Phase I/II Study of Low Dose Cyclophosphamide, Tumor Associated Peptide Antigen-Pulsed Dendritic Cell Therapy and Imiquimod, in patients with Progressive and/or Refractory Solid Malignancies.

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Principal Investigator Signature Page

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ABSTRACT

Patients diagnosed with progressive and/or refractory solid malignancies (SM), who have failed conventional therapy, and have no available, potentially curative therapeutic options, will be candidates for this Phase I/II study. Following confirmation of disease progression and/or refractoriness, eligible patients who agree to participate and sign a consent form will have their tumor cells/tissues and/or blood analyzed for the expression of a specific panel of Tumor Associated Peptide Antigens (TAPAs), including Sp17, ropporin, AKAP-4, PTTG1, Span-xb, Her-2/neu, HM1.24, NY-ESO-1 and MAGE-1. Patients whose tumors express one (1) or more of these TAPAs will undergo phlebotomy and/or leukapheresis performed for generation of autologous DCs. Patient's DCs will be generated in Kiromic's Cell Processing GMP facility, according to established Standard Operating Procedures, and activated by pulsing/loading them with the TAPA(s) relevant for each particular patient. Patients will receive five (5) days of low-dose cyclophosphamide prior to each vaccination with TAPA-pulsed DCs to decrease Treg activity. TAPA-pulsed DCs will be administered at a fixed dose of up to 1 X 10^7 DCs following cyclophosphamide administration. DC vaccination schedule will be once every seven (7) days via intradermal (ID) injections for a total of 3 vaccinations. Topical Imiquimod will also be administered once after the TAPA-pulsed DC vaccination, to optimize immune responses. Patients will be followed on a weekly basis (or more frequently if required) to evaluate treatment-related toxicity. Immune efficacy and anti-tumor responses will be evaluated per protocol specifications. Continuation and stopping rules for the study will be defined based on toxicity/tolerability (Phase I) and immune efficacy (Phase II).

1.0 BACKGROUND AND SIGNIFICANCE

Despite advances in understanding the biology of solid malignancies (SM), and the availability of new treatment options, the vast majority of patients with SM remain incurable (*1*). This is especially true for elderly patients and those whose disease proves refractory and/or relapses following standard therapy. Therefore, therapeutic options for this patient population are quite limited and novel strategies are desperately needed for patients with progressive and/or refractory SM.

Immunotherapy has recently emerged as a promising treatment strategy for patients with SM (*2*). Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) and are necessary for the efficient priming and activation of naïve T-cells against specific antigens. DCs undergo maturation and activation in the presence of specific immunogenic antigens and serve as a critical link between innate and adaptive immunity resulting in antitumor responses (*3*). It is now possible to generate human DCs *in-vitro* from monocytes exposed to GM-CSF and IL-4, and DC-based treatments have become one of the most attractive immunotherapeutic strategies for cancer in the last decade. Indeed, DC vaccination studies have been conducted in several different neoplasms (*4, 5*) with variable results.

Since most patients with progressive SM display a defective immune response to tumor antigens, the *ex-vivo* activation of DCs, through their exposure to tumor associated antigens, is an attractive and active area of investigation (*6, 7*). A variety of early clinical studies have demonstrated the feasibility and promise of using antigen-pulsed DC therapy in patients with SM malignancies including metastatic prostate, hepatocellular, colorectal, renal, cervical, and breast carcinomas, as well as melanomas and glioblastomas (*8-13*). The fundamental strategies tested have included pulsing DCs with whole tumor cell lysates or specific tumor-associated antigens (*8-13*). Although safe, DC vaccination strategies have resulted in unsatisfactory clinical outcomes attributed to either the poor immunogenicity of the target antigens, suboptimal DC-dosing, generation of tolerogenic DC subtypes and activity of suppressor regulatory T-cells (Treg). Thus, results of these preliminary clinical studies indicate the need to optimize DC-based immunotherapy to improve responses in patients with SM.

DCs can be safely generated from blood-derived monocytes in the presence of GM-CSF and IL-4 and administered safely to cancer patients (*14, 15*). Moreover, DCs pulsed with specific Tumor Associated Peptide Antigens (TAPAs) are capable of eliciting immune and antitumor responses, without prohibitive toxicity (*8-13, 16, 17*). Despite these advances many questions remain on how to improve this immunotherapeutic strategy, including how to best activate DCs with various antigens and cytokine combinations, the characterization of "ideal" TAPAs capable of eliciting strong and durable immune responses in patients with SM, the best vaccine immune adjuvants, the optimal dosing and schedule of DC treatments, how to inhibit inhibitory signals from Tregs and what specific group of patients with SM are best suited for this particular DC-based vaccination approach. To solve these problems several potential strategies have been proposed including the use of more potent and immunogenic TAPAs and/or Th1 polarizing cytokines (*18, 19*), enhancement *ex-vivo*

DC maturation and activation through Toll-like receptor (TLR) signaling (*20*), cross presentation of neoplastic cell-associated antigens (*21*), stimulation of natural killer cell and/or T-helper activity to generate Th1 polarization in DCs (*22*), inhibition of immunosuppressive Treg cell populations (*23*) and regulation of DC migration to regional lymph nodes (*24*), among others.

We and others have explored several potential TAPAs as immunotherapeutic targets in different malignancies. These highly immunogenic proteins include Sp17, Ropporin, AKAP-4, PTTG1, Span-xb, Her-2/neu, HM1.24, NY-ESO-1, MAGE-1, and are thought to be potential targets for DC-based immunotherapy based on the following observations:

Sperm protein 17 (Sp17)

Sp17 is a highly immunogenic spermatozoan protein which has been considered a potential therapeutic target for immunocontraception in the last few years (*25*). We have identified Sp17 as a novel Cancer-Testis Antigen (CTA) in hematologic and SM. Using RT-PCR, Sp17 transcripts were detected in more than 70% of tumor-cell enriched bone marrow from multiple myeloma (MM) patients. Sp17 gene expression was associated with Sp17 protein translation as demonstrated by Western blot analysis. Importantly, Northern blot analysis and RT-PCR, demonstrated Sp17 expression in normal testis and tumor cells, but not in normal tissues, suggesting Sp17 could serve as an excellent immunotherapeutic target in MM (*26*). We have cloned the full-length coding sequence of the Sp17 cDNA and successfully generated Sp17-specific HLA-class I restricted cytotoxic T lymphocyte (CTL) after "pulsing" monocyte-derived DCs with the E. coli-derived recombinant Sp17 protein (*14*). These Sp17-specific CTLs were able to lyse autologous target cells pulsed with recombinant Sp17 protein as well as Sp17-expressing fresh MM cells. Moreover, we have generated a synthetic Sp17 peptide (103-111) (ILDSSEEDK) capable of inducing HLA-A1 restricted CTL recognition and activation (*27*). We have also expanded our evaluation of Sp17 potential as an immunotherapeutic target by recently demonstrating its strong immunogenicity in an ovarian cancer animal model (*28*) and have recently found Sp17 is expressed in human lung cancer cell lines and tumor tissues, but not in normal lung (*29*). More importantly, we have shown Sp17-loaded DCs activate CTLs capable of eliciting antitumor responses, *in vitro* (*29*). Finally, Dadabayev *et al* have reported the safety and clinical response of Sp17-pulsed DCs in patients with MM and ovarian cancer, validating the clinical potential of this protein as an immunotherapeutic target (*30*). Taken together, these findings suggest the feasibility of exploiting Sp17-pulsed DC immunotherapy as a promising treatment option for patients with both hematologic and SM.

AKAP-4

A-kinase anchoring protein 4 (AKAP-4) is a member of a growing family of scaffolding proteins involved in the control of signal transduction by targeting cyclic adenosine monophosphate-dependent protein kinase-A, and directing its actions (*31, 32*). We have shown that AKAP-4 is expressed in lung cancer and MM cells, at both the transcriptional

and the protein level, with no evidence of expression in human normal tissues, other than the testis (*29, 33*). The lack of expression in normal tissues and the presence of AKAP-4 antibodies in lung cancer and MM patient sera supports the protein's immunogenicity and indicates AKAP-4 is a novel TAPA in these two malignancies. Additionally, we have also demonstrated AKAP-4 serves as a marker of disease status in a murine model of MM, supporting its importance in the biology of this disease (*34*). Thus, our findings suggest AKAP-4 is a potential target for developing specific immunotherapeutic strategies against MM, lung cancer and possibly other SM (*29*).

