A Randomized Placebo-Controlled Phase III Trial of Yeast Derived GM-CSF VS Peptide Vaccination VS GM-CSF Plus Peptide Vaccination VS Placebo in Patients with "No Evidence of Disease" after Complete Surgical Resection of "Locally Advanced" and/or Stage IV Melanoma: A trial of the ECOG-ACRIN Cancer Research Group (E4697)

Lawson, et al

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3. Selection of Patients

NOTE: For SWOG and CALGB institutions: All questions regarding eligibility should be directed to the ECOG Coordinating Center at (617) 632-3610.

3.1 Patients must have HLA-A2 status known prior to randomization. Typing may be obtained through a local laboratory facility or through a reference lab utilized by the initiating institution. If typing is not available through these means, it may be obtained from the University of Pittsburgh (see Section 5.1).

HLA-A2 status known prior to randomization? Yes _____ No _____

Date of randomization _____ HLA-A2 status _____

3.2 All patients must have disease completely resected with one of the following in order to be eligible:

3.2.1 Completely resected disease? Yes _____ No _____

a. Any locoregional recurrence after prior adjuvant interferon or failure on S008?

Yes _____ No _____ Reason _____

b. Any local recurrence of disease after adequate surgical excision of the original primary?

Yes _____ No _____

c. Mucosal melanoma?

Yes _____ No _____

d. Stage IV melanoma (cutaneous, ocular, mucosal, or unknown primary)?

Yes _____ No _____ Type _____

3.2.2 The following groups of patients may be entered onto this trial only if they are ineligible for S0008 or are, in the opinion of the managing physician, medically unfit to receive standard high-dose interferon.

Ineligible for S0008? Yes ____ No _____

Medically unfit to receive standard high-dose interferon?

Yes _____ No _____

Managing physician's signature _____

a. Any clinically evident satellite or intransit disease?

Clinically evident satellite? Yes ____ No ____

Intransit disease? Yes _____ No _____

b. Stage II disease with gross extracapulsar extension? Stage II disease?

Yes _____ No _____

Gross extracapulsar extension? Yes _____ No _____

c. Recurrence in a previously resected nodal basin?

Yes _____ No _____

d. Four or more involved lymph nodes or matted lymph nodes.

Four or more involved lymph nodes? Yes _____ No _____

Matted lymph nodes? Yes _____ No _____

e. Ulcerated primary melanoma and any involved lymph nodes?

Ulcerated primary melanoma? Yes _____ No _____

Involved lymph nodes? Yes _____ No _____

NOTE: Patients who are eligible for S0008 will be strongly encouraged to participate in that study in preference to this one.

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3.3 Patients must have been surgically rendered free of disease with negative margins on resected specimens. Patients rendered free of disease by non-surgical means are not eligible.

Surgically rendered disease-free? Yes _____ No _____

Negative margins? Yes _____ No _____

3.4 Patients must be randomized within 112 days (16 weeks) of surgical resection. If more

than one surgical procedure is required to render the patient disease-free, all required surgeries must be accomplished within this 16 week time period.

Date of Randomization: _____ Date of surgical resection _____

More than 1 surgery required to render patient disease-free?

Yes ____ No _____ Number of surgeries _____

Dates of surgeries _____, _____, _____, _____

3.5 Patients must not have received any adjuvant treatment (chemotherapy, biotherapy, or limb perfusion) **after the resection(s) that make(s) them eligible for this trial.** One systemic treatment after a **prior** surgery is allowed, and must have been completed **>** 8 weeks prior to randomization. [When chemotherapy and biotherapy are given together as one planned treatment (biochemotherapy), this counts as one regimen.]

NOTE: Previous radiation therapy, including after the resection, is allowed as long as 30 days elapse between the radiation and initiation of therapy.

Adjuvant treatment (chemotherapy, biotherapy, limb perfusion) received after resection?

Yes ____ No _____

Systemic treatment after a prior surgery? Yes _____ No _____

Date of systemic treatment _____

Previous radiation therapy (including after resection)? Yes _____ No _____

Date of radiation _____

3.6 Prior treatment with GM-CSF or any peptides used in this protocol, is not allowed.

Prior treatment with GM-CSF or any other peptides used in this protocol?

Yes ____ No _____

3.7 Patients must be **>** 18 years of age. Age _____

3.8 Patients must have ECOG performance status of 0-1.

ECOG performance status _____

3.9 Patients must not have an active infection requiring treatment with parenteral antibiotics.

Active infection? Yes _____ No _____

Treatment with parenteral antibiotics? Yes _____ No _____

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3.10 Patients must not have other significant medical, surgical, or psychiatric conditions or require any medication or treatment that may interfere with compliance on any of the E4697 treatment regimens.

