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A Phase II Trial of High Dose Interleukin-2 (HDIL-2) with Recombinant MAGE-A3 Protein Combined with Adjuvant System AS15 (recMAGE-A3 + AS15) in Patients with Unresectable or Metastatic Melanoma

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1 BACKGROUND

1.1 Cutaneous melanoma

Metastatic melanoma continues to have a dismal prognosis with a median survival of 6 to 9 months in large randomized trials (1). Interleukin-2 (IL-2) is an active agent with modest clinical benefit in melanoma (response rate <20%) (2). Melanoma vaccines, though capable of inducing tumor-specific T cells, have not shown significant clinical benefit until gp100 peptide vaccine has been used in combination with high dose IL-2 (HDIL-2) and shown encouraging results (3). A recent prospective randomized phase III trial showed gp100 peptide vaccine + HDIL-2 to be superior to HDIL-2 alone in increasing overall response rate and progression free survival in locally advanced stage III and stage IV melanoma patients (4).

1.2 recMAGE-A3 + AS15 Antigen-Specific Cancer Immunotherapeutic

MAGE genes and, particularly the gene coding for the MAGE-A3 protein, are silent in all normal adult tissues with the exception of spermatogonia and placenta, but are reactivated in many cancers, including melanoma. The MAGE-A3 antigen is strictly tumor-specific, a finding supported by the fact that its expression is not detected by RT-PCR in any normal adult tissue with the exception of testis and placenta (5,6). Although expressed in testis and placenta, the MAGE-A3 antigen is believed to be strictly tumor-specific as the cells where it is expressed (spermatogonia in testis and trophoblasts in placenta) do not bear Major Histocompatibility Complex (MHC) molecules on their surface and therefore do not present any MAGE-A3 antigen on the cell surface (7). Therefore, immunization against the MAGE-A3 protein is not expected to induce autoimmune effects in humans and will result solely in an immune response against MAGE-A3 expressing cancer cells. The study investigational product is an Antigen-Specific Cancer Immunotherapeutic (ASCI) comprising the recombinant protein ProtD-MAGE-A3/His (abbreviated as recMAGE-A3 in the rest of this document) and the GSK proprietary immunological Adjuvant System AS15. The recMAGE-A3 antigen used in the study is a 450-amino acid fusion protein containing an 18-amino acid signal sequence, 109 residues of Protein D, a lipoprotein present on the surface of *Haemophilus influenzae* B (ProtD), the MAGE-A3 protein, and a polyhistidine tail (His). For details of this recombinant protein, see the Investigator's Brochure (Appendix M).

1.2.1 The expression of MAGE-A3 in human tumors

The expression of the *MAGE*-A3 gene has been detected in substantial fractions of human tumors of different histological types, including melanoma, up to 76%; bladder cancer, 62%; hepatic cancer, 48%; non-small cell lung cancer (NSCLC), 35 - 50%; esophageal cancer, 47%; leukemia, 29%; prostate cancer, 18%, ovarian cancer, 30% (8-12). In addition to its interesting tumor-

specific expression profile, a link between expression of the MAGE-A3 gene and the outcome of disease has been suggested. Using tumor samples from 447 patients for whom clinical data available were able to identify MAGE-A3 as being one of two indicators of a poor prognosis in adenocarcinoma (but not in squamous or bronchoalveolar) NSCLC (13).

1.2.2 The need for Adjuvant Systems containing immunostimulants

Early clinical studies made it clear that to efficiently achieve a strong and persistent immune response, the MAGE-A3 protein must be combined with one or more potent immunostimulants formulated in an Adjuvant System. A recent booster study, in which a booster injection of recMAGE-A3 combined with an Adjuvant System (AS02B) was given to patients previously immunized (primed) with either recMAGE-A3 alone or combined with the Adjuvant System AS02B, showed that the absence of an Adjuvant System at the time of initial immunization may lead to development of tolerance to the administered antigen (14). In addition, this study showed the potential for cross-reactivity of the treatment, as the patients immunized with recMAGE-A3 combined with AS02B developed antibodies and T-cell responses against various new and known epitopes not present in the study product.

1.2.3 The immunological Adjuvant System AS15

The AS15 Adjuvant System is a novel combination of the Adjuvant System AS01B (liposomes containing MPL combined with QS21 in phosphate-buffered saline) and mixed with the AS07A Adjuvant System (containing the immunostimulatory oligonucleotide CpG). For further details and description of the pre-clinical safety and toxicology tests of AS15, refer to the Investigator's Brochure. AS15 is a strong immunological Adjuvant System in mice, monkeys and humans, and is able to induce a more powerful Th1 response and subsequently better protect against tumor challenge than previously tested immunological Adjuvant Systems (15).

The decision to develop the recMAGE-A3 combined with AS15 is based on pre-clinical data and on the preliminary results of a Phase II proof-of-concept trial evaluating two formulations of the MAGE-A3 ASCI in metastatic melanoma (GSK 249553/008). Preclinical data suggesting that AS15 is able to induce strong immune and anti-tumor responses have been reported by several investigators (15-19). The preliminary results of the GSK 249553/008 clinical Phase II trial suggest that the recMAGE-A3 + AS15 ASCI induces a

stronger immunological response and more frequent clinical activity: among the 36 eligible patients in the recMAGE-A3 + AS15 ASCI arm, 3 complete responses and one partial response were observed, vs. 1 partial response in the recMAGE-A3 + AS02B arm, and the treatment was well tolerated (20).

1.2.4 Using recombinant proteins for immunotherapy

The use of recombinant protein presumably containing several epitopes, both known and unknown, potentially elicits immune responses to multiple antigenic sites and thereby could broaden the repertoire of the induced response. The protein also carries CD4+ Th1 helper type epitopes. The Th1 effect is critical for the activity of the ASCI because (i) Th1 cells promote initiation of the production of antigen-specific CD8+ T-cells that exert anti-tumor activity, (ii) Th1 cells amplify and sustain CD8+ T-cell function, by secreting cytokines such as IL-2, and (iii) Th1 cells can inhibit tumor growth even in the absence of CD8+ T-cells, by releasing interferon-gamma (IFN gamma; a macrophage activating and anti-angiogenic cytokine) or by potential direct cytotoxicity.

1.2.5 Gene signature associated with clinical benefit

Microarray gene profiling has been shown to be a powerful technique predicting treatment response. In the Phase II melanoma trial (GSK 249553/008), an attempt was made to identify from a tumor biopsy a gene signature that may predict a favorable clinical outcome for patients treated with the recMAGE-A3 + AS15 ASCI (20). Biopsies from 69 patients were tested. In a first step, a supervised comparison was performed in order to find a group of genes able to cluster 11 patients considered as presenting clinical benefit (complete response, partial response, mixed response or stable disease) in one group, and 11 patients with progressive disease in the other group. As a result, 2 clusters were clearly identified: one associated with patients who have progressive disease, and another one associated with clinical benefit. Estimated Kaplan-Meier (KM) curves of the Time to Treatment Failure for the recMAGE-A3 + AS15 group of patients from the GSK 249553/008 melanoma trial also suggest that the presence of the gene signature correlates with a favorable clinical benefit in response to the recMAGE-A3 + AS15 therapy. In a Phase II study of stage IB/II MAGE-A3 positive NSCLC patients (study GSK 249553/004), the same gene signature was found to be predictive of clinical activity (12).

1.2.6 Melanoma peptide vaccine enhances response rate of standard high dose IL-2 (HDIL-2)

A number of murine tumor models have demonstrated synergy between antigen-specific vaccination and HDIL-2 administration. Recent evidence supports this in melanoma patients.

A prospective randomized phase III trial was conducted at 21 centers with 185 patients (4). Eligibility included stage IV or locally advanced stage III cutaneous melanoma, HLA A0201, no brain metastases, and suitability for HDIL-2. Arm 1 received HDIL-2 alone (720,000 IU/kg/dose) and Arm 2 gp100:209-217(210M) peptide + Montanide ISA followed by HDIL-2. The primary objective was clinical response. Toxicities were consistent with HDIL-2. Investigator assessed response rate showed significant improvement in overall response for Arm 2: 16% vs/ 6%. (P = 0.03) and progression free survival in favor of Arm 2: 2.2 months (1.7-3.9) vs 1.6 (1.5-1.8) (P=0.01). Median overall survival favored Arm 2: 17.8 months (11.9-25.8) vs 11.1 (8.7-16.3) (P=0.06). Blinded response review confirmed the findings. Thus, response rate and progression free survival were superior with peptide vaccine and HDIL-2 compared to HDIL-2 alone, providing rationale for additional approaches of vaccine + HDIL-2.

1.2.7 MAGE-A3 protein to metastatic melanoma patients

Several groups have administered the recMAGE-A3 protein to metastatic melanoma patients, used alone or in combination with the immunological Adjuvant System AS02B or AS15. The MAGE-A3 immunizations were well tolerated and led to a specific immune response (18, 21, 22). Pilot studies have shown anecdotal but long-term objective clinical responses in patients with metastatic disease (23).

The preliminary results of a Phase II study, GSK 249553/008, suggest that development of the recMAGE-A3 + AS15 ASCI in metastatic melanoma may improve the clinical outcome for these patients. The study showed that the treatment was well tolerated, induced a strong immune response and led to a partial or complete clinical response in some of the patients.