Ropporin

Ropporin is a rhophilin-associated protein normally expressed in the inner fibrous sheath of sperm flagella. Ropporin has previously been found to interact with other fibrous sheath proteins, including Sp17 and AKAP-110, suggesting a common or related biological function. A study by Li *et al* has demonstrated a very restricted RNA expression of ropporin in normal tissues, with the exception of testicular and fetal liver tissue (*35*). Ropporin expression was also detected in tumor cells derived from the bone marrow in 6 of 16 (37.5%) patients with MM, 6 of 14 (43%) cases of CLL and 2 of 11 (18%) cases of acute myeloid leukemia. No ropporin transcripts were detected in the peripheral blood mononuclear cells of 17 healthy donors. Importantly, these investigators detected high titers of antibodies against ropporin in 8 of 30 MM (26.7%), 7 of 24 AML (29.2%), 18 of 31 chronic lymphocytic leukemia (CLL) (58.1%), 9 of 27 chronic myelogenous leukemia (CML) (33.3%) and 1 of 3 acute lymphoblastic leukemia (ALL) (33.3%), compared to healthy donors. The presence of ropporin antibodies in patients with a variety of malignancies indicates this CTA is a highly immunogenic protein. Taken together, these findings support the notion that ropporin is a tumor-restricted CTA with a potential role in the biology of certain neoplasms. The restricted expression of ropporin in neoplastic cells, testes and fetal liver, makes it a suitable candidate as a target for immunotherapy including DC vaccine applications.

PTTG-1

PTTG-1 is a novel oncogene involved in transcriptional and cell cycle regulation with expression in the normal testis and thymus (*36*). PTTG-1 has been shown to be highly expressed in different hematologic malignancies (HM) including promyelocytic leukemia (PML) cell line HL-60, CML cell line K-562, ALL cell line MOLT-4 and Burkitt's lymphoma cell line Raji (*37*). PTTG-1 has also been shown to be associated with tumorigenesis, angiogenesis and cancer progression, making it a logical therapeutic target (*36*). We and others have found PTTG-1 is expressed at the transcriptional level in MM, with PTTG-1 being expressed in 63% of MM patients and 66% of human MM cell lines studied, but not in normal tissues (*38*). We also documented cytoplasmic and surface PTTG-1 expression in plasma cells from MM patients supporting PTTG-1 potential role as a therapeutic target in patients with HM. More recently, we have also demonstrated PTTG-1 expression in lung cancer tissues and cell lines and showed PTTG-1-loaded DCs

can activate CTL-mediated lysis of human lung cancer cells, i*n vitro (29)*. Therefore, our data indicates the suitability of using PTTG-1 as a potential target for immunotherapy in both HM and SD.

Span-xb

Span-xb is a novel CTA expressed in CML and other neoplasms. Using RT-PCR, we have detected Span-xb transcripts in 20% of MM patients, 33% of patients with CLL, 29% of CML patients and 50% of patients with AML. In contrast, Span-xb expression was not detected in peripheral blood or bone marrow samples from healthy donors (*39*). Importantly, span-xb gene expression has also been found in a variety of SM, including melanoma and carcinomas of the lung, colon and breast, making it a target for immunotherapeutic interventions (*40*).

Her-2/neu

HER-2/neu is a trans-membrane tyrosine-kinase involved in aberrant signal transduction in a variety of neoplasms (*41, 42*). HER-2/neu amplification has been demonstrated in certain HM and its functional inhibition, using anti-sense oligonucleotides, results in a reduced tumor cell proliferative rate. The observation that Her-2/neu is expressed at very low levels in normal tissues and preferentially expressed in neoplastic cells, suggest it could serve as an immunotherapeutic target in HER-2/neu-expressing SM. In fact, Scardino et al have demonstrated HLA-restricted CTL activation by high several high affinity HER-2/neu peptides capable of HLA-restricted killing of neoplastic cells of diverse origin (*43*). Thus, specific Her-2/neu peptides may serve as good candidates for immunotherapy in HER-2-expressing SM.

HM1.24

The HM1.24 protein is a novel, 29-33 kDa membrane glycoprotein expressed in mature B-cells. We have proposed HM1.24 as a new antigen for CTLs activation against MM (*44*). HM1.24 expression has been found in all five human MM cell lines assayed, as well as in mature, Ig-secreting B-cells (plasma cells and lymphoplasmacytoid cells), but not in non-B-Cells in the peripheral blood, bone marrow, liver, spleen, kidney, or heart of normal individuals or patients with non-plasma-cell-related malignancies. Thus, HM1.24 protein represents a specific marker of late-stage B-cell maturation and may potentially serve as a target antigen for the development of immunotherapeutic strategies specific against MM. HM1.24 is also expressed in SM including brain tumors, renal, hepatocellular, breast, ovarian, and breast carcinomas, with some expression in a few normal organs including liver and kidney (*45*). Although HM1.24 function is unknown at this time, its promise as a therapeutic target has been demonstrated using a specific HM1.24 monoclonal antibody (MoAb) (*46*).

NY-ESO-1

NY-ESO-1 is one of the most immunogenic tumor antigens known to date. Spontaneous humoral and cellular immune responses against NY-ESO-1 are detected in a substantial proportion of patients with NY-ESO-1 expressing malignancies and NY-ESO-1 antibody titers correlate with clinical development of disease (*47*). Moreover, the development of NY-ESO-1 serum antibody is associated with detectable NY-ESO-1-specific CD8+ T cell reactivity, suggesting this antigen is an excellent immunogen and potential therapeutic target, *in vivo (48)*.

MAGE-1

MAGE-1 is expressed in hematologic malignancies, including human MM cell lines and malignant plasma cells, as well as melanomas (*49, 50*). Both RNA and protein expression has been demonstrated in MM cells, but not in polyclonal, reactive plasma cells. Moreover, anti-MAGE-1 HLA-A1 cytotoxic T lymphocytes can efficiently kill MAGE-1 HLA-A1 expressing MM and melanoma cells, suggesting MAGE-1 represents a specific and potential immunotherapeutic target for patients with these malignancies.

Using an HLA binding prediction algorithm, we have identified peptides, within the sequence of the above-mentioned antigens, predicted to have high binding affinity to HLA-class I and class II molecules. Peptide sequences from each antigen were linked together using the furin-sensitive linker, RVKR. This linker is used to connect peptides with different HLA restrictions to form a unique long peptide, and it was shown to be safe and effective in previous clinical trials linking the MAGE-A3 or human papillomavirus (HPV)-16 derived peptides, and the a "penetrin" peptide sequence derived from HIV-TAT (*51, 52*) (IND#10927, #NCT00257738).

The rationale for using long peptides (synthetic long peptides, SLP) is based on the evidence that dendritic cells present SLP better than whole proteins, and that the anti-SLP immune repose lasts longer than that of short peptides (*53, 54*).
The SLP sequences are as follows (the linker peptide, RVKR, is evidenced in **bold**).

1) SP17

SP17- peptide A IPQGFGNLLEGLTREIL**RVKR**DNIPAFAAAYFESLLE**RVKR**KTNFDPAEW

SP17-peptide B KEEETSVTILDSSEEDKEKEEVAAVKIQAAFRGHIAR**RVKR**AKKMKTNSL

2) AKAP4

AKAP4- peptide A
MMAYSDTTMMSDDIDWL**RVKR**YALGFQHALSPSTST**RVKR**YADQVNIDYLMNRPQNLRLEM**RVKR**CSID DLSFYVNRLSSLVIQMA

AKAP4- peptide B
FLYSELSNK**RVKR**PASVVLKRVLLRHTKEI**RVKR**MEAMLKRLVSALIGE**RVKR**QSLSYASLK

AKAP4- peptide C GEHILKEGL**RVKR**MSNIVLMLI**RVKR**WIAASQFNVPMLY**RVKR**EAVGKVARK

3) PTTG1

PTTG1- peptide A KLGSGPSIK**RVKR**RSQVSTPRF**RVKR**LPKATRKALGTVNRATEK

PTTG1- peptide B
FFPFNPLDF**RVKR**EHQIAHLPL**RVKR**RELEKLFQLGPPSPVKM**RVKR**ILSTLDVELPPV

4) ROPPORIN-1

ROPN1- peptide A DLIQWAADYFEAL**RVKR**LLKILHSQVAGRLIIRAEELAQMW

ROPN1- peptide B IEWLKFLALACSALGVTIT**RVKR**SPRIPFSTFQFLYTYIAKVDGEI**RVKR**HVSRMLNYM

5) Spanx-B1

SPNXB1 SESSTILVVRYRRNVKRTS**RVKR**MEEEEFIEI

6) NY-ESO-1

NYESO1- peptide A APRGPHGGA**RVKR**RLLEFYLAMPFATPM**RVKR**SLAQDAPPL

NYESO1- peptide B ILTIRLTAADHRQLQLSISSCLQQLSLLMWI**RVKR**FLPVFLAQPPSGQRR

7) MAGE-1

MAGE1- peptide A SAFPTTINF**RVKR**ILESLFRAVITKKVADLVGFL**RVKR**IMPKTGFLI

MAGE-1 peptide B EIWEELSVM**RVKR**AETSYVKVLEYVIKVSARVRFFFPSLREAAL

8) Her2/Neu

Her2/Neu peptide A

CRWGLLLAL**RVKR**HLDMLRHLY**RVKR**LELTYLPTNASLSFL**RVKR**QLFEDNYAL

Her2/Neu peptide B

ETLEEITGY**RVKR**SVFQNLQVIRGRILH**RVKR**RELGSGLAL**RVKR**FRNPHQALL**RVKR**LTSIISAVV**RVKR**LIKRR
QQKIRKYTMRRLLQ

Her2/Neu peptide C

SGAFGTVYK**RVKR**VMAGVGSPY**RVKR**MQIAKGMSY**RVKR**GKVPIKWMALESILRRRFT**RVKR**SYGVTVWE
L**RVKR**AARPAGATL**RVKR**PAFDNLYYW

9) HM1.24

KRCKLLLGI**RVKR**PLIIFTIKANSEACR**RVKR**HTVMALMASLDAEKA**RVKR**KLQDASAEV**RVKR**VLSVRIADK

Being more specific, and potentially less toxic than chemotherapy, immunotherapeutic strategies, such as TAPA-pulsed DC vaccination, may prove an ideal treatment for patients with a variety of SM. In fact, such an approach has recently gained FDA approval for

patients with advanced prostate cancer (*55*). Therefore, studies using novel TAPAs are clearly warranted in patients with limited therapeutic options, such as those with progressive and/or refractory SM.