Significant medical, surgical or psychiatric conditions? Yes _____ No _____

Condition requiring any medication or treatment interfering with compliance?

Yes _____ No _____

3.11 Patients must not have a diagnosis or evidence of organic brain syndrome or significant impairment of basal cognitive function or any psychiatric disorder that might preclude participation in the full protocol.

Diagnosis or evidence of organic brain syndrome? Yes _____ No _____

Diagnosis or evidence of significant impairment of basal cognitive function?

Yes _____ No _____

Diagnosis or evidence of any psychiatric disorder?

Yes ____ No _____

3.12 Patients must be able to self-administer or arrange for administration of subcutaneous injections.

Ability to self-administer subcutaneous injections? Yes _____ No _____

Ability to arrange for administration of subcutaneous injections?

Yes _____ No _____

3.13 Patients who have other current malignancies are not eligible.

Other current malignancies? Yes ___ No _____

3.14 Patients with prior history at any time of any in situ cancer, lobular carcinoma of the breast in situ, cervical cancer in situ, atypical melanocytic hypeplasia or Clark I melanoma in situ are eligible.

Any in situ cancer? Yes ____ No____

Atypical melanocyctic hyperplasia? Yes _____ No_____

Clark melanoma in situ? Yes _____ No_____

3.15 Patients with prior history of basal or squamous skin cancer are eligible.

Basal or squamous skin cancer? Yes _____ No _____

3.16 Patients who meet either eligibility criteria 3.15 or 3.16 must be disease-free at time of randomization.

Disease-free at time of randomization? Yes _____ No _____

3.17 Patients who have had multiple primary melanomas are eligible.

Multiple primary melanomas? Yes _____ No _____

3.18 Patients with other malignancies are eligible if they have been continuously disease free for > 5 years prior to the time of randomization.

Other malignancies? Yes ____ No ____

Disease-free? Yes _____ No _____

Date of last treatment _____

3.19 Patients must not have autoimmune disorders, conditions of immunosuppression or treatment with systemic corticosteroids, including oral steroids (i.e., prednisone, dexamethasone), continuous use of topical steroid creams or ointments, or any steroid containing inhalers. Replacement doses of steroids for patients with adrenal insufficiency are allowed. Patients who discontinue use of these classes of medication for at least 2 weeks prior to randomization are eligible if, in the judgement of the treating physician, the patient is not likely to require these classes of drugs during the study.

Autoimmune disorder? Yes _____ No _____

Condition of immunosuppresion? Yes _____ No _____

Treatment with systemic corticosteroids (including oral steroids)?

Yes _____ No _____

Continuous use of topical steroid creams/ointments? Yes _____ No _____

Use of steroid containing inhalers? Yes _____ No _____

Adrenal insufficiency? Yes _____ No _____

Date of last dose of steroid containing medicines _____

3.20 Women of childbearing potential must not be pregnant (negative bHCG within 2 weeks prior to randomization) or breast-feeding due to the unknown effects of peptide vaccines and/or GM-CSF on a fetus.

Female of child-bearing potential? Yes _____ No _____

Date of pregnancy test:_____

Breast-feeding? Yes _____ No _____

3.21 Women of childbearing potential and sexually active males must be counseled to use an accepted and effective method of contraception (including abstinence) while on treatment and for a period of 18 months after completing or discontinuing treatment.

Method: _____

3.22 All patients must have brain CT or MRI, chest CT or CXR, and abdominal (liver) CT or MRI within 4 weeks prior to randomization (see Sec. 3.181 for exceptions). PET scans are also acceptable in place of CT, CXR and/or abdominal MRI if obtained within 4 weeks prior to randomization. Patients with lesions on the lower extremity must also have pelvic imaging within this time period; this is also strongly recommended for patients with lesions on the lower trunk. PET scans are acceptable.

CT scan/MRI? Yes _____ No _____ Date _____

Chest CT/CXR? Yes _____ No _____ Date _____

Abdominal liver CT or MRI? Yes _____ No _____ Date _____

PET scan? Yes _____ No _____ Date _____

Lesions on the lower extremities/lower trunk?

Yes _____ No _____

Pelvic imaging? Yes _____ No _____ Date _____

PET scan? Yes _____ No _____ Date _____

3.22.1 Patients with resection of visceral disease must have imaging of the affected area/organ documenting disease-free status within 2 weeks prior to randomization.

Resection of visceral disease? Yes _____ No _____

Type of imaging: _____ Date _____

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3.23 Patients must have WBC > 3,000/mm³ and platelet count > 100,000/mm³ obtained within

4 weeks prior to randomization.

