

**Protocol Title:** Multi-center phase I/IIa trial of an autologous tumor lysate (TL) + yeast cell wall particles (YCWP) + dendritic cells (DC) vaccine in addition to standard of care checkpoint inhibitor of choice in metastatic melanoma patients with measurable disease.

**Study Drugs:** Autologous TLPLDC vaccine (tumor lysate, particle-loaded, dendritic cells)

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**1.0 PROTOCOL SYNOPSIS**

<b>Brief Title</b>	Multi-center phase I/IIa trial of an autologous tumor lysate (TL) + yeast cell wall particles (YCWP) + dendritic cells (DC) vaccine in addition to standard of care checkpoint inhibitor of choice in metastatic melanoma patients with measurable disease.	
<b>Test Article</b>	Name	Autologous TLPLDC vaccine (tumor lysate, particle loaded, dendritic cells)
	Activity	Induction of anti-cancer cell-mediated immune response
	Dosage form	Intradermal injection of $1 \times 10^6$ autologous tumor lysate-loaded dendritic cells monthly x 4 followed by boosters at 6 and 9 months.
<b>Development Phase</b>	Phase I/IIa	
<b>Indication</b>	To prevent disease progression in metastatic melanoma patients	
<b>Rationale</b>	<p><b>Setting:</b> Responsible for approximately 5% of all skin cancers, melanoma is the cause of 80% of skin cancer related deaths according to the World Health Organization<sup>1</sup>. The 5-year median survival rate for purely local disease is 98% versus a rapid decline to approximately 10-15% in patients with metastatic disease<sup>1,2</sup>. Surgical excision plays a major role in loco-regional treatment for melanoma; regarding metastatic disease, there exists a 20-40% 5-year survival benefit with metastectomies, although truly limited to surgically accessible tumors. Radiation has a limited role in treatment of melanoma, as this cancer is considered to be radio-resistant. Despite multi-modal treatment regimens, the population continuing to experience suboptimal treatment outcomes involves stage IV disease. Until recently, most treatments for metastatic melanoma were considered palliative treatments, and patients were encouraged to enroll into clinical trials once they reached this stage.</p> <p>Checkpoint inhibitors (CPI) are now approved for use in metastatic melanoma (MM), and a small proportion of treated pts will achieve a clinical complete response (CR) and another substantial portion will achieve durable partial responses (PR) and stable disease (SD). However, the majority of pts will demonstrate progressive disease (PD). One hypothesis is that these patients lack sufficient numbers/types of tumor reactive T cells to mount an endogenous, protective, anti-tumor immune response.</p> <p>Compared to the fusion technology, our new technology is more efficient and utilizes a yeast cell wall particle (YCWP) loading system to deliver tumor lysate (TL) to the cytoplasm of DC and requires only 120 mL of blood (for DC isolation) and as little as 1 mg of autologous tumor. The TL, particle-loaded, DC (TLPLDC) vaccine can be produced in 48 hrs. This new technology has been tested in preclinical animal models with superior results compared to the prior fusion technology, which showed clinical benefit (SITC 2015). These results led to a phase I/IIa (n=20) clinical trial of the TLPLDC vaccine in multiple solid tumors demonstrating minimal toxicity and a 60% clinically beneficial (CR+PR+SD) response rate and a 30% objective tumor response rate (ICIC 2015).</p> <p>T cell-eliciting vaccines like our TLPLDC vaccine could be used in combination with CPI to theoretically improve the clinical efficacy of standard of care (SoC) CPI in MM.</p> <p><b>Vaccine:</b> 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 x4 followed by boosters at 6 and 9 months.</p>	

<b>Rationale (continued)</b>	<p><b>Prior Results:</b></p> <p>To date, we have vaccinated a total of 36 patients with varying malignancies with the dendritoma fusion technology, 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 of the dendritoma fusion technology, 25 stage IV patients were vaccinated. The median overall survival (OS) was 16.1 months (compared to a historic rate of 8-10 months), and there was a 29.3% OS at 5 years. There was a dose response with an OS improvement in patients receiving <math>\geq 3</math> inoculations compared to patients receiving less (log rank <math>p=0.02</math>).</p> <p>The fusion technology has now been replaced by the TLPLDC vaccine technology to create personalized autologous tumor/DC vaccines. To date, 20 pts have been enrolled to the TLPLDC trial with a median follow-up of 7 mos. The study population is 60% male, 90% Caucasian, 95% stage III/ IV disease, with a median age of 58 years. Thirteen different tumor types have been treated. Of the 20 pts, 17 had measurable disease on initiation of treatment, and 3 were vaccinated adjuvantly. Twelve patients have completed treatment, 6 are in treatment, and 2 are consented but not initiated. The 12 pts who have completed treatment received a median of 4 inoculations, and no grade <math>\geq 3</math> toxicities have been noted. Flu-like symptoms have been seen in 20%. Of the 17 pts with measurable disease, 10 have completed treatment with 6 demonstrating clinical benefit (1 CR, 2 PR, 3 SD). In the 3 adjuvant pts, 2 have completed treatment, and all 3 remain disease-free at a median follow-up of 11 months.</p>
<b>Primary Endpoint</b>	<p>1) To determine the safety of adding the TLPLDC vaccine to SoC CPI.</p> <p>2) To determine tumor response with the addition of the vaccine per RECIST and iRECIST criteria.</p>
<b>Secondary Endpoints</b>	<p>1) To compare combination therapy with currently known rates of AEs and ORR of CPI monotherapy.</p>
<b>Exploratory Endpoints</b>	<p>1) To document the immunologic response to the vaccine through T-cell assays recognizing known melanoma-specific antigens.</p> <p>2) To correlate vaccine immune response to clinical outcome.</p>
<b>Patient Population</b>	<p>Metastatic melanoma patients eligible for (or currently on) SoC CPI with measurable disease.</p>
<b>Treatment</b>	<p>Vaccinated group = autologous tumor cell lysate + yeast cell wall particles + autologous DC</p>
<b>Number of Patients</b>	<p>N= up to 45</p>
<b>Sample Size Justification</b>	<p>45 patients will allow an 80% power of detecting an absolute 25% increase in the objective tumor response rate compared to current SoC CPI monotherapy with an alpha of 0.05 (one sided).</p>
<b>Number of Sites</b>	<p>4-8 sites will be established.</p>

<b>Duration of Trial</b>	With 4-8 sites enrolling, we anticipate enrollment to be complete in 12 months. With a primary endpoint of 1 year to monitor the safety of the vaccine and tumor response, the trial duration is expected to be 2 years.
<b>Trial Design and Conduct</b>	<p>Metastatic melanoma patients eligible for (or currently on) CPI therapy per SoC will be identified and screened for inclusion/exclusion criteria. Eligible patients will be counseled and consented for tissue procurement. They will undergo excisional or core needle biopsy as clinically indicated and this tissue will be shipped in liquid nitrogen shippers through FedEx to our central facility in Greenville, SC. The tumor will be stored frozen until vaccine preparation. Vaccine development requires 48 hours for preparation. Upon verification that adequate tissue was obtained, these patients will then be counseled and consented for participation in the trial.</p> <p>The patients who qualify for participation in this trial will continue their treatment of CPI. Once consented, patients will receive a single injection of Neupogen (G-CSF) 300 µg SQ 24-48 hrs prior to having 70 mL of blood collected and sent to our central facility for DC isolation and preparation. Those who cannot tolerate Neupogen 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 <math>1 \times 10^6</math> TLPLDC and will be labeled with the patient's unique study number.</p> <p>The frozen autologous TLPLDC will be sent back to the site with a total of 6 single dose vials after the vaccine has completed QA/QC testing and lot-release (usually 3 weeks). The primary vaccination series will include monthly inoculations at 0, 1, 2, 3 months followed by boosters at 6 and 9 months in the same lymph node draining area (preferably the anterior thigh). Once received, the first inoculation should occur within 4 weeks.</p> <p>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.</p> <p>Patients will follow-up at their respective sites for evaluation of metastatic disease per SoC. They will under imaging, CT/PET-CT, to meet Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1 and iRECIST to monitor disease.</p> <p>Blood (50 mL) will be collected from all patients prior to each inoculation and at 12 months from enrollment for a total of 7 time points or a total of 350 mL of blood over 1 year. The collected blood will be sent to our central facility for immunologic testing of T-cell responses.</p>

