential, agreement to use adequate birth control (abstinence, hysterectomy, bilateral oophorectomy, bilateral tubal ligation, oral contraception, IUD, or use of condoms or diaphragms)
- Signed informed consent

5.1.3 Exclusion criteria

Patients will be excluded from the study based on the following criteria:

- Evidence of residual disease after surgery and SoC adjuvant therapies
- Insufficient tumor available to produce vaccine
- ECOG ≥2 performance status (Appendix E)
- Immune deficiency disease or known history of HIV, HBV, HCV
- Receiving immunosuppressive therapy including chronic steroids, methotrexate, or other known immunosuppressive agents
- Pregnancy (assessed by urine HCG)
- Breast feeding
- Active pulmonary disease requiring medication to include multiple inhalers (>2 inhalers and one containing steroids)
- Involved in other experimental protocols (except with permission of the other study PI)

5.2 Method of Treatment Assignment

If volunteers meet all inclusion criteria and none of the exclusion criteria and agree to participate, they will continue in the study. After tissue procurement, if adequate tumor is available for vaccine production (1 cm^3 preferred, 1 mg minimum) and patients are rendered clinically disease-free, then they will be consented and randomized for treatment assignment.

Patients will be randomly assigned by the CRO using a computer-generated randomization table to receive the autologous TLPLDC vaccine or placebo (empty YCWP + autologous DC). Randomization between the two treatment groups will occur in a 2:1 allocation ratio using an institutional balancing algorithm. After the 120^{th} patient is randomized, the TLPO phase I/IIa trial will commence with a 2:1 (TLPO phase I/IIa:TLPLDC) randomization for an additional 60 patients (refer to Appendix B). This randomization will use the same computer-generated institutional balancing algorithm and the patients will be randomly assigned by the CRO. Patients, treating physicians, and site PIs will be blinded as to the treatment assignment. Active vaccine will be made available to those patients assigned to the control group at time of recurrence or after they have completed the trial, including all follow-ups and primary and secondary endpoints, if they have not recurred and wish to receive the active vaccine.

5.3 Study Treatment

5.3.1 Vaccine production

Vaccines will be produced by Orbis Health Solutions (Greenville, SC). Tumor lysate from the patient's tumor is loaded into YCWP which are phagocytized by the patient's dendritic cells in vitro to generate TLPLDC. The TLPO vaccine is produced from the patient's tumor lysate loaded into YCWP and capped with silicate.

The final vaccine contains 1-1.5 $x10^6$ TLPLDC or 1.0 x 10^8 TLPO per dose and will be provided in frozen single dose vials in 250 µl of freezing media for TLPLDC and saline for TLPO. The final vaccine is prepared for injection by thawing the single dose vial and diluting the vaccine (TLPLDC or TLPO) by adding 500 µl of sterile saline for injection.

For the open label portion of the study, new vaccine will be made for all patients from frozen tumor and a new blood draw.

5.3.1.1 Preparation of dendritic cells

Dendritic cells will be generated from the patient's peripheral blood monocytes (PBMC) obtained from the post-Neupogen (or its equivalent) 70 mL blood draw or from 120 mL of blood drawn if Neupogen (or its equivalent) not used after consent #2. The PBMCs are initially diluted and placed in a plastic culture flask for 2 hours.

Non-adherent cells are washed away and collected. These cells are rich in lymphocytes and will be frozen for future assays.

The adherent cells will be incubated at 37˚C in 5% CO_2 for two days in serum-free cell culture medium with the appropriate cytokines added to generate immature monocyte-derived dendritic cells.

5.3.1.2 Tumor tissue preparation

A minimum of 1 mg sample of viable tumor tissue is required to be collected at the time of primary surgery. The specimens will be collected and placed in sterile freezing vials that will be provided and then shipped in specialized FedEx shippers designed to keep the specimens frozen (also provided). The cells of the tumor tissue will be lysed by multiple freeze/thaw cycles in lysis buffer to produce tumor lysate.

5.3.1.3 YCWP preparation

YCWP are prepared by NaOH digestion of all non-cell wall material from Fleishman's Bakers' yeast, Saccharomyces cerevisiae, followed by thorough aqueous, ethanol, and acetone washings.

5.3.1.4 Loading of YCWP with tumor lysate

TLPLDC are generated by incubating tumor lysate loaded YCWP in the presence of dendritic cells at a specific time during day 2 of the DC isolation/incubation/maturation process. The timing is essential in that the immature DC are given the YCWP at their most phagocytic phase. For control patients, empty YCWP will be incubated in the presence of DC at the same point on day 2 of the DC incubation.

5.3.1.5 Capping of TL-Loaded of YCWP with Silicate

TLPO vaccine is generated by capping tumor lysate-loaded YCWP with silicate. The fully loaded, dried YCWP are added to a solution of ammonium hydroxide, tetraethylorthosilicate and 100% ethanol and shaken vigorously for 15 minutes in order to "cap" the particles with a silicate coating. Following "capping," the loaded and "capped" YCWP are washed multiple times in PBS with sonication to remove all "capping" reagents. After washing, the suspension of "capped" tumor lysate-loaded YCWP (TLPO) is aliquoted into individual doses each containing $1x10^8$ TLPO in 250 uL of sterile saline and frozen.

5.3.2 Storage

Six doses of 1-1.5 x 10^6 autologous TLPLDC or 1.0 x 10^8 TLPO will be cryopreserved in patient-specific labeled single dose vials and maintained at the central production facility at -80˚C until shipped. Once approved for release, the entire vaccine series (all six doses) will be shipped to the site frozen in specialized

LN shippers. The patient-specific dose series will be stored at the site at least -70˚C in the research pharmacy until serially thawed for use. Any temperature excursion for more than 30 minutes at -55˚C or warmer needs to be reported. Outside of preparation for patient injection, the vaccine may not, at any time, be allowed to thaw. The isolated 250ul vial will thaw in less than one minute at room temperature.

5.3.3 Inoculation series – administration

Patients will receive six intradermal inoculations on the anterior or medial side of the same thigh or arm. The general area of inoculation will be at a location midway between the inguinal ligament and the knee.

Before injection, this cellular vaccine will either be thawed slowly in a 37^0 C water bath or the vaccine vial can be held in a gloved hand until thawed if water bath is not available. The vial should not be left lying out to thaw at ambient temperature. The 250 µl of thawed TLPLDC or TLPO will be diluted with 500 µl of sterile saline for injection. The final 750 µl volume will be drawn up into a 1 mL syringe and should be injected within 4 hours. The entire contents of the syringe will be injected intradermally in two approximately equal inoculums at two different sites 5 cm from each other on the anterior or medial thigh or arm. Inoculations will be administered in the same lymph node draining area (same arm or leg) (see also Appendix C).

The research nurse coordinator will administer the inoculations sterilely in the clinic facility located at each study site. For female patients with childbearing potential, a urine pregnancy test will be performed before each inoculation. If this test is positive at any time, the patient will be discontinued from the study.

Initial inoculations will be performed at 0, 1, and 2 months ± 1 week followed by booster inoculations at 6, 12, and 18 months ± 2 weeks from initial inoculation for a total of six doses.

For the open label portion of the study, inoculations will be performed at 0, 1, 2, and 3 months, ± 1 week and at 6 and 9 months, ± 2 weeks for a total of six doses.

5.3.4 Dose reduction for TLPO

Patients experiencing robust local reactions or unacceptable toxicities (see section 4.4 for definitions) will have their TLPO dose reduced by 50%. This will be accomplished by doubling the sterile saline diluent and maintaining the same inoculation volume.

5.4 Study Assessments

Signed, IRB approved informed consent must be obtained from patients prior to any pretreatment assessments.

5.4.1 Pretreatment assessments

This trial requires two consents: one for tissue procurement and one for randomization/treatment. The second consent will be signed once a patient has been rendered clinically disease-free after surgery, adequate tumor tissue has been acquired for vaccine production, and all eligibility criteria are met. Consent #2 will be signed as close to completion of SoC therapies as possible but may overlap completion of systemic and/or radiation therapy by up to a month. For those patients on CPI therapy, they must have completed 3 doses of therapy and demonstrated clinical tolerance before continuing with consent #2. Initiation of vaccination cannot begin until SoC therapy has been completed (and/or demonstration of 3 doses of CPI tolerance, as applicable), but must begin 3 weeks to 3 months from completion of SoC (or after 3 doses of CPI therapy demonstrating tolerance).

All patients must have a CBC with differential, CMP, and LDH within two months of trial initiation and, for female patients, a urine pregnancy test (upon consent to study), for screening and immediately prior to any vaccine inoculations. If the pregnancy test is positive the patient will be excluded from the study. Women who have had a hysterectomy, bilateral oophorectomy, tubal ligation, documented absence of menses for two years, or FSH hormonal laboratory results that verify menopause, will not be required to have pregnancy testing.

For patients who have a complete metastatic evaluation (CBC, LFTs, CXR, chest and abdomen CT, bone scan, and PET scan) available, all studies will be screened but these studies are not required for enrollment. Disease-free status will be assured by the patients' primary treating/referring physician. Overall health screen will be assessed utilizing the ECOG performance status grading system (Appendix E).

All patients regardless of randomization will be assessed for baseline and post-vaccination immunologic responses. Blood samples will be obtained prior to each inoculation (0, 1, 2, 6, 12, 18 months) and at study completion at 24 months from randomization. The protocol schedule of events is listed in Appendix G. Specimen handling, processing, and assays are described in Sections 5.4.4, 5.4.5, and 5.4.6.

5.4.2 Assessments during treatment

Any laboratory evaluations or imaging studies ordered by the treating oncologist as part of standard practice will be reviewed by the research nurses or study coordinators and any abnormal results will be reviewed by the study investigators.

Research nurses or study coordinators will assess the patients prior to and during a 30 minute observation after vaccine inoculation, and will assess the patient on an "as needed" basis.

For both the primary inoculations and booster series, the patients will be monitored for 30 minutes after the inoculation with vital signs taken as needed. Local or systemic toxicities will be collected and graded by the research staff at the patient's next visit. The NCI CTCAE v4.03 graded toxicity scale (Appendix C) will be utilized to assess local and systemic toxicity.

Blood samples will be obtained from all patients, regardless of randomization, to determine induction of anticancer immune responses as described in Sections 5.4.4, 5.4.5, and 5.4.6.

5.4.3 Follow-up assessments

The clinical endpoint of recurrence will be determined by the patients' own physicians at the individual study sites during routine follow-up. This will include history and physical examination every 3-6 months for all patients. Additional laboratory and radiographic surveillance will be performed as indicated and directed by the patients treating physicians. The determination of recurrence will be per standard practice by the treating physicians and communicated to the study investigators on a routine basis. Documentation of any recurrence will be obtained. Whenever possible, pathologic confirmation of recurrence will be obtained.