WBC count: _____ Date _____

Platelet count: _____ Date _____

3.24 Patients must have SGOT (AST) and bilirubin < 2 x institutional upper limit (IUL) of normal and serum creatinine < 1.8 mg/dl, all obtained within 4 weeks prior to randomization.

AST Level _____ IUL _____ Date _____

Bilirubin level _____ IUL _____ Date _____

Serum creatinine level _____ Date _____

3.25 Alkaline phosphatase and LDH must be performed within 4 weeks prior to randomization. LDH must be normal. Patients with abnormal alkaline phosphatase which is < 1.25 times the institutional upper limit of normal who have a negative CT or MRI of the liver and negative bone scan or a negative PET scan (see Sec. 3.18) are eligible.

LDH level _____ Date _____

Alk Phos must be < 1.25 ULN.

Alk Phos level _____ Date of test_____ ULN_____

Negative CT or MRI of liver? Yes _____ No _____ Date _____

Negative bone scan? Yes _____ No _____ Date _____

Negative PET scan? Yes _____ No _____ Date _____

3.26 Patients with bone pain must have a bone scan within 4 weeks prior to randomization to document the absence of tumor.

Bone pain? Yes _____ No _____

If yes, date of bone scan _____

_____

_____

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SCHEMA IN FIGURES

5. Treatment Plan

NOTE: For SWOG and CALGB institutions: all questions regarding treatment or dose modifications should be directed to the ECOG study chairs.

5.1 HLA-A2 positive patients will be randomized to receive either GM-CSF plus peptide vaccination, GM-CSF placebo plus peptide vaccination, GM-CSF plus peptide placebo,

or GM-CSF placebo plus peptide placebo. HLA-A2 negative patients will be randomized to receive either GM-CSF or GM-CSF placebo.

NOTE: If HLA typing cannot be performed at the registering institution, it can be done at the University of Pittsburgh free of charge. Institutions can contact the ECOG Study Coordinator at (412) 624-3277 to request that typing be done. Institutions must allow one week for testing results to be ready.

5.2 Treatment Administration

NOTE: 1 cycle = 28 days

5.2.1 GM-CSF Plus Peptide Vaccination

GM-CSF will be administered at a fixed dose of 250 µg subcutaneously for 14 days followed by 14 days off every 28 days for one year (13 cycles) or until disease recurrence.

Each peptide vaccine will be individually prepared using Montanide ISA-51 or Montanide ISA-51 VG and administered subcutaneously in two contiguous 1 ml aliquots (i.e., choose a center location, inject first 1 ml slightly to right of center location with syringe needle directed to right [i.e., away from the center location] inject second 1 ml slightly to left of center location with syringe needle directed to left [i.e., away from the center location]). Peptide vaccines should be injected in the vicinity of one of the major nodal basins. The basin must not have been dissected. Each peptide vaccine should be placed in a separate extremity or truncal location and the sites should be rotated. Vaccinations (6 shots, 3 locations) will be given on days 1 and 15 of the first cycle (1 cycle = 28 days) and on day 1 of each subsequent cycle for one year (13 cycles) or until disease recurrence.

After recurrence, GM-CSF and peptide vaccines will be administered as described in Section 5.3.

5.2.2 GM-CSF Placebo Plus Peptide Vaccination

GM-CSF placebo will be administered at a fixed dose of 250 µg subcutaneously for 14 days followed by 14 days off every 28 days for one year (13 cycles) or until disease recurrence.

Each peptide vaccine will be individually prepared using Montanide ISA-51 or Montanide ISA-51 VG and administered subcutaneously in two contiguous 1 ml aliquots (i.e., choose a center location, inject first 1 ml slightly to right of center location with syringe needle directed to right [i.e., away from the center location], inject second 1 ml slightly to left of center location with syringe needle directed to left [i.e., away from the center location]). Peptide vaccines should be injected in the vicinity of one of the major nodal basins.

The basin must not have been dissected. Each peptide vaccine should be placed in a separate extremity or truncal location and the sites should be rotated. Vaccinations (6 shots, 3 locations) will be given on days 1 and 15 of the first cycle (1 cycle = 28 days) and on day 1 of each subsequent cycle for one year (13 cycles) or until disease recurrence.

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After recurrence, GM-CSF placebo and peptide vaccines will be administered as described in Section 5.3.

5.2.3 GM-CSF Plus Peptide Placebo

GM-CSF will be administered at a fixed dose of 250 µg subcutaneously for 14 days followed by 14 days off every 28 days for one year (13 cycles) or until disease recurrence.