<b>Inclusion Criteria</b>	<ol style="list-style-type: none"> <li>1) 18 years or older</li> <li>2) Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1 (Appendix A)</li> <li>3) Metastatic melanoma eligible for (or currently on) standard of care CPI therapy (treating physician's choice) with measurable disease.</li> <li>4) Approximately 1 cm<sup>3</sup> preferred but 1 mg minimum of accessible and dispensable tumor (minimum of 3 passes with a core needle)</li> <li>5) Able to tolerate CPI treatment regimen (if already started)</li> <li>6) Adequate organ function as determined by the following laboratory values: <ul style="list-style-type: none"> <li>• ANC <math>\geq</math> 1,000/<math>\mu</math>L</li> <li>• Platelets <math>\geq</math> 75,000/<math>\mu</math>L</li> <li>• Hgb <math>\geq</math> 9 g/dL</li> <li>• Creatinine <math>\leq</math> 1.5 x upper limit of normal (ULN) or Creatinine clearance <math>\geq</math> 50% of lower limit of normal (LLN)</li> <li>• Total bilirubin <math>\leq</math> 1.5 x ULN</li> <li>• ALT and AST <math>\leq</math> 1.5 x ULN</li> </ul> </li> <li>7) 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)</li> <li>8) Signed informed consent</li> </ol>
<b>Exclusion Criteria</b>	<ol style="list-style-type: none"> <li>1) Inability to tolerate CPI therapy (if already started)</li> <li>2) Rapidly progressive, multi-focal metastatic disease</li> <li>3) Insufficient tumor available to produce vaccine</li> <li>4) ECOG <math>\geq</math> 2</li> <li>5) Immune deficiency disease or HIV, active HBV, or active HCV</li> <li>6) Receiving immunosuppressive therapy including chronic steroids (except physiologic maintenance doses), methotrexate or other known immunosuppressive agents.</li> <li>7) Pregnancy (assessed by urine HCG)</li> <li>8) Breast feeding</li> <li>9) Active pulmonary disease requiring medication to include multiple inhalers (&gt;2 inhalers and one containing steroids)</li> <li>10) Involved in other experimental protocols (except with permission of the other study PI)</li> </ol>

<b>Statistical Analysis</b>	Toxicities will be monitored and categorized according to CTCAE v.4.03 continuously. Each patient's tumor response will be assessed per RECIST criteria version 1.1 and iRECIST at multiple time points. Overall objective tumor response rates will be analyzed among all patients and compared to expected response rates to CPI monotherapy.
<b>Planned Analyses</b>	<ol style="list-style-type: none"> <li>1) Safety will be monitored continuously.</li> <li>2) Objective tumor response rates will be assessed in real time.</li> <li>3) The interim analysis of response rates and comparisons will be completed 12 months after initiation of this trial to assess the vaccine safety profile and tumor response trends.</li> <li>4) The final analysis will be completed after 12 months follow-up from the last enrolled patient.</li> </ol>

## 2.0 INTRODUCTION

Responsible for approximately 5% of all skin cancers, melanoma is the cause of 80% of skin cancer related deaths according to the World Health Organization<sup>1</sup>. The 5-year median survival rate for purely local disease is 98% versus a rapid decline to approximately 10-15% in patients with metastatic disease<sup>1, 2</sup>. Surgical excision plays a major role in loco-regional treatment for melanoma; regarding metastatic disease, there exists at 20-40% 5-year survival benefit with metastectomies, although truly limited to surgically accessible tumors. Radiation has a limited role in treatment of melanoma, as it is considered to be radio-resistant. For resections with inadequate margins, positive nodal disease status post lymphadenectomy, and brain metastases are instances in which radiation can be used for further loco-regional control of disease<sup>2, 3</sup>.

The addition of radiation has been associated with a 10% rate of local recurrence compared to a 40.6% rate in those who did not receive radiation in high risk node positive patients. These results are associated with the risk of development of lymphedema<sup>2</sup>. The risk of recurrence is the highest within the first 5 years of diagnosis. Despite multimodal treatment regimens, the population continuing to experience poor treatment outcomes involves stage IV disease. To date, most treatments for metastatic melanoma are considered palliative treatments, and patients are encouraged to enroll into clinical trials once they have reached this stage.

The mainstay of treatment for metastatic melanoma is systemic therapy. The only FDA approved chemotherapeutic medication is dacarbazine, and its oral preparation temozolomide. These medications have shown minimal overall survival (OS) benefit, median OS 6.4 and 7.7 months, respectively with a 12% and 14% objective response rate (ORR), respectively<sup>4</sup>. Despite poor outcomes with monotherapy, all other chemotherapeutic drugs, such as taxanes and vinca alkaloids, have similar poor results. Cytokine therapy is another modality, which has showed notable shifts in management of metastatic melanoma. Interleukin-2 (IL-2), known to stimulate T-cell proliferation and further release of cytokines, when used



in high doses was associated with a 16% objective response rate (complete response (CR) 6%, and partial response (PR) 10%)<sup>4, 5</sup>. This data was obtained via retrospective analysis of several trials from 1985-1993 where high dose IL-2 was given over a 5 day, as tolerated, with multiple cycles occurring every 6-12 weeks. Of the patient's whom experienced tumor response, there was no evidence of disease progression after 5-years<sup>5</sup>. Interferon-alpha (IFN- $\alpha$ ), also involved in systemic inflammation, is the only FDA approved drug for adjuvant therapy with metastatic melanoma<sup>3, 4</sup>. Four trials have studied this drug, the most significant by the European Organization for Research and Treatment of Cancer (EORTC) randomizing patients with resected stage III melanoma to 5 years of pegylated-IFN $\alpha$ , pegylated to increase the drugs half-life, versus observation. After median follow-up of 7.6 years, the reduction in relative risk of recurrence was 39.1% versus 34.6% in the observation group<sup>3, 6</sup>. The results with these medications were not devoid of side effects. Many include fever, chills, myalgias and malaise<sup>4</sup>. IL-2 has been associated with hypotension, arrhythmias and even systemic inflammatory response requiring close monitoring during treatment. Neither of these medications has shown a significant improvement in overall survival (OS). In addition, combination therapy, termed biochemotherapy, also demonstrated significant toxicities with no survival benefit. Interestingly, the use of IL-2 and IFN- $\alpha$  did reveal that stimulation of the immune system can effectively destroy tumors in metastatic melanoma patients. Creating an immune system capable of eradicating metastatic tumor burden and preventing recurrence; therein lies the ideology behind further immunotherapy development for the management of stage IV melanoma.

Targeted therapies have been developed as further research has revealed specific mutations associated with melanoma. Medications targeting B-RAF signal mutations in melanoma tumors include vemurafenib and dabrafenib.