However, a patient will also be considered to have a recurrence if highly suggested on radiographic evaluation and oncologic treatment for the recurrence is initiated by the treating physician. Documentation of the latter will be obtained. For the purposes of this trial, DFS will be calculated from the date of randomization until date of last follow-up or recurrence. If records are not available, patients, or their referring physicians, will be contacted to discern their disease status and every effort will be made to obtain documentation.

Once a patient recurs, they will be offered the opportunity to participate in the open label portion of the study. The patients will be vaccinated at 0, 1, 2, 3, 6, and 9 months. Assessments during inoculations and blood draws will be the same as above. These patients will be treated per SoC for their recurrence. The vaccinations will be performed in the background and will not interfere with SoC treatments. Additional laboratory and radiographic surveillance will be performed as indicated and directed by the patients treating physicians and per standard of care. The determination of tumor response will be assessed on these SoC scans per RECIST and iRECIST (Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors) (Appendix F). These results will be communicated by the primary study investigators on a routine basis.

All enrolled patients, regardless of randomization, will be followed for clinical recurrence and overall survival for a minimum of three years from randomization. The open label patients will be followed for 12 months.

5.4.4 Blood collection and processing

Multiple blood draws will be required for this trial. All blood tubes will be labeled only with the patients' unique study number. Approximately 50 mL will be collected for immunologic assessments prior to each inoculation (0, 1, 2, 6, 12, and 18 months) and at 24 months from randomization. Thus, a total of approximately 350 mL of blood will be drawn over the two-year course of the study. De-identified patient blood samples showing only the unique study ID number will be sent from study sites via overnight delivery to our central lab facility in Greenville, SC where they will be stored until used for immunologic assays (Section 5.4.6). At no point will laboratory personnel have access to patient identifiers. Blood will be frozen and stored under unique study numbers for up to five years for additional immunologic studies related to this protocol (for example, to repeat assays or perform new immunologic assays that do not exist at present but may become available) as needed and then destroyed. No genetic testing will be performed on this material. Study participants will not be contacted in the future for additional use of these stored blood specimens. If study participants want their blood specimens removed from storage and destroyed, they may do so by contacting the PI or research nurse at any time. Additionally, any stored blood may also be utilized to assess new generations of vaccines. A similar schedule will be followed for the open label portion of the study.

The 50 mL of blood will be collected as 10 mL into a BD Vacutainer Rapid Serum tube (BD, Franklin Lakes, NJ) which contain a clot activator and silicone coated interior. After receipt and centrifugation, serum will be collected, aliquoted in vials and frozen. The remaining 40 mL will be collected into four BD Vacutainer Heparin tubes (green tops), which contain an anticoagulant (sodium heparin). Once received, the heparinized blood will be pooled, diluted, and added to FICOLL HYPAQUE density gradient fluid containing tubes to allow for the separation of PBMC from the red blood cells by a single step centrifugation process. The PBMC fraction will be collected, aliquoted, and frozen for future use in immunologic assays.

5.4.5 Phenotypic assay (for example, dextramer assay) for melanoma-specific antigens

Thawed and cultured PBMC will be stained with aqua live/dead stain (Invitrogen) and the following antibodies: CD8 APC-H7 (BD Biosciences), CD3 PE Cy7 (BD Biosciences), gp100-APC-conjugated dextramer (Immudex), and the following pacific blue conjugated lineage antibodies: CD14 (BD Biosciences), CD16 (BD Biosciences), and CD19 (Biolegend). Cells will be analyzed on a Canto flow cytometer (BD Biosciences).

The frequency of gp100-specific $CD8^+$ T-cells will be determined as the percentage of cells that are alive, lineage-, $CD3^+$ $CD8^+$ and gp100-dextramer+. This assay may be performed for other known $CD8^+$ epitopes from known melanoma-specific antigens.

5.4.6 Functional assay (for example, ELISPOT assay) for melanoma-specific antigens

Thawed and isolated PBMC are cultured/stimulated overnight in complete medium (RPMI + 5 %FCS + PSG) supplemented with IL-7 (20 ng/mL) with the individual peptides at 25 µg/mL (gp100, MART-1, etc.) or PMA + Ionomycin in flat-bottom anti-human IFN-γ ELISPOT plates (BD PharMingen) at 5 x 10^5 cells/well/200 µL in duplicate wells. The plate is incubated at 37ºC overnight after which the wells will be washed and incubated with the biotinylated-anti-IFN-γ mAb for two hours. The wells will be washed again and incubated with streptavidin-conjugated HRP for one hour. After a final wash the AEC-substrate solution will be added to the wells and allowed to develop for approximately 5-10 minutes at which time the wells will be washed with deionized water to stop the reaction. The number of spots present in each well will be enumerated using the CTL ELISPOT analyzer (CTL Analyzers LLC, Cleveland, OH).

5.5 Discontinuation of Protocol-Specific Therapy

Protocol-specific therapy may be discontinued for any of the following reasons:

- Progressive disease
- Unacceptable toxicity as described in the protocol:
    - Please refer to Section 4.4 (Safety Considerations). Adverse events that do not respond to clinical management of the reaction will result in discontinuation from the study.
    - Please refer to Section 5.6 (Subject Discontinuation) for a list of severe adverse reactions warranting discontinuation from the study.
- Patient election to discontinue therapy (for any reason)
- Physician's judgment

5.6 Subject Discontinuation

Those patients who display significant reactions (e.g., anaphylactic reaction immediately after vaccine administration) or serious toxicities will be discontinued from the study as discussed below and as determined by the PI and/or Sponsor. They will be followed by the study investigator until resolution of the adverse event.

Inoculations will be immediately halted if any serious adverse reactions occur to include: death, life-threatening adverse drug experience (e.g.,, severe anaphylactic reaction immediately after vaccine administration), inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, a congenital anomaly/birth defect, or other important medical events that may not result in death, be life-threatening, or require hospitalization

but which, when based upon appropriate judgment of the PI, be determined to jeopardize the patient or require medical or surgical intervention to prevent an outcome listed above. Any death or grade 4 adverse drug experience found to be directly related to the experimental vaccine will result in suspension of patient enrollment to the study.

Patients may withdraw from the study at any time and for any reason. A patient may be asked to withdraw from the study by the PI if they are not compliant with the timing of the inoculation series, observation period, or return visits to monitor for study-associated toxicities.

Additionally, if the PI determines that it is no longer safe for a patient to continue in the trial for any reason, he/she may be withdrawn.

Because it is not known whether these inoculations might harm an unborn child, patients who are pregnant, plan on becoming pregnant, or who are breast-feeding will not be enrolled into the study. Women of childbearing potential will take a urine pregnancy test before starting this study and prior to each inoculation; a positive test result will terminate the patient's participation in the study. Patients will be counseled to avoid becoming pregnant while participating in this study, and that in order to prevent pregnancy they should either have no sexual relations or use a reliable type of birth control. They will be counseled that with the exception of hysterectomy, bilateral oophorectomy, or tubal ligation, birth control methods are not totally effective in preventing pregnancy, and that the only ways to completely avoid the risk of the vaccine or immunoadjuvant alone to an unborn baby are (1) avoid becoming pregnant, or (2) do not receive these inoculations. Patients will be counseled to avoid becoming pregnant for at least six months after receiving the inoculations, as pregnancy within this time after inoculation administration may be a risk to an unborn baby.

If a patient is discontinued from the study for an adverse event or pregnancy, they will continue to be followed for resolution of adverse event and clinical recurrences unless the patient withdraws consent for further study evaluation.

The reason for any premature discontinuation of a patient from the study will be recorded on the appropriate Case Report Form.

5.7 Study Discontinuation

The IND sponsor, the Data Safety Monitoring Board, and the overall study Principal Investigator have the right to suspend or terminate this study entirely or at a specific site at any time, and for any reason. Reasons for suspending or terminating the study may include, but are not limited to, the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to subjects
- Subject enrollment is unsatisfactory

- Data recording are inaccurate or incomplete
- Study protocol not followed

5.8 Data Collection

Basic demographic, pathologic, and relevant clinical information will be gathered on each patient and entered into an Electronic Data Capture (EDC) database. The URL for the EDC system is https://cancerinsight.eclinicalhosting.com/OpenClinica. The EDC system is accessible from any major web browser. User name and password will be generated by the Data Manager of Cancer Insight, LLC and sent via email to appropriate site personnel prior to enrolling their first patient. Clinical nurses at the site will be provided with Source Document Flow Sheets to capture data at enrollment and for each study visit. Although some data fields on the Flow Sheets will not be entered into EDC, they must be captured on the Flow Sheets for monitoring purposes.

All data must be submitted within 72 hours of the data collection visit. Data entry will begin with the patient's randomization with enrollment date to be date of signed informed consent.

Edit checks will fire in real time as data is being entered in the EDC system to ensure quality data is provided. In addition to edit checks, queries will be generated as Discrepancy Notes from the Data Manager and Monitors. Sites will have ten business days to update a query. It is the responsibility of the coordinator at each site to ensure that data has been submitted. Cancer Insight personnel or the Sponsor's representative will perform an audit of the site-specific Flow Sheets and will match them against source documents to ensure the quality of data coming to the Data Manager in the EDC per the internal monitoring plan.

The database, hosted by OpenClinica, resides in a SAS 70 Type II data center and meets ISO 17799 standards for information security. The EDC system is HIPAA and 21 CFR Part 11 compliant with robust audit logs, controlled user access, and electronic signature/password management. In addition to the site user, the Data Manager, the Monitors, the study PI and Sponsor/Sponsor representative have access to the database. Each user is assigned a role, which grants limited access and functionalities dependent upon that specific role.

5.9 Statistical Methods

5.9.1 Rationale for study design

Based on our prior phase I/IIa clinical trials in stage IV melanoma, the resected stage IV patients enjoyed the greatest benefit from vaccination. As such, stage III and IV (resected) patients are the target population for this adjuvant trial. Prevention of recurrence is the primary objective.

5.9.2 Sample size determination

Assuming a baseline recurrence rate of 60% (corresponding to a DFS of 40%) at two years based on published data in this mixed group of stage III (predominantly IIIB and IIIC) and stage IV (resected) melanoma patients, a sample of size of 120 will have 80% power to detect a statistical difference between treatment proportions controlling the type I error at alpha = 0.05 (two-sided).

A blinded evaluation of the first 75 patients enrolled discovered an early recurrence rate of 12% prior to completion of primary vaccine series. To compensate for this early recurrence rate and preserve the power of the trial, the enrollment will be extended by at least an additional 20 patients, now totaling at least 140.

After randomization of the 120th patient, the randomization will transition to 2:1 (TLPO phase I/IIa:TLPLDC) for an additional 60 patients. The TLPO vaccine group (n=40) will be assessed primarily for tolerability of the TLPO vaccine based on CTCAE v4.03 graded local and systemic toxicities. Given that the patients will be randomized between the TLPO phase I/IIa and TLPLDC, there will be an opportunity to directly compare the toxicity profile of these two vaccines.