A promising way of optimizing the efficacy of therapeutic vaccines would be the inhibition of suppressive T-cell populations, such as Tregs. It has been recognized for many years that cyclophosphamide (CYP) can exert both cytotoxic and immunosuppressive effects, depending on the dose used. For example, studies have shown that low-dose cyclophosphamide (CYP) decreases Treg number and functionality (*56*). Greten and colleagues evaluated single-agent CYP doses of 150, 250, and 350 mg/m^2 in patients with hepatocellular carcinoma and reported that the two (2) lower doses induced a decrease in the absolute and relative frequency of Tregs in the blood of patients, and the 250 mg/m^2 dose impaired suppressor function and showed decreased Treg frequency up to day 71. Alpha-fetoprotein–specific T-cell responses were also induced in the lower treatment arms (*57*). In a different study, breast cancer patients received CYP, 50 mg orally daily for 3 months. Tregs were reduced within 14 days of treatment and remained decreased until day 42, returning to pretreatment levels by day 84. Interestingly, endogenous breast tumor–reactive T cells were detected in 27% of patients before CYP treatment and increased to 73% on day 14, 80% on day 42, and 88% on day 84, indicating enhanced T-cell function after the use of metronomic doses of CYP (*58*). More recently, the use of metronomic CYP combined with active immunotherapy has been reported (*59*). In this study, patients with advanced solid tumors were treated with 3 different regimens of low-dose CYP in combination with an oncolytic adenovirus. CYP was given either as oral metronomic (50 mg/day), a single intravenous (i.v.) injection (1,000 mg), or both. Metronomic CYP was given starting 1 week before the adenovirus, and i.v. cyclophosphamide was given 1 hour prior to the adenovirus. All CYP regimens resulted in higher rates of disease control when compared with the rates for the adenovirus vaccine only, and the metronomic groups were most effective in decreasing Treg numbers. Based on these encouraging results numerous studies are in progress combining metronomic doses of CYP with active vaccination strategies (ClinicalTrials.gov) for a variety of cancers. For an excellent discussion on the role of low-dose CYP as a suppressor of Treg activity and its potential in cancer immunotherapy, see the review by Le and Jaffee (*60*).

Two additional strategies to optimize the efficacy of anti-tumor cellular vaccines would be to improve the *ex-vivo* generation of DCs with an immunogenic phenotype and to select appropriate adjuvant molecules or cytokines to enhance DC migration and activation, *in vivo.* For example, the use of type I interferons and Toll-like receptor agonists to induce DC maturation *ex-vivo* have been shown to stimulate generation of immunogenic, rather than tolerogenic, DCs (*6, 61*).
Activation of Toll-Like Receptor 7 (TLR7) has been shown to improve the survival and immunogenicity of DC (*62-64*) (#NCT01792505). Additionally, topical usage of the imiquimod cream meaningfully improved DC migration and increased numbers of emigrated DCs. By contrast, intradermal injection of soluble imiquimod or the TLR7/8

ligand R848 did not result in the same effect (*65*). Of note, TLR7 adjuvant is available as a topical cream, i.e. Imiquimod (Aldara cream), with evident advantages over other classical DC adjuvants such as GM-CSF, which requires injections (*66*).

In this phase I/II study we will examine the feasibility, toxicity, immune response and antitumor activity of TAPA-pulsed DC therapy in patients with progressive and/or refractory SM. To improve the efficacy of this strategy, selected TAPAs will be used to pulse DCs *ex-vivo*, in the presence of a highly immunogenic maturation cocktail (*9, 12, 19, 20*). Patients will also be treated with low-dose CYP prior to each DC vaccination, in an attempt to decrease the number and activity of Tregs, and topical Imiquimod will be administered following each DC vaccination, in order to optimize immune responses in patients with relapsed/refractory SM. A similar strategy has recently been safely explored by others in patients with refractory SM (*9*).

2.0 HYPOTHESIS AND OBJECTIVES

2.1 Hypothesis

We hypothesize that treatment of patients with progressive and/or refractory SM, without available, potentially curative therapeutic options, and whose tumor cells express at least one (1) TAPA of a defined panel of TAPAs, using low-dose CYP followed by an autologous, monocyte-derived, TAPA-pulsed DC vaccine and topical Imiquimod, will result in TAPA-specific CD4+ T-cell and CD8+ CTL responses without significant toxicities. We also hypothesize CD4+ T-cell and CD8+ CTL responses generated against specific TAPAs may translate into clinical antitumor activity.

2.2 Primary Objective: Phase I

To determine the toxicity of low-dose CYP followed by TAPA-pulsed DC therapy and topical Imiquimod, in patients with progressive and/or refractory SM.

2.3 Secondary Objectives: Phase II

To determine immune responses associated with low-dose CYP followed by TAPA-pulsed DC therapy and topical Imiquimod, in patients with progressive and/or refractory SM.

3.0 STUDY DESIGN

3.1 Study Population

3.1.1 Number and Characteristics of Patients Enrolled

1. The study population will be drawn from patients at various institutions in USA following contractual agreements with Kiromic.

2. There will be no patient restrictions based on race or gender.

3. Up to six (6) subjects may be enrolled and receive DC vaccination to evaluate toxicity of the proposed vaccination strategy (Phase I stage).

4. Up to an additional eleven (11) patients, for a total of seventeen (17) patients, may be enrolled for determination of immune efficacy (Phase II stage), assuming no overt toxicity is encountered during the safety evaluation.

3.2 Eligibility Criteria

Patients must have baseline evaluations performed prior to administration of the first dose of study vaccine and must meet all inclusion and exclusion criteria. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule, required evaluations, and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled on the study unless otherwise specified.

3.2.1 Inclusion Criteria

1. Ability to provide informed consent.

2. Patients with histologically proven progressive and/or refractory SM, s/p conventional salvage therapy, completed at least 3 weeks prior to study vaccination, will be eligible for enrollment.

3. Expression of one (1) or more of the following TAPAs: Sp17, AKAP-4, Ropporin, PTTG-1, Span-xb, Her-2/neu, HM1.24, NY-ESO-1 and MAGE-1, by either RT-PCR and/or immunocytochemistry, Western blotting or ELISA, in neoplastic cells. For HER-2/neu expression, positive FISH results are acceptable.

4. Presence of measurable or evaluable disease.

5. Patients must not have any active infectious process.

6. Patients must not have a history of HIV, or active Hepatitis A, B, or C.

7. Patients must not be receiving active immunosuppressive therapy.

8. Patients must have discontinued systemic cytotoxic or radiation therapy at least three (3) weeks prior to vaccination and toxicities from previous therapies must be grade 1 or less. All other FDA approved forms of antineoplastic therapy are allowed such as immunotherapy, targeted therapies, or hormonal therapies (*67, 68*).

9. Patients may not have any known allergy to CYP and/or Imiquimod.

10. Patients must be willing to provide at least 250 mL, and up to 500 mL, of whole blood obtained by phlebotomy and/or consent to leukapheresis for DC generation.

11. Adequate renal and hepatic function (creatinine ≤ 2.0 mg/dl, bilirubin ≤ 2.0 mg/dl, AST and ALT ≤ 4X upper limit of normal range).

12. Adequate hematologic function (Platelets ≥ 60,000/mm^3, lymphocytes ≥ 1,000 mm^3, neutrophils ≥ 750/mm^3, hemoglobin ≥ 9.0 g/dl).

13. Karnofsky performance status ≥ 70%.

14. Expected survival ≥ 6 months.

15. Either a female or male of reproductive capacity wishing to participate in this study must be using, or agree to use, one or more types of birth control during the entire study and for 3 months after completing the study. Birth control methods may include condoms, diaphragms, birth control pills, spermicidal gels or foams, anti-gonadotropin injections, intrauterine devices (IUD), surgical sterilization, or subcutaneous implants. Another choice is for a subject's sexual partner to use one of these birth control methods. Women of reproductive capacity will be required to undergo a urine pregnancy test before completion of the post-screening informed consent process.