Each peptide placebo will be individually prepared using Montanide ISA-51 or Montanide ISA-51 VG and administered subcutaneously in two contiguous 1 ml aliquots (i.e., choose a center location, inject first 1 ml slightly to right of center location with syringe needle directed to right [i.e., away from the center location] inject second 1 ml slightly to left of center location with syringe needle directed to left [i.e., away from the center location]). Peptide placebos should be injected in the vicinity of one of the major nodal basins. The basin must not have been dissected. Each peptide placebo should be placed in a separate extremity or truncal location and the sites should be rotated. Vaccinations (6 shots, 3 locations) will be given on days 1 and 15 of the first cycle (1 cycle = 28 days) and on day 1 of each subsequent cycle for one year (13 cycles) or until disease recurrence.

After recurrence, GM-CSF and peptide placebo will be administered as described in Section 5.3.

5.2.4 GM-CSF Placebo Plus Peptide Placebo

GM-CSF placebo will be administered at a fixed dose of 250 µg subcutaneously for 14 days followed by 14 days off every 28 days for one year (13 cycles) or until disease recurrence.

Each peptide placebo will be individually prepared using Montanide ISA-51 or

Montanide ISA-51 VG and administered subcutaneously in two contiguous 1 ml aliquots (i.e., choose a center location, inject first 1 ml slightly to right of center location with syringe needle directed to right [i.e., away from the center location] inject second 1 ml slightly to left of center location with syringe needle directed to left [i.e., away from the center location]). Peptide placebos should be injected in the vicinity of one of the major nodal basins. The basin must not have been dissected. Each peptide placebo should be placed in a separate extremity or truncal location and the sites should be rotated. Vaccinations (6 shots, 3 locations) will be given on days 1 and 15 of the first cycle (1 cycle = 28 days) and on day 1 of each subsequent cycle for one year (13 cycles) or until disease recurrence.

After recurrence, GM-CSF placebo and peptide placebo will be administered as described in Section 5.3.

5.2.5 GM-CSF

GM-CSF will be administered at a fixed dose of 250 µg subcutaneously for 14 days followed by 14 days off every 28 days for one year (13 cycles) or until disease recurrence.

After recurrence, GM-CSF will be administered as described in Section 5.3.

5.2.6 GM-CSF Placebo

GM-CSF placebo will be administered at a fixed dose of 250 µg subcutaneously for 14 days followed by 14 days off every 28 days for one year (13 cycles) or until disease recurrence.

After recurrence, GM-CSF placebo will be administered as described in Section 5.3.

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5.3 Treatment after Disease Recurrence (All Patients)

If surgically resectable, resect (complete E4697 Mid-Treatment Surgery Form #1380). Patients will then be given the option to continue treatment. This treatment will be with the blinded therapy to which the patient was initially assigned. Treatment will continue for 6 cycles or until they complete one year of protocol treatment (whichever is longer).

If not surgically resectable, or in the event of a **second recurrence**, discontinue protocol treatment and treat at the discretion of the physician (complete ECOG Melanoma Recurrence Form #1379). Patients will not be unblinded in the case of discontinuation of

protocol treatment due to progressive disease. All patients will be followed for survival until death.

No dose modifications

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6. Measurement of Effect
6.1 Overall survival, time to disease progression and survival at 2 years are the primary criteria of treatment effect. Patients will be evaluated for recurrence and survival as indicated in Section 7.0 (footnote 2).

2 Once off treatment, all patients will be followed per the standard ECOG follow-up schedule. Once disease has progressed, patients are to be followed for survival only per the standard ECOG follow-up schedule. Every 3 months if patient is < 2 years from study entry, every 6 months if patient is 2-5 years from study entry, and every 12 months if patient is > 5 years from study entry.

6.2 Documentation of recurrence will require specification of all sites involved to establish the pattern of recurrence.
6.3 Histological or cytological evidence of recurrence should be attempted in all cases except for brain metastases. Strong consideration should be given to resection of solitary brain lesions in the absence of other systemic disease.
6.4 The following criteria of treatment failure constitute the only acceptable evidence of disease recurrence.
6.4.1 Lung
Positive cytology or biopsy in the presence of a single new lesion or the appearance of multiple lesions consistent with metastatic disease.
6.4.2 Liver
Positive cytology or biopsy in the presence of a single new lesion or the appearance of multiple lesions consistent with metastatic disease.
6.4.3 Central Nervous System
A positive brain CT or MRI scan or CSF cytology.
6.4.4 Cutaneous, Subcutaneous and Lymph Node Recurrence
Positive cytology or biopsy in the presence of a single new lesion or the appearance of multiple lesions consistent with metastatic disease.
6.4.5 Bone and Other Organs
Positive cytology or biopsy in the presence of a single new lesion or the appearance of multiple lesions consistent with metastatic disease identified on two different radiologic studies: i.e., positive gallium scan and contrast GI series or ultrasound, x-ray or CT of abdomen for abdominal disease.