Also, the 20 additional TLPLDC vaccine patients will be assessed with the original 120 randomized patients to account for the 140 patients mentioned above (Appendix B).

5.9.3 Data analysis

For the TLPLDC phase IIb, the intention-to-treat (ITT) population will include all subjects who were randomly assigned. ITT analysis will be performed with each randomized patient evaluated in the treatment arm to which they are randomized regardless of actual treatment received. The per-protocol (PP) population is a subset of the ITT population.

Subjects may be excluded from the PP population for the following reasons and others as determined prior to database lock: violations of eligibility criteria, development of second malignancies, early recurrences occurring prior to completion of the first four inoculations, receiving alternative disease-directed therapy without evidence of recurrence, or major deviation from prescribed vaccination schedule (to include boosters).

5.9.3.1 Study patient characteristics

Demographic characteristics of all patients in the ITT and PP populations will be summarized including age, race, disease histology, tumor location, tumor depth, ulceration, number of mitoses, nodal status (number and micro- vs macro-metastasis), AJCC clinical stage, location and number of distant metastases (if present), surgery extent, and other disease-directed therapies to include chemotherapy, radiation therapy, and biologic therapy. Continuous variables will be summarized using the number of patients, mean, standard deviation, median,

minimum, and maximum; and categorical variables will be summarized using the frequency count and the percentage of patients in each category. Differences between treatment groups will be determined using a chi-square test for categorical variables and a t-test for continuous variables.

5.9.3.2 Primary efficacy analysis

For the TLPLDC phase IIb, the primary efficacy endpoint of the trial is 24 month DFS when analyzed as a proportion using Pearson chi-square test. The primary efficacy analysis will be performed on the ITT and the PP population as equally important analyses given the high early recurrence rate.

The primary efficacy analysis will also be conducted by site for the ITT and PP populations in order to explore the possibility of a treatment by investigative site interaction. Investigative sites that individually represent fewer than five subjects will be combined for this exploratory analysis; geographic region will be substituted if the average number of subjects per site is fewer than ten.

The primary analysis will be conducted once the 120th patient enrolled completes 24 months of follow-up.

An interim analysis of DFS and compared between treatment groups will be performed 6 months after the 120th patient is enrolled. No early stopping rule is included.

5.9.3.3 Secondary efficacy analysis

For the TLPLDC phase IIb, DFS and overall survival (OS) will be compared between the vaccinated (TLPLDC and TLPO) and control groups at 36 months from the 120th patient enrolled. Both DFS and OS will be measured from time of randomization to date of recurrence and death, respectively. DFS and OS will be determined using the Kaplan-Meier method and differences between treatment groups will be assessed using the log-rank test.

In addition, Cox proportional hazards regression models will be fit to determine the association between DFS and OS and treatment group. The secondary efficacy analysis will be performed on the ITT and the PP population as equally important analyses given the high early recurrence rate.

Due to changes in standard of practice, specifically the FDA approval of checkpoint inhibitors (CPI) in the adjuvant setting, the protocol was modified to allow for the inclusion of patients who had demonstrated initial tolerability to CPI. These patients were randomized and received concurrent CPI + either TLPLDC or placebo. Therefore, as a part of the overall secondary efficacy analysis, an additional analysis will specifically assess DFS and OS in this subgroup of patients who received concurrent therapy.

During the course of the trial, it has been noted that the production method of the TLPLDC vaccine may impact clinical outcome. As such, as a part of the overall secondary efficacy analysis, an analysis will be performed for DFS and OS comparing patients who received TLPLDC produced from 120 mL of blood vs TLPLDC produced after G-CSF. Additionally, these groups will be compared to the placebo group in three arm analyses as well as exploratory two arm analyses by grouping like arms.

5.9.3.4 Safety analysis

For both trials, the incidence of treatment-emergent AEs, SAEs, severe AEs, AEs related to study drug, (defined as not, unlikely, possibly, probably, or definitely related to study drug), and AEs which lead to study discontinuation will be summarized by treatment group. Differences between treatment groups will be determined using Fisher's exact tests.

Time to AE onset will be estimated using the Kaplan-Meier method and treatment differences will be determined using the log-rank test. In addition, duration of any AE with >10% incidence, total number of AEs, SAEs, and related AEs will be summarized and treatment group differences assessed using a t-test or Wilcoxon rank sum test, depending on the distribution of the data.

5.9.3.5 Immunologic analysis

For both trials, immunologic responses will be determined using phenotypic and functional assays (*ex vivo*).

Expression percentages will be compared using a chi-square test while continuous outcomes will be compared using a paired t-test (within treatment group) and a two-sample t-test (between treatment groups). For the *ex vivo* response evaluated by the phenotypic and functional assays, the number of peptide-specific CTL will be determined at multiple time points (see Sections 5.4.4, 5.4.5, and 5.4.6) during the trial.

Changes from pre-vaccination levels to each time point will be compared, and differences within treatment groups will be evaluated using a paired t-test while between treatment group differences will be evaluated using two-sample t-tests.

In a series of exploratory analyses, the different immunologic response indicators (*in vivo* and *ex vivo*) at different time points will be correlated with clinical outcome in order to assess for any predictors of outcome. Additional exploratory outcomes will compare clinical and immunological outcomes based on the dose of vaccine (TLPLDC and TLPO) and number of inoculations received.

5.9.3.6 *Open Label Study*

At the time of recurrence, all patients will be offered the opportunity to participate in the open label portion of the study regardless of their randomization. All patients

will have new vaccine produced, and they will receive inoculations at 0, 1, 2, 3, 6, and 9 months. They will receive the vaccinations in the background of their SoC therapies initiated to treat their recurrence. These patients will be followed for safety and tumor response in combination with their SoC therapies and assessed per iRECIST and RECIST of their SoC follow-up scans.

5.9.4 Withdrawal

Subjects may withdraw or be discontinued from the study as discussed in Section 5.6. Subjects who do withdraw or are discontinued will be included in the efficacy analyses unless a subject withdraws consent to participate. In the instance of a subject withdrawing consent, any data collected will be excluded from analysis.

5.9.5 Missing data

Every reasonable attempt will be made to recover any missing data. If any data remains missing that data point will be excluded from analysis for that patient. A sensitivity analysis using the tipping point approach is the imputation strategy for missing data as described in the SAP, Section 4.7.

6.0 ADVERSE EVENTS

Reporting of adverse events (AEs) will be performed in accordance with the Data Safety Monitoring Plan (DSMP) (Appendix H), this protocol, and federal regulations.

6.1 Adverse Event and Reporting Definitions

With the occurrence of an AE, the first concern will be for the safety of the subject. An adverse event (AE) is any untoward medical occurrence in a subject participating in an investigational study or protocol regardless of causality assessment.

An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome, or disease associated with or occurring during the use of an investigational product whether or not considered related to the investigational product.

Serious Determination:

Serious adverse events (SAEs) are AEs occurring at any dose which meet one or more of the following serious criteria:

- Results in death (i.e., the AE caused or led to death)
- Is life-threatening (i.e. the AE placed the subject at immediate risk of death; it does not apply to an AE which hypothetically might have caused the death if it were more severe)

- Requires or prolongs inpatient hospitalization (i.e., the AE required at least a 24-hour hospitalization. (Inpatient hospitalization or prolonged a hospitalization beyond the expected length of stay; hospitalizations for elective medical/surgical procedures, scheduled treatments, or routine check-ups are not SAEs by this criterion.)

- Is disabling (i.e., the AE resulted in a substantial disruption of the subject's ability to carry out normal life functions)

- Is a congenital anomaly/birth defect (i.e., an adverse outcome in a child or fetus of a subject exposed to the study drug prior to conception or during pregnancy)

- Does not meet any of the above serious criteria but may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

Expected/ Unexpected Determination:

Expected AEs are those AEs that are listed or characterized in the current Investigator Brochure (IB) and/or are listed in the informed consent document. Unexpected AEs are those not listed in the current IB and/or informed consent document. This includes AEs for which the specificity or severity is not consistent with the description in the IB. For example, under this definition, hepatic necrosis would be unexpected if the Investigator Brochure only referred to elevated hepatic enzymes or hepatitis.

Study Drug Relationship:

The Principal Investigator will determine if there is a possibility that the study drug caused the adverse event. In the report to the sponsor, the investigator must include an assessment of causality, i.e., whether there is a possibility that the drug caused the event. Some factors that may be considered in determining if an AE should be regarded as possibly related to the use of the drug components are as follows:

- There is a clinically plausible time sequence between onset of the AE and administration of drug components;
- There is a biologically plausible mechanism for drug components to cause or contribute to the AE;
  The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.

6.2 Reporting of Adverse Events Associated with This Study

Investigators are required to report to the sponsor and/or sponsor's designated representative:

- Any adverse event that may be regarded as caused by, or possibly caused by, the study drug
- All serious adverse events
- All unanticipated problems involving risks to human subjects or others (UPIRSOs), including any adverse event that falls into this category
- All serious and unexpected adverse events for which there is a reasonable possibility that the adverse event was caused by the study drug (SUSARs)

Investigators are required to report to the Institutional Review Board (IRB):

- All unanticipated problems involving risks to human subjects or others (UPIRSOs)
- All adverse events that falls into the category of UPIRSO. An adverse event is a UPIRSO when it is unexpected, possibly related to participation in the research, and places the subject or others at a greater risk of harm than was previously known.
- All serious and unexpected adverse events for which there is a reasonable possibility that the adverse event was caused by the study drug (SUSARs)

If an event is reportable under 21 CFR §312.32, i.e., if the event is a SUSAR, the IND Sponsor or sponsor's representative will report these to the FDA per the requirements of 21 CFR § 312.32.

All events meeting the outlined criteria will be reported for the time period beginning with any amount of exposure to vaccine components through the protocol-defined follow-up period.

6.3 Reporting Requirements for IND Sponsor

6.3.1 MedWatch 3500A reporting guidelines

For sponsors of IND Studies, there are additional reporting requirements for the FDA in accordance with the regulations set forth in 21 CFR § 312.32. If an event is reportable under the U.S. Code of Federal Regulations, the sponsor will submit a Form FDA 3500A (MedWatch), as required by regulation. Form FDA 3500A Supplement provides general instructions for filling out the Form FDA 3500A, and should be used as guidance. Events meeting the following criteria need to be submitted to the FDA as expedited IND Safety Reports according to the following guidance and timelines:

7 Calendar-Day Telephone or Fax Report: The Sponsor is required to notify the FDA of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible, but no later than 7 calendar days after the sponsor's initial receipt of the information.

15 Calendar-Day Written Report: The Sponsor is required to notify the FDA and all participating investigators, in a written IND Safety Report, of any suspected

adverse reaction that is both serious and unexpected. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event.

- Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed 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 and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g., summary letter).