These results were encouraging and GSK took the decision to further develop recMAGE-A3 + AS15 in the adjuvant NSCLC setting. GSK has recently initiated two Phase III trials in patients with stage IB, II or IIIA, completely resected NSCLC, either directly after surgery or after surgery and chemotherapy. The study is called MAGRIT (MAGE-A3 as Adjuvant Non-Small Cell LunG

Cancer ImmunoTherapy) and a Phase III trial of the recMAGE-A3 + AS15 ASCI in patients with stage IIIB or IIIC cutaneous melanoma and macroscopic lymph node involvement after complete surgical resection. The study is called DERMA (Adjuvant immunotherapy with MAGE-A3 in melanom).

Most of the adverse events observed in all studies were to be anticipated and consist of local and systemic reactions (i.e., injection site reactions and constitutional symptoms such as myalgia, artrhalgia and fatigue). Almost all such events have been of Grade 1 or 2. Systemic Grade 3 events have been occasionally observed and, except for fatigue, they were rarely considered to be related to the treatment. In addition, the only serious adverse events deemed to be possibly related to the treatment have been: an allergic reaction, a case of injection site pain (Grade 3) followed by nausea and vomiting, a case of exacerbation of COPD and a fatality due to disseminated intravascular coagulation in conjunction with major visceral tumor progression. Such events have remained isolated cases, and almost all serious adverse events in these studies were assessed by the investigators to be unrelated to the study immunization.

Biological indicators of auto-immunity have been observed infrequently. Very rare local clinical reactions (one transient uveitis and localized vitiligo) have been recorded. A systematic serological investigation of such reactions in Phase I studies (recMAGE-A3 + AS15 in melanoma) led to the conclusion that the study treatment was not associated with auto-immune disorder in the patients who were included. This conclusion is supported by independent studies with other antigens combined with AS15: P501 + AS15 in prostate cancer and dHER2 + AS15 in breast cancer.

2 RATIONALE

Patients with unresectable stage III/IV malignant cutaneous melanoma have a poor prognosis and there is currently no effective treatment to extend survival. New approaches to improve clinical outcome and eventually the life expectancy for these patients are needed. HDIL-2 is an active and an FDA approved therapy in this stage of disease. The use of recombinant protein presumably containing several epitopes, potentially elicits immune responses to multiple antigenic sites and thereby could broaden the repertoire of the induced response. A previous report suggests that vaccination against a melanoma antigen, gp100, can double the objective response rate to IL-2 in HLA-A0201+, metastatic melanoma patients. The active immunization such as recMAGE-A3 + AS15 ASCI against

tumor antigens has the potential to increase the response rate of HDIL-2 as a combination therapy in this disease.

3 STUDY OBJECTIVES

3.1 Primary Objectives

3.1.1 To evaluate the objective response rate induced by the concurrent administration of HDIL-2 and recMAGE-A3 + AS15 ASCI in patients with MAGE-A3-positive, unresectable or metastatic melanoma.

3.1.2 To evaluate the safety and toxicity profile of HDIL-2 in combination with recMAGE-A3 + AS15 ASCI in patients with MAGE-A3-positive, unresectable or metastatic melanoma.

3.2 Secondary objectives

3.2.1 To evaluate the rate of stable disease, progression-free survival and the overall survival of patients with MAGE-A3- positive, unresectable or metastatic melanoma who received the combination of HDIL-2 and recMAGE-A3 + AS15 ASCI.

3.2.2 To evaluate the immune response generated by the treatment of HDIL-2 in combination with recMAGE-A3 + AS15 ASCI in patients with MAGE-A3-positive unresectable or metastatic melanoma.

3.2.3 To evaluate the correlation of the predictive value of the gene signature with the clinical response to the study treatment and the clinical activity of those presenting the predictive gene signature.

4 ELIGIBILITY & ENROLLMENT

4.1 Inclusion Criteria

Step 1

4.1.1 Written informed consent has been obtained from the patient before the performance of any protocol-specific procedure.

4.1.2 Male or female patient with histologically proven, measurable unresectable or metastatic cutaneous melanoma.

4.1.3 Patient is >/= 18 years of age

4.1.4 Formalin-fixed paraffin-embedded (FFPE) tumor tissue must be available for MAGE-A3 expression screening test from cutaneous, subcutaneous, lymph node lesion, lung or liver lesion. Archival FFPE tumor tissue can

be provided for the MAGE-A3 screening test, as long as the FFPE tumor tissue was obtained from a biopsy or resection and no systemic chemotherapy, immunotherapy or targeted therapy has been received by the patient between the tumor collection and the MAGE-A3 screening test.

Fresh tumor tissue in RNAlater must be also available for gene signature testing. Patients must have at least one biopsible cutaneous, subcutaneous, lymph node lesion, lung or liver lesion and willing to undergo a punch or a CT or US guided biopsy of this lesion. The tumor sample should be preferably from the same lesion as the FFPE tumor tissue Cutaneous lesions must measure $\geq$ 4mm and lymph nodes, subcutaneous, lung or liver lesions must measure $\geq$ 1cm.

Step 2

4.1.5 ANA (antinuclear antibody) titer <1:80

4.1.6 The patient's tumor shows expression of MAGE-A3 gene

4.1.7 ECOG performance status of 0 or 1

4.1.8 WBC $\geq$ 3000/mm^3 and Hemoglobin $\geq$ 9 g/dl

4.1.9 Platelet count $\geq$ 100,000/mm^3

4.1.10 Normal AST and ALT except for patients with liver metastases, in which serum ALT and AST $\leq$ 2.5 X upper limit of normal (ULN) will be permitted.

4.1.11 Creatinine $\leq$ 1.5 mg/dL

4.1.12 Normal total bilirubin except for patients with liver metastases, in which total bilirubin $\leq$ 1.5 X ULN will be permitted (patients with Gilbert's syndrome must have a total bilirubin less that 3.0 mg/dL).

4.1.13 LDH $\leq$ 2 X ULN

4.1.14 Stress cardiac test (stress thallium, stress MUGA, dobutamine echocardiogram or other stress test that will rule out cardiac ischemia) with estimated ejection fraction >50% within 6 months of signing consent form

4.1.15 Pulmonary function tests showing FEV1 > 65% or FVC > 65% of predicted within 6 months of signing consent form

4.1.16 Women of childbearing potential (WOCBP) must be using an adequate method of contraception prior to treatment, throughout the study, and for up to 8 weeks after the last dose of investigational product, in such a

manner that the risk of pregnancy is minimized. In general, the decision for appropriate methods to prevent pregnancy should be determined by discussions between the investigator and the study subject. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not post-menopausal. Post-menopause is defined as:

Amenorrhea for 12 consecutive months without another cause, or
For women with irregular menstrual periods and taking hormone replacement therapy (HRT), a documented serum follicle stimulating hormone (FSH) level 35 mIU/mL.
Women who are using oral contraceptives, other hormonal contraceptives (vaginal products, skin patches, or implanted or injectable products), or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy, or are practicing abstinence or where their partner is sterile (eg, vasectomy) should be considered to be of childbearing potential.

WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 14 days before the start of treatment.

4.1.16 Men must also agree to use an adequate method of contraception.

4.2 Exclusion Criteria

4.2.1 The patient has at any time received systemic chemotherapy, immunotherapy or targeted therapy (except for isolated limb perfusion, interferon, or radiation in the adjuvant setting, as long as this was performed at least 4 weeks before first study treatment administration).

4.2.2 Brain metastasis or history of brain metastasis.

4.2.3 Any types of melanoma other than cutaneous, i.e. ocular or mucosal

4.2.4 The patient received any cancer immunotherapeutic containing a MAGE-A3 antigen

4.2.5 Patients with a history of second malignancies are eligible provided that they have been free of recurrence from secondary malignancy for at least 3 years, does not include squamous cell carcinoma, basal cell carcinoma or carcinoma in situ.

4.2.6 The patient has a history of an autoimmune disease such as, but not limited to, multiple sclerosis, lupus, rheumatoid arthritis, and inflammatory bowel disease or an antinuclear antibody (ANA) titer > 1:80.

4.2.7 The patient has a history of allergic disease or reactions likely to be exacerbated by any component of the study investigational compound

4.2.8 The patient has a family history of congenital or hereditary immunodeficiency.

4.2.9 Known to be positive for viral hepatitis B or C (HBsAg or Anti HCV) or HIV (HIV antibodies) Patients should have a negative test within 6 months of starting treatment.

4.2.10 Systemic steroid therapy, steroid-containing compounds or any other immunosuppressive agents or to be used for more than 7 consecutive days (at a dose of prednisone or equivalent of ≥ 0.125 mg/kg/day).

4.2.11 The patient has psychiatric or addictive disorders that may compromise his/her ability to give informed consent, or to comply with the trial procedures. Each patient will be evaluated by the principal investigator or his designee.

4.2.12 The patient has concurrent severe medical problems, unrelated to the malignancy, that would significantly limit full compliance with the study or expose the patient to unacceptable risk. Each patient will be evaluated by the principal investigator or his designee.