3.2.2 Exclusion Criteria

1. Patients without confirmed progressive and/or refractory SM using standard RECIST criteria.

2. Patients without measurable or evaluable disease.

3. Patients receiving cytotoxic or radiation therapy, within three (3) weeks of vaccination.

4. Active immunosuppressive therapy, including non-physiologic systemic steroids (excluding topical, intraocular, inhaled, and intranasal steroids) for any other condition.

5. Persistent fever (>24 hours) documented by repeated measurement or active, uncontrolled infection within 4 weeks of enrollment.

6. Active ischemic heart disease or history of myocardial infarction within six months.

7. Active autoimmune disease, including, but not limited to, Systemic Lupus Erythematosus (SLE), Multiple Sclerosis (MS), Ankylosing Spondylitis (AS), Inflammatory Bowel Disease (IBD), and Rheumatoid Arthritis (RA).

8. Pregnancy or breast feeding.

9. Active second invasive malignancy, other than basal cell carcinoma of the skin.

10. Life expectancy of less than 6 months.

11. Patients with contraindications to known allergies to CYP and/or Imiquimod.

12. Patients who have received organ transplants.

13. Patients with psychological or geographic conditions that prevent adequate follow-up or compliance with the study protocol.

3.3 Withdrawal Criteria

3.3.1 Reasons for Withdrawal of Patients from Study

1. Patients with clinically significant biochemical, histological and/or radiographic evidence of increasing disease burden since entry into the study may be removed from the study.

2. Patients may be withdrawn from the study in the event of dose limiting toxicities.

3. Patients may be withdrawn from study in the event of protocol non-compliance.

4. Patients may voluntarily withdraw from study, if they decide to do so, for any reason.

3.3.2 Follow-up of Withdrawn Patients

1. A subject withdrawn by virtue of progressive disease will continue to be followed as clinically indicated, as determined by the patient's physician and will continue to receive standard of care follow-up, as medically indicated and at the discretion of the patient's physician.

2. A subject withdrawn by virtue of limiting treatment-related toxicity will be followed as clinically indicated, as determined by the patient's physician per standard of care, and/or until resolution of the adverse event(s).

3. All subjects will be requested to adhere to the protocol schedule for clinic visits as far as is reasonable and as clinically indicated.

4. Blood draws and protocol related data will be requested and collected according to the protocol schedule as is reasonable and as clinically indicated.

5. As far as is reasonable, data will continue to be collected at the indicated protocol time-points.

3.4 Patient Replacement

3.4.1 Reasons for Replacement

1. In the event of screening failures post-enrollment (i.e., patients without confirmed

progressive and/or relapsed SM diagnosis or failure to meet other screening or inclusion criteria), patients will be replaced.

2. Inability of enrolled subjects to tolerate phlebotomy or leukapheresis.

3. Voluntary withdrawal of subjects prior to initiation of treatment.

4. Failure to complete three (3) DC vaccinations.

3.5 Study Schedule

3.5.1 Summary of Pre-treatment Evaluations

1. Obtain informed consent to enroll patients with refractory SM s/p conventional salvage therapy.

2. Completion of screening procedures per study calendar (including determination of TAPA expression in neoplastic cells and/or blood) and pre-treatment evaluation for clinical trial enrollment.

3. Completion of systemic therapy and confirmation of refractory, progressive, and/or relapsed SM.

4. CBC, differential leukocyte counts, and baseline biochemical and/or radiographic evaluation of disease status ~~no more than~~ per study calendar.

5. Pregnancy evaluation.

6. Completed baseline delayed-type hypersensitivity response (DTH) skin tests with specific TAPA(s) seven (7) days +/- 3 days prior to 1st DC vaccination.

7. Negative infectious disease history, for safe handling of blood/leukapheresis products (including a negative history of HIV, and a negative history of active hepatitis A, B, or C).

8. Phlebotomy/leukapheresis within 2 weeks +/- 7 days prior to beginning DC vaccination schedule.

9. Manufacturing of DC vaccine bank.

10. Baseline Immune Assays and tissue TAPA levels.

11. Initiation of treatment a 5-day course of CYP, which may be taken at home, beginning 5-7 days prior to each DC vaccination.

3.5.2 Summary of Treatment Visits and Procedures

1. Please refer to Appendix 1, Study Calendar

3.6 *Safety Endpoints*

The main endpoints for this Phase I/II trial are safety and immune efficacy. Up to six (6) patients will be enrolled in the Phase I/toxicity stage of the study. Early stopping rules will be in place to protect patients from excessive toxicity. ***The trial will be halted if Dose Limiting Toxicity (DLT, defined below) occurs in two (2) or more of six (6) patients receiving DC vaccination.***

If DLTs are observed in no more than 1/6 patients during the Phase I/toxicity stage, the treatment will advance to the Phase II/immune efficacy stage. Immune efficacy will be evaluated in all patients (**see below for stopping rule regarding lack of immune efficacy**).

Toxicity will be graded according to NCI-CTCAE, version 4.0.

3.6.1 Rules Based on Dose Limiting Toxicity

Patients with DLTs	Action
0-1/6	Proceed to the immune efficacy stage of the study
≥ 2/6	Stop study

3.6.2 Definition of Adverse Event and Adverse Reaction

1. An adverse event is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. More specifically, an adverse event can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

2. An adverse reaction is defined as any adverse event caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

3.6.3 Causality

1. Suspected

   A suspected adverse reaction is defined as any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" indicates that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

2. Unexpected

An adverse event or suspected adverse reaction is considered unexpected if it is not listed in the investigator brochure or package insert(s), or is not listed at the specificity or severity that has been observed, or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. Adverse events that would be anticipated to occur as part of the disease process are considered unexpected for the purposes of reporting because they would not be listed in the investigator brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the investigator brochure. Some adverse events are listed in the Investigator Brochure as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered unexpected until they have been observed with the drug under investigation. For example, although angioedema is anticipated to occur in some patients exposed to drugs in the ACE inhibitor class and angioedema would be described in the investigator brochure as a class effect, the first case of angioedema observed with the drug under investigation should be considered unexpected for reporting purposes.

3. Serious

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

a. Death

b. Life-threatening adverse event

c. Inpatient hospitalization or prolongation of existing hospitalization

d. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function

e. Congenital anomaly/birth defect

f. Important medical events that may not result in death, are life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room

or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

4. Life-threatening

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

3.6.4 Recording of Adverse Events (AE)

1. All grade 3 and above AE will be recorded whether or not the event is believed to be associated with use of the study drug. Data about these events and their severity will be recorded using the NCI CTCAE version 4.0.

2. The Investigator will assign attribution of the possible association of the event with use of the investigational drug, and this information will be recorded using the classification system listed below:

   a. Unrelated to investigational drug/intervention: The AE is clearly NOT related to the intervention

   b. Unlikely related to investigational drug/intervention: The AE is doubtfully related to the intervention

   c. Related to investigational drug/intervention:

      - Possible: The AE may be related to the intervention
      - Probable: The AE is likely related to the intervention
      - Definite: The AE is clearly related to the intervention

3. Signs or symptoms reported as AE will be graded and recorded by the Investigator according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v. 4.0. When specific AE are not listed in the NCI-CTCAE v. 4.0, they will be graded by the Investigator as none, mild, moderate, or severe according to the following grades and definitions:

   a. Grade 0: No AE (or within normal limits)

   b. Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

   c. Grade 2: Moderate; minimal, local, or noninvasive intervention (e.g.,

packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL)

d. Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL

e. Grade 4: Life-threatening consequences; urgent intervention indicated

f. Grade 5: Death related to AE

3.6.5 Follow-up of Adverse Events

1. All adverse events will be followed with appropriate medical management until resolved.

2. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

3. For selected adverse events for which administration of the investigational drug was stopped, a re-challenge of the subject with the investigational drug may be conducted if considered both safe and ethical by the Investigator.

3.6.6 Adverse Event Monitoring

1. All AE, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be recorded, as noted above.

2. The Principal Investigator (PI) will assess all AE and determine reportability requirements to the Food and Drug Administration (FDA), if it meets the FDA reporting criteria.

3. All AE recorded will be reviewed by the study PI on a weekly basis and the Institutional Protocol Review Committee (IPRC) and/or the Institutional Review Board (IRB) on a monthly basis, or as deemed necessary by the study PI. The PI/IPRC will review and discuss at each meeting the selected toxicity, the toxicity grade, and the attribution of the relationship of the AE to study drug/treatment.

3.6.7 Definition of Dose-Limiting Toxicity (DLT)

1. Grade III or higher allergic reaction. Grade III is defined as symptomatic bronchospasm requiring medication, edema or angioedema, and Grade IV is defined as anaphylaxis.

2. Grade II or higher autoimmune reaction. Grade II is defined as an autoimmune reaction involving a non-essential organ or function requiring treatment.