A phase III trial comparing vemurafenib to dacarbazine, in patients with metastatic melanoma with no prior treatments, revealed increase in progression free survival (PFS) 6.9 months versus 1.6 months and median OS 13.6 months versus 9.7 months (both  $p < 0.001$ )<sup>7</sup>. Dabrafenib was also evaluated in a phase III trial against dacarbazine revealing a PFS of 5.1 months versus 2.7 months ( $p < 0.0001$ )<sup>2</sup>. An additional drug trametinib, an oral medication which inhibits a downstream molecule, MEK1 and MEK2, has shown efficacy in a phase III trial versus chemotherapy revealing a PFS of 4.8 months versus 1.5 months<sup>2</sup>. Toxicities for these medications include arthralgias, development of squamous cell carcinoma and photosensitivity. Although effective, these medications can only be used in approximately 50% of patients with melanoma who have this mutation<sup>2, 7</sup>.

The final group of developed drugs for treatment of metastatic melanoma includes the check point inhibitors (CPI). These medications are human monoclonal antibodies used to prevent interaction of tumor cell surface molecules used to downregulate the immune system thus preventing activation of T-cells. The initial CPI used for metastatic melanoma, ipilimumab, is an anti-cytotoxic T lymphocyte antigen-4 (anti-CTLA-4) antibody. This medication has been extensively studied, and received FDA approval in 2011. A phase III trial was completed on patients

with stage III/IV melanoma, where patients were randomized into three groups, Ipilimumab alone, a peptide vaccine, gp100 alone, or ipilimumab and gp100. Median OS was noted to be 10.1 months, 6.4 months and 10 months, respectively. There were no statistically significant differences between the two groups involving ipilimumab<sup>1</sup>. In addition, this medication was compared to dacarbazine in a phase III trial. The two treatments included ipilimumab and dacarbazine versus dacarbazine alone in untreated metastatic melanoma patients. The overall survival was 11.2 months versus 9 months, respectively. As evidenced in a CTLA-4 knock-out animal model, the animals all died from severe lymphoproliferative disease; it is not unexpected that significant autoimmune toxicities are associated with this medication<sup>1</sup>. These are characterized mostly as skin and gastrointestinal (GI) toxicities, with 8-23% of patient's experiencing grade III/IV GI toxicities. Specifically, patients may experience severe diarrhea/colitis, hypophysitis, uveitis, and hepatitis. Although reported to be managed with corticosteroids and anti-interferon gamma (anti-IFN- $\gamma$ ), these significant toxicities definitely reflect likely poor compliance with the medication<sup>1,8</sup>.

The second class of CPI drugs includes monoclonal anti-PD1 antibodies, again functioning to block suppression of T-cell activation, nivolumab and pembrolizumab. Both drugs have been approved by the FDA for treatment of metastatic disease that has failed management with anti-CTLA-4 antibody or mutated BRAF inhibitor therapy if indicated. Also extensively studied, a phase III trial comparing nivolumab to dacarbazine in stage III/IV unresectable melanoma without the BRAF mutation revealed significant differences; a total of 68% of the patient population were previously treated with chemotherapy prior to enrollment in the trial, evenly distributed between the two treatment arms.

The 12 month OS was 72.9% versus 42.1% in the two arms, respectively, with an ORR of 40% with nivolumab (CR 7.6%) and 13.9% with dacarbazine (CR 1.0%) ( $p<0.001$ )<sup>9</sup>. Another phase III trial revealed similar promising results when comparing two dosing regimens of pembrolizumab (10mg/kg every 2 weeks versus every 3 weeks) to ipilimumab. This trial included unresectable stage III/IV melanoma patients. Patients in the pembrolizumab treatment arms experienced 12 month OS of 74.1% and 68.4% in the every 2 week versus every 3 week dosing regimens. They were compared to a 58.6% 12 month OS associated with ipilimumab treatment ( $p=0.0005$ )<sup>10</sup>. Interestingly, the PD1 inhibitors appear to be superior to the CTLA-4 inhibitors in terms of toxicity. Although the patients treated with pembrolizumab did experience grade 3 toxicities (13.3% and 10.1% according to treatment dosing every 2 weeks versus 3 weeks), the anti-PD1 therapy compared favorably to the 19.9% with ipilimumab. The majority of the toxicities were related to cutaneous toxicities, thyroid derangements and diarrhea<sup>11</sup>.

In September 2015, the FDA approved a new treatment regimen using combination therapy with two CPI for metastatic melanoma, ipilimumab and nivolumab followed by nivolumab maintenance therapy. This new approval was obtained after a phase II double-blinded trial randomizing 142 patients with no prior treatment to combination therapy with ipilimumab plus nivolumab versus ipilimumab plus

placebo revealed a 61% ORR in the combination arm compared to 11% in the ipilimumab plus placebo arm<sup>12</sup>. Approximately 39% patients had a partial response on combination therapy, with potentially 80% of those partial responses being durable. However, the combination did produce significant toxicities with 54% of patients experiencing grade 3 or 4 toxicities in the combination arm versus 24% with ipilimumab alone. The most common cause for discontinuation of the trial was toxicity in the combination arm (45%) and disease progression<sup>12</sup>.

During this same period of targeted therapy development, recognition of the high potential for dendritic cells (DC) to aid in the development of appropriate immune responses for management of solid tumors also occurred. These cells innately possess receptors necessary for T-cell stimulation. Initially introducing specific tumor associated antigens to dendritic cells in order to stimulate MHC Class I-mediated cytotoxic T-cells (CTL) was evaluated. Due to a system limited by the number of identifiable antigens, we have pursued DC fusion with tumor as a vaccine strategy. Through the strong immunogenicity produced by DC, in addition to creating a vaccine with multiple antigens by utilizing a complete sample of the solid tumor, more effective vaccines were produced<sup>2</sup>. Pilot studies were conducted in stage IV melanoma and stage IV renal cell carcinoma patients testing the fusion of DC with tumor cells termed dendritoma and used with IL-2<sup>2</sup>. These pilot studies revealed the dendritomas to be immunogenic, leading to a significant increase in interferon-gamma (IFN- $\gamma$ ) producing T-cells. Clinically, stabilization or partial responses were observed in 40% of stage IV RCC patients involved. In the completed phase I/IIa melanoma trial of the dendritoma fusion technology, 25 stage IV patients were vaccinated. The median overall survival (OS) was 16.1 months (compared to a historic rate of 8-10 months), and there was a 29.3% OS at 5 years. There was a dose response with an OS improvement in patients receiving  $\geq 3$  inoculations compared to patients receiving less (log rank  $p=0.02$ )<sup>16</sup>.

Multiple DC-based vaccines have now been studied along with a multitude of other vaccine strategies, but the only FDA approved cancer vaccine, Spileucel – T or Provenge, is a DC-based vaccine where DC are incubated with a fusion protein PA2024 (prostatic acid phosphatase and granulocyte macrophage colony-stimulating factor). The vaccine is approved for use in castration-resistant metastatic prostate cancer patients. Results from the phase III study of this vaccine versus placebo resulted in a median OS of 25.9 months versus 21.4 months in the placebo group ( $p<0.02$ )<sup>8</sup>. Despite the efficacy, significant effort is required in order to produce these autologous cell-based vaccines. The final vaccines are not available for several weeks due to long incubation periods, in addition to quality assurance protocols, and the requirement for a large volume of blood greatly affect the efficiency of production.

Thus, we have developed a new highly efficient technique for introducing tumor lysate into the cytoplasm of DC using denatured yeast cell wall particles (YCWP). These tumor lysate-loaded particles are incubated and phagocytized by autologous DC forming the tumor lysate, particle-loaded, dendritic cell (TLPLDC) vaccine.