4.2.13 Initiation of another anti-cancer therapy

4.2.14 For female patients: the patient is pregnant or lactating

4.2.15 WOCBP who are unwilling or unable to use an acceptable contraceptive method to avoid pregnancy

5 PRE-TREATMENT EVALUATION

In order to be eligible for this study, the patients must have a ANA titer <1:80 and MAGE-A3-positive tumor. Therefore, the MAGE-A3 expression of FFPE tumor tissue from cutaneous, subcutaneous, lymph node lesion, lung or liver lesion in unresectable or metastatic melanoma will be tested before enrolling patients in the treatment part of the study. A tumor biopsy at the time of screening is preferred but well preserved archival tumor tissue will be acceptable for the MAGE-A3 expression screening test if the tumor tissue was taken from a biopsy or resection of the lesion within 1 year of the MAGE-A3 screening test and no systemic chemotherapy, immunotherapy or targeted therapy has been received by the patient between the time of tumor biopsy and the MAGE-A3 screening testAll patients must sign an informed consent before performing the MAGE-A3 testing and must be registered in CORE/PDMS. If the ANA titer is <1:80 and the MAGE-A3 is positive, the patient will be eligible to proceed to the treatment part of the protocol. A second informed consent will need to be signed to complete the screening and before starting the study treatment.

Fresh tumor tissue (minimum volume recommended is 10 mm^3 conserved in RNAlater after biopsy) must be also available for gene signature testing. Patients must have at least one biopsible cutaneous, subcutaneous, lymph node lesion, lung or liver lesion and willing to undergo a punch or a CT or US guided biopsy of this lesion. The tumor sample should be preferably from the same lesion as the FFPE tumor tissue.

5.1 MAGE-A3 Expression Screening

A formalin-fixed paraffin-embedded (FFPE) tumor tissue block sample of at least 10 mm^3 or alternatively 15-20 unstained slides freshly cut from FFPE tumor blocks (a minimum of 14 slides of 10 μm and 1 slide of 5 μm for a total of at least 50 mm^2 tumor tissue) - will be provided by the study center to GSK Biologicals or contracted lab. The laboratory will then extract the RNA from these samples and assess the MAGE-A3 expression by quantitative PCR. Upon reception of the tumor tissue sample, the MAGEA3 screening will be done within 5 working days. The cut-off value for the MAGE-A3 expression assay is defined as 1% of the positive MAGE-A3 control included in the assay. If there are several melanoma blocks, it is preferred that 2 of these are sent for determination of MAGE-A3 expression. For each block, a paraffin-embedded tissue block sample of at least 10 mm^3 - or alternatively but less preferable 15-20 unstained slides freshly cut

from FFPE tumor blocks (a minimum of 14 slides of 10 µm and 1 slide of 5 µm are required for a total of at least 50mm^2 tumor tissue). The second block will only be tested, in case the first tested is MAGE-A3-negative (in this case results might be delayed from 5 to 7 working days). The patient will be considered eligible on this criterion, if at least one tested block is MAGE-A3- positive. If tissue from the primary tumor is also provided to the laboratory, this may be tested to examine whether it expresses MAGE-A3. At the end of the recruitment period, the study center may request that all residual tumor blocks they shipped for MAGE-A3 screening are returned to them.

5.2 Patient Screening for MAGE-A3 Positive Patients

Once the MAGE-A3 expression status is determined, patients will proceed to the screening visit. At the screening visit, patients will sign a second informed consent and will be assessed for study eligibility. If some tests or imaging studies were done before signing the informed consent as part of standard of care and the date they were done falls within the time limit allowed by protocol before starting treatment, these test will not be repeated. The following baseline studies must be completed within 14 days of signing the second consent form:

- History, physical examination (including vital signs: blood pressure, heart rate, respiration rate, temperature and medication use history), weight, height, and performance status will be obtained

A pregnancy test (urine or serum) must be performed for women of childbearing potential prior to treatment. FSH will also be performed if needed.

- CBC, differential, PT/PTT, platelet count, HLA typing (if necessary).
- Chemistries to include serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, AST, LDH, calcium, and total bilirubin, magnesium, phosphorous, and creatinine kinase.
- EKG
- Concomitant medications
  Toxicity assessment

The following baseline studies must be completed within 30 days of signing the second consent form:
Radiological studies, chest x-ray, CT scan of the chest, abdomen and pelvis, and CT/MRI of brain and when clinically indicated a bone scan

- Evaluation of disease by photography if needed
- A biopsy will be obtained within 30 days of treatment. The biopsy will be obtained by CT or US-guided, or a punch biopsy of a cutaneous, subcutaneous mass, lymph node lesion, lung or liver lesion (10 mm^3 is the minimum volume recommended of fresh tumor tissue stored in

RNAlater for gene signature testing) . This will be used for genetics studies on the tumor tissue (See section 17.5)

The following baseline studies must be completed within 6 months of signing the second consent form for treatment:

- PFTs
- Cardiac Stress Test

6 STUDY IMPLEMENTATION

6.1 Study Design

This is a Phase II study in patients with measurable unresectable or metastatic cutaneous melanoma who have not received systemic chemotherapy, immunotherapy or targeted therapy (First-line metastatic treatment). We expect to enroll 30 patients in a 2-year period.

Cycles 1-8:
Treatment needs to be started within 14 days of signing the second informed consent. All patients will be seen in clinic on Day 1 of week 1 of cycle 1; Day 1 (+/-2 business days) of weeks 3, 5, and 7 (+/- 2 business days) of cycle 2; Day 1 (+/- 2 business days) of weeks 9,11,15 of cycles 3 and 4; Day 1 of weeks 18,21, and 24 of cycles 5 and 6, and Day 1 (+/-2 business days) of weeks 27 and 30 of cycles 7 and 8 (Figure 1 and 2). Patients will receive recMAGE-A3 + AS15 ASCI in CTRC on the same days as stated above. After the injection on Day 1 (+/- 2 business days) of weeks 1,3,9,11,18,21,27 and 30, patients will be admitted to the hospital to receive HDIL-2. Treatment of HDIL-2 will start on Day 2 and within 24 hours of receiving recMAGE-A3 + AS15 ASCI.

Patients will receive a maximum of 12 ASCI administrations (PI will monitor creatinine levels) in combination with HDIL-2 during cycles 1-8 for up to 33 weeks:

- 6 ASCI administrations, with a 2-week interval (weeks 1, 3, 5, 7, 9, 11)
- 6 ASCI administrations, with a 3-week interval (weeks 15, 18, 21, 24, 27, 30)

See section 9 for re-treatment in cycles 3-8.

Maintenance phase (ASCI monotherapy):
Patients continuing on the study beyond completing 8 cycles of HDIL-2 will receive the ASCI injection (recMAGE-A3 + AS15) on Day 1 (+/- 2 business days) every 6 weeks for 4 doses (weeks 34,40,46, and 52) then on Day 1 (+/- 2 business days) every 12 weeks (weeks 64, 76, 88, and 100) for 4 doses and then every 24 weeks for 4 doses (PI will monitor creatinine levels). Patients who had their HDIL-2 discontinued before completing 8 cycles because of HDIL-2 related

toxicities without showing signs of progression of disease or those who had their HDIL-2 discontinued before completing 8 cycles because of achieving complete response will be allowed to continue on the ASCI injection alone following the same schedule described in Figure 1.

Patients will receive a maximum of 12 ASCI (PI will monitor creatinine levels) administrations in the maintenance phase (beyond cycle 8) for up to 187 weeks from first study treatment:

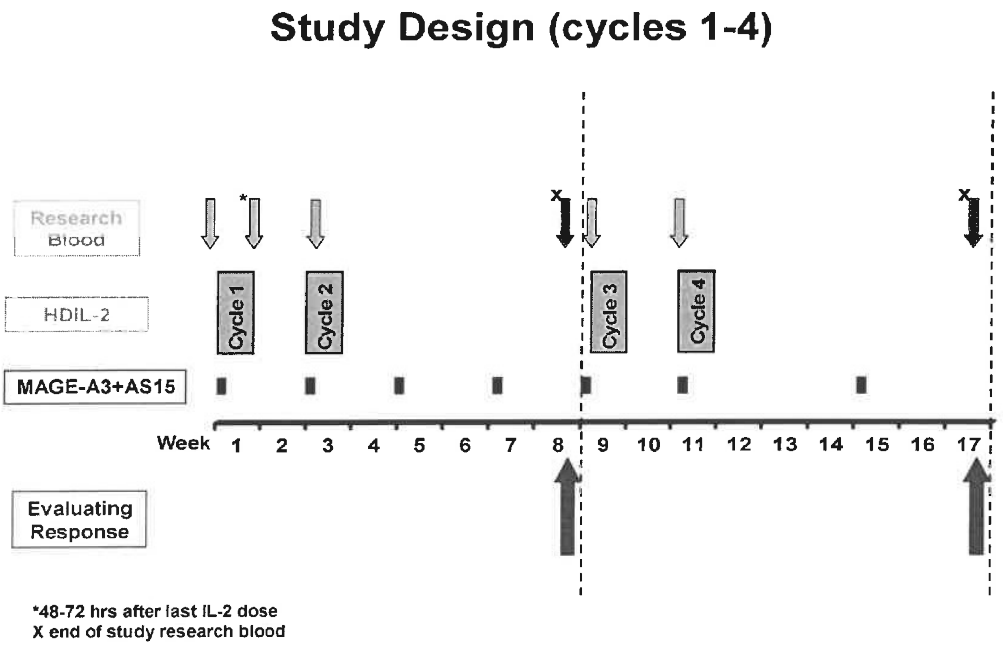
- 4 ASCI administrations, with a 6-week interval (weeks 34, 40, 46, 52)
- 4 ASCI administrations, with a 12-week interval (weeks 64, 76, 88, 100)
- 4 ASCI administrations, with a 24-week interval (weeks 124, 148, 172, 187)

On the weeks when both the ASCI injection and HDIL-2 are administered, all patients will receive the ASCI injection in CTRC following routine laboratory tests, research blood specimen, and physical examination. The patient will be observed for at least 30 minutes following the ASCI injection. The injections will be given intramuscularly (IM) in the deltoid or lateral region of the thigh. Sequential injections will be administered alternately on the left and right sides. All patients will then be admitted to the hospital, either the ICU or nursing area which specializes in the administration of HDIL-2. HDIL-2 will be administered on Day 2 and repeated every 8 hours as tolerated for up to 14 doses per cycle.