REASONS FOR EARLY CESSATION OF TREATMENT WERE UNRESECTABLE DISEASE PROGRESSION OR PATIENT REFUSAL OR NONCOMPLIANCE, NOT SPELLED OUT IN ANY SECTION OF THE PROTOCOL

2. Objectives

Patients are divided into HLA-A2 positive and negative groups. Since peptide vaccination is an option only for the A2 positive patients, they will be randomized, using a 2 x 2 factorial design,

into one of four treatment regimens. The A2 negative patients will be randomized to receive GMCSF
or placebo. Objectives for the laboratory correlate are also listed.
2.1 The primary objective of this study is to compare overall survival and disease-free survival of patients with completely resected stage IV melanoma or stage III melanoma with gross extranodal extension, satellites, and/or intransit lesions, treated with GM-CSF vs. no GM-CSF, or other high risk patients listed in the eligibility section (Section 3.0).
2.2 The secondary objective is to compare, using a 2 x 2 factorial design, overall survival and disease-free survival of HLA-A2 positive patients treated with peptide vaccination vs. no peptide vaccination.
2.3 The following descriptive evaluations of survival and disease-free survival are planned for the HLA-A2 positive patients:
(1) GM-CSF plus peptide vaccination vs. peptide vaccination alone;
(2) GM-CSF plus peptide vaccination vs. GM-CSF alone;
(3) GM-CSF plus peptide vaccination vs. placebo.
In addition, for descriptive purposes, survival and disease-free survival of HLA-A2 positive patients not receiving peptide vaccination will be compared to that of HLA-A2 negative patients not receiving peptide vaccination.
2.4 The objectives of the laboratory correlates of this study are:
1. To determine the influence of GM-CSF on circulating dendritic cell numbers and subpopulations in peripheral blood of patients receiving and not receiving GM-CSF;
2. To determine, in HLA-A2 positive patients, whether immunization with peptides with or without GM-CSF elicits a measurable T-cell response as assessed by ELISPOT and the MHC tetramer assay, and to determine the functionality of these cells by intracellular cytokine staining.
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9. Statistical Considerations

9.1 Objectives
In this phase III study, HLA-A2 positive patients will be randomized to peptide vaccination plus GM-CSF, peptide vaccination alone, GM-CSF alone, or Placebo. The HLA-A2 negative patients will be randomized between GM-CSF alone and Placebo only.

The primary endpoint in this study is overall survival. We denote the treatment arms containing GM-CSF by GM-CSF+ and the arms not containing GM-CSF are denoted by GM-CSF-. The main objective, and hence primary comparison, in this study is to compare GM-CSF- vs. GM-CSF+ with respect to overall survival in the entire population (i.e., HLA-A2 positive and HLA-A2 negative patients).

The secondary objective in this study is to compare, in a 2 x 2 factorial design, overall survival and disease-free survival of HLA-A2 positive patients treated with peptide vaccine versus those not receiving peptide vaccine. For the HLA-A2 positive patients, we denote the two arms containing peptide vaccine by PEP+ and the two arms not containing peptide vaccine by PEP-. In addition, exploratory analyses will be conducted as outlined in Objective 2.3.

9.2 Summary of New Study Design

The study design was modified in March 2005. The sample size has increased from 600 to 800. The median survival of the control group (GM-CSF-) was longer than what was assumed in the design stage of this study. Based on the recommendation of the ECOG DMC in April 2004, the sample size of this study has been increased to have an adequate power for the primary comparison of overall survival(OS) between GM-CSFand GM-CSF+. The study objectives remain the same.

Briefly the main changes include:

• The sample size has been increased to 800 patients. This will provide 80% power for the comparison of overall survival of GM-CSF- vs. GM-CSF+ groups. The median survival of the control group (GM-CSF-) was estimated to be 40 months and a relative increase of 33% in the median survival was used for the GM-CSF+ group. The percent improvement is the same as in the original design.

• It was estimated that 55% of the study population will be HLA-A2+ patients. Thus the new sample sizes by treatment arm are 110 for arms A-D and 180 for arms E-F.

• Interim analysis plan of study has been updated per current ECOG policies. The truncated O'Brien-Fleming boundaries will be used. The first interim analysis will be performed at 25% information time and subsequently the interim analysis will be repeated every 6 months. The study will be monitored for early stopping in favor of the null hypothesis using Jennison-Turnbull's repeated confidence interval.