Follow-up information:

If any information necessary to evaluate the suspected unexpected serious adverse reaction is missing or unknown, or if new information becomes available, that information should be submitted as a Follow Up IND Safety Report as soon as the information is available, but no later than 15 calendar days after the sponsor receives the information.

Study Drug Relationship Determination for Reporting Purposes:

For reporting purposes, suspected adverse reactions are those where there is a reasonable possibility that the study drug caused the adverse event. For reporting purposes, “reasonable possibility” means there is evidence to suggest a causal relationship between the drug and the adverse event. See 21 CFR 312.32.

7.0 INVESTIGATOR REQUIREMENTS

7.1 Study Initiation

Before the start of this study, the following documents must be on file with the Sponsor or Sponsor’s representative:

- Original U.S. Form FDA 1572 for each Principal Investigator (for all studies conducted under U.S. Investigational New Drug [IND] regulations), signed by the Principal Investigator. The names of any sub-investigators must appear on this form, as well as all other information required by the form.

- Current *curriculum vitae* of the Principal Investigator

- Written documentation of IRB approval of protocol and informed consent document

- A copy of the IRB-approved informed consent document
- A signed Clinical Research Agreement
- A signed investigator's agreement (Appendix K)
- Laboratory Documentation
- All other essential documents required to be obtained under the CFR and ICH GCP, as applicable to this study

7.2 Study Completion

The following materials are requested by the sponsor when the study is considered complete or terminated:

- A summary, prepared by the Principal Investigator, of the study, and/or a study manuscript, and/or a study abstract submitted to scientific conferences.

7.3 Informed Consent

Informed consent form templates will be provided, and the final IRB-approved document must be provided to the Sponsor or Sponsor's representative for regulatory purposes. The informed consent document must be signed by the subject or the subject's legally authorized representative before his or her participation in the study. The case history for each subject shall document that informed consent was obtained prior to participation in the study.

Copies of the informed consent form documents must be provided to the subject or the subject's legally authorized representative. If applicable, it will be provided in a certified translation of the local language.

Signed consent forms must remain in each subject's study file and must be available for verification by study monitors at any time.

7.4 Institutional Review Board or Ethics Committee Approval

This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB for review and must be approved before the study is initiated. The study will be conducted in accordance with U.S. FDA, applicable national and local health authorities, and IRB requirements.

The Principal Investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case the IRB must be updated at least once a year. Investigators are required to promptly notify their respective IRB of events as described in this protocol in Sections 6.0, 6.1, and 6.2. Some IRBs may have other

specific adverse event requirements to which investigators are expected to adhere. Investigators must immediately forward to their IRB any written safety report or update provided by the Sponsor (e.g., IND safety report, IB, safety amendments and updates, etc.).

Ethics and Regulatory Considerations

The protocol will be reviewed and approved by the IRB or Independent Ethics Committee (IEC) of each participating center prior to study initiation. A list of IRB/IEC members should be obtained by the investigator and provided to the sponsor and sponsor representative. Any documents that the IRB/IEC may need to fulfill its responsibilities, such as protocol amendments and/or information from the sponsor will be submitted to the IRB/IEC. The IRB/IEC's written unconditional approval of the study protocol and the informed consent form will be in the possession of the PI and the Sponsor before the study is initiated. The IRB/IEC's unconditional approval statement will be transmitted by the investigator or designee to the sponsor/sponsor representative prior to shipment of study drug supplies to the site. This approval document must refer to the study by exact protocol title and protocol version number/date and must identify the documents reviewed and the date of review.

Protocol modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazard to the patients. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and IRB/IEC acknowledgement/approval should be obtained and transmitted to the Sponsor/Investigator or Sponsor/Investigator's representative. The IRB/IEC must be informed by the principal investigator of any changes or revisions of informed consent forms or other documents originally submitted for review; adverse events and other reportable events as described in this protocol in Sections 6.0, 6.1, and 6.2; any new information that may affect adversely the safety of the patients or the conduct of the study; annual updates and/or request for re-approval; and when the study has been completed.

7.5 Study Monitoring Requirements

Site visits may be conducted by authorized Sponsor representative or CRO representatives to inspect study data, subjects' medical records, and CRFs in accordance with current U.S. GCPs and the respective local and national government regulations and guidelines (if applicable).

The Principal Investigator will permit authorized representatives of Sponsor, CRO, the FDA, and the respective national or local health authorities to inspect facilities and records relevant to this study.

7.6 Data Safety Monitoring Plan

A DSMP (Appendix H) describing the CRO internal monitoring plan includes data safety and integrity and site initiation/QA monitoring, as well as external monitoring plan, the Data Safety Monitoring Board (DSMB) charter and responsibilities.

7.7 Study Medication Accountability

The study drug will be provided by the Sponsor. The recipient will acknowledge receipt of the drug by returning the INDRR-1 form indicating shipment content and condition. Damaged supplies will be replaced. Accurate records of all study drug dispensed from and returned to the study site should be recorded by using the institution's drug inventory log or the NCI drug accountability log. All partially used or empty containers should be disposed of at the study site according to institutional standard operating procedure. Return unopened, expired, or unused study drug with the Inventory of Returned Clinical Material form as directed by the Sponsor.

7.8 Disclosure of Data

Subject medical information obtained by this study is confidential, and disclosure to third parties other than those noted above is prohibited. Upon the subject's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. Data generated by this study must be available for inspection upon request by representatives of the FDA, national and local health authorities, the Sponsor, and the IRB for each study site, if appropriate.

7.9 Retention of Records

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for two years after marketing application approval. If no application is filed, these records must be kept two years after the investigation is discontinued and the FDA and the applicable national and local health authorities are notified. The Sponsor will notify the Principal Investigator of these events.

For studies conducted outside the United States under a U.S. IND, the Principal Investigator must comply with U.S. FDA IND regulations and with the record retention policies of the relevant national and local health authorities.

7.10 Publications

The investigator must agree to send to the Sponsor or Sponsor's representative, for review all manuscripts, abstracts and presentations using data from this study prior to their submission. The Sponsor or Sponsor's representative reserves the

right to delete from such materials any part or parts deemed to be confidential or proprietary.

7.11 Changes to Protocol

The protocol may not be modified without written approval of the Sponsor or Sponsor's representative, or the Study Director. All changes to the protocol must be submitted to the FDA, the overseeing IRB, and local IRB/IEC. Additionally, changes must be approved by overseeing IRB prior to their implementation. Documentation of IRB/IEC approval must be sent to the Sponsor or Sponsor's representative, and the Study Director immediately upon receipt. Any changes and modifications to the informed consent language must be reviewed and approved by the Sponsor or Sponsor's representative, and the Study Director prior to submission to the local IRB.

REFERENCES

1. Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. N Engl J Med 2004; 351:998-1012.
2. DR P, EA G. Cytokines: biology and applications in cancer medicine. In: al JHEFIRBJe, ed. Cancer Medicine 4th Editions, 1997:1213-26.
3. Philip PA, Flaherty L. Treatment of malignant melanoma with interleukin-2. Semin Oncol 1997; 24:S32-8.
4. Smith FO, Downey SG, Klapper JA, Yang JC, Sherry RM, Royal RE, Kammula US, Hughes MS, Restifo NP, Levy CL, et al. Treatment of metastatic melanoma using interleukin-2 alone or in conjunction with vaccines. Clin Cancer Res 2008; 14:5610-8.
5. Atkins MB, Kunkel L, Sznol M, Rosenberg SA. High-dose recombinant interleukin-2 therapy in patients with metastatic melanoma: long-term survival update. The cancer journal from Scientific American 2000; 6 Suppl 1:S11-4.
6. Legha SS, Ring S, Bedikian A, Plager C, Eton O, Buzaid AC, Papadopoulos N. Treatment of metastatic melanoma with combined chemotherapy containing cisplatin, vinblastine and dacarbazine (CVD) and biotherapy using interleukin-2 and interferon-alpha. Ann Oncol 1996; 7:827-35.
7. Legha SS, Ring S, Eton O, Bedikian A, Buzaid AC, Plager C, Papadopoulos N. Development of a biochemotherapy regimen with concurrent administration of cisplatin, vinblastine, dacarbazine, interferon alfa, and interleukin-2 for patients with metastatic melanoma. J Clin Oncol 1998; 16:1752-9.
8. O'Day SJ, Boasberg PD, Piro L, Kristedja TS, Wang HJ, Martin M, Deck R, Ames P, Shinn K, Kim H, et al. Maintenance biotherapy for metastatic melanoma with interleukin-2 and granulocyte macrophage-colony stimulating factor improves survival for patients responding to induction concurrent biochemotherapy. Clin Cancer Res 2002; 8:2775-81.
9. Atkins MB, Hsu J, Lee S, Cohen GI, Flaherty LE, Sosman JA, Sondak VK, Kirkwood JM, Eastern Cooperative Oncology G. Phase III trial comparing concurrent biochemotherapy with cisplatin, vinblastine, dacarbazine, interleukin-2, and interferon alfa-2b with cisplatin, vinblastine, and dacarbazine alone in patients with metastatic malignant melanoma (E3695): a trial coordinated by the Eastern Cooperative Oncology Group. J Clin Oncol 2008; 26:5748-54.
10. Ives NJ, Stowe RL, Lorigan P, Wheatley K. Chemotherapy compared with biochemotherapy for the treatment of metastatic melanoma: a meta-analysis of 18 trials involving 2,621 patients. J Clin Oncol 2007; 25:5426-34.
11. Bajetta E, Del Vecchio M, Nova P, Fusi A, Daponte A, Sertoli MR, Queirolo P, Taveggia P, Bernengo MG, Legha SS, et al. Multicenter phase III randomized trial of polychemotherapy (CVD regimen) versus the same chemotherapy (CT) plus subcutaneous interleukin-2 and interferon-alpha2b in metastatic melanoma. Ann Oncol 2006; 17:571-7.
12. Keilholz U, Punt CJ, Gore M, Kruit W, Patel P, Lienard D, Thomas J, Proebstle TM, Schmittel A, Schadendorf D, et al. Dacarbazine, cisplatin, and interferon-alfa-2b with or without interleukin-2 in metastatic melanoma: a randomized phase III trial (18951) of the European Organisation for Research and Treatment of Cancer Melanoma Group. J Clin Oncol 2005; 23:6747-55.
13. Ridolfi R, Chiarion-Sileni V, Guida M, Romanini A, Labianca R, Freschi A, Lo Re G, Nortilli R, Brugnara S, Vitali P, et al. Cisplatin, dacarbazine with or without subcutaneous interleukin-2, and interferon alpha-2b in advanced melanoma outpatients: results from an Italian multicenter phase III randomized clinical trial. J Clin Oncol 2002; 20:1600-7.