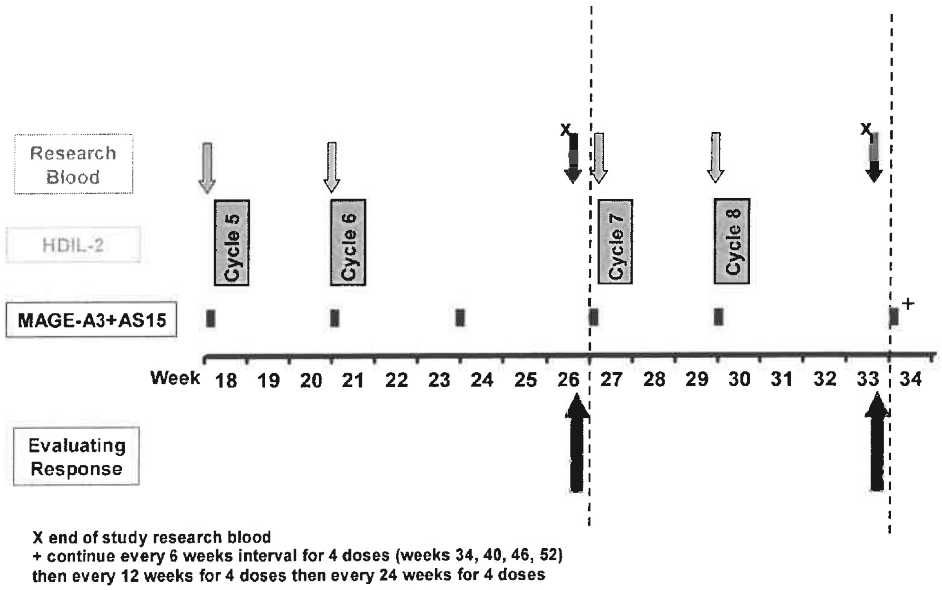
On the weeks when the ASCI injection is administered alone, all patients will receive the ASCI injection following routine laboratory tests. The patient will be observed for at least 30 minutes following the ASCI injection. The injections will be given intramuscularly (IM) in the deltoid or lateral region of the thigh. Sequential injections will be administered alternately on the left and right sides. Every effort should be made to administer the HDIL-2 and the ASCI injection as described, however the weeks when ASCI is administered alone +/- 2 business days will be allowed.

CT scans of chest, abdomen, and pelvis, chest x-ray and MRI/CT of the brain will be done at the end of Weeks 8,17,26,33,39, 51, 63, 75, 87, 99, 123, 147, 171 and 195 (+/-1 week) CXR is only required before each cycle of treatment in which the patient receives HDIL-2, which would require confirmation of line placement prior to treatment.

Figure 1


Study Design (cycles 1-4)
Research Blood
HDIL-2
MAGE-A3+AS15
Evaluating Response
Cycle 1
Cycle 2
Cycle 3
Cycle 4
Week 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
*48-72 hrs after last IL-2 dose
X end of study research blood

Study Design (cycles 5-8)



X end of study research blood
+ continue every 6 weeks interval for 4 doses (weeks 34, 40, 46, 52)
then every 12 weeks for 4 doses then every 24 weeks for 4 doses

Scans will be done every 8 weeks until week 39, then every 12 weeks until week 100, then every 24 weeks until week 187 (end of study). Physical exams are done on the weeks the patient receives the vaccine.

6.2 Drug Administration and Dose Modification

6.2.1 Interleukin-2 (IL-2)

IL-2 (Novartis Corporation, East Hanover, NJ) will be administered at a dose of 720,000 IU/kg as an intravenous bolus over an approximate 15 minute period every eight hours, for a maximum of 14 doses per cycle (documentation of IL-2 infusion beginning time is required but not the stop time). Patients will begin HDIL-2 the day after ASCI injection. Doses may be delayed depending on patient tolerance (no IL-2 dose reduction will take place on this study). Doses will be delayed if patients reach grade III or IV toxicity that is not reversible within 24 hours of holding HDIL-2. The most common grade III and IV toxicities seen with IL-2 are summarized in Appendix D. If this toxicity is easily reversed by supportive measures, then additional doses may be given, as outlined in the guidelines for IL-2 administration (Appendix E). If a patient's dose is delayed by greater than 24 hours, no further doses will be administered in that cycle. The principal investigator or his designee will evaluate patients with grade II toxicities in order to make the decision as to continuing the HDIL-2. Detailed guidelines for HDIL-2 administration will direct the administration and discontinuation of HDIL-2 (Appendix F). HDIL-2 will be administered as an inpatient and will be obtained through the hospital pharmacy. HDIL-2 administration schedule is shown in Figure 1 and described in section 6.1 and will start within 24 hours from ASCI

administration. HDIL-2 treatment will be delayed (for a maximum of 24 hours) in cycle 2, 3, 4, 5, 6, 7 or 8 until all HDIL-2 related toxicities are reversed to grade 1 or less.

6.2.2 **recMAGE-A3 + AS15 (ASCI)**

The candidate ASCI to be used in this study has been developed and manufactured by GSK Biologicals. In the study, the recMAGE-A3 + AS15 ASCI will be administered by using a sterile two vial set comprising:

• One vial with the lyophilized preparation containing 300 µg recMAGE-A3 antigen plus 420 µg of CpG7909 (a part of the Adjuvant System AS15),

• One vial with liquid adjuvant diluent AS01B (liposomes containing 50 µg of MPL®, 1mg of DOPC, 250 µg of cholesterol and 50 µg of QS21 in phosphate-buffered saline), making up the remainder of the Adjuvant System AS15.

The final recMAGE-A3 + AS15 ASCI for administration is obtained by reconstitution of the lyophilized preparation with the adjuvant diluent. One recMAGE-A3 + AS15 ASCI dose consists of 0.5 ml of this mixture.

A standard dose of recMAGE-A3 (300 µg) and AS15 will be used, irrespective of the patient's body weight or body surface area. No dose modification is allowed. 0.5 ml corresponding to 300 µg of recMAGE-A3 antigen and 420 µg CpG reconstituted in AS01B. Route and site: IM Deltoid or lateral region of the thigh. The study drug administration schedule is shown in Figure 1. When given with HDIL-2, the ASCI will be administered within 24 hours from the first dose of HDIL-2.

The needles used for study treatment administration should be suitable for intramuscular injection. The liquid content of the diluent vial is to be transferred aseptically into the vial containing the lyophilized preparation. The vial is to be shaken gently until complete dissolution of the pellet. The two solutions should be completely combined within 5 minutes. To avoid added discomfort to the patient, the reconstituted mixture must be at a suitable temperature (i.e., no longer chilled), when it is administered. When reconstituted, the recMAGE-A3 + AS15 ASCI can be kept at a temperature between 4°C and 25°C/39°F and 77°F for a maximum of 4 hours. The investigator or designate will subsequently withdraw the reconstituted mixture, change the needle, and 0.5 ml will be injected slowly (over approximately 30 seconds) intramuscularly (IM) into the deltoid or the lateral regions of the thighs. Sequential injections will be administered alternately on the right and left sides. The patients will be observed closely for at least 30 minutes following the administration of the study medication, with appropriate medical treatment readily available in case of a rare anaphylactic reaction.

All investigational products must be stored in a safe and locked place with no access by unauthorized personnel. The treatments will be stored at the defined temperature range (i.e., +2 to +8°C/ 36°F to 46°F). The storage temperature of treatments will be monitored daily by means of validated temperature monitoring devices and the temperature measurements will be recorded during working days, preferably at the same time of the day (e.g., at the beginning of the day).

Any temperature deviation, meaning temperature outside the defined range (i.e. +2 to +8°C/36 °F to 46°F), must be reported immediately to Principal Investigator and refer to instructions provided by GSK Biologicals (manufacturer of recMAGE-A3 + AS15 ASCI study drug) , amongst others the Pharmacy Manual and the "Product / Study specific range (PSR)" defined from the product thermo-stability data.

Any unused investigational product will be destroyed according to institutional policy.

Anticipated toxicity profile for recMAGE-A3 + AS15 ASCI is generalized chills, fever, fatigue, nausea, myalgia, arthralgia, and headache. At the injection site redness, swelling and pain may occur.