• The primary comparison in HLA-A2+ population has been changed from overall survival to disease-free survival by PEP treatment status.

• The power calculations for the secondary endpoints have been updated.

• We have conducted an interim analysis of laboratory results including T-cell reactivity, and circulating DC (lineage negative DR+) cells per protocol. The melanoma committee decided to continue monitoring the changes between day 43

and baseline values of T-cell reactivity, and circulating DC by GM-CSF treatment status.

9.3 Study Design

9.3.1 Sample Size and Accrual

The primary comparison will be an intent to treat analysis of overall survival (OS) between GM-CSF- vs. GM-CSF+ groups in eligible patients. The primary analysis of OS will be performed using a two-sided log-rank test stratified on HLA-A2 status, site of metastases, number of metastatic lesions, using an overall type I error of 0.05.

Allowing for the interim analysis plan discussed in section 9.3.2, a total of 800 patients are planned and 400 deaths are anticipated. Of the 800 patients, 440 (55%) patients are expected to be HLA-A2+ and will be randomized to treatment arms A-D. The remaining 360 (45%) patients are expected to HLA-A2- and will be randomized to treatment arms E and F. With an anticipated accrual rate of 15 patients per month, accrual would be completed by September 2006.

The current data indicates that the ineligibility rate in this study has been less than 5%. Thus we assume that of the 800 patients enrolled to this study, 768 (96%) patents will be eligible.

9.3.2 Interim Analysis

It is expected that the median survival of patients who do not receive GM-CSF will be 40 months. It is assumed GM-CSF will improve the median survival to 53.3 months. The proposed design will provide 80% power to detect 33% relative increase in the median overall survival from 40 months to 53.3 months. Interim analyses of OS will be performed for all semi-annual DMC meetings beginning when approximately 25% of the planned full information (400 deaths among eligible patients) has occurred, continuing until either criteria for early stopping are met or full information is reached. To preserve the overall type I error rate, critical values at the interim analyses will be determined using a truncated version of of the Lan-DeMets spending function corresponding to the O'Brien-Fleming boundary. Boundary values at a nominal significance less than 0.0005 will be truncated at 0.0005, with the boundary also adjusted to preserve the overall two-sided type I error rate of 5%. Under the accrual and failure rate assumptions above, interim analyses would be expected to occur at 32, 38, 44, 50, 56, 62, 68, 74 and 77.3 months which correspond to information times of

25%, 33%, 44%, 55%, 66%, 77%, 87%, 96% and 100%. Table 1 below summarizes operating characteristics of the proposed monitoring plan.

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Table 1. Operating Characteristics of Interim Analysis for OS

Repeated Analyses	Information Time	No. of Deaths under H1	Upper Boundary	Nominal Significance
1	0.25	97.94	3.2905	0.500000E-03
2	0.33	133.85	3.2905	0.500000E-03
3	0.44	174.02	3.2905	0.500000E-03
4	0.55	218.03	2.9293	0.169860E-02
5	0.66	264.67	2.5843	0.487923E-02
6	0.77	307.86	2.3835	0.857436E-02
7	0.87	346.77	2.2442	0.124105E-01
8	0.96	381.85	2.1406	0.161551E-01
9	1.00	399.64	2.1197	0.170149E-01

This study will also be monitored for early stopping in favor of the null hypothesis using Jennison-Turnbull repeated confidence interval (RCI). At each interim analysis the RCI on the hazard ratio will be computed using the critical value from the error spending rate function. If this RCI does not include the target alternative hazard ratio of 1.33 (GM-CSF-/ GM-CSF+ groups), then the study may be stopped early for lack of benefit.

If criteria for early stopping are not met, then the final analysis will be performed when approximately 400 deaths have been observed. If for any reason that does not occur within 30 months after completion of accrual, then the final analysis will be performed 30 months after the study is closed to accrual.

Disease-free survival (DFS) will be also compared between GM-CSF+ and GMCSF- groups. The DFS is defined as time from randomization to date of recurrence or death, whichever comes first. We assume that the median DFS is 11 months in GM-CSF- group and 13.6 months in GM-CSF+ group. There will be 80% power to detect 24% relative increase in the median DFS. The stratified logrank test, stratifying on HLA-A2 status, site of metastases, number of metastatic lesions will be used for this comparison. A two-sided type I error rate of 0.05 will be used.