3. Grade III or higher hematologic or non-hematologic toxicity including fever (> 40° C for > 24 hours).

4. Grade III injection site reaction (severe or prolonged ulceration or necrosis).

5. Toxicity will be graded according to NCI-CTCAE, version 4.0.

3.7 Immune Efficacy Endpoint

3.7.1 Immune Efficacy (IE) Rules

The main secondary endpoint of this Phase I/II clinical study is immune efficacy (IE), defined as *"detection of a TAPA-specific immune response in SM patients receiving DC vaccination"*. Immune responses will be evaluated, as described, in all patients enrolled on the study. Up to six (6) patients will receive the planned ~~six~~ three (3) DC vaccinations ~~before~~ with an interim analysis of IE performed on Day 22 and Day 45 of the sixth patient (*69*). With regard to IE, the accrual goal will be up to seventeen (17) patients. Stopping criteria, with regard to IE, will be based on defined immune responses and will follow a Simon 2-stage design with a null hypothesis of 5% IE ($p_0 = 0.05$) and alternative hypothesis of 35% IE ($p_1 = 0.35$) with desired significance level (α) and desired power ($1-\beta$) of 0.05 and 0.9, respectively. Interim analysis of immune response will be conducted after accrual of six (6) subjects completing the planned three (3) DC vaccine doses. **If no immune responses are observed at interim analysis (0/6 patients), early stopping will be considered based on lack of efficacy of the intervention.** With this rule, the probability of early termination (if the alternative hypothesis is true) is seven and one-half percent (7.5%). If three (3) or more patients, out of a total of seventeen (17) develop TAPA-specific immune responses, then the null hypothesis of 5% IE can be rejected, and results will be deemed favorable to move to a formal phase II study. Analysis to determine IE will be performed in all patients after receiving ~~six~~ three (3) DC vaccine doses.

Patients who are unable to complete the planned ~~six~~ three (3) DC vaccine doses will be withdrawn from the study.

1. *Immune Efficacy Endpoint will be determined as follows:*

   a. Positive T-cell cytokine IFN-ɣ, and/or TNF-α, and/or IL-17 ELISpot assay for *ex vivo* T cell cytokine expression following stimulation with relevant TAPA. For each patient sample the IFN-γ ELISpot assay (and/or TNF-α or IL-17) will be performed in triplicate using a minimum of three wells per replicate, as described by the manufacturer.

   b. Positive DTH skin tests with relevant TAPA (DTH skin tests will be performed on subject's forearm or within 5 cm from the site of prior DC vaccination, if possible).

3.8 Reasons for Discontinuation of Study

1. High frequency of limiting toxicities.

2. Lack of immune response.

3. Non-availability of reagents required for DC manufacture (including non-availability of acceptable alternative reagents).

4. Non-availability of Imiquimod and/or oral CYP, in the absence of an available alternative.

3.9 Potential Risks and Benefits

3.9.1 Known Toxicities Associated with DC Vaccination

1. DC vaccination strategies have been studied clinically in many different diseases. This particular vaccine has been tested in very few patients. Both monocyte-derived DCs (as being proposed for this study) and CD34+-derived DCs have been used in the presence of serum-free or fetal calf serum-containing mediums. Because these cells have been generated from autologous cells, their administration either intravenously (IV), subcutaneously (SC) or intradermally (ID) has not been associated with any significant adverse effects, with the exception of low grade fever and local reactions, such as erythema, at the sites of injection.

2. In a recently conducted phase I clinical trial of DC vaccination of patients with early-stage cervical cancer (BB-IND 11307), no adverse side effects were observed or reported by subjects following immunization beyond the immediate discomfort associated with injection (*10*). However, local reactions (mild erythema, swelling/induration, pruritus) were noticed at the subcutaneous vaccination sites that increased with the number of vaccinations in most of the patients. Also, a slight enlargement in the draining lymph node in the groin after DC injections was observed in some of the patients. The patients were monitored during treatment with complete blood counts and serum chemistries that included liver and renal function tests and electrolytes. No alterations in liver and renal function were detected. The safety of DC vaccination was also recently demonstrated in a study combining autologous DC vaccine against the melanoma antigen MART-1 and the cytotoxic T lymphocyte associated antigen 4 (CTL-4) antagonist tremelimumab (*70*). The only toxicity reported in this study was related to grade I-II pruritic skin eruptions previously associated with CTLA4 antagonists. No toxicity attributed to DC vaccination alone was reported.

3. One of the major concerns regarding DC vaccination with self-tumor antigens is the possible induction of autoimmunity. Vitiligo has been seen in some melanoma

patients, but no cases of severe autoimmune reactions have been reported. Some TAPAs used in this study have not been explored as targets for immunotherapy in large numbers of patients. Therefore, the clinical data supporting their safety in humans is limited (*30*). Nevertheless, the available data indicating these TAPAs are selectively expressed in tumor cells and normal testis (but not in any normal tissues) suggest the immune responses generated by TAPA-pulsed DC therapy will only affect tumor cells. Further evidence supporting the safety of TAPA immuno-targeting is the lack of toxicity associated with spontaneous development of anti-TAPA antibodies following vasectomy (*25*). Despite these observations, the possible risks of stimulating Th17 responses through DC vaccination in SM patients, are not known. Interestingly, a number of studies have documented contradictory results on the effects of stimulating Th17 T cell responses, with some showing potent anti-tumor activity for Th17 T cells, while others reporting pro-angiogenic and tumor-promoting properties ascribed to Th17 immune responses (*71*). Moreover, since Th17 responses are pro-inflammatory, they may be associated with as yet unknown side effects. Despite these potential problems, the available evidence suggests the risk of developing autoimmune complications/disease should be minimal. Also, since the majority of these TAPAs are expressed in spermatids, there is also a remote possibility that normal testicular function may be affected. All subjects will be monitored closely for treatment-related adverse events and dose-limiting toxicities.

4.0 METHODS AND ASSESSMENTS

4.1 *Contraception and Pregnancy Testing*

Either a female or male of reproductive capacity wishing to participate in this study must be using, or agree to use, one or more types of birth control during the entire study and for 3 months after completing the study. These may include condoms, diaphragms, birth control pills, spermicidal gels or foams, anti-gonadotropin injections, intrauterine devices (IUD), surgical sterilization, or subcutaneous implants. Another choice is for a subject's sexual partner to use one of these birth control methods. Women of reproductive capacity will be requested to undergo a urine pregnancy test before completion of the post-screening informed consent process.

4.2 *Screening, Study Visits and Procedures*

4.2.1 *Screening Visits and Procedures*

1. Subjects must be adult patients with confirmed progressive and/or refractory SM and no available, potentially curative therapeutic option. Informed consent will be sought for completion of screening procedures.

2. Screening evaluation will include the following:

a. History and physical examination.

b. Karnofsky performance status.

c. Evaluation of concomitant medications.

d. CBC and differential leukocyte count.

e. Tests for hepatic function and renal function.

f. Pregnancy evaluation and birth control agreement.

g. Negative infectious disease history, for safe handling of blood/leukapheresis products (including a negative history of HIV, and a negative history of active hepatitis A, B, or C).

h. Diagnostic studies, including biopsies (if indicated), appropriate imaging studies, serum and/or urine tumor marker measurements (if indicated), will be obtained within three (3) weeks ±14 days prior to vaccination.

i. Confirmation of expression of one (1) or more TAPA, by either RT-PCR, immunohistochemistry, Western blotting, or ELISA, in neoplastic cells prior to enrollment. For HER-2/Neu expression a positive FISH will be considered adequate.

4.2.2 Study Visits and Procedures

1. Peripheral blood mononuclear cells will be harvested by phlebotomy and/or leukapheresis, via peripheral or central venous access.

   Patients must be willing to provide at least 250 mL, and up to 500 mL, of whole blood obtained by phlebotomy and/or consent to leukapheresis for DC generation.

   If leukapheresis is required, this procedure will require a median duration of 2-3 hours on a leukapheresis machine and a median volume of processed whole blood of 150 ml/kg body weight. This can be done with a large bore peripheral IV but in select patients it may require a temporary central line during the procedure. The end result is a rich collection of nucleated white blood cells from which to extract DC precursors. The total volume of removed blood product is a small bag containing approximately 100 cc of plasma which contains the desired cells.

   Central venous access will be placed and used for phlebotomy/leukapheresis only if blood cannot be obtained by peripheral access. Placement of central venous access will be done at the hospital as an outpatient and with a separate surgical consent.

Phlebotomy/leukapheresis will be conducted within two (2) weeks +/- 7 days of the first DC vaccination.

2. The patient will receive a 5-day course of CYP (1.6 mg/Kg/day, with a maximum of 100 mg/day), which may be taken at home, beginning 5-7 days prior to each DC vaccination.