Figure 2 Study Calendar for MAGE-A3 Positive Patients

PROCEDURES	W/IN 30 D	W/IN 14 D	CYC 1			CYC 2**						CYC 3**		CYC 4**				
			WK 1			WK 3		WK 5	WK 7	WK 8	EOS	WK 9		WK 11		WK 15	WK 17	EOS
			DAY 1	DAY 2 [a]	48-72 HR	DAY 1	DAY 2 [a]	DAY 1	DAY 1			DAY 1	DAY 2 [a]	DAY 1	DAY 2 [a]	DAY 1		
CONSENT		X																
H/P*		X	X			X		X	X		X	X		X		X		X
VITAL SIGNS		X	X			X		X	X		X	X		X		X		X
CBC, PLTS		X	X	X		X	X	X	X		X	X	X[c]	X	X[c]	X		X
PT/PTT		X	X	X[c]		X	X	X	X		X	X	X	X	X	X		X
CHEMISTRIES[df]		X	X	X	X	X	X	X	X		X	X	X	X	X	X		X
ANA titer	X										X							X
PREGNANCY		X																
FSH		X																
HLA	X																	
PHOSPHOROUS		X		X			X	X	X				X		X	X		
CREATINE KINASE		X		X			X	X	X				X		X	X		
MAGNESIUM		X		X			X	X	X				X		X	X		
STRESS CARDIAC	X(6mos)																	
EKG		X																
PFT's	X(6mos)																	
MRI/CT BRAIN	X									X							X	
CT (C,A,P), CXR	X									X							X	
BONE SCAN (if symptomatic)	X																	
RESEARCH BLOOD[g] (humoral immunity)			X (C1-WK1)		X (C1-WK1*-post HDIL2)	X (C2-WK3)					X (EOS-WK8)	X (C3-WK9)		X (C4-WK11)				X (EOS-WK17)
MAGE-A3 + AS15 ASCI			X			X		X	X			X		X		X		
Start HDIL-2				X			X						X		X			
CLINIC VISIT			X			X		X	X		X	X		X		X		X
Toxicities		X	X	X		X	X	X	X		X	X	X	X	X	X		X
Concomitant meds		X	X	X		X	X	X	X		X	X	X	X	X	X		X
Tumor biopsy	X									X[b]								
Photographs (prn)		X								X							X	

* if H/P is already done within 14 days, then it does not need to be repeated on C1Wk1D1

** procedures will be administered within +/- 2 business days.

Figure 2 Study Calendar (Continued)

PROCEDURES	CYC 5		CYC 6					CYC** 7		CYC** 8					
	WK 18**		WK 21**		WK 24**	WK 26**	EOS	WK 27**		WK 30**		WK 33**	EOS	Week 8, 17, 26, 33, 39, 51, 63,75, 87, 99, 123, 147, 171, 195 (+/- 1 week)	Week 34, 40, 46, 52, 64, 76, 88, 100, 124, 148, 172, and 187 (+/-2) business days**
	DAY 1	DAY 2 [a]	DAY 1	DAY 2 [a]	DAY 1			DAY 1	DAY 2 [a]	DAY 1	DAY 2 [a]			DAY 1	DAY 1
H/P	X		X		X		X	X		X			X		X
VITAL SIGNS	X		X		X		X	X		X			X		X
CBC, PLTS	X	X	X	X	X		X	X	X	X	X	X	X		X
PT/PTT	X	X[c]	X	X[c]	X		X	X	X[c]	X	X[c]	X	X		X
CHEMISTRIES[d,e]	X	X	X	X	X		X	X	X	X	X	X	X		
ANA Titer							X						X		
PREGNANCY															
MRI/CT BRAIN						X						X		X	
CT (C,A,P),						X						X		X	
BONE SCAN															
RESEARCH BLOOD[g] (humoral immunity)	X (C5-WK18)		X (C6-WK21)				X (EOS-WK26)	X (C7-WK27)		X (C8-WK30)			X (EOS-WK33)		
MAGE-A3 + AS15 ASCI	X		X		X			X		X					X[h]
Start HDIL-2		X		X					X		X				
Toxicities	X	X	X	X	X		X	X	X	X	X		X		X
Concomitant Meds	X	X	X	X	X		X	X	X	X	X		X		
CLINIC VISIT	X		X		X		X	X		X			X		X
Photographs (prn)						X						X			

a. Labs on day 2 is repeated daily until the patient is being discharged. The patients will have a complete blood count, and a comprehensive metabolic panel, including as a minimum: electrolytes, creatinine, blood urea nitrogen, glucose, AST, ALT, total bilirubin, creatinine kinase, LDH, calcium, phosphorus, magnesium and albumin measured daily. PT and PTT will be obtained starting on the third day of IL-2.
b. This is an optional biopsy preferably from the same lesion as for baseline biopsy for the gene signature analysis (fresh tumor tissue in RNAlater)
c. PT/PTT will be obtained on the 3rd day of HDIL-2
d. chemistries include serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT/AST, LDH, Calcium, Total bilirubin, magnesium, phosphorous and creatinine kinase. BUN + Serum creatinine will be tested one day after every vaccine and can be done at an outside lab.
e. Patient with history of Hepatitis B and C and HIV should have a negative (-) test results within 6 months of starting treatment.
f. see Section 8.2
g. Then every 24 weeks for up to 4 additional doses (week 124, 148, 172, 187)
h. Scans will be done every 8 weeks until week 39, then every 12 weeks until week 100, then every 24 weeks until week 187 (end of study). Physical exams are done on the weeks the patient receives the vaccine.

* Scans can be done (+/- 1 week)
* CXR only needed prior to each cycle of HDIL-2 to check for line placement.

7 SUPPORTIVE CARE

Acetaminophen (650 mg q4h), indomethacin (50-75 mg q8h) and ranitidine (50mg IV q8h) or an H2 Blocker are utilized routinely during HDIL-2 administration and are started before the first IL-2 dose. It is imperative that these medications be administered to prevent several of the common side effects seen with IL-2 administration. Supportive therapy for chills, nausea, emesis, diarrhea, hypotension or other side effects of therapy is carefully detailed in Appendix G. If patients require systemic steroid therapy for more than 7 consecutive days, they will be taken off protocol. However, the use of prednisone, or equivalent, at a dose of <0.125 mg/kg/day (absolute maximum 10 mg/day), inhaled or topical steroid is permitted.

8 EVALUATION DURING AND POST TREATMENT

8.1 Clinical Evaluation

Labs on Day 2 are repeated daily during HDIL-2 treatment until the patient is discharged. The patients will have a complete blood count, and a comprehensive metabolic panel, including as a minimum: electrolytes, creatinine, blood urea nitrogen, glucose, ALT, AST, total bilirubin, creatinine kinase (CK), LDH, calcium, phosphorus, magnesium and albumin measured daily. PT and PTT will be obtained starting on the third day of IL-2. All patients will have routine laboratory tests before each clinic visit.

8.2 Immunological Evaluations

60 ml whole of blood will be collected into heparinized green top tubes (for PBMC isolation) and 10 ml of blood in red top tubes (for serum isolation) from each enrolled patients 3 times in total during the first 2 cycles (once before the study drug administration on Day 1 and once 48-72 hours after the last HDIL-2 administered during cycle 1) and one time before study drug administration on Day 1 of cycle 2, for immunologic testing (See section 17 for details on the immunologic testing and Figure 1 and 2 for blood collection time points). An additional sample will be collected at the end of the study visit. The blood PBMC and serum processing and immunological evaluations of T-cell responses will be performed in the immunomonitoring core lab (IMCL) of MD Anderson Cancer Center (Dr. Laszlo Radvanyi directs this lab).

The analysis of humoral response (serum antibodies titers) will be done by GSK Biological contracted laboratory after aliquots of serum are sent from the IMCL to the GSK labs batched before the end of the study or at any other times as mutually agreed.

8.3 Post Treatment Biopsy (optional)

An optional biopsy will be obtained any day in Week 8 regardless of treatment response. The benefit for the post biopsy is to evaluate for gene signature after treatment. Preferably the same lesion as baseline or any other lesion will be biopsied. The biopsy will be obtained by CT or US-guided, or a punch biopsy of a cutaneous, subcutaneous mass, lymph node, lung or liver lesion (10 mm^3 is the minimum volume of fresh tumor tissue) and conserved in RNAlater.. This will be used for genetics studies on the tumor tissue (See section 17). This will be compared to the biopsy obtained at baseline.

8.4 Post Treatment Disease Assessment

Tumor measurements and CT/MRI scans and photographs will be at the end of Weeks 8,17,26,33, 39, 51, 63, 75, 87, 99, 123, 147, 171, 195 (+/- 1 week). All measurable visceral lesions must be evaluated by CT/MRI scans. The same method used to document measurable and non-measurable disease at baseline should be used consistently for all evaluations throughout the study. Cutaneous lesions that are used to follow disease response will be photographed at baseline and with every tumor measurement.

End of study visit will be performed at the time the patient is taken off study for meeting any of the Off-study Criteria or completing the study. End of study evaluation will include physical examination (including vital signs: blood pressure, heart rate, respiration rate and temperature), weight, performance status, CBC/differential, platelet count, PT/PTT, routine chemistry panel (must include

ALT and AST, LDH, alkaline phosphatase, total bilirubin, creatinine, research blood and ANA titer).

9 RE-TREATMENT

9.1 Patients will be eligible for re-treatment based on the evaluation which is performed at the end of Week 8 (+/- 2 business days). If patients have no progression, and HDIL-2 related toxicities are reversed to grade 1 or less (CTCAEv4), they may be re-treated with the same treatment that they had received previously following the schedule described in Figure 1 to a maximum of 8 HDIL-2 cycles.