These vaccines require less tissue, small quantities of blood, and are produced within 48 hours<sup>14</sup>. This new method was used in a B16 metastatic melanoma murine model. The TLPLDC vaccine was created with lysed tumor cells from B16F0 melanoma and each mouse injected with 3 weekly doses for up to 4 weeks. All mice were sacrificed at the end of the observation period, and lungs harvested to identify and count pulmonary metastasis. All mice who received the TLPLDC vaccinations showed no evidence of metastatic disease compared to controls ( $p < 0.01$ ). The median survival for mice in the control arm was 21 days compared to 29 days with TLPLDC and 41 days with TLPLDC with CpG adjuvant. Additional preclinical data has demonstrated that the TLPLDC vaccine is actually superior to the fusion dendritoma technology in the same murine metastatic melanoma model<sup>14</sup>. Therefore, the fusion technology has now been replaced by the TLPLDC vaccine technology to create personalized autologous tumor/DC vaccines. To date, 20 pts have been enrolled to the TLPLDC trial with a median follow-up of 7 mos. The study population is 60% male, 90% Caucasian, 95% stage III/ IV disease, with a median age of 58 years. Thirteen different tumor types have been treated. Of the 20 pts, 17 had measurable disease on initiation of treatment, and 3 were vaccinated adjuvantly. Twelve patients have completed treatment, 6 are in treatment, and 2 are consented but not initiated. The 12 pts who have completed treatment received a median of 4 inoculations, and no grade  $\geq 3$  toxicities have been noted. Flu-like symptoms have been seen in 20%. Of the 17 pts with measurable disease, 10 have completed treatment with 6 demonstrating clinical benefit (1 CR, 2 PR, 3 SD). In the 3 adjuvant pts, 2 have completed treatment, and all 3 remain disease-free at a median follow-up of 11 months.

Both the fusion dendritoma technology and the current TLPLDC vaccine technology, both autologous tumor/DC vaccines, have shown promise in patients with metastatic melanoma. From these trials arose an on-going randomized phase IIb trial utilizing the TLPLDC vaccine in the adjuvant setting to prevent recurrence in high risk melanoma patients.

Recognizing the utility of the CPI and the immunogenicity of the TLPLDC vaccination, merging these two treatment methods may lead to a more robust and effective anti-tumor immune response. The tumor-reactive T cells stimulated by the TLPLDC vaccine may become inactivated in the tumor microenvironment due to T-cell downregulation by the tumor. In fact, this may explain the failures of most cancer vaccines in general that have been tested in the metastatic setting. CPI could aid in overcoming this mechanism of tumor cell evasion by blocking these inhibitory signals and allowing the T cells to attack the tumor. Likewise, CPIs are limited in their effectiveness based on the availability of tumor-reactive T-cells. Therefore, the use of CPI + vaccines makes intuitive sense.

There may be some concern regarding the toxicity associated with the combination of these modalities; however, one could also argue that the combination could theoretically reduce the toxicity of the CPI. First, the vaccines are inherently safe and have never been shown to produce autoimmunity; therefore, if toxicity were to

increase with the combination, it would likely be related to the increased activity of tumor destruction not autoimmunity. On the other hand, since the vaccine will induce larger numbers of tumor-reactive T cells, the dose of the CPI may be decreased perhaps resulting in less toxicity. Furthermore, the larger number of tumor-reactive T cells may bind a higher proportion of the CPI leaving less to bind to auto-reactive T cells. Either mechanism may lead to lower, not higher, toxicity with the combination of CPI and TLPLDC vaccine.

This prospective, open label, multicenter phase I/IIa trial will assess the effects of utilizing both a CPI and the TLPLDC vaccine in metastatic melanoma patients with measurable disease eligible for (or currently on) standard of care CPI therapy. By developing an adaptive immunity to melanoma via the TLPLDC vaccine, and inhibiting the down regulation of T-cells via tumor-induced immunosuppression with the CPI, the tumor response rates would be expected to increase relative to the results experienced when these modalities are used individually.

### **3.0 OBJECTIVES**

In this study, we intend to assess the safety and tumor response of utilizing an autologous tumor lysate, particle-loaded, dendritic cell (TLPLDC) vaccine given in combination with standard of care (SoC) checkpoint inhibitors (CPI) in patients with stage IV melanoma with measurable disease.

#### **3.1 Primary Objectives**

- To determine the safety of adding the TLPLDC vaccine to SoC CPI.
- To determine tumor response with the addition of the vaccine per the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria<sup>15, 18</sup> and RECIST criteria specific for cancer immunotherapy trials (iRECIST)<sup>26</sup>.

#### **3.2 Secondary Objectives**

- To compare combination therapy with currently known rates of AEs and ORRs of CPI monotherapy.

#### **3.3 Exploratory Objectives**

- To document the immunologic response to the vaccine through T-cell assays recognizing known melanoma-specific antigens.
- To correlate vaccine immune response to clinical outcome.

### **4.0 STUDY DESIGN**

#### **4.1 Description of Study**

This will be a prospective, open label, multi-center phase I/IIa trial utilizing the

TLPLDC vaccine added to SoC CPI for treatment of metastatic melanoma. The vaccine to be used in this study remains an investigational drug under the IND 16101. The Sponsor of this IND is Elios Therapeutics, LLC.

The target study population includes metastatic melanoma patients eligible for (or currently on) CPI therapy per their provider's choice and with measurable disease. Up to 45 patients will be enrolled over approximately one year at 4-8 sites. These patients must be able to tolerate CPI treatment if already started.

Each patient must have biopsy proven evidence of metastatic disease and measurable on imaging studies.

Prior to participation in the trial, eligible patients will be counseled and consented on the process of tissue procurement. These patients will have tumor acquired for vaccine production via core needle biopsy or excisional biopsy (need not occur at the study site), and a portion (approx. 1 cm<sup>3</sup> preferred but 1 mg minimum) of their melanoma sterilely frozen in provided freezing vials and storage tubes. This tissue will then be shipped in liquid nitrogen shippers through FedEx to our central facility in Greenville, SC and stored frozen until vaccine preparation.

Upon verification of adequate amount of tumor procured, patients will then be counseled and consented for full participation in the trial. Each patient will receive a single injection of Neupogen (G-CSF) 300 µg subcutaneously 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 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. Excess tumor, serum and blood will be placed into a tissue repository and stored under the patient's unique study number for future use in re-creating the vaccine and performing immunologic assays. Additionally, any stored tumor, serum or blood may also be utilized to assess new generations of vaccines.

During the time between enrollment and the first vaccine inoculation (approximately 3-7 weeks after enrollment), each patient should undergo imaging to determine baseline metastatic disease and baseline labs, including lactate dehydrogenase. Each patient must obtain a cross sectional chest, abdomen and pelvis imaging with contrast, MRI Brain/CT Head with intravenous contrast, and/or total body PET per institutional preference per standard of care. Baseline imaging studies must be completed within 4 weeks of first vaccine dose. At this point, a maximum of 5 tumor foci (maximum of 2 foci per organ) will be identified by the site primary investigator that are easily measurable and designated as the target lesions. These tumors must be at least 10mm in size per RECIST 1.1 criteria (Appendix B). Baseline non-target lesions will also be documented. Additionally, iRECIST criteria will be applied (Appendix B)

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 isolated DC for phagocytosis; thus creating the TLPLDC vaccine which will be frozen in single dose vials. Each vial will contain  $1 \times 10^6$  TLPLDC 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. Excess tumor will be maintained for each patient in a tissue repository.

This tissue may be used in later research to assess new generations of vaccines or to re-create vaccine for the patient if necessary.

The frozen autologous TLPLDC vaccine will be sent back to the site with a total of 6 single dose vials for intradermal injections. The vaccination series must be initiated within 4 weeks of receipt of the vaccination series at the site. The primary vaccination series will include 4 monthly inoculations at 0, 1, 2, and 3 months followed by boosters at 6 and 9 months in the same lymph node draining area (preferably the anterior thigh).

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 C).