3. TAPA-pulsed DC will be administered at a vaccine dose of up to 1 x 10^7 DC, in injection-grade saline solution to bring total volume to 1 mL, if necessary. The vaccine total volume will be up to 1.0 mL and will be administered in 4 separate intradermal (ID) injections (0.25ml per injection) in the upper anterior thigh (inguinal node region) or the upper outer arm (axillary node region) in order to increase proximity to local lymph node draining basins and optimize propagation of TAPA-pulsed DCs to secondary lymphoid organs (*72*). If clinically feasible, DC vaccination will be alternated between the right and left sides of either the upper anterior thigh, or the upper outer arm. If there is the history of a prior inguinal lymphadenectomy, lower extremity lymphedema, prior inguinal hernia repair, or, the history of an axillary lymph node dissection, or upper extremity lymphedema, then the non-affected site/anatomic region would be preferred. The three (3) DC vaccines will be administered at 7-day intervals to maximize patient convenience and protocol adherence. Patients will be observed for up to one (1) to three (3) hours following each vaccine dose administration.

4. Vaccination Schedule:

Day 1	Day 8	Day 15
Vaccine 1	Vaccine 2	Vaccine 3

Imiquimod cream will be administered at a dose of ~10 mg after each intradermal injection. Immediately after the injections, the area will be cleaned by gently tapping with an alcohol swab (such as BD Alcohol Swabs). When the area is dry, Imiquimod (Aldara cream) will be applied over the injection sites, at the dose of about 4 mm of cream, corresponding to 10 mg Imiquimod. The area will be covered with a Band-Aid type patch and the patient will be instructed not to wash it or wipe it until the following day, when the patient will remove the medication by washing the area with mild soap and water. If itchiness is present, the patient will be instructed to apply topical anti-histamine cream as appropriate. Steroid-based medication at the site of the injection are to be avoided.

5. Blood for immune assays and TAPA levels (up to 100 mL) will be drawn ~~1 to~~ 7 days +/- 3 days before the 1st DC vaccination, and on days twenty-two (22) and day forty-five (45) +/- 3 days after the third vaccination.

6. DTH skin tests to assess cellular immune responses against relevant TAPAs will be conducted at the same time of blood immune assays (see above). DTH response will be determined and the response measured, between 2 and 3 days following the skin test, the results of which can be read by the study coordinator or by an appropriate study coordinator designated health care professional.

7. Evaluation of tumor responses:

   Immune responses shall be evaluated 7 days (Day 22) and 30 days (Day 45) following the last (3rd) DC vaccination, with imaging as clinically indicated (See Appendix 3). Patients may undergo evaluation of disease status anytime, at the discretion of PI, if disease progression is suspected.

8. Patient Enrollment

   Once the Phase I stage of the study is completed (subject 6 receives the 3rd vaccination dose and is evaluated for toxicity) the Phase II stage of the study will begin with enrollment of subject # 7.

9. All clinical laboratory tests will be performed in a CLIA and/or ISO-certified facility. Non-clinical tests (i.e., IHC/PCR/ELISA tests for evaluation of TAPA expression, ELISPOT for evaluation of immune response, etc.) will be performed in CLIA and/or ISO-certified facilities whenever possible.

4.2.3 Final Study Visit

1. The final study visit will take place 30 days (Day 45) +/- 3 days after the last DC vaccination. Procedures will include a blood draw and DTH skin tests for responsiveness to TAPAs.

2. Laboratory tests at the final study visit will include serum tumor markers (if indicated), hepatic and renal profile, CBC, and differential leukocyte counts.

3. Appropriate imaging studies and other relevant tests for determining disease status (i.e., cytogenetics, etc.) will be also obtained as clinically indicated.

4.2.4 Post-Study Follow-up

1. Blood for immune assays (up to 100 ml) will be drawn 7 days prior to the first vaccination and again at day 22 and day 45.

4.2.5 Discontinuation Visit

1. Will be identical to final study laboratories/procedures. Procedures will include a blood draw and skin tests for responsiveness to TAPAs.

2. Laboratory tests at the final study visit will include hepatic and renal profile, CBC and differential leukocyte counts.

3. Imaging studies and other relevant test for determining disease status will be obtained, if necessary.

5.0 STUDY MATERIALS AND PROCEDURES

5.1 *Products*

5.1.1 TAPA-Pulsed Dendritic Cells (DCs)

The procedure for generating TAPA-pulsed DCs suitable for clinical use will be carried out at Kiromic's Cell Processing laboratory specifically designed for the generation of GMP clinical grade cellular materials. DCs will be derived from monocyte precursors present in peripheral blood mononuclear cells (PBMC) cultures following phlebotomy and/or leukapheresis. PBMC will be separated by Ficoll gradients and/or CliniMACs separation system and cultured in CellGro medium (CellGenix, USA), IL-4 and GM-CSF (CellGenix, USA) for up to seven (7) days. DCs will be grown in culture flasks and/or G-Rex growth system and vaccine will be prepared by exposing DCs to a maturation cytokine cocktail (IL-1β and TNFα (CellGenix, USA), poly (I: C) (InvivoGen, USA) and INFα for 16-72 hrs. DCs will then be "pulsed" with relevant, recombinant TAPAs for 2-4 hours. Successful generation of mature DCs will be confirmed in each case by immunophenotyping for classical DC markers, including CD1a, CD58, CD80, CD83, CD86 and HLA-DR. Recombinant, clinical grade TAPAs will be obtained from CS Bio Company, Inc. a company specialized in manufacturing cGMP peptides located at 20 KELLY CT. Menlo Park, CA 94025. Following the maturation period DCs will be aliquoted into six or more equal volumes for cryopreservation until further use. Alternatively, one DC vaccine dose will be manufactured, with additional doses prepared subsequently from cryopreserved PBMNCs. DCs may also be generated following the same but inverted procedure (TAPA pulsing prior to exposure to maturation cytokine cocktail, See SOP).

All reagents and plastics to be used for this purpose will be stored at Kiromic's GMP laboratory and used solely for the DC generation and maturation process. All procedures will be carried out in sterile conditions and the final products will be evaluated by an independent CLIA-certified laboratory for sterility, including presence of endotoxin, mycoplasma contamination, fungal, aerobic and anaerobic bacterial cultures and Gram staining, prior to human administration. Results of these tests will be obtained prior to release of the final product for human administration. Mature DCs (or PBMCs for later generation of fresh mature DCs) will be cryopreserved and stored in a dedicated liquid

nitrogen container at Kiromic, until being used.

5.2 *Storage and Drug Accountability*

5.2.1 TAPA-Pulsed Dendritic Cells (DCs)

1. A minimum of three (3) and a maximum of six (6) vials of TAPA-pulsed DCs (containing up to 1 X 10^7 DCs each) will be cryopreserved as a master vaccine bank for each patient. TAPA-pulsed DC vaccine will be frozen in 90% heat-inactivated normal human AB serum and 10% dimethyl sulfoxide. Alternatively, an aliquot of the original pool of PBMCs will be cryopreserved, as described above, for subsequent thawing and generation of fresh DCs prior to each vaccination schedule (see SOP). This process may improve the viability of DCs. A dedicated liquid nitrogen storage container will be used exclusively for patient PBMCs and DC vaccine preparations for this study.

2. All specimens and vaccines will be transported to/from the participating institution in compliance with standard biohazard protocol.

6.0 TREATMENT

6.1 *Selection of TAPA-Pulsed DC Dose Level*

6.1.1 Rationale

A phase I dose escalation clinical trial of DC vaccination in patients with cervical cancer indicated optimal stimulation of tumor antigen-specific cytotoxic T cell responses with a dose of up to 1.0 x 10^7 DCs and delivered SC and ID at 21 day intervals (*10*). Thus, in this study we will explore one (1) dose of TAPA-pulsed DC vaccination (up to 1 X 10^7 DCs) and determine the toxicity, immune efficacy (IE) and clinical response in patients with progressive and /or refractory SM without available, potentially curative therapeutic options.

Preliminary studies indicate that leukapheresis (10-liter volume) followed by CliniMACS isolation of CD14+ cells and high-density DC culture in G-Rex flasks provides an optimal yield of 2.5 x10^8 mature DCs from 10^9 CD14+ cells. This would be sufficient for cryopreservation of 12 vials at 1 x10^7 DC/vial. Phlebotomy of 250 mL of whole blood yields approximately 1.2 X 10^8 monocytes and 3 X 10^7 mature DCs (see Section 7.2). The CliniMACS separation system and the G-Rex DC growth system may be used as an alternative to Ficoll-gradient monocyte separation and standard DC growth protocol using flasks.

6.1.2 TAPA-Pulsed DC Vaccination

This is a phase I/II safety and efficacy study. Only one dose level of TAPA-pulsed DC will be used.

Dose Level	Minimal Number of Patients	DCs per Vaccination
1	6	1 x 10^7

The first six (6) patients will receive up to 1 x 10^7 DCs divided into 4 intradermal (ID) injections, every seven (7) days, for up to a maximum of ~~six~~ three (3) treatments. The (ID) DC vaccinations will be administered in an approximate volume of 0.25 ml per injection site (total vaccination dose-volume 1.0 ml). Thirty minutes prior to receiving the DC vaccination, all patients will receive premedication with diphenhydramine (12.5-50 mg) and acetaminophen (325-1000 mg) orally. Topical lidocaine based anesthetic applications may also be administered prior to the vaccination at the discretion of the study coordinator. During the Phase I stage of this study up to six (6) consecutive subjects will be enrolled.