We mention here that the immunobiologic constraints of peptide recognition dictate the design chosen, and thus there is no scientific basis for randomizing HLA-A2 negative patients to peptide interventions. Moreover, there is no scientific rationale for assuming differences in survival for the HLA-A2 positive and HLA-A2 negative patients, and there is no literature or scientific basis for assuming that the effect of GM-CSF would be different in the HLA-A2 positive or HLA-A2 negative groups.

9.3.3 Secondary Objectives

The main secondary comparison will be DFS of PEP+ vs. PEP- groups in HLAA2+ patients. We will use the cure rate model for this endpoint. The cure rate model specifies that the target population is a mixture with proportion p who will be cured and 1-p who will fail according to an exponential distribution with rate l. The DFS function at time t can be expressed as:

$S(t)= p + (1-p) \exp(-lt)$.

We assume that the cure rate is 20% in PEP- group and 28% in PEP+ group. The median DFS of patients considered not cured is 9 months in PEP- group and 12 months in PEP+ group. With 220 (for 211 eligible) patients in each group and the follow-up time of 24 months, there will be 80% power to detect 40% relative increase in the cure rate and 25% relative increase in the median DFS of not cured population.

Interim analyses of DFS will be performed when the interim analysis of the primary comparison for OS (Section 9.3.2) is conducted. Based on the cure rate model, the full information corresponds to 316 events. The first interim analysis for the DFS endpoint is expected to occur at 39% information time. Subsequent analyses will be at all semi-annual meetings. The truncated O'Brien-Fleming boundaries will be used for the interim analyses with an overall two-sided type I

error rate of 0.05. The stratified log-rank test, stratifying on the presence and absence of GM-CSF, will be used in this comparison. Table 2 below summarizes operating characteristics of the proposed monitoring plan for DFS.

Table 2. Operating Characteristics of Interim Analysis for DFS in HLA-A2+ Patients

Repeated Analyses Information Time	No. of Events	Upper Boundary	Nominal Significance
1 0.39	122	3.2905	0.500000E-03
2 0.50	157	3.0499	0.114475E-02
3 0.61	192	2.6996	0.347095E-02
4 0.72	228	2.4672	0.680938E-02
5 0.83	263	2.2886	0.110514E-01
6 0.91	286	2.1979	0.139769E-01
7 0.960	303	2.1604	0.153692E-01
8 0.990	312	2.1478	0.158641E-01
9 1.000	316	2.1643	0.152188E-01

Since PEP vaccine may not be effective in HLA-A2+ patients, we will consider an early suspension of PEP treatment in HLA-A2+ patient population in favor of null hypothesis. In particular, we will compute the conditional power of rejecting the null hypothesis at full information at each interim analysis. If the conditional power is less than 10%, a suspension of PEP vaccine or placebo treatment will be considered in HLA-A2+ patients for lack of benefit.

Based on the interim analysis of DFS by PEP treatment in HLA-A2+ patients, an early suspension of PEP vaccine or placebo can be considered. However, the study will continue with GM-CSF or GM-CSF placebo treatment in case of the suspension of PEP treatment.

In addition, OS of PEP+ vs. PEP- groups in the HLA-A2+ population will be compared. The stratified log-rank test, stratifying on the GM-CSF status, will be used at a two-sided significance level of 0.05.

9.4 Correlative Studies

9.4.1 T-cell Reactivity and Circulating DC

All patients are eligible for the analysis of T-cell reactivity and circulating DC cells. The primary endpoint is a change in number of T-cells and circulating DC cells between baseline and Day 43. We wish to determine if there is a significant difference in this change betweenGM-CSF- and GM-CSF+ groups. Of the 800 patients, we assume 540 patients (67.5%) will provide samples for both baseline and day 43.

Let "d" denote the population median of the difference in change of T-Cell or Lineage negative DR (Day 43- baseline) between the two groups. The statistical analysis for this endpoint will involve doing a two-sample Wilcoxon rank sum test for the null hypothesis that d equals zero against the alternative that d does not equal zero. We can determine the power for such a test using the technique outlined in equation 2 of Section 2.3 of Noether (1987, JASA). Let N- denote the change the GM-CSF- group, and let N+ denote the change in the GM-CSF+ group for an arbitrary patient in the population. For this endpoint, it has been determined that a clinically important difference for detecting the effect of treatment is that a true rate of 60% of the cases have N+ > N-. Assuming a twosided significance level of .05 and a sample size of 540 analyzable patients, the power for detecting such a difference is, 80%.

9.4.2 Immune Response

There will be approximately 440 HLA-A2+ patients who can potentially receive the multi-epitope vaccine. Of these, we assume 400 patients (100 from each arm A-D) will be analyzable for the immune response.