Patients will follow-up at their respective sites for evaluation of metastatic disease per NCCN guidelines<sup>2</sup>. They will undergo imaging, with the same modality used for baseline measurements, to meet Response Evaluation Criteria in Solid Tumors (RECIST) criteria<sup>15</sup> to monitor disease approximately every 3 months for 1 year. Time to the best overall response will be from the date of first inoculation until the first day that the tumor meets RECIST criteria for a particular tumor response (Appendix B). Additionally, iRECIST criteria will be applied (Appendix B).

In addition to the initial blood collection (70-120 mL) for vaccine preparation, 50 mL of blood will be collected from all patients prior to each of the six inoculations and at 12 months from enrollment for a total of seven time points or a total of 420 - 470 mL of blood over one year. 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.

## **4.2 Rationale for Study Design**

Individually, CPI have been approved for use in metastatic melanoma and the TLPLDC vaccine appears to provide benefit to these patients as well in small studies. By stimulating the immune system to develop tumor-reactive T cells while simultaneously preventing down-regulation of these T cells in the tumor microenvironment could result in a highly effective multimodality immunotherapy that could significantly impact clinical outcomes of patients with metastatic melanoma. While CPI have been proven to be effective in metastatic melanoma patients in terms of tumor response as well as survival, the majority of metastatic

melanoma patients still succumb to their disease. In response to CPI treatment, metastatic melanoma lesions may show no response (de novo PD), an initial response followed by progressive disease (delayed PD), or may stabilize but persist (SD). In all of these situations, the CPI monotherapy is not sufficient alone.

One theory is that these patients lack adequate numbers of tumor-reactive T cells on which the CPI can work. The TLPLDC vaccine generates tumor-reactive T cells as its primary mechanism of action.

In order to assess both the safety as well as the efficacy of adding the TLPLDC vaccine to SoC CPI therapy in metastatic melanoma patients, up to 45 patients will be enrolled over approximately one year at 4-8 sites into this study. This number will allow an overall assessment of the safety and potential efficacy of this novel combination treatment regimen compared to the currently known and accepted rates of response to CPI monotherapy in these patients.

### **4.3 Outcome Measures**

#### *4.3.1 Primary outcome measures*

Standard local and systemic toxicities will be collected and graded per the NCI CTCAE v4.03 graded toxicity scale (Appendix C). For both the primary vaccine series and the booster inoculations, patients will be monitored closely for 30 minutes after each inoculation with questioning, serial exams and vital signs as needed to observe for a hypersensitivity reaction. 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.

Evidence of tumor response to the proposed treatment regimen will be determined by the primary investigator at the individual study sites during their routine follow-up screening and assessed by the Guidelines for the Evaluation of Immune Therapy Activity in Solid tumors criteria (iRECIST) in addition to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria (Appendix B). This will occur for all enrolled patients, approximately every three months for 1 year. Follow-up will include history, full dermatologic clinical exam, laboratory and radiographic surveillance per NCCN guidelines. Surveillance imaging should be conducted with the same modality as used for baseline imaging. If patients develop contraindications to certain modalities during the trial, their cases must be discussed with a radiologist to determine the best alternative imaging study to used, otherwise they must be deemed 'not evaluable' from that time period. If records are not available, patients, or their referring physicians, will be contacted to discern their disease status.

The primary outcome for this trial includes safety of the TLPLDC vaccine used in conjunction with CPI in addition to overall tumor response to this treatment plan. While the safety and tumor response in individual patients will be assessed continuously, the assessment of the primary endpoints will be determined and analyzed 12 months after the last patient is enrolled into this trial.



An interim analysis will be performed 12 months after the initiation of this trial for both safety and tumor response trends. No action regarding stopping the trial will be taken based on this analysis unless there is a safety concern. It is anticipated that all patients will have been enrolled with approximately 6 months of median follow-up at this interim analysis.

#### **4.3.2    Secondary outcome measures**

The secondary outcome measures for this trial will compare treatment to the currently known rates of AEs and ORR of CPI monotherapy. These assessments would be completed at both the interim and final analyses.

#### **4.3.3    Exploratory outcome measures**

Immune responses will be primarily documented using CTL assays on stored blood and assessment of stored serum pre- and post-vaccination. Detailed descriptions of these assays/tests are described in Sections 5.3.5 and 5.3.6.

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 and at study completion at 12 months from enrollment. Comparisons will be made between the immunologic responses within and between each treatment group.

In a series of exploratory analyses, the results of these immunologic measures will be correlated with clinical outcome within and between each treatment group.

### **4.4    Safety Considerations**

In our previous trials, the safety of the autologous tumor/DC vaccines has been excellent. According to the preclinical trial, mice behavior was observed for evidence of possible toxicities. No abnormalities were appreciated within the vaccination group, nor were there differences between the vaccinated or controls<sup>23</sup>.

In the completed melanoma phase I/IIa in stage IV melanoma patients, where the autologous tumor/DC dendritoma vaccine was given with IL-2, the most common adverse events experienced by patients included fever (60%), chills (32%), nausea (28%), arthralgias/myalgias (28%), erythema (20%), headache (16%), anemia (16%), hypotension (16%), weight gain (12%), stomatitis (12%), edema (12%), rash (12%), pain (12%), and fatigue (12%). There were a total of four severe adverse reactions, which occurred in this trial, although they were deemed

unrelated to the dendritoma vaccine. Many of these toxicities were attributable to IL-2, which will not be used in the current study<sup>24</sup>. In the phase I/IIa trial of the TLPLDC vaccine, there were only mild toxicities (grade  $\leq 2$ ) in 20% of the patients.

Previous trials evaluating the toxicity associated with CPI have resulted in various immune related adverse events. The most common toxicities included fatigue, rash, diarrhea, and pruritus<sup>11</sup>. Additional toxicities have been associated with specific CPIs at varying rates<sup>17</sup>. Ipilimumab is characterized by an overall 72.3% occurrence of immune-related AE. Grade 3 or 4 irAEs were observed in 25.2% of patients, affecting the GI tract (12%), liver (7%), skin (3%), and endocrine system (3%). These AE are noted to occur at predictable times based on duration of treatment – skin related events within the first 3 weeks, GI/hepatic AE after 6-7 weeks of treatment, and endocrinopathies after 9 weeks of treatment. AE also varies based on dosing, with approximately 7% of Grade 3 and 4 toxicities occurring at the FDA approved 3mg/kg dose<sup>17</sup>. Similarly, pembrolizumab had an overall AE rate between 72.9 – 79% based on dosing; 10mg/kg every 2 or 3 weeks. This CPI is noted to have a lower AE profile compared to ipilimumab, 13.3% versus 19.9% respectively. Grade 3 and 4 toxicities included colitis and hepatitis within 1- 2.5% of treated patients<sup>11</sup>.

In this trial, all patients will continue their treatment with CPI of choice by the primary investigator/study site. There are inherently known toxicities associated with each CPI, and thus baseline toxicities associated with these medications will be assessed prior to the initiation of the vaccines. We anticipate similar local responses as seen in our prior phase I/IIa trials utilizing the TLPLDC vaccines. We anticipate a similar favorable safety profile for the vaccine in this trial; however, meticulous documentation will be performed on the impact of the vaccine on baseline toxicities associated with the CPI.

Patients will be monitored after each inoculation, and 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 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.

#### **4.5 Compliance with Laws and Regulations**

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

### **5.0 MATERIALS AND METHODS**

#### **5.1 Subjects**

Up to 45 patients over the age of 18 years with a diagnosis metastatic melanoma,

with evidence of measurable disease, and eligible for (or being treated with) CPI therapy may be included. Patients should have completed biochemotherapy or targeted therapy per standard of care as indicated. Each patient must be currently eligible for (or being treated with) a FDA-approved CPI. 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 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, the research nurse coordinator and/or study coordinator, or Principal Investigator (PI), will explain the study and review the consent form with the patient. Prospective participants will be provided a copy of the consent form to read and given ample time to ask and have all questions answered prior to signing the consent form. After all questions have been answered, and the patient wishes to participate in the trial, they will sign the consent form to undergo tissue procurement (consent#1).