If one (1) or less than six (6) patients develops DLT, the Phase II/immune efficacy stage will proceed. If two or more (≥ 2) of the first six (6) patients develop DLTs, the study will be terminated. A minimum of six (6) patients will be treated for evaluation of safety/toxicity (Phase I level). If toxicity criteria for continuation is fulfilled during the phase I stage, patient enrollment in the Phase II/immune efficacy stage may proceed. Up to seventeen (17) patients will be treated in the study for evaluation of immune efficacy and possible clinical response (Phase II stage), with IE determined following completion of three (3) DC vaccine doses (day 22 and day 45).

6.1.3. Imiquimod Treatment

Following each DC vaccination, the patient will receive a single dose of Imiquimod cream.

6.2 Study Drug Administration

6.2.1 Cyclophosphamide (CYP)

The patient will receive a 5-day course of CYP (1.6 mg/kg/day with maximum dose of 100 mg/day), which may be taken at home, beginning 5-7 days prior to each DC vaccination.

6.2.2 TAPA-Pulsed Dendritic Cells (DC)

TAPA-pulsed DC will be administered at a vaccine dose of up to 1 x 10^7 DC, in injection-grade saline solution to bring total volume to 1 mL, if necessary. The vaccine volume will be up to 1.0 mL and will be administered in 4 separate intradermal (ID) injections at the 12 o'clock, 3 o'clock, 6 o'clock and 9 o'clock positions in the upper outer arm or the upper anterior thigh region) in order to increase proximity to local lymph node draining basins and optimize propagation of TAPA-pulsed DCs to secondary lymphoid organs. Thus approximately 0.25 mL will be injected at each injection site (total volume 1.0 mL). Axillary lymph node region ~~area~~ is the preferred vaccination site for this study. DC vaccination will be alternated between the upper outer arm, and upper anterior thigh of both limbs. Three (3) DC vaccines will be administered at 7-day intervals. Patients may be observed for up to one (1) to three (3) hours following each vaccine dose administration.

6.3 Study Drug Quality and Safety Measures

6.3.1 TAPA-Pulsed Dendritic Cells (DCs)

1. TAPA-pulsed DCs will be administered ID at 7-day intervals. ~~plus or minus 2 days~~.
2. Safety tests before release of TAPA-pulsed DCs:
    a. Bacterial and fungal sterility test.
    b. Mycoplasma test.
    c. Endotoxin test: Endotoxin must be less than 1 IU/ml by the LAL method.
3. Plan of action in the event of DC vaccine test failures:
    a. If a sterility and/or endotoxin test is positive, the vaccine dose will be discarded. A second dose of DC vaccine will be thawed and tested. If test results on the second dose are negative, the patient will receive treatment. If test results on the second dose are positive, the entire batch of DC vaccine will be discarded, and the patient will be withdrawn from study.
    b. In the unlikely event that a dose gives a positive result by bacterial growth test (agar sterility test) after administration to the patient, the patient's physician will be notified immediately, with the recommendation that the patient be treated with antibiotics, even if the patient shows no clinical signs of infection. A second DC vaccine dose will be thawed and subjected to the bacterial growth test. If the second dose tests positive, the DC vaccine batch will be discarded, and treatment discontinued. If the second dose tests negative, patient treatment will proceed.
    c. If an entire DC vaccine batch is discarded, treatment may proceed if it is possible to prepare a new vaccine batch (usually contingent on a second leukapheresis/phlebotomy procedure).
4. All clinical laboratory tests will be performed in a CLIA certified facility.

6.4 Specific Restrictions and Requirements

6.4.1 Concomitant Therapy

1. Immunosuppressive or anti-inflammatory drugs (including hydrocortisone) that inhibit cellular immune responses should not be taken, unless otherwise indicated for the management of study-related toxicities, adverse events, symptomatic

disease progression, or pre-existing conditions (i.e., chronic adrenal cortical insufficiency). All medications (prescription and over the counter), vitamin and mineral supplements, and/or herbs taken by the study subjects will be documented. Topical steroids are permissible.

6.4.2 Compliance with Therapy

1. All treatments and study procedures will be administered in the clinic except for the cyclophosphamide that can be taken at home as per the protocol schedule.

7.0 SAMPLE SIZE AND STATISTICAL METHODS

7.1 Determination of Sample Size

7.1.1 Sample Size for Safety Evaluation

An initial cohort of up to six (6) subjects will receive low-dose CYP, TAPA-pulsed DC vaccination and topical Imiquimod for determination of safety/toxicity (phase I stage).

7.1.2 Sample Size for Evaluation of Immune Efficacy

Up to seventeen (17) patients may be enrolled for evaluation of immune efficacy (Phase II stage). Enrollment will begin if toxicity criteria are fulfilled and interim evaluation of IE is completed during the phase I stage (first 6 patients). Stopping criteria for the study, based on IE, will follow the Immune Efficacy Rules described in 3.7.1.

7.2 Statistical Methods

7.2.1 Safety and Toxicity

A single dose of CYP, TAPA-pulsed DC vaccine and topical Imiquimod will be evaluated in this study. Six (6) consecutive patients will be treated as proposed for toxicity evaluation (Phase I stage). If no limiting toxicity is encountered, additional patients will be enrolled to determine immune efficacy (Phase II stage).

7.2.2 Immune Efficacy (IE)

For the IE part of the study, a Simon 2-stage design with a null hypothesis of 5% IE (p_0 = 0.05) and alternative hypothesis of 35% IE (p_1 = 0.35), with desired significance level (α) and desired power (1-β) of 0.05 and 0.9, respectively, will be utilized. An interim analysis of immune response will be conducted after accrual and treatment of the first six (6) patients, following completion of the planned ~~six~~ three (3) DC vaccine doses, with immune response evaluation on day 22 and day 45. Patients who are unable to complete the planned ~~six~~ three (3) DC vaccine doses will be withdrawn from the study.

If no immune responses are observed at interim analysis, termination of the study will be considered based on lack of efficacy of the intervention. The probability of early termination, if no immune responses are observed in the first six (6) patients and the alternative hypothesis is true, is 7.5%. If 3 or more responses out of a total of 17 patients

are observed, the null hypothesis can be rejected and DC vaccination may move to a formal Phase II/III developmental stage.

8.0 ETHICAL CONSIDERATIONS

8.1 Protection of Patients from Unnecessary Harm

Safety of subjects involved in human experimentation is protected by both the participating clinical investigators and independently by the Institutional Protocol Review Committee (IPRC) and/or the Institutional Review Board (IRB). The IRB reviews all proposed studies involving human experimentation and ensures that the subject's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The IRB also reviews the informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

8.2 Privacy and Confidentiality

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign the HIPAA form and informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document. The use and disclosure of protected health information will be limited to the individuals described in the informed consent document.

In order to protect patient's privacy, all study data will be identified only by initials and a specifically assigned study number. However, in compliance with Good Clinical Practices, the PI will allow the independent study monitor(s) to review that portion of the patient's medical record that is directly related to the study. This shall include all relevant study documentation, including patient medical histories to verify eligibility, laboratory test results, x-ray reports, and admission/discharge summaries while the patient is on-study, and autopsy reports for deaths occurring during the study (if available).

8.3 Informed Consent

In obtaining and documenting informed consent, the investigators will comply with the IRB guidelines and the ethical principles originating in the Declaration of Helsinki.

8.4 IRB Approval

This protocol and the associated informed consent documents must be submitted to the IRB for review and approval.

9.0 SAFETY CONSIDERATIONS

9.1 TAPA-Pulsed DC Vaccination

DC-based treatments have been applied and studied clinically in many different diseases. Both monocyte-derived DCs (as being proposed for this study) and CD34+-derived DCs have been used in the presence of serum-free or autologous serum-containing mediums. Because these cells have been generated from autologous cells, their administration intravenously (IV), subcutaneously (SC) or intradermally (ID) has not been associated with any significant adverse reactions, with the exception of low grade fever and local reactions, such as erythema, at the sites of injection (See discussion above).

9.2 Adverse Immune Responses Against TAPA-Pulsed DC Vaccinations

The specific panel of TAPAs used in this study is novel and has not been explored extensively as a target for immunotherapy in humans. Therefore, there is no significant clinical data to support its safety in patients. Nevertheless, the available data showing these TAPAs are selectively expressed in tumor cells and normal testis (but not in normal tissues), it is expected the immune responses generated by TAPA-pulsed DC therapy will only affect tumor cells (and possibly normal testis). Further evidence supporting the safety of TAPA immunotargeting is the lack of toxicity associated with spontaneous development of anti-TAPA antibodies following vasectomy (*25*).

9.3 Regulatory Aspects

This study will be conducted as part of an Investigational New Drug (IND) Application approved by the FDA (IND #12612). The study will not commence until full approval is obtained from either the central and/or local IRB.