14. NCCN Practical Guidelines in Oncology: Melanoma. National Comprehensive Cancer Network 2014; Version 3.2014.
15. Cascinelli N, Belli F, MacKie RM, Santinami M, Bufalino R, Morabito A. Effect of long-term adjuvant therapy with interferon alpha-2a in patients with regional node metastases from cutaneous melanoma: a randomised trial. Lancet 2001; 358:866-9.
16. Hancock BW, Wheatley K, Harris S, Ives N, Harrison G, Horsman JM, Middleton MR, Thatcher N, Lorigan PC, Marsden JR, et al. Adjuvant interferon in high-risk melanoma: the AIM HIGH Study--United Kingdom Coordinating Committee on Cancer Research randomized study of adjuvant low-dose extended-duration interferon Alfa-2a in high-risk resected malignant melanoma. J Clin Oncol 2004; 22:53-61.
17. Pehamberger H, Soyer HP, Steiner A, Kofler R, Binder M, Mischer P, Pachinger W, Aubock J, Fritsch P, Kerl H, et al. Adjuvant interferon alfa-2a treatment in resected primary stage II cutaneous melanoma. Austrian Malignant Melanoma Cooperative Group. J Clin Oncol 1998; 16:1425-9.
18. Grob JJ, Dreno B, de la Salmoniere P, Delaunay M, Cupissol D, Guillot B, Souteyrand P, Sassolas B, Cesarini JP, Lionnet S, et al. Randomised trial of interferon alpha-2a as adjuvant therapy in resected primary melanoma thicker than 1.5 mm without clinically detectable node metastases. French Cooperative Group on Melanoma. Lancet 1998; 351:1905-10.
19. Kirkwood JM, Strawderman MH, Ernstoff MS, Smith TJ, Borden EC, Blum RH. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. J Clin Oncol 1996; 14:7-17.
20. Kirkwood JM, Ibrahim JG, Sondak VK, Richards J, Flaherty LE, Ernstoff MS, Smith TJ, Rao U, Steele M, Blum RH. High- and low-dose interferon alfa-2b in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. J Clin Oncol 2000; 18:2444-58.
21. Kirkwood JM, Manola J, Ibrahim J, Sondak V, Ernstoff MS, Rao U, Eastern Cooperative Oncology G. A pooled analysis of eastern cooperative oncology group and intergroup trials of adjuvant high-dose interferon for melanoma. Clin Cancer Res 2004; 10:1670-7.
22. Eggermont AM, Suciu S, Santinami M, Testori A, Kruit WH, Marsden J, Punt CJ, Sales F, Gore M, Mackie R, et al. Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: final results of EORTC 18991, a randomised phase III trial. Lancet 2008; 372:117-26.
23. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363:711-23.
24. Robert C, Thomas L, Bondarenko I, O'Day S, M DJ, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011; 364:2517-26.
25. Ipilimumab or High-Dose Interferon Alfa-2b in Treating Patients With High-Risk Stage III-IV Melanoma That Has Been Removed by Surgery. http://www.clinicaltrials.gov/ct2/show/NCT01274338?term=A+Phase+III+Randomized+Study+of+Adjuvant+Ipilimumab+Anti-CTLA4+Therapy&rank=1: ClinicalTrials.gov.
26. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366:2443-54.
27. Taneja SS. Re: Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. The Journal of urology 2012; 188:2148-9.
28. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Current opinion in immunology 2012; 24:207-12.

29. Mario Sznol HMK, F. Stephen Hodi, David F. McDermott, Richard D. Carvajal, Donald P. Lawrence, Suzanne Louise Topalian, Michael B. Atkins, John D. Powderly, William Howard Sharfman, Igor Puzanov, David C. Smith, Jon M. Wigginton, Georgia Kollia, Ashok Kumar Gupta, Jeffrey Alan Sosman. Survival and long-term follow-up of safety and response in patients (pts) with advanced melanoma (MEL) in a phase I trial of nivolumab (anti-PD-1; BMS-936558; ONO-4538). J Clin Oncol 31, 2013 (suppl; abstr CRA9006^) 2013.
30. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013; 369:122-33.
31. ClinicalTrials.gov. Phase 3 Study of Nivolumab or Nivolumab Plus Ipilimumab Versus Ipilimumab Alone in Previously Untreated Advanced Melanoma (CheckMate 067). http://clinicaltrials.gov/ct2/show/NCT0184450.
32. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010; 363:411-22.
33. Stockert E, Jager E, Chen YT, Scanlan MJ, Gout I, Karbach J, Arand M, Knuth A, Old LJ. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med 1998; 187:1349-54.
34. Sahin U, Tureci O, Schmitt H, Cochlovius B, Johannes T, Schmits R, Stenner F, Luo G, Schobert I, Pfreundschuh M. Human neoplasms elicit multiple specific immune responses in the autologous host. Proc Natl Acad Sci U S A 1995; 92:11810-3.
35. Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, Kavanaugh D, Carbone DP. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med 1996; 2:1096-103.
36. Ishida T, Oyama T, Carbone DP, Gabrilovich DI. Defective function of Langerhans cells in tumor-bearing animals is the result of defective maturation from hemopoietic progenitors. J Immunol 1998; 161:4842-51.
37. Gong J, Nikrui N, Chen D, Koido S, Wu Z, Tanaka Y, Cannistra S, Avigan D, Kufe D. Fusions of human ovarian carcinoma cells with autologous or allogeneic dendritic cells induce antitumor immunity. J Immunol 2000; 165:1705-11.
38. Song W, Kong HL, Carpenter H, Torii H, Granstein R, Rafii S, Moore MA, Crystal RG. Dendritic cells genetically modified with an adenovirus vector encoding the cDNA for a model antigen induce protective and therapeutic antitumor immunity. J Exp Med 1997; 186:1247-56.
39. Specht JM, Wang G, Do MT, Lam JS, Royal RE, Reeves ME, Rosenberg SA, Hwu P. Dendritic cells retrovirally transduced with a model antigen gene are therapeutically effective against established pulmonary metastases. J Exp Med 1997; 186:1213-21.
40. Li J, Holmes LM, Franek KJ, Wagner TE, Wei Y. Murine tyrosinase expressed by a T7 vector in bone marrow-derived dendritic progenitors effectively prevents and eradicates melanoma tumors in mice. Cancer gene therapy 2000; 7:1448-55.
41. Wei Y, Li J, Chen WY, Yu X, Sticca RP, Wagner TE. Enhanced transgene expression and effective in vivo antitumor immune responses initiated by dendritic progenitors transfected with a nonviral T7 vector expressing a model tumor antigen. J Immunother 2000; 23:75-82.
42. Trefzer U, Weingart G, Chen Y, Herberth G, Adrian K, Winter H, Audring H, Guo Y, Sterry W, Walden P. Hybrid cell vaccination for cancer immune therapy: first clinical trial with metastatic melanoma. Int J Cancer 2000; 85:618-26.
43. Rosenberg SA, Yang JC, Schwartzentruber DJ, Hwu P, Marincola FM, Topalian SL, Restifo NP, Dudley ME, Schwarz SL, Spiess PJ, et al. Immunologic and therapeutic

evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nat Med 1998; 4:321-7.
44. Wallack MK, Sivanandham M, Balch CM, Urist MM, Bland KI, Murray D, Robinson WA, Flaherty LE, Richards JM, Bartolucci AA, et al. A phase III randomized, double-blind multiinstitutional trial of vaccinia melanoma oncolysate-active specific immunotherapy for patients with stage II melanoma. Cancer 1995; 75:34-42.
45. Bystryn JC. Clinical activity of a polyvalent melanoma antigen vaccine. Recent Results Cancer Res 1995; 139:337-48.
46. Mitchell MS. Perspective on allogeneic melanoma lysates in active specific immunotherapy. Semin Oncol 1998; 25:623-35.
47. Morton DL, Hoon DS, Nizze JA, Foshag LJ, Famatiga E, Wanek LA, Chang C, Irie RF, Gupta RK, Elashoff R. Polyvalent melanoma vaccine improves survival of patients with metastatic melanoma. Annals of the New York Academy of Sciences 1993; 690:120-34.
48. Berd D, Kairys J, Dunton C, Mastrangelo MJ, Sato T, Maguire HC, Jr. Autologous, hapten-modified vaccine as a treatment for human cancers. Semin Oncol 1998; 25:646-53.
49. Berd D, Maguire HC, Jr., Schuchter LM, Hamilton R, Hauck WW, Sato T, Mastrangelo MJ. Autologous hapten-modified melanoma vaccine as postsurgical adjuvant treatment after resection of nodal metastases. J Clin Oncol 1997; 15:2359-70.
50. Gong J, Chen D, Kashiwaba M, Kufe D. Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. Nat Med 1997; 3:558-61.
51. Wang J, Saffold S, Cao X, Krauss J, Chen W. Eliciting T cell immunity against poorly immunogenic tumors by immunization with dendritic cell-tumor fusion vaccines. J Immunol 1998; 161:5516-24.
52. Lespagnard L, Mettens P, Verheyden AM, Tasiaux N, Thielemans K, van Meirvenne S, Geldhof A, De Baetselier P, Urbain J, Leo O, et al. Dendritic cells fused with mastocytoma cells elicit therapeutic antitumor immunity. Int J Cancer 1998; 76:250-8.
53. Cao X, Zhang W, Wang J, Zhang M, Huang X, Hamada H, Chen W. Therapy of established tumour with a hybrid cellular vaccine generated by using granulocyte-macrophage colony-stimulating factor genetically modified dendritic cells. Immunology 1999; 97:616-25.
54. Zhang JK, Li J, Zhang J, Chen HB, Chen SB. Antitumor immunopreventive and immunotherapeutic effect in mice induced by hybrid vaccine of dendritic cells and hepatocarcinoma in vivo. World J Gastroenterol 2003; 9:479-84.
55. Kawada M, Ikeda H, Takahashi T, Ishizu A, Ishikura H, Katoh H, Yoshiki T. Vaccination of fusion cells of rat dendritic and carcinoma cells prevents tumor growth in vivo. Int J Cancer 2003; 105:520-6.
56. McConnell EJ, Pathangey LB, Madsen CS, Gendler SJ, Mukherjee P. Dendritic cell-tumor cell fusion and staphylococcal enterotoxin B treatment in a pancreatic tumor model. The Journal of surgical research 2002; 107:196-202.
57. Xia J, Tanaka Y, Koido S, Liu C, Mukherjee P, Gendler SJ, Gong J. Prevention of spontaneous breast carcinoma by prophylactic vaccination with dendritic/tumor fusion cells. J Immunol 2003; 170:1980-6.
58. Chen D, Xia J, Tanaka Y, Chen H, Koido S, Wernet O, Mukherjee P, Gendler SJ, Kufe D, Gong J. Immunotherapy of spontaneous mammary carcinoma with fusions of dendritic cells and mucin 1-positive carcinoma cells. Immunology 2003; 109:300-7.
59. Gong J, Avigan D, Chen D, Wu Z, Koido S, Kashiwaba M, Kufe D. Activation of antitumor cytotoxic T lymphocytes by fusions of human dendritic cells and breast carcinoma cells. Proc Natl Acad Sci U S A 2000; 97:2715-8.