Tumor procurement may be completed using core needle biopsy or excisional biopsy, depending on the location of the tumor.

After tissue procurement (which need not occur at the study site), if sufficient tumor is available for vaccine production (1 cm<sup>3</sup> preferred, 1mg minimum) (minimum 3 passes with core needle), and the patient is able to continue treatment with a CPI, they will then be consented for participation in the clinical trial (consent#2). Blood will be obtained from the patient per below.

The patient will receive a single injection of Neupogen (G-CSF) 300 µg subcutaneously 24-48 hours 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 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 vaccine will be produced at one time after tumor and blood is received at the central laboratory facility. The production and QA testing for vaccine release will take approximately three weeks at which point the entire vaccine series and boosters will be shipped back to the site for storage until use. Frozen tumor, serum and blood will be maintained on all patients under their unique study number for re-creation of the vaccine as needed. Vaccinations must begin within 4 weeks after vaccine is received at the site.

### *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 or 1 (Appendix A)
- Metastatic melanoma eligible for (or on) standard of care CPI (treating physician's choice) with measurable disease.
- Approximately 1 cm<sup>3</sup> preferred but 1 mg minimum of accessible and dispensable tumor (minimum of 3 passes with a core needle)
- Able to continue their CPI treatment regimen (if already started)
- Adequate organ function as determined by the following laboratory values:
  - ANC  $\geq$  1,000/ $\mu$ L
  - Platelets  $\geq$  75,000/ $\mu$ L
  - Hgb  $\geq$  9 g/dL
  - Creatinine  $\leq$  1.5 x upper limit of normal (ULN) or Creatinine clearance  $\geq$  50% of lower limit of normal (LLN)
  - Total bilirubin  $\leq$  1.5 ULN
  - ALT and AST  $\leq$  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:

- Inability to continue treatment with CPI (if already started)
- Rapidly progressing multi-focal metastatic melanoma
- Insufficient tumor available to produce vaccine
- ECOG  $\geq$  2 performance status (Appendix A)
- Immune deficiency disease or known history of HIV, active HBV, or active HCV

- Receiving immunosuppressive therapy including chronic steroids (except physiologic maintenance doses), 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 Study Treatment**

### *5.2.1 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 final vaccine contains  $1 \times 10^6$  TLPLDC per dose and will be provided in frozen single dose vials in 250  $\mu$ l of freezing media. The final vaccine is prepared for injection by thawing the single dose vial and diluting the TLPLDC by adding 500  $\mu$ l of sterile saline for injection.

#### *5.2.1.1 Preparation of dendritic cells*

Dendritic cells will be generated from the patient's peripheral blood monocytes (PBMC) obtained from the post-Neupogen 70 mL blood draw or from 120 mL of blood drawn if Neupogen not used. 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<sub>2</sub> for two days in serum-free cell culture medium with the appropriate cytokines added to generate immature monocyte-derived dendritic cells.

#### *5.2.1.2 Tumor tissue preparation*

A minimum of 1 mg sample of viable tumor tissue is required to be collected and verified prior to consent#2 for study participation. 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.

#### *5.2.1.3 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.

#### *5.2.1.4 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.

#### *5.2.2 Storage*

Six doses of  $1 \times 10^6$  autologous TLPLDC will be cryopreserved in patient-specific labeled single dose vials and maintained at the central production facility at  $-80^{\circ}\text{C}$  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  $-70^{\circ}\text{C}$  in the research pharmacy until serially thawed for use.

#### *5.2.3 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  $37^{\circ}\text{C}$  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  $\mu\text{L}$  of thawed TLPLDC will be diluted with 500  $\mu\text{L}$  of sterile saline for injection. The final 750  $\mu\text{L}$  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 D).

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, 2 and 3 months  $\pm$  1 week followed by booster inoculations at 6 and 9 months  $\pm$  2 weeks from initial inoculation for a total of six doses.

### **5.3    Study Assessments**

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

#### *5.3.1    Pretreatment assessments*

This trial requires two consents; the first for tumor tissue procurement, and a second for blood draw and treatment. The consents will be signed once the patient has been adequately and appropriately counseled and all eligibility criteria are met. The second consent will be signed once adequate tissue is received for vaccine production.

All patients must have a CBC, CMP, and LDH within 4 weeks 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.

All patients must have a complete metastatic evaluation within 4 weeks of vaccine initiation as a baseline evaluation. Overall health screen will be assessed utilizing the ECOG performance status grading system (Appendix A).

All patients will be assessed for baseline and post-vaccination immunologic responses. Blood samples will be obtained prior to each inoculation (0, 1, 2, 3, 6, and 9 months) and at study completion at 12 months from enrollment. The protocol schedule of events is listed in Appendix E. Specimen handling, processing, and assays are described in Sections 5.3.4, 5.3.5, and 5.3.6.

#### *5.3.2    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.

For both the primary inoculations and booster series, the patients will be monitored closely for 30 minutes after inoculation with questioning, serial exams, and vital signs as needed to observe for a hypersensitivity reaction. 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 to determine induction of anticancer immune responses as described in Sections 5.3.4, 5.3.5, and 5.3.6.

### *5.3.3 Follow-up assessments*

The clinical endpoint of objective tumor response as defined as progressive disease (PD), stable disease (SD), partial response (PR), and complete response (CR) will be determined by the primary investigator at the individual study sites during routine follow-up. This will include history and physical examination every 3 months for all patients for 1 year.

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 CR, PR, SD, or PD will be assessed per RECIST criteria and iRECIST (Appendix B). These results will be communicated by the primary study investigators on a routine basis.

For the purposes of this trial, the timing associated with tumor response will be calculated from the date of initial inoculation to the first date that the tumor meets RECIST criteria and iRECIST criteria. 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.

All enrolled patients will be followed for 1 year after enrollment.

### *5.3.4 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, 3, 6, and 9 months) and at the final study visit.

Thus, including the initial blood draw for vaccine production (70-120 mL), a total of approximately 420-470 mL of blood will be drawn over the one-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 or for additional studies for melanoma treatment (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.

The 50 mL of blood will be collected as follows: 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, aliquotted, and frozen for future use in immunologic assays.

#### *5.3.5 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<sup>+</sup> T-cells will be determined as the percentage of cells that are alive, lineage-, CD3<sup>+</sup> CD8<sup>+</sup> and gp100-dextramer+. This assay may be performed for other known CD8<sup>+</sup> epitopes from known melanoma-specific antigens.

#### *5.3.6 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<sup>5</sup> cells/well/200 µL in duplicate wells. The plate is incubated at 37°C 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).

### **5.4 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 5.5 (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

## **5.5 Subject Discontinuation**

Those patients who display significant reactions (i.e. 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 (i.e., 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.

In the event a patient develops a severe adverse reaction to the CPI that is unable to be managed with immunosuppressive medications may also be discontinued from 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, they 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.

## **5.6 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. Reasons for suspending or terminating the study may include 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

## **5.7 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 can be accessed 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 when the patient signs the second informed consent, 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 QA 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.

## **5.8    Statistical Methods**

### *5.8.1    Rationale for study design*

Individually, CPI therapies have been approved for use in metastatic melanoma and the TLPLDC vaccine appears to provide benefit to these patients as well in small studies.