9.2 Patients continuing on the study beyond completing 8 cycles of HDIL-2 will receive the ASCI on Day 1 (+/- 2 business days) every 6 weeks for 4 doses (weeks 34,40,46 and 52) then on Day 1 (+/- 2 business days) every 12 weeks (weeks 64, 76, 88 and 100) for 4 doses, then on Day 1 (+/- 2 business days) every 24 weeks (weeks 88, 100, 124, 148, 172, and 187). The patients will be seen in clinic on Day 1 (+/- 2 business days) of each of the stated weeks. The patient will have a history, physical examination, vital signs, and laboratory tests including CBC, platelets, PT/PTT, chemistries, and liver function tests. Patients who had their HDIL-2 discontinued before completing 8 cycles because of HDIL-2 related toxicities without showing signs of progression of disease (as described in the Response Criteria Section) or those who had their HDIL-2 discontinued before completing 8 cycles because of achieving complete response will be allowed to continue on the ASCI injection alone following the same schedule described in Figure 1. Re-treatment benefits and risks will be carefully explained to the patient.

10 FOLLOW-UP

10.1 Patients with no progression of their disease while on study treatment, will have long term follow-up for survival after all treatments have stopped (i.e. up to week 187 after last subject first dose). Scans will be done every 8 weeks until week 39, then every 12 weeks until week 100, then every 24 weeks until week 187 (end of study). For patients who progressed, survival data should be collected if possible every two months while patients are off-study and in long term-follow up, either by a clinic visit or by phone. All cancer related therapies after study discontinuation will be recorded when possible.

10.3 Patients will be contacted either by phone or by clinic visit at 30 days (+/- 2 business days) from last dose of drug to evaluate any toxicities which were present at the end of study.

11 POSTPONEMENT OF TREATMENT

If an acute disease occurs at the time of the study treatment, the patient may be treated at a later date (i.e., the entire program of study visits and immunizations is interrupted), within the time window specified below, or withdrawn at the discretion of the investigator. The patient must be followed until resolution of the event, as with any AE. Acute disease is defined as the presence of a moderate or severe illness with or without fever or any ≥ CTCAE v 4 grade II adverse event possibly related to the study treatment. Study treatment can be administered to persons with a minor illness such as diarrhea or mild upper respiratory infection if the serious adverse event is < grade II (CTCAEv4).

- During the combination treatment phase, the maximum delay for postponement of ASCI study treatment administration is 3 weeks.
- During the maintenance phase (ASCI monotherapy), the maximum delay for postponement of ASCI study treatment administration is 3 months.

Postponement of ASCI study treatment will be delayed until treatment related toxicities are grade I or less.

When an ASCI study treatment administration has to be postponed, a visit to administer the missed treatment should be planned as soon as possible to catch up with the originally planned schedule. If the administration occurs within the specified delay, the treatment dose to administer corresponds to the next one in the initial order of administration. If the administration does not occur within the delay stated above the patient will be taken off the study.

12 OFF-STUDY CRITERIA

12.1 Disease progression

All patients who develop progression of disease will be taken off the study. Please see progression of disease criteria.

12.2 Withdrawal of consent

The patient's desire to withdraw from the study may occur at any time. If a patient withdraws consent, the investigator will assess whether the reason for withdrawal is actually an adverse event, in which case the adverse event should be noted as the reason for withdrawal.

12.3 Adverse event

Any intolerable adverse event or any persistent moderate adverse event that could be worsened by subsequent administration of the study treatment, at the investigator's discretion. For example, any grade III or greater toxicity, or a serious adverse event attributable to the ASCI or HDIL2.

ASCI- related or possibly ASCI- related ≥Grade 2 as per CTC v.4 Criteria for Adverse Events allergic reaction/ hypersensitivity toxicity (i. e. generalized rash, flushing, urticaria and dyspnea).
Grade ≥ 2 (as per CTCAE, v4.) autoimmune reaction
Grade ≥ 3 (as per CTCAE, v 4.0) injection site reaction (i. e. ulceration or necrosis that is severe; operative intervention indicated).

Participants who are withdrawn from the study due to toxicity will be followed weekly with either a phone call or visit until the toxicity is a grade 1 or less.

12.4 Withdrawal by the physician

For clinical reasons not related to treatment.

12.5 Violation of the study protocol

Including patient failure to return for required visits. Every effort should be made to contact such patients to determine their reason for withdrawal, and to assess any adverse events that may have contributed to the patient's failure to return. Patients who miss dose(s) because of holidays and/or clinic closures will not be withdrawn from the study.

12.6 Other Criteria

12.6.1 Treatment with an investigational product or non-registered product other than the study treatment (recMAGE-A3 + AS15 ASCI and HDIL-2) or any other anticancer treatments, including but not limited to chemotherapeutic or immunomodulating agents and radiotherapy.

12.6.2 Clinical signs or symptoms indicative of vasculitis, glomerulonephritis or any autoimmune disorder; in such cases, appropriate clinical and laboratory testing will be performed to identify and characterize that disorder.

12.6.3 Appearance of any confirmed or suspected immunosuppressive or immunodeficient condition, including human immunodeficiency virus (HIV) infection, or any medical condition requiring the administration of immunosuppressive agents or systemic corticosteroids.

12.6.4 For female patients, pregnancy or decision to become pregnant.

13 DATA COLLECTION

For the purposes of this study at M.D. Anderson Cancer Center, the Protocol Data Management System (PDMS) will be employed. All patients will be registered in PDMS/CORE.

All laboratory and clinical data gathered in this protocol will be stored in a password-protected database. All patient information will be handled using anonymous identifiers. Linkage to patient identity is only possible after accessing a password-protected database. Access to the database is only available to individuals directly involved in the study.

Once the research
has been completed, identifiers will be retained for as long as is required by law and by institutional regulations, and at that point will be destroyed.

- Adverse events will be recorded in PDMS.
- Disease progression will not be recorded as a SAE.

Concomitant Medicament Information/Therapy Administration.

1) Concomitant therapy administrations during the study are to be recorded as follow:

    - Scheduled Prophylaxis: All antibiotic, antiviral and antifungal agents given during the treatment schema.
    - Empiric Antibiotics: All antibiotic, antiviral and antifungal agents given with unexplained fever or any infectious complications.
    - Blood Product Support: Granulocyte Colony Stimulating Factors (GCSF).
    - Therapeutic dosing with anticoagulants.

2) Concomitant drugs such as: Diphenhydramine, Famotidine, Palonosetron or Ondansetron, given as Treatment-premedication will be reviewed and documented in the subjects medical record, but will not be captured in the case report form.

3) Concomitant medications given as part of necessary supportive care will be reviewed and documented in the subjects medical record, but will not be captured in the case report form. Medicaments are included but not limited as follows:

    - Analgesics and antipyretics
    - Antihistaminics
    - Appetite stimulants

- Sleeping medication and other sedatives
- Antacids and laxatives
- Anti-emetics and anti-diarrheal drugs
- Analeptics and other stimulants
- Electrolytes: potassium, calcium, magnesium.
- Vasopressors and antihypotensive agents

4) Vitamins, nutritional supplements, hormone replacement therapy (HRT), pro re nata (PRN), and topical medications will be reviewed and documented in the subjects medical record, but will not be captured in the case report form.

14 RESPONSE CRITERIA

Primary evidence of antineoplastic activity will be evaluated as a function of objective tumor response. Objective response is either a complete response or a partial response which is further defined in section 14.5. An overall objective assessment of all measurable and non-measurable disease will be performed according to the Visit Schedules (see Figure 1). Tumor response will be defined by the Response Evaluation Criteria in Solid Tumors (RECIST) solid tumor response criteria (24).

Tumor assessments should be performed by the MRI or CT scan, throughout the study. The treating physicians will perform tumor measurement. Radiological studies must account for all lesions that were present at baseline and must use the same techniques as used at baseline. All complete and partial responses must be confirmed by a second assessment at least four weeks later.

14.1 Measurable Disease

Lesions that can be accurately measured in at least one dimension with longest diameter ≥ 20 mm or ≥ 10 mm with spiral CT scan.

Note: Clinical lesions will only be considered measurable when they are superficial (i.e., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended. Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung (however, CT is preferable).

Measurable cutaneous/subcutaneous lesions: lesions that can be accurately measured in at least one dimension. Minimal longest diameter required for one target lesion is:

	Clinical detection (color photography)	Ultrasound
Cutaneous lesion	Lesion must be ≥ 5 mm	–
Subcutaneous lesion	–	Lesion must be ≥ 5 mm

Measurable cutaneous confluent lesion: if cutaneous lesions are too numerous and too small (< 5 mm) but are confluent, they can be recorded as a specific measurable confluent lesion. Non-measurable lesion: all other lesions, including small lesions (longest diameter < 5 mm). All measurements should be recorded in metric notation by use of a ruler or calipers.

14.2 Non-Measurable Disease

All other lesions, including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm with spiral CT scan) and other non-measurable lesions. These include: bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques.

14.3 Baseline Documentation of "Target" and "Non-Target" Lesions

All measurable lesions up to five lesions in any one organ and 10 lesions in total, representative of all involved organs should be identified as ***target lesions*** and recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).

A sum of the longest diameter (LD) for all target lesions will be calculated and reported as baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.

All other lesions (or sites of disease) should be identified as ***non-target lesions*** and should also be recorded at baseline. Measurements of these lesions are not required but the presence or absence of each should be noted throughout follow-up.
The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

14.4 Methods of measurement

- Clinically detected lesions will only be considered measurable when they are superficial (e.g. skin nodules, palpable lymph nodes). For the case of skin lesions, documentation by color photography - including a ruler to estimate the size of the lesion - is required.