10.0 FINANCIAL CONSIDERATIONS

The expenses and costs incurred by this study for the generation and processing of TAPA-pulsed DC vaccines, oral CYP dose, topical Imiqumod, and leukapheresis/phlebotomy, will be provided by financial support entirely or partially from Kiromic, and/or external funding sources. It is expected that standard of care treatments and studies during and after the course of the study plus the follow-up monitoring (as part of the standard of care for patients with SM) will be paid either by the patient or their insurance companies. This arrangement will be clarified in the Patient Consent Form.

11.0 POTENTIAL CONFLICT OF INTEREST

It is possible that in the future Kiromic, may apply for a patent covering the potential use of some TAPAs in cancer therapeutics and diagnostics. The investigators will declare this potential conflict of interest in the Patient Consent Form so that all patients recruited to this study will be aware that some or all data obtained may be used to support future application that may result in commercial value of the DC vaccination strategy. A panel of independent

physicians/investigators will serve as an external Independent Clinical and Scientific Review Board monitor team (ICSRB) that will review the clinical and immunologic data generated in the study in order to guarantee the un-biased evaluation of study results.

12.0 QUALITY CONTROL AND QUALITY ASSURANCE

The quality of the vaccine product will be determined and guaranteed by strict adherence to the Standard Operating Procedure (SOP) for DC Vaccine Production following Good Manufacturing (GMP) and Good Laboratory Processing (GLP).

13.0 PUBLICATION POLICY

Results of this clinical study may be presented and/or published in National Meetings and/or peer-reviewed medical or scientific journals, partially or completely. Patient's personal data will remain anonymous during such presentations/publications and integrity of data preserved and guaranteed during the dissemination process.

14.0 DIRECT ACCESS TO SOURCE DATA AND DOCUMENTS, DATA HANDLING, AND RECORD KEEPING AND RETENTION OF STUDY DOCUMENTS

Only the Study Chair, PI and Kiromic's Clinical Research personnel may have access to the study source data and documents. Data will be handled exclusively by the Study Chair, PI, designated Clinical Research personnel and/or an officially designated person or persons such as one of the study sub-investigators and members of the ICSRB. Data will be handled under strict confidentiality and kept in a secure place at Kiromic. Only the Study Chair, PI, designated Clinical Research personnel and members of the ICSRB may have access to the study data, including patient information and study outcomes. Study documents will be retained at Kiromic for at least 5 years following completion and closure of the study.

APPENDIX 1: STUDY CALENDAR

PROCEDURE	-28	-14	-7	1	3	8	10	15	17	22	45
Informed consent	X										
Documentation of confirmed refractory disease	X										
Confirm completion of salvage therapy > 3wks prior to vaccination	X										
Pregnancy Evaluation	X										
Birth Control agreement	X										
Evaluation of disease status	X										
TAPA expression	X										
Infectious Disease history evaluation (HIV, Hepatitis A, B,or C)	X										
Medical History	X										
History & Physical	X			X		X		X		X	X
Karnofsky Performance Status	X			X		X		X		X	X
Concomitant Medications	X										
CBC with diff	X			X		X		X		X	X
CMP	X			X		X		X		X	X
Phlebotomy/Leukapheresis		X									
Immune Assays			X							X	X
DTH skin test			X							X	X
Oral Cyclophosphamide Max 100mg/day x 5 days			X		X		X				
Topical Imiquimod				X		X		X			
TAPA-pulsed DC Vaccine				X		X		X			
Dose limiting toxicity assessment					X	X	X	X	X	X	X

Study calendar is designed to track the administration and application of the study protocol parameters and may adjusted as logistically needed as allowed protocol variances.

APPENDIX 2: KARNOFSKY PERFORMANCE STATUS SCALE

DEFINITIONS	RATING (%)	CRITERIA
Able to carry on normal activity and to work; No special care needed.	100	Normal no complaints; no evidence of disease
Unable to work; able to live at normal home and care for most personal needs; varying amount of assistance needed.	90	Able to carry on normal activity; Minor signs or symptoms of disease.
	80	Normal activity with efforts; some signs or symptoms of disease
Unable to work; able to live at normal home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; Requires equivalent of institutional or hospital care; diseases may be progressing rapidly.	40	Disabled; requires special care and assistance
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; Active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

APPENDIX 3: RESPONSE CRITERIA

Immune-Related Response Criteria (irRC) (73)

Baseline tumor assessment is determined by the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (five lesions per organ, up to 10 visceral lesions and five cutaneous index lesions) is calculated.

At each subsequent tumor assessment, the SPD of the index lesions and of new, measurable lesions (≥ 5 × 5 mm; up to 5 new lesions per organ) are added together to provide the total tumor burden:

Tumor Burden = SPD (index) lesions + SPD (new) lesions

Time-point response assessment using irRC. Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear.

At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out irPD). Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening).

Overall response using the irRC

The overall response according to the irRCis derived from time-point response assessments (based on tumor burden) as follows:

- **irCR**: complete disappearance of all lesions (whether measurable or not, and no new lesions) confirmation by a repeat, consecutive assessment no less than 4 wk from the date first documented
- **irPR:** decrease in tumor burden ≥50%relative to baseline confirmed by a consecutive assessment at least 4 wk after first documentation
- **irSD:** not meeting criteria for irCR or irPR, in absence of irPD
- **irPD**: increase in tumor burden ≥25% relative to nadir (minimum recorded tumor burden) confirmation by a repeat, consecutive assessment no less than 4 wk from the date first documented

Derivation of irRC overall responses

MEASURABLE RESPONSE	NONMEASURABLE RESPONSE		OVERALL RESPONSE
Index and new, measurable lesions (tumor burden), *%	***Non-Index lesions***	***New, nonmeasurable lesions***	***Using irRC***
↓100	Absent	Absent	irCR†
↓100	Stable	Any	irPR†
↓100	Unequivocal progression	Any	irPR†
↓≥50	Absent/Stable	Any	irPR†
↓≥50	Unequivocal progression	Any	irPR†
↓<50 to <25↑	Absent/Stable	Any	irSD
↓<50 to <25↑	Unequivocal progression	Any	irSD
≥25?	Any	Any	irPD†

*Decreases assessed relative to baseline, including measurable lesions only (>5 × 5 mm).
†Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 weeks apart.

Patients are considered to have irPR or irSD even if new lesions were present, as long as they met the respective thresholds of response as described above.

Patients are not considered to have irPD if new lesions were present and the tumor burden of all lesions did not increase by ≥25%.

In contrast to irCR, irPR, and irPD, a response of irSD does not require confirmation.

It is important to note that irCR, irPR, and irSD include all patients with CR, PR, or SD by WHO criteria as well as those patients that shift to these irRC categories from WHO PD.

Patients with irSD, particularly those with slow-declining tumor burden ≥25% from baseline at the last tumor assessment, are considered clinically meaningful because they show an objectively measurable reduction in tumor burden without reaching the 50% threshold that defines irPR.

If a patient is classified as having irPD at a post-baseline tumor assessment, then confirmation of irPD by a second scan in the absence of rapid clinical deterioration is required. The definition of confirmation of progression represents an increase in tumor burden ≥25% compared with the nadir at two consecutive time points at least 4 wk apart. It is recommended that this be done at the discretion of the investigator because follow-up with observation alone may not be appropriate for patients with a rapid decline in performance status.

Confirmation of irPD allows for the capture of all observed responses using the irRC, as most of these late responding patients have a trend toward response within 4 wk after initial irPD. Whereas WHO criteria consider any new measurable lesion to indicate PD, determination of immune-related best overall response (irBOR) is based on changes in total tumor burden from the baseline (nadir, for irPD) tumor assessment, regardless of any initial increase in baseline lesions or the appearance of new lesions.

Wolchok,J, Hoos A, O'Day S, et al. Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria. Clin Cancer Res 15(23):7412–20, 2009

APPENDIX 4: NCI CTCAE V4.0

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.04_20100-06-14_QuickReference_8.5x11.pdf

APPENDIX 5: ADVERSE EVENT LOG

STUDY TITLE			
SITE		Principal Investigator (PI)	
Patient Number		Patient Initials	

Has the participant had any AE during this study? Yes No If Yes, how many? _____
Dates: _____

ADVERSE EVENT	START DATE/TIME	STOP DATE/TIME

Brief Summary:

Severity	Relation to Study Drug	Action taken with Investigational drug	Corrective Action	Outcome	Expected	Serious
1= Mild 2= Moderate 3= Severe 4= Life Threatening	1= Definitely 2= Possibly 3= Not related 4= Unknown	1= None 2= Discontinued permanently 3= Discontinued temporarily 4= Reduced Dose 5= Increased Dose 6= Delayed Dose	1=Remedial Therapy (Pharmacologic) 2= Remedial Therapy (Non pharmacologic) 3=Hospitalization 4=Medication	1= Resolved 2= Resolved with sequel 3= Not resolved 4= Residual effects present 5= Death	1=Yes 2=No	1=Yes 2=No

_____	_____	_____	_____
Clinician Name	Clinician Initials	Clinician Signature	Date
_____	_____	_____	_____
PI Name	PI Initials	PI Signature	Date

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