By stimulating the patients' immune system to produce tumor-reactive T-cells against their personal cancer, while simultaneously preventing down-regulation of these newly induced/amplified T cells in the tumor microenvironment could result in a highly effective multimodality immunotherapy that could significantly impact clinical outcomes of patients with metastatic melanoma. While CPI have been proven to be effective in metastatic melanoma patients in terms of tumor response as well as survival, the majority of metastatic melanoma patients still succumb to their disease. Thus, the CPI monotherapy is not sufficient alone. One theory is that these patients lack adequate numbers of tumor-reactive T cells on which the CPI can work. The TLPLDC vaccine generates tumor-reactive T cells as its primary mechanism of action. Therefore, intuitively there is rationale for combining the two forms of immunotherapy.

### *5.8.2    Sample size determination*

In order to assess both the safety as well as the efficacy of adding the TLPLDC vaccine to SoC CPI in metastatic melanoma patients, a total of up to 45 patients will be enrolled into this study. The primary endpoint of efficacy is defined as the objective tumor response rate (ORR) (CR + PR) per RECIST criteria and iRECIST. With 45 patients, the study will have an 80% power to detect an absolute 25%

improvement in ORR compared to SoC CPI monotherapy in these patients with an  $\alpha = 0.05$  (one-tailed). Furthermore, this number of patients will allow for a similar level of detection of safety/toxicity parameters.

### *5.8.3 Data analysis*

Tumor response to the CPI+TLPLDC vaccine will be evaluated by the site primary investigators for each patient at multiple time points (approximately every 3 months for a year).

The time to best tumor response for each patient will be determined from the date of first inoculation until they meet a specified tumor response based on the RECIST criteria and iRECIST (Appendix B). ORR (CR + PR) as well as the clinical beneficial response rate (CBR) (CR + PR + SD) will be calculated and represented in waterfall plots and line graphs. The progression free survival and overall survival will be determined using the Kaplan-Meier method.

#### *5.8.3.1 Study patient characteristics*

Demographic characteristics of all patients 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), and other disease-directed therapies to include chemotherapy, radiation therapy, and biologic therapy including the checkpoint inhibitor used. 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.

#### *5.8.3.2 Primary efficacy analysis*

The primary endpoint for efficacy is the overall study-wide objective tumor response rate in response to the TLPLDC vaccine being added to SoC CPI therapy.

The primary investigators at each site will determine each patient's tumor response to the TLPLDC vaccine + CPI and classify the patient as a CR, PR, SD, or PD per RECIST criteria and iRECIST criteria as delineated in Appendix B. Each patient will be assessed approximately every 3 months for a year. Durability of response will also be reported.

The primary efficacy analysis will be an accumulation of the individual patients' responses. The overall study ORR (CR + PR) will be calculated and reported as well as the median durability of response. Additionally, the CBR (CR + PR + SD) will also be reported with median durability of response.

#### *5.8.3.3 Secondary efficacy analysis*

The ORR and rate/type of AEs will be compared to currently available CPI monotherapy ORR and rate/type of AEs using proportional statistical analysis.

#### 5.8.3.4 Safety analysis

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) will be summarized.

Time to AE onset will be estimated using the Kaplan-Meier method. In addition, duration of any AE with >10% incidence, total number of AEs, SAEs, and related AEs will be summarized..

#### 5.8.3.5 Immunologic analysis

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.

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 5.3.4, 5.3.5, and 5.3.6) during the trial.

Changes from pre-vaccination levels to each time point will be compared, and differences will be evaluated using a paired t-test.

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.

#### 5.8.3.6 Planned analyses

- 1) Safety will be monitored continuously.
- 2) Objective tumor response rates will be assessed in real time.
- 3) The interim analysis response rates and comparisons will be completed 12 months after initiation of this trial to assess the vaccine safety profile and tumor response trends. No action regarding stopping the trial will be taken based on this analysis unless there is a safety concern.
- 4) The final analysis will be completed after 12 months follow-up from the last enrolled patient.

#### 5.8.4 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.

#### *5.8.5 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.

### **6.0 ADVERSE EVENTS**

Reporting of adverse events will be performed in accordance with the Data Safety Monitoring Plan (DSMB) (Appendix F) and Sections 6.2 and 6.3.

#### **6.1 Adverse Event and Reporting Definitions**

With the occurrence of an AE, the first concern will be for the safety of the subject. Investigators are required to report to the IRB, medical monitor, clinical research organization (CRO) QA Officer, or designee and CRO Regulatory Affairs Officer any serious adverse event, whether expected or unexpected, and which is assessed by the investigator to be possibly or probably related to or caused by the vaccine components. 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. The Regulatory Affairs Officer will then report the event to the Sponsor. The IND Sponsor or Sponsor's representative will report these to the FDA per the requirements of 21 CFR Section 312.32. Serious criteria, definitions, and guidance for reporting follow.

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 adverse events (SAE) 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 AEs are those AEs that are listed or characterized in the current Investigator Brochure.

Unexpected AEs are those not listed in the current Investigator Brochure (IB).

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.

## **6.2 Reporting of Serious Adverse Events Associated with This Study**

Each site PI will within 24 hours of notification of the event report all related or unrelated serious AEs occurring in subjects enrolled at their respective study site to the Internal Review Board (IRB) of their site (see AE Reporting Algorithm, Appendix G). This will be accomplished by submitting an AE report memorandum to the IRB per the IRB's site-specific standard operating procedures.

The site PI will also, within 24 hours of notification of the event, forward a copy of the serious adverse event report (CRF Form 3.1) to the CRO QA Officer or designee, and to the CRO Regulatory Affairs Officer.

The QA Officer or designee will then forward the report to the Sponsor. The Sponsor or Sponsor's representative will review the serious AE to determine the need for expedited reporting to the FDA. If expedited reporting is required, the site nurse coordinator will be notified by the QA Officer or designee to complete a MedWatch FDA Form 3500 (Appendix H).

The Regulatory Affairs Officer will then submit the final report to the Sponsor or Sponsor's representative who will then report to the FDA.



### 6.2.1 MedWatch 3500A reporting guidelines

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

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the AE to each investigational product and suspect medication

#### **Follow-up information:**

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

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

The CRO or Sponsor may contact the reporter for additional information, clarification, or current status of the subject for whom an AE was reported. For questions regarding SAE reporting, you may contact the CRO QA Officer or designee.

#### **Study Drug Relationship:**

The PI will determine which events are associated with the use of study drug. For reporting purposes, an AE should be regarded as possibly related to the use of vaccine components if the PI believes:

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

### 6.3 Reporting Requirements for IND Sponsor

For sponsored IND Studies, there are additional reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR § 600.80. 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 fatal or life-threatening AE that is unexpected and assessed by the investigator to be possibly related to the use of the investigational product. An unexpected AE is one that is not already described in the IB for the vaccine. Such events are to be reported by the sponsor to the FDA and within seven calendar days of first learning of the event.

*15 Calendar-Day Written Report:* The Sponsor is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of the study agents. An unexpected AE is one that is not already described in the Investigator Brochure for vaccine.

- 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).

## 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. FDA Form 1572 for each site (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. Investigators must also complete all regulatory documentation as required by local and national regulations.
- 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 I)

## **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 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. 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. The Principal Investigator must also keep the IRB informed of any significant adverse events. Investigators are required to promptly notify their respective IRB of all adverse drug reactions that are both serious and unexpected. This generally refers to serious adverse events that are not already identified in the IB and that are considered possibly or probably related to the molecule or study drug by the investigator.

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; serious and/or unexpected adverse experiences occurring during the study; 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 F) 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.

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## Appendix A

### 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 B

### 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  $\geq 10\text{mm}$  by CT (CT must use 5mm cuts or smaller)
  - If tumor lesions are measured on clinical exam, the lesion should be superficial,  $\geq 10\text{mm}$  in diameter when measured by a caliber
  - Tumors must be  $\geq 20\text{mm}$  by chest xray.