60. Marten A, Renoth S, Heinicke T, Albers P, Pauli A, Mey U, Caspari R, Flieger D, Hanfland P, Von Ruecker A, et al. Allogeneic dendritic cells fused with tumor cells: preclinical results and outcome of a clinical phase I/II trial in patients with metastatic renal cell carcinoma. Hum Gene Ther 2003; 14:483-94.
61. Holmes LM, Li J, Sticca RP, Wagner TE, Wei Y. A Rapid, Novel Strategy to Induce Tumor Cell-Specific Cytotoxic T Lymphocyte Responses Using Instant Dendritomas. Journal of immunotherapy : official journal of the Society for Biological Therapy 2001; 24:122-9.
62. Li J, Holmes LM, Franek KJ, Burgin KE, Wagner TE, Wei Y. Purified hybrid cells from dendritic cell and tumor cell fusions are superior activators of antitumor immunity. Cancer Immunol Immunother 2001; 50:456-62.
63. Wei YC, Sticca RP, Li J, Holmes LM, Burgin KE, Jakubchak S, Bouton-Verville H, Williamson J, Meyer K, Evans L, et al. Combined treatment of dendritoma vaccine and low-dose interleukin-2 in stage IV renal cell carcinoma patients induced clinical response: A pilot study. Oncol Rep 2007; 18:665-71.
64. Wei Y, Sticca RP, Holmes LM, Burgin KE, Li J, Williamson J, Evans L, Smith SJ, Stephenson JJ, Wagner TE. Dendritoma vaccination combined with low dose interleukin-2 in metastatic melanoma patients induced immunological and clinical responses. Int J Oncol 2006; 28:585-93.
65. Sun W, Schuchter LM. Metastatic melanoma. Current treatment options in oncology 2001; 2:193-202.
66. Han KR, Pantuck AJ, Bui MH, Shvarts O, Freitas DG, Zisman A, Leibovich BC, Dorey FJ, Gitlitz BJ, Figlin RA, et al. Number of metastatic sites rather than location dictates overall survival of patients with node-negative metastatic renal cell carcinoma. Urology 2003; 61:314-9.
67. Avigan D, Vasir B, Gong J, Borges V, Wu Z, Uhl L, Atkins M, Mier J, McDermott D, Smith T, et al. Fusion cell vaccination of patients with metastatic breast and renal cancer induces immunological and clinical responses. Clin Cancer Res 2004; 10:4699-708.
68. Nair SK, Morse M, Boczkowski D, Cumming RI, Vasovic L, Gilboa E, Lyerly HK. Induction of tumor-specific cytotoxic T lymphocytes in cancer patients by autologous tumor RNA-transfected dendritic cells. Ann Surg 2002; 235:540-9.
69. Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat Med 1998; 4:328-32.
70. Banchereau J, Palucka AK, Dhodapkar M, Burkeholder S, Taquet N, Rolland A, Taquet S, Coquery S, Wittkowski KM, Bhardwaj N, et al. Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. Cancer Res 2001; 61:6451-8.
71. Timmerman JM, Singh G, Hermanson G, Hobart P, Czerwinski DK, Taidi B, Rajapaksa R, Caspar CB, Van Beckhoven A, Levy R. Immunogenicity of a plasmid DNA vaccine encoding chimeric idiotype in patients with B-cell lymphoma. Cancer Res 2002; 62:5845-52.
72. Geiger JD, Hutchinson RJ, Hohenkirk LF, McKenna EA, Yanik GA, Levine JE, Chang AE, Braun TM, Mule JJ. Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression. Cancer Res 2001; 61:8513-9.
73. Schuler-Thurner B, Schultz ES, Berger TG, Weinlich G, Ebner S, Woerl P, Bender A, Feuerstein B, Fritsch PO, Romani N, et al. Rapid induction of tumor-specific type 1 T helper cells in metastatic melanoma patients by vaccination with mature,

cryopreserved, peptide-loaded monocyte-derived dendritic cells. J Exp Med 2002; 195:1279-88.
74. Eisenhauer E a., Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-247.
75. Seymour L, Bogaerts J, Perrone A, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. The Lancet Oncology. 2017;18(3):e143-52.

Appendix A

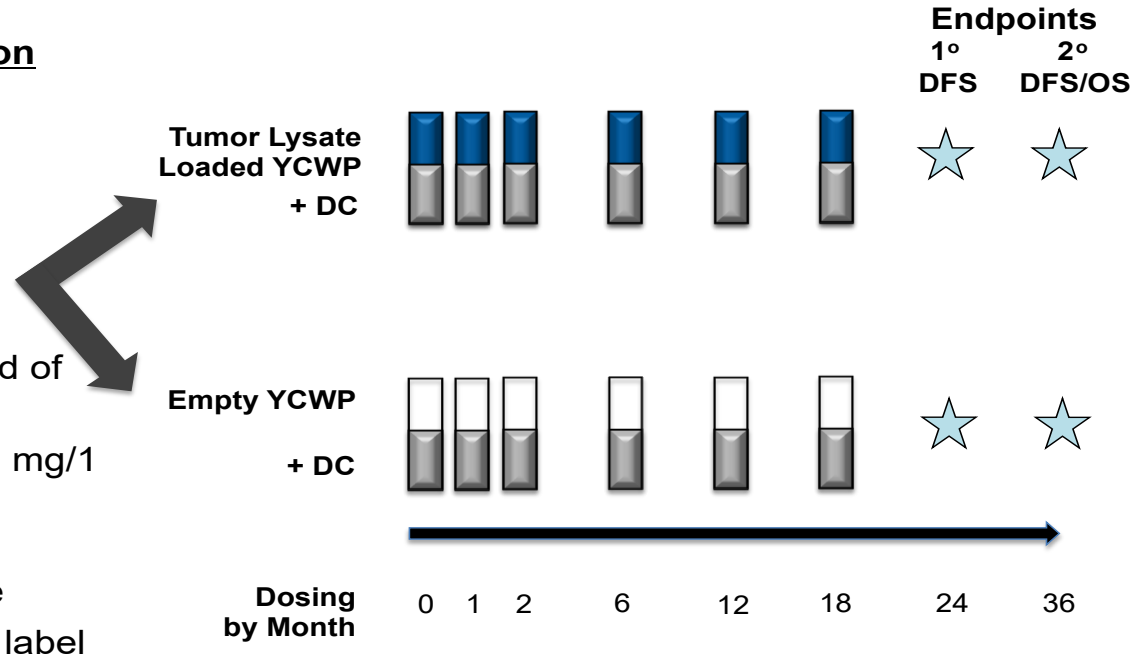
Trial Schema

A prospective, randomized, blinded, placebo-controlled, phase IIb trial of an autologous tumor lysate (TL)+yeast cell wall particles (YCWP)+dendritic cells (DC) vaccine vs unloaded YCWP+DC in stage III and stage IV (resected) melanoma to prevent recurrence

Study Population

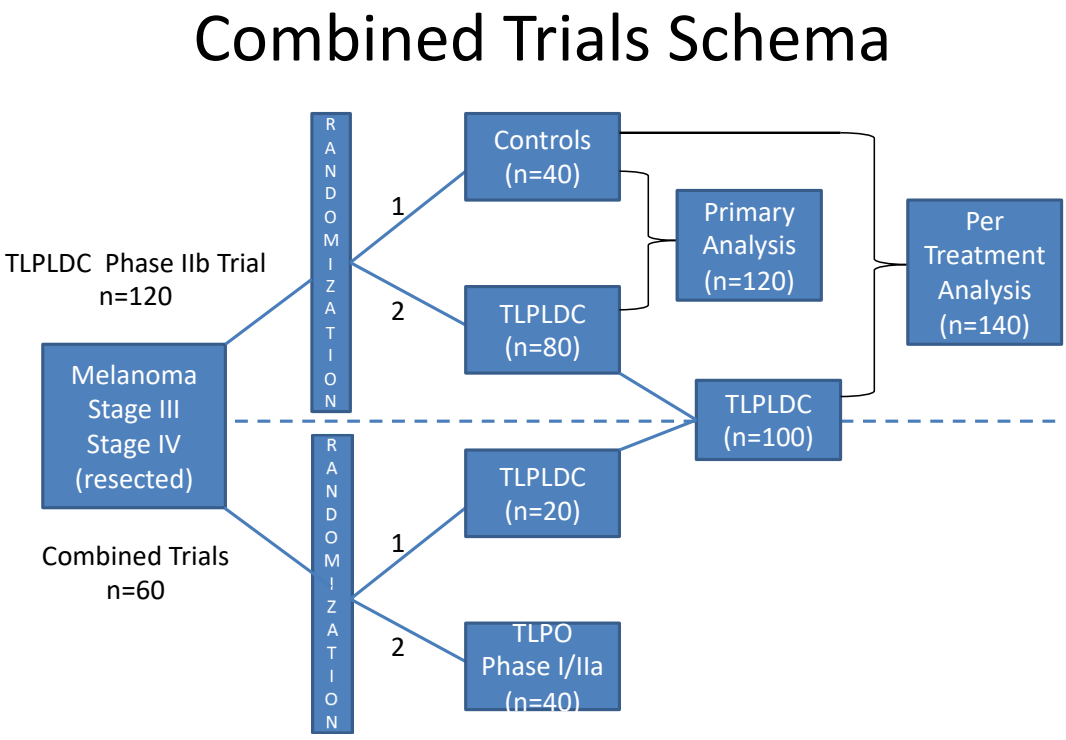
Adjuvant Melanoma patients, n=140, randomized 2:1 (vaccine:control)

- Stage III and IV (resected)
- NED after standard of care (SOC)
- Sufficient tumor (1 mg/1 cm^3) available to produce vaccine
- Stratified by Stage
- Crossover – open label treatment of recurred controls