- CT-scan and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT-scan and MRI should be performed with contiguous cuts of 10 mm or less in slice thickness. Spiral CT-scan should be performed using a 5 mm contiguous reconstruction algorithm; this specification applies to the tumors of the chest, abdomen and pelvis while head and neck tumors and those of the extremities usually require specific protocols. PET/CT scans will be accepted only if a patient is allergic to contrast materials or has any other contraindication to conventional CT-scans.
- Ultrasound (US) should not be used to measure tumor lesions that are clinically not easily accessible. It may be used as a possible alternative to clinical measurements of superficial palpable nodes, subcutaneous lesions. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination. US should preferentially be performed by the same investigator.

14.5 Response Criteria: Evaluation of Target Lesions

Complete Response (CR)

Disappearance of all target lesions.

Partial Response (PR)

At least a 30% decrease in the sum of the LD of target lesions, taking as a reference the baseline sum LD.

Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

Disease Progression (PD)

At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions (please see mixed response and slow progressive disease criteria)

14.6 Response Criteria: Evaluation of Non-Target Lesions

Complete Response (CR)

Disappearance of all non-target lesions and normalization of tumor marker level.

Incomplete Response/Stable Disease (SD)

Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits.

Disease Progression (PD)

Appearance of one or more new lesions and/or unequivocal progression or existing non-target lesions.

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesion	Non-Target Lesion	New Lesion	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

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

14.7 Confirmation

The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.

In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol.

14.8 Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

14.9 Duration of Stable Disease

SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.

The clinical relevance of the duration of SD varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

14.10 Mixed Response Criteria and Slow Progressive Disease

In 2004 and 2005, approximately 200 oncologists, immunotherapists, and regulatory experts convened in a series of workshops to discuss their experience with immunotherapeutic agents in cancer patients (24,25). These discussions resulted in the following conclusions: (a) The appearance of measurable antitumor activity may take longer for immune therapies than for cytotoxic therapies; (b) responses to immune therapies may occur after conventional PD; (c) discontinuation of immune therapy may not be appropriate in some cases, unless PD is confirmed (as is usually done for response); (d) allowance for "clinically insignificant" PD (e.g., small new lesions in the presence of other responsive lesions) is recommended; and (e) durable SD may represent antitumor activity. The workshop participants proposed a new clinical paradigm and recommended that existing response criteria be refined to address these issues. This was evaluated in a series of large, multinational studies, representing a clinical trial program of 487 patients with advanced melanoma who received ipilimumab, a fully human monoclonal antibody that blocks CTL antigen-4 (CTLA-4).

Mixed Response (MR)

The concept of a Mixed Response (MR) is based on the observation that some melanoma patients present with tumor lesions that indubitably regress after ASCI administration, whereas other lesions remain unchanged or progress or with new lesions appearing. As one example, consider a case with complete regression of all initial target lesions but with appearance of one new lesion - this must certainly be considered as a PD. However, it is important that the detailed

information about such a case is recorded in an early clinical study as a MR, because such a situation could be explained by an adequate biological activity of ASCI but with a resistance of the new tumor lesion to the immune response

Cases of MR will NOT be considered as meeting the criteria of the definition of an objective response (for assessment of the primary endpoint), as all MR are either a stable disease (SD) or a progressive disease (PD). However, the recorded information about MRs will be useful in the descriptive assessment of the biological activity of the treatment.

The MR criteria are defined as follows:

- At least 30% decrease in the longest diameter (LD) occurring in at least one target lesion recorded and measured at baseline. Such response occurring in otherwise SD or PD status of the LD of target lesions and without the appearance of one or more new lesions will be classified as "SD with target lesion regression" or "PD with target lesion regression", respectively.
- The appearance of new lesion(s) in otherwise PR status of the LD of target lesions will be classified as "PR with new lesion".

Patients with MR will be allowed to continue on the study if all of the following criteria are met:

1. The patient's ECOG performance status is 0 or 1.
2. The patient's LDH value is not greater than twice the Upper Limit of Normal (ULN), if normal at baseline, or 3 times the ULN if above normal at baseline.
3. The patient does not meet any of the criteria for permanent stopping of study treatment.

Slow Progessive Disease (SPD)

The concept slow progressive disease (SPD) has been defined to allow for the time required for eliciting a stable and effective immune response and to avoid premature withdrawal from the study treatment of some patients who might benefit from continued immunization. For patients meeting the criteria for SPD status, disease progression is therefore not to be considered as a criterion for withdrawal from the study treatment.

It is to be noted that SPD is ***not*** to be considered as a success criterion of the study, and time to progression will be determined as the time of first evidence of disease progression notwithstanding that the patient may have been allowed to continue the study treatment because of having SPD status.

Therefore, patients with tumor volume increase detected but without rapid clinical deterioration should continue to be treated and clinically observed with a stringent imaging schedule to allow detection of a subsequent tumor response.

This will improve the overall assessment of the clinical activity of the combination and more likely capture its true potential to induce clinical responses.

All of the following criteria must be met to continue with the treatment:

4. The patient's ECOG performance status is 0 or 1.
5. The patient's LDH value is not greater than twice the Upper Limit of Normal (ULN), if normal at baseline, or 3 times the ULN if above normal at baseline.
6. The patient does not meet any of the criteria for permanent stopping of study treatment.

15 ADVERSE EVENTS/SERIOUS ADVERSE EVENTS

HDIL-2 side effects have been extensively reported and is listed in Appendix D Severe (grade 4 and 5) and unusual toxicities related to IL-2 will be reported. The treating physician will make the determination to whether or not a toxicity is related or unrelated to HDIL-2.

Toxicities will be assessed on day 1 and Day 2 of each cycle and on Days of ASCI administration. Toxicity will be graded according to the NCI Common Toxicity Criteria (CTC), Version 4.0.
All toxicities will be reviewed monthly at the departmental research meeting.

All adverse events will be recorded according to this table:

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

- General therapy related events:

  i. Catheter related events
  ii. Rash related to antibiotic use

- The electronic database will capture all events and/or lab values that either require intervention or are grade 3/4.

Serious Adverse Events Reporting Requirements

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

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience 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 (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).
- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.
- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.
- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have

returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

· Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Reporting to FDA:
· Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

Reporting Clinical Safety data to GSK Biologicals Clinical Safety and Pharmacovigilance (BCSP) Department:
Fax: + 32 (0) 2 656 51 16 or + 32 (0) 2 656 80 09

The Investigator will be responsible for reporting AEs and SAEs to GSK Bio:

All SAEs arising during the study in patients exposed to the study drug will be reported to GSK Bio by facsimile (using a mutually agreed SAE form within 24 hours of first becoming aware of the event; Any new pIMD of any grade, whether serious or not (section 15.1.1) or exacerbation of a pre-existing pIMD must be reported to GSK Bio using a mutually agreed reporting form within 24 hours of first becoming aware whether it is considered serious or not; pregnancy information on any female patient who becomes pregnant while participating in this study or female partner of a male subject participating in this study, and following exposure to study drug will be reported to GSK Bio using a mutually agreed pregnancy reporting form and within 24 hours of first becoming aware of the pregnancy.

Careful evaluation to ascertain the toxicity, immunologic effects and anti-tumor efficacy of therapy will be performed. The principal investigator will monitor the data and toxicities to identify trends. The principal investigator will be responsible for revising the protocol as needed to maintain safety. The MD Anderson IRB will review adverse events as they are submitted. The principal investigator will also review adverse events and evaluate trends. Whenever a trend is identified, the principal investigator will determine an appropriate follow up plan.

Study Stopping Criteria:

Safety, including SAE's, will be monitored monthly in the Department of Melanoma Medical Oncology Research meeting. The following will be monitored;

1. Any deaths
2. Any grade 4 events possibly, probably, or definitely related to ASCI have occurred; enrollment into the trial will stop pending a review of the data with the MDACC IRB and/or DSMB.
3. Additionally, if in the opinion of the study's Principal Investigator, the grade -3/4 toxicity rate in this study exceeds expected rates, enrollment will stop pending a review with the MDACC IRB and/or DSMB.

All drug-related toxicities must be followed until resolution or until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event. Patients are to be followed for 30 days after last drug administration for adverse events, and/or pregnancy regardless of causal relationship.
The investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

15.1 Adverse events of specific interest

15.1.1 Potential immune-mediated diseases

Potential immune-mediated diseases (pIMDs) are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology. AEs that need to be recorded and reported as pIMDs include those listed in the table below.

However, the investigator will exercise his/her medical and scientific judgement in deciding whether other immune-mediated diseases have an autoimmune origin (i.e. pathophysiology involving systemic or organ-specific pathogenic autoantibodies) and should also be recorded as a pIMD.