##### II. Lymph nodes must be measured along its *shortest* axis

- Nodes must be  $> 15\text{mm}$  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 measureable. 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  $\leq 10\text{mm}$  (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  $< 10\text{mm}$  (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  $< 10\text{mm}$  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 tumours: Revised RECIST guidelines – version 1.1 for further details<sup>15</sup>.*

## 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<sup>26</sup>

	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 $\geq 5$ 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 $\geq 5$ 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.<sup>26</sup>

## **Appendix C**

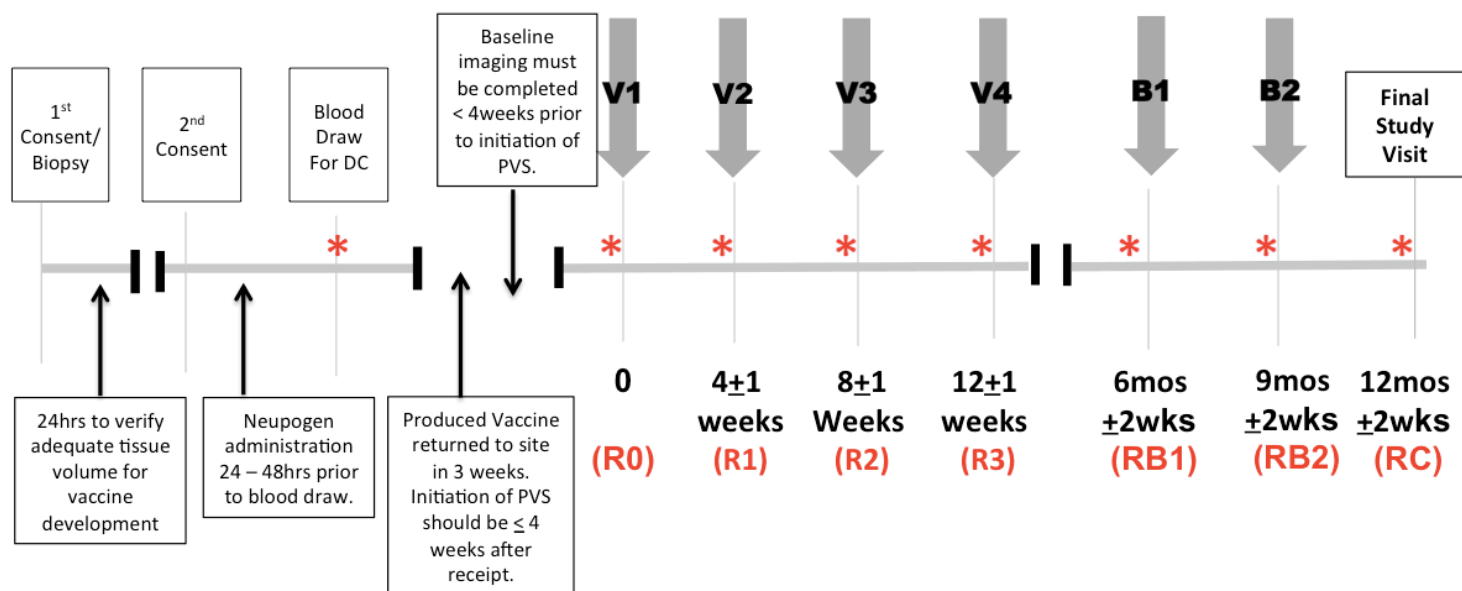
### **NCI Common Terminology Criteria for Adverse Events, v4.03**

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](http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06_14_QuickReference_8.5x11.pdf)

## Appendix D- Study Timeline

### Phase I/IIa Study Timeline



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



## Appendix E - Protocol Schedule

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

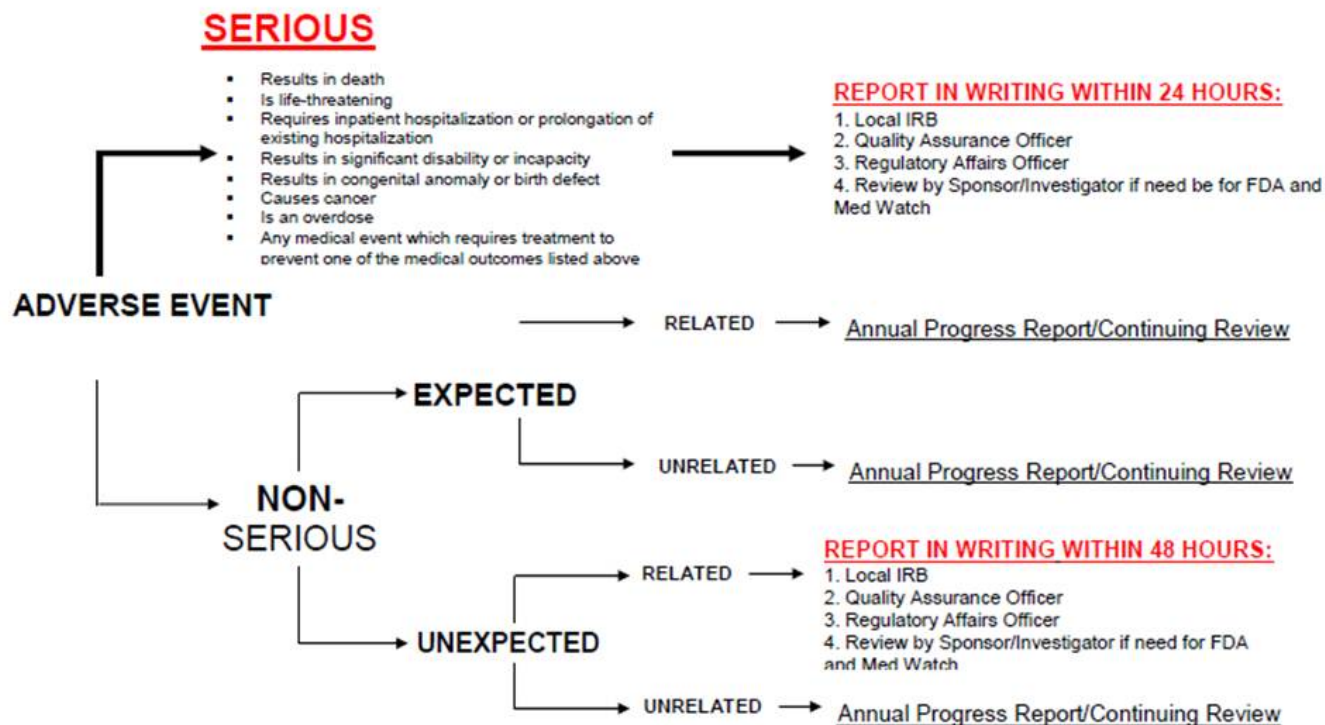
Total Number of Visits: 10 / Total Number of Inoculation Visits: 7 (1 Neupogen injection, 6 vaccines) / Total Number of Blood Draws: 8 (1 for dendritic cells, 6 prior to inoculation, 1 at end of study)

## **Appendix F**

### **Data Safety Monitoring Plan**

This is a free standing document

## Appendix G Adverse Events Reporting Algorithm



## **Appendix H**

### **MedWatch FDA Form 3500 Link**

<http://www.fda.gov/downloads/Safety/MedWatch/DownloadForms/ucm082728.pdf>

## Appendix I

**PROTOCOL TITLE:**

**STUDY DRUG:** Autologous tumor lysate (TL) + yeast cell wall particles (YCWP) + dendritic cells (DC)

**PRINCIPAL INVESTIGATOR:**

**PROGRAM DIRECTOR:** George E. Peoples, MD, FACS

**PROTOCOL VERSION/DATE:** 2.0/23 June 2017

### 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 InCITe. 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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