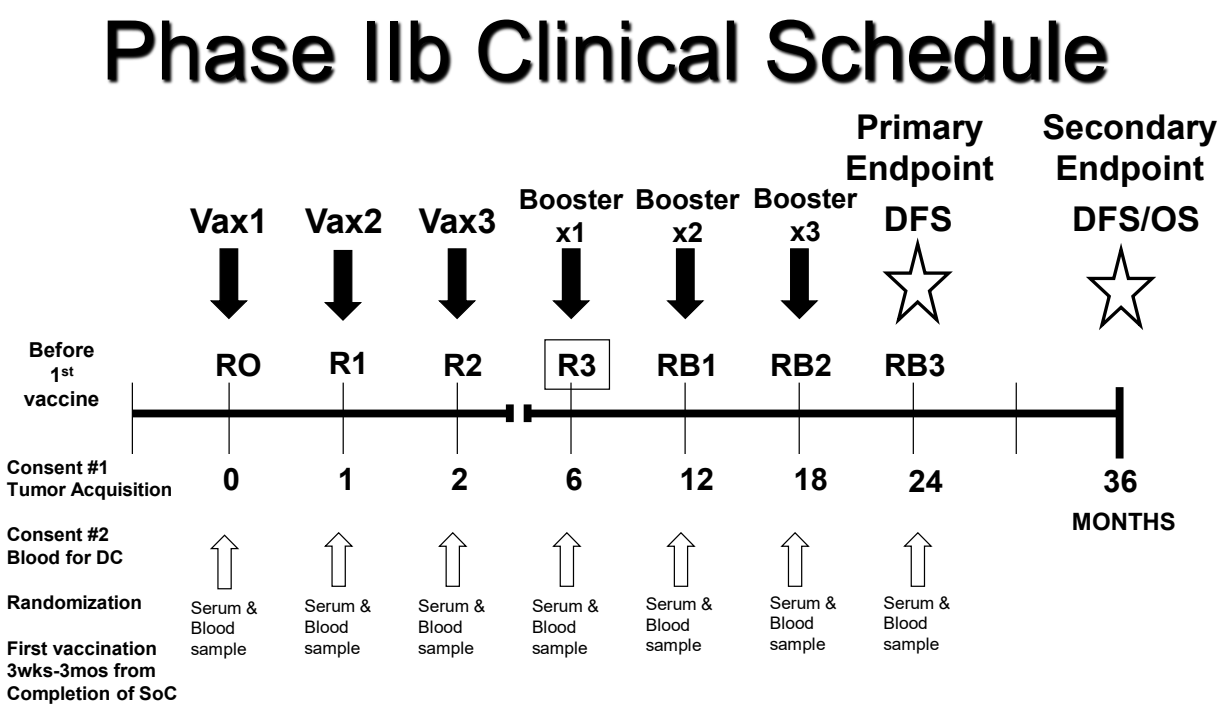
Appendix B

Combined Trial Schema



Appendix C

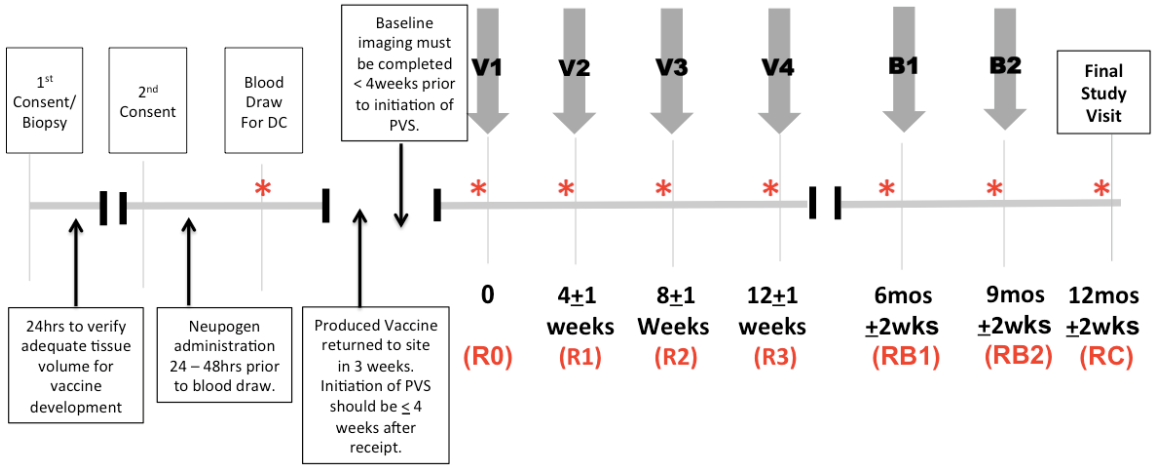
Vaccination Timeline



Appendix C

Open Label Vaccination Timeline

Phase I/IIa Study Timeline



* Blood draws completed immediately prior to each inoculation and at the 12 month follow-up appointment.

Appendix D

NCI Common Terminology Criteria for Adverse Events, v4.03

obtained from http://ctep.cancer.gov/forms/CTCAEv4.03.pdf
http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06 14_QuickReference_8.5x11.pdf

Appendix E

ECOG Performance Status Criteria

Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

Appendix F

1. Quick Reference for the Response Evaluation Criteria in Solid Tumors (RECIST Criteria)

A. Indications for use of RECIST Criteria
- The RECIST criteria is useful in all trials where objective response is the primary study endpoint, as well as in trials where assessment of stable disease, tumor progression or time to progression analyses are undertaken, since all of these outcome measures are based on an assessment of anatomical tumor burden and its change on the study.

B. Identification of Lesions

I. Measurable disease
- All patients must have measurable disease to be enrolled into this trial.
- All baseline imaging for determining these target lesions must be completed no more than *4 weeks* prior to beginning treatment
- Measurable disease includes:
    - Tumors that can be accurately measured along the *longest* diameter
    - Tumors must be ≥10mm by CT (CT must use 5mm cuts or smaller)
    - If tumor lesions are measured on clinical exam, the lesion should be superficial, ≥10mm in diameter when measured by a caliber
    - Tumors must be ≥20mm by chest xray.

II. Lymph nodes must be measured along its *shortest* axis
    - Nodes must be >15mm by CT scan (CT must use 5mm cuts or smaller)

III. Target lesions
- Target lesions must be determined by each primary investigator
- Baseline imaging must be completed no more than 4 weeks before initiation of trial
- All target lesions must be measurable tumors that are easily monitored with reproducible repeated measurements.
    - Maximum of 5 target lesions
    - Maximum of 2 lesions per organ
    - A sum of all the diameters for each target lesion is recorded and monitored throughout the study forming a *baseline sum of diameters*.
- All other lesions will be deemed non-target lesions. They may be followed throughout the trial, but will not be incorporated into the baseline sum of diameters. These non-target tumors must be recorded

separately.

IV. Imaging Modalities

- All lesions must be measurable per iRECIST 1.1 Criteria. It is preferred to monitor each lesion with an imaging study if able.
    - CT scans must be completed as IV contrast and a 5mm cuts.
    - MRI may also be used.
    - FDG-PET useful in monitoring lesions. It should be combined with a CT scan for complete evaluation of tumors.

C. Evaluation of Tumor Response

- Tumor response of target lesions include:
    - Complete response (CR): Disappearance of all target lesions. Any pathologic lymph nodes must be ≤10mm (target or non-target nodes).
    - Partial Response (PR): At least a *30% decrease* in the sum of diameters for target lesions in comparison to baseline sum of diameters.
    - Progressive Disease (PD): At least a *20% increase* in the sum of diameters for target lesions. In addition to this increase, the sum of diameters must increase by at least 5mm. The development of new lesions is also evidence of PD.
    - Stable Disease (SD): Tumors neither fit criteria for PR or PD.
- Tumor response of non-target lesions include:
    - CR - Disappearance of all target lesions. Any pathologic lymph nodes must be <10mm (target or non-target nodes).
    - Non-CR/Non-PD - Persistence of one or more non-target lesions and maintenance of tumor marker level above the normal limits.
    - PD – Unequivocal progression of existing non-target tumors, or the appearance of new lesions.
- The primary investigator at each site will verify the presence of changes in target/non-target lesions.
- Target lesions which shrink, and are deemed too small to measure should be given a default measurement of 5mm.
- If target lesions have completely disappeared is should be measured as 0mm.
- Lymph nodes may regress to <10mm but because they are still measurable, the sum of diameters may never equal 0mm in the setting of CR when they are included as target lesions.
- Please see table below regarding the Best overall response

Target lesions	Non-Target lesions	New Lesions	Overall response

CR	CR	No	CR
CR	Incomplete response/SD	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

** Please refer to the complete New response evaluation criteria in solid tumors: Revised RECIST guidelines – version 1.1 for further details[74].*

2. Quick Reference for iRECIST Criteria

A. Indications:

- The guidelines continue to recommend use of RECIST 1.1 to define whether tumor lesions are measurable or non-measurable and the principles used to determine objective tumor response are largely unchanged from RECIST 1.1. The concept change for iRECIST is the resetting the bar if RECIST 1.1 progression is followed at the next assessment by tumor shrinkage.

-iRECIST defines unconfirmed progress (iUPD) on the basis of RECIST 1.1 principles; however, iUPD requires confirmation, which is done on the basis of observing either a further increase in size (or in the number of new lesions) in the lesion category in which progression was first identified in (ie, target or non-target disease), or progression (defined by RECIST 1.1) in lesion categories that had not previously met RECIST 1.1 progression criteria. However, if progression is not confirmed, but instead tumor shrinkage occurs (compared with baseline), which meets the criteria of iCR, iPR, or iSD, then the bar is reset so that iUPD needs to occur again (compared with nadir values) and then be confirmed (by further growth) at the next assessment for iCPD to be assigned. If no change in tumor size or extent from iUPD occurs, then the time point response would again be iUPD. This approach allows atypical responses, such as delayed responses that occur after pseudoprogression, to be identified, further understood, and better characterized.

-However, many aspects of new lesion assessment are unique to iRECIST. If a new lesion is identified (thus meeting the criteria for iUPD) and the patient is clinically stable, treatment should be continued. New lesions should be assessed and categorized as measurable or non-measurable using RECIST 1.1 principles.

-The algorithm for patients with no previous iUPD is identical to RECIST 1.1. For patients with iUPD at the last timepoint response, the next timepoint

response is dependent on the status of all lesions, including target, non-target, new lesion target, and new lesion non-target; on whether any increase in size has occurred (either a further increase in size or a sufficient increase to assign a new iUPD if the criteria were not previously met); or the appearance of additional new lesions.

B. Identification of tumors, refer to RECIST 1.1

C. Assignment of time point response using iRECIST[26]

	Time point response with no previous iUPD in any category	Time point response with previous iUPD in any category *
Target lesions: iCR; non-target lesions: iCR; new lesions: no	iCR	iCR
Target lesions: iCR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iPR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iSD; non-target lesions: non-iCR/non-iUPD; new lesions: no	iSD	iSD
Target lesions: iUPD with no change, or with a decrease from last timepoint; non-target lesions: iUPD with no change, or decrease from last timepoint; new lesions: yes	Not Applicable	New lesions confirm iCPD if new lesions were previously identified and they have increased in size (≥5 mm in sum of measures for new lesion target or any increase for new lesion non-target) or number; if no change is seen in new lesions (size or number) from last timepoint, assignment

		remains iUPD
Target lesions: iSD, iPR, iCR; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in the size of non-target disease (does not need to meet RECIST 1.1 criteria for unequivocal progression)
Target lesions: iUPD; non-target lesions: non-iCR/non-iUPD, or iCR; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in sum of measures ≥5 mm; otherwise, assignment remains iUPD
Target lesions: iUPD; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed based on a further increase in previously identified target lesion iUPD in sum of measures ≥5 mm or non-target lesion iUPD (previous assessment need not have shown unequivocal progression)

Target lesions: iUPD; non-target lesions: iUPD; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in previously identified target lesion iUPD sum of measures ≥5 mm, previously identified non-target lesion iUPD (does not need to be unequivocal), or an increase in the size or number of new lesions previously identified
Target lesions: non-iUPD or progression; non-target lesions: non-iUPD or progression; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of an increase in the size or number of new lesions previously identified
Target lesions, non-target lesions, and new lesions defined according to RECIST 1.1 principles; if no pseudoprogression occurs, RECIST 1.1 and iRECIST categories for complete response, partial response, and stable disease would be the same. *Previously identified in assessment immediately before this timepoint. “i” indicates immune responses assigned using iRECIST. iCR=complete response. iPR=partial response. iSD=stable disease. iUPD=unconfirmed progression. non-iCR/non-iUPD=criteria for neither CR nor PD have been met. iCPD=confirmed progression. RECIST=Response Evaluation Criteria in Solid Tumors.		