List of potential immune-mediated diseases

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve disorders, including paralyses/paresis (e.g. Bell's palsy), and neuritis (e.g. optic neuritis) • Multiple sclerosis (including variants) • Transverse myelitis • Guillain-Barré syndrome, (including Miller Fisher syndrome and other variants) • Other demyelinating diseases (including acute disseminated encephalomyelitis)	• Systemic lupus erythematosus • Scleroderma (including, CREST syndrome and morphoea) • Systemic sclerosis • Dermatomyositis • Polymyositis • Antisynthetase syndrome • Rheumatoid arthritis, • Juvenile chronic arthritis, (including Still's disease) • Polymyalgia rheumatica • Reactive arthritis	• Psoriasis • Vitiligo • Raynaud's phenomenon • Erythema nodosum • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) • Cutaneous lupus erythematosus • Alopecia areata • Lichen planus • Sweet's syndrome

• Myasthenia gravis (including Lambert-Eaton myasthenic syndrome) • Non-infectious encephalitis/ encephalomyelitis • Neuritis (including peripheral neuropathies)	• Psoriatic arthropathy • Ankylosing spondylitis (including undifferentiated spondylarthritides) • Relapsing polychondritis • Mixed connective tissue disorder	
Liver disorders	**Gastrointestinal disorders**	**Metabolic diseases**
• Autoimmune hepatitis • Primary biliary cirrhosis • Primary sclerosing cholangitis • Autoimmune cholangitis.	• Crohn's disease • Ulcerative colitis • Ulcerative proctitis • Celiac disease	• Autoimmune thyroiditis (including Hashimoto thyroiditis) • Grave's or Basedow's disease • Diabetes mellitus type I • Addison's disease

Vasculitides	**Others**
• Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis. • Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome, thromboangiitis obliterans (Buerger's disease), necrotizing vasculitis, allergic granulomatous angiitis, Henoch-Schonlein purpura, anti-neutrophil cytoplasmic antibody positive vasculitis, Behcet's syndrome, leukocytoclastic vasculitis. • Vasculitides secondary to other immune mediated diseases such as lupus vasculitis and rheumatoid vasculitis.	• Autoimmune hemolytic anemia • Autoimmune thrombocytopenias • Antiphospholipid syndrome • Pernicious anemia • Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis) • Uveitis • Autoimmune myocarditis/cardiomyopathy • Sarcoidosis • Stevens-johnson syndrome • Sjögren's syndrome • Idiopathic pulmonary fibrosis • Goodpasture syndrome

When there is enough evidence to make any of the above diagnoses, the AE must be reported as a pIMD. Symptoms, signs or conditions which might (or might not) represent the above diagnoses, should be recorded and reported as AEs but not as pIMDs until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

16 STATISTICAL CONSIDERATIONS

The primary efficacy endpoint of this trial is the response rate (RR) as defined in Section 14 measured at week 8. A 2-stage Fleming design will be used to assess the response rate, assuming H0: RR≤5% and Ha: RR≥20%. In the first stage, 15 patients will be treated. If 0 out of 15 patients respond, the trial will be terminated due to inadequate efficacy. Accrual will be suspended until all 15 patients in Stage I have been fully evaluated for response if there are fewer than 4 responses by the time the 15th patient is enrolled. If a patient drops out of the study prior to the evaluation of the primary endpoint, the patient will be counted as a failure. Should 4 or more of the first 15 patients respond to the treatment, the trial will continue with the second stage of the trial. Otherwise, 15 additional patients will be treated in the second stage. If 4 or more of the 30 patients respond to treatment, a RR greater than 20% cannot be rejected and HDIL2 in combination with recMAGE-A3 + AS15 will be taken to a randomized phase II or phase III trials. If however, fewer than 4 out of 30 patients respond to treatment,

we will reject the alternative hypothesis that the RR is greater than or equal to 20%. The overall type I error rate of this design is 5.8%, and the power to detect a response rate of 20% is 86.5%.

Safety Monitoring

The safety profile of HDIL-2 is well known. We do not expect the administration of ASCI to increase the toxicity of HDIL-2 as most toxicity reported from using ASCI have been related to local site reaction. Safety including SAE will be monitored monthly in department of Melanoma Medical Oncology Research meeting. According CTCAE v4.0

Analyses

The association between response and disease and demographic characteristics of interest will be assessed using logistic regression.

Adverse events and laboratory parameters will be summarized using standard statistical measures, including the mean, standard deviation, median, quartiles, and range for continuous measurements and frequency distribution for categorical variables.

The distribution of overall survival, progression-free survival, and the duration of response will be estimated using the Kaplan-Meier method. Cox proportional hazards regression will be used to assess the association between survival parameters and covariates of interest.

Logistic regression will be used to assess the association between immune response and clinical response.

17 LABORATORY CORRELATES

17.1 Measurement of MAGE-A3-specific antibody

Serum at pre-treatment and the indicated post-treatment time points will be analyzed for the titer of antibodies (anti-MAGE-A3 and if possible Protein D IgG) using ELISA. The recMAGE-A3 protein will be used as the target protein. This ELISA assay will be performed by GSK Biologicals or a sub-contracted lab as a batch analysis of all collected serum samples at the end of the clinical trial. The IMCL at MD Anderson Cancer Center will collect and store all the serum samples during the clinical trial and will then ship aliquots of serum to the GSK Bio labs for this analysis.

17.2 Measurement of MAGE-A3-specific effector $CD4^+$ T-cell

MAGE-A3-specific CD4+ T-cell responses will be monitored in PBMC preparations isolated at the indicated time points after the start of ASCI injection

and after the different HDIL-2 therapy cycles as indicated in the treatment and sampling schema. The overall strategy of sampling and antigen-specific T-cell response testing is designed to test the following key questions: 1) whether ASCI injection induces an overall MAGE-A3-specific CD4+ T-cell response, and 2) how HDIL-2 therapy cycles alter the levels of MAGE-A3-specific CD4+ T-cells by measuring samples from vaccinated patients before and after HDIL-2 therapy cycles. In all cases, PBMC from pre-immunization blood samples will be baseline controls to gauge the extent of the induced antigen-specific T-cell response.

The MAGE-A3 protein-specific CD4+ T-cell responses will be determined using intracellular staining (ICS) for IFN-γ and TNF-α production in CD4+ T cells in PBMC samples *ex vivo* and after a 2-week *in vitro* re-stimulation with recMAGE-A3 protein.

In a subset of patients carrying a heterozygous or homozygous HLA-DP-0401/0402 (DP4) allele, we will also track changes in the frequency of MAGE-A3-specific CD4+ T cells using flow cytometry after staining with a MAGE-A3 peptide HLA-DP4 multimer. This multimer stains CD4+ T-cells specific for the MAGE-A3 DP4 epitope KKLLTQHFVQENYLEY (amino acid sequence 243-258). We have this reagent on-hand in our laboratory and have already successfully tracked MAGE-A3 DP4 peptide epitope responses using this multimer. The frequency of DP4 allele carriers in the human population is between 60-70%, thus, most of the patients recruited into this clinical trial will be able to be tested using this approach.

17.3 Measurement of MAGE-A3-specific CD8+ T-cell

A similar set of ICS assays used for CD4+ T-cell responses will be used to study MAGE-A3-specific CD8+ T-cell responses. Here, we will measure changes in CD8+ T-cell activity against the HLA-A2.1-restricted epitope FLWGPRALV (MAGE-A3 amino acid sequence 271-279) in PBMC collected from HLA-A2.1+ patients (about 40-45% of the patient population). An HLA-A24 MAGE-A3 epitope (VAELVHFLL) has also been identified and can be used to monitor CD8+ T-cell responses in HLA-A24+ patients (about 10-15% of the patient population). Thus, together these epitopes will cover the majority of patients recruited into the clinical trial. CD8+ T-cell responses against these peptides in will be assayed ex vivo and after a 7-day in vitro stimulation of PBMC with the peptide. Using an HLA-A2.1 tetramer containing the FLWGPRALV peptide, we will also track changes in the frequency of antigen-specific CD8+ T cells in HLA-A2.1+ patients during the vaccination treatment.

17.4 Detection of MAGE-A3-specific CD4+, CD25+, Foxp3+ T-regulatory cells

A critical issue in tumor antigen immunization, especially whole protein and HLA class II peptide vaccines is the activation and expansion of vaccine antigen-specific CD4+, CD25+, Foxp3+ T-regulatory cells (T-regs) that inhibit anti-tumor effector T cells. T-regs have been shown in cancer vaccine clinical trials to expand in response to multiple antigen boosting and accumulate over time as boosting continues. Secondly, HDIL-2 therapy potently stimulates and induces T-reg cell expansion in melanoma patients and other cancer patients. Thus, an important question in this clinical trial is whether the combination of MAGE-A3 protein vaccination together with HDIL-2 expands MAGE-A3-specific T-regs or T-regs of other melanoma specificities (due to antigen/epitope spreading induced by the vaccination). The first approach we will use to monitor MAGE-A3-specific T-regs will be to use our MAGE-A3 peptide DP4 multimer reagent and track changes in this T-reg population in DP4+ immunized patients.

17.5 Gene profiling of tumor tissue

Testing will be performed by GSK Biologicals or a contracted laboratory on RNA extracted from fresh tumor tissue obtained from the biopsy at baseline and optional one at week 8 and stored in RNA later. Gene expression profiling the presence or absence of the predictive gene signature will be assessed by an appropriate technology such as qRT-PCR, microarray, or ICH on these samples and correlated with the patient's clinical data. Mutational analysis will also be performed. Additional gene profiling might be performed by GSK Biologicals (or contracted lab) in the FFPE tumor samples used for MAGE-A3 expression analysis, including MAGE-A3 negative samples.

18 Administrative Procedures

18.1 Changes to the protocol

Any change or addition to this protocol requires a written protocol amendment that must be approved by the IRB. A copy of the written approval of the IRB must be received before implementation of any changes. The IRB must review and approve all amendments to the protocol.

18.2 Ethics and good clinical practice:

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

18.2.1 ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.

18.2.2 US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).

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

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

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