** Please refer to the complete Guidelines for iRECIST.*[75]

Appendix G

Schedule of Events

CLINIC VISIT				PROCEDURES						TOTAL	
Visit #	Time Req (hr)	Time Point	Purpose	Screening	History	Nursing Visit	UPT	Blood Draws	Inoc	# of Shots This Visit	Time for Inoc + Follow-Ups
1	2	Pre-treatment	Consent/Tissue Procurement Gather Baseline Data	x	x	x	x			0	N/A
2	2	Baseline	Consent/Randomization & Treatment Neupogen Injection Gather Current Baseline Data	x	x	x	x			1	N/A
3	1	Baseline	Draw Blood to Obtain Dendritic Cells		x	x		x		0	N/A
START PRE-INOCULATION BLOOD DRAWS AND ADMINISTRATION OF INOCULATIONS											
4	1.5	Starting Time Point	Draw blood for Immunologic Sample R0 Administer Inoculation #1		x	x	x	x	x	2	1.5 hrs
5	1.5	Week 4 ± 1 week	Draw blood to record immunologic levels pre-inoculation #2 Administer Inoculation #2		x	x	x	x	x	2	1.5 hrs
6	1.5	Week 8 ± 1 week	Draw blood to record immunologic levels pre-inoculation #3 Administer Inoculation #3		x	x	x	x	x	2	1.5 hrs
START PRE-BOOSTER BLOOD DRAWS AND ADMINISTRATION OF BOOSTERS											
7	1.5	Month 6 from Starting Time Point (±2 weeks)	Draw blood to record immunologic levels pre-booster #1 Administer Booster #1		x	x	x	x	x	2	1.5 hrs
8	1.5	Month 12 (±2 weeks)	Draw blood to record immunologic levels pre-booster #2 Administer Booster #2		x	x	x	x	x	2	1.5 hrs
9	1.5	Month 18 (±2 weeks)	Draw blood to record immunologic levels pre-booster #3 Administer Booster #3		x	x	x	x	x	2	1.5 hrs
10	0.75	Month 24	Draw Blood for final Immunologic Sample Final Study Assessment		x	x		x	x	0	0.75 hrs

Total Number of Visits: 10

Total Number of Inoculation Visits: 7 (1 Neupogen injection, 6 vaccines)

Total Number of Blood Draws: 9 (1 baseline set of labs + 8 during vaccine series)

Open Label Protocol Schedule of Events

CLINIC VISIT				PROCEDURES						TOTAL	
Visit #	Time Req (hr)	Time Point	Purpose	Screening	History	Nursing Visit	UPT	Blood Draws	Inoculation	# of Shots This Visit	Time for Inoc + Follow-Ups
1	2	Pre-treatment	Consent/Tissue Procurement Gather Baseline Data	X	X	X	X			0	N/A
2	2	Baseline	Consent/Randomization & Treatment Neupogen Injection Gather Current Baseline Data	X	X	X	X		X (Neupogen injection)	1	N/A
3	1	Baseline	Draw Blood to Obtain Dendritic Cells		X	X		X		0	N/A
START PRE-INOCULATION BLOOD DRAWS AND ADMINISTRATION OF INOCULATIONS											
4	1	Starting Time Point	Draw blood for Immunologic Sample R0 Administer Inoculation #1		x	x	x	x	x	2	1 hr
5	1	Week 4 ± 1 week	Draw blood to record immunologic levels pre-inoculation #2 Administer Inoculation #2		x	x	x	x	x	2	1 hr
6	1	Week 8 ± 1 week	Draw blood to record immunologic levels pre-inoculation #3 Administer Inoculation #3		X	X	X	X	X	2	1 hr
7	1	Week 12 ± 1 week	Draw blood to record immunologic levels pre-inoculation #4 Administer Inoculation #4		X	X	X	X	X	2	1 hr
START PRE-BOOSTER BLOOD DRAWS AND ADMINISTRATION OF BOOSTERS											
8	1	Month 6 from Starting Time Point (±2 weeks)	Draw blood to record immunologic levels pre-booster #1 Administer Booster #1		X	X	X	X	X	2	1 hr
9	1	Month 9 (± 2 weeks)	Draw blood to record immunologic levels pre-booster #2 Administer Booster #2		X	X	X	X	X	2	1 hr
10	1	Month 12 (±2 weeks)	Draw Blood for final Immunologic Sample Final Study Assessment		X	X	X	X		0	1 hr

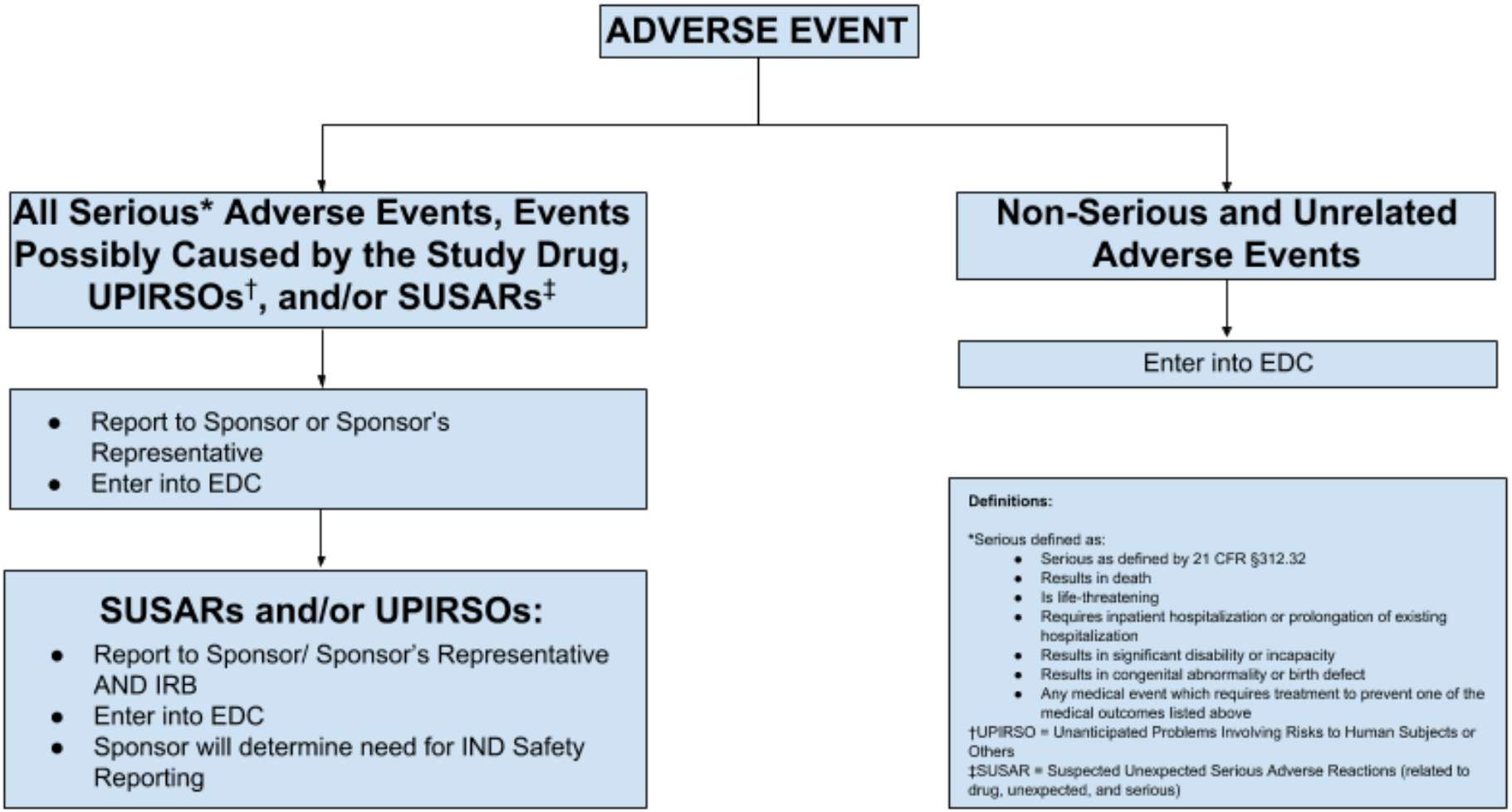
Appendix H

Data Safety Monitoring Plan

This is a free-standing document

Appendix I

Adverse Events Reporting Algorithm



Appendix J

MedWatch FDA Form 3500A Link

Form FDA 3500A Link

Appendix K

PROTOCOL TITLE:	A prospective, randomized, blinded, placebo-controlled, phase IIb trial of an autologous tumor lysate (TL) + yeast cell wall particles (YCWP) + dendritic cells (DC) vaccine vs unloaded YCWP + DC and embedded phase I/IIa trial with tumor lysate particle only (TLPO) vaccine in stage III and stage IV (resected) melanoma to prevent recurrence
PROTOCOL NUMBER:	20141932
STUDY DRUG:	Autologous tumor lysate (TL) + yeast cell wall particles (YCWP) + dendritic cells (DC) and Autologous tumor lysate (TL) + yeast cell wall particles only (PO)
PRINCIPAL INVESTIGATOR:	Mark B. Faries, MD, FACS
PROGRAM DIRECTOR:	George E. Peoples, MD, FACS
PROTOCOL VERSION/DATE:	2.4/ 15 February 2021

INVESTIGATOR'S AGREEMENT / INVESTIGATOR'S SIGNATURE PAGE
I have read the protocol described above. I have fully discussed the objectives of this trial and the content of this protocol with the sponsor or sponsor's representative. I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the ethical review of the trial, without written authorization from Cancer Insight. It is, however, permissible to provide information to a patient in order to obtain consent. I agree to conduct this trial according to the protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the trial in accordance with all applicable regulations, and guidelines as stated in the protocol and other information supplied to me. I understand that the sponsor may decide to suspend or prematurely terminate the trial at any time, for whatever reason. Such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the trial, I will communicate my intention immediately, in writing to the Sponsor.

Signed: _____ **Date:** _____

Investigator's Name and Address:

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