Patients will be eligible for the vaccine, if serotyped (+) for HLA-A2 using a panel of MA2.1 and BB7.2 monoclonal antibodies in conjunction with w6/32, anticipating the majority to be HLA-A2*0201 for study in this analysis (118-120). HLA-DR4 is the MHC class II allele that serves as the restriction element for the CD4 T-cell response to the Melan-A/MART-1:27-35 51-73 epitope we have initially planned to analyze for this study. The restriction elements for T-cell recognition of this epitope beyond DR4 are as yet undefined. However, conservatively limiting our analysis to the population that is DR4 positive, we project approximately 33% of patients to be appropriate on the basis of those we

have studied in the current intergroup E1690/E2690 trials. It is therefore anticipated that at least 33 patients per arm in E4697 will be informative for the CD4 T-cell response analysis. Up to 100 patients per arm will be informative for the CD8 T-cell response analysis.

CD8 T-cell response will serve as the chief endpoint, but CD4 T-cell responses and antibody responses will be evaluated to ascertain the correlation of CD4 with CD8 reactivity. The exploratory studies we have conducted in 6 patients reported in Zarour et al. (121), show that 5 of 6 with CD8 T-cell reactivity to Melan-A/MART-1:27-35 HLA-A2 presented Class I epitope 27-35 have CD4 Tcell reactivity to Melan-A/MART-1:27-35 HLA-DR presented Class II epitope 51-73. The correlation of antibody response with CD4 (or CD8) response has not previously been sought, but the presence of antibody reactivity to both lineage and cancer-testis antigens of melanoma is well recognized (122,123).

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We estimate that 90-95 out of 100 HLA-A2+ analyzable patients in each arm (Arms A-d) will be HLA-2*0201. In the primary analysis, the ELISPOT assay will be scored as positive if the number of CD8+ T-cells (averaged over triplicate assays) reactive to an antigen increases by 26/50,000 at either the one or threemonth evaluation as compared to baseline. This definition of a positiveassay is based on data from previous studies. These data indicate that the increase in spots after vaccine treatment is additive, rather than being proportional to the pre-vaccination number of spots. Previous data indicate that an immune response is any increase in ELISPOT counts that is at least 30 spots. Using this criterion, the numbers of responders from previous data is 2/4 or 50% for P1 (Melan-A/MART-1:27-35), 9/22 or 41% for gp-100, 2/13 or 15% for tyrosinase, 6/21 or 29% for Melan-A/MART-1:27-35 (124-132), and 4/14 or 29% for influenza matrix. Similar proportions of responders are expected using the tetramer assay. We will compare the proportion of PEP+ GM-CSF+ subjects who have positive assays to the proportion of PEP-, GM-CSF+ subjects with positive assays. This comparison will be made separately for each of the peptide antigens Melan-A/MART-1, gp-100, and tyrosinase. We anticipate that at most 5% of subjects not receiving peptide vaccine will show such an increase in CD8+ reactivity. We will be interested in any peptide that has a response rate of at least 30% among

vaccinated subjects. These three comparisons will be repeated with subjects who are not receiving systemic GM-CSF therapy. We anticipate a 2% response rate in PEP-, GM-CSF-patients, and we will be interested in an increase to a 20% response rate among PEP+, GM-CSF-patients. We will perform two-sided level 0.05/6 z-tests, using a Bonferroni correction for these six comparisons. For each of the three comparisons with GM-CSF+ patients, there will be 97.9% power to detect an increase from a 5% response rate among PEP-, GM-CSF+ subjects to a 30% response rate among PEP+ GM-CSF+ subjects. For the three comparisons involving GM-CSF-patients, there will be 92% power to detect an increase from a 2% response rate among PEP-, GM-CSF- patients to a 20% response rate among PEP+, GM-CSF- patients. All calculations assume 100 analyzable patients in each treatment arm. Similar analyses will be done with data generated using the tetramer assay.

In secondary analysis, we will also compare proportions of CD8+ responders among those receiving peptide vaccine and GM-CSF to those receiving peptide vaccine and no GM-CSF. We anticipate the proportion of responders among PEP+, GM-CSF- patients to be 20%. In order for the improvement in response rate due to GM-CSF+ to be of clinical relevance, the response rate should increase to at least 40%. There will be 85% power to detect such a difference, using a two-sided level 0.05 z-test. We will perform this comparison for each of the immunizing peptides. We will also compare the proportion of responders among PEP-, GM-CSF- patients to the proportion among PEP+GM-CSF+ patients. We will have over 99% power to -detect a change from 2% responders in the control group to 30% in the treatment group. Similar analyses will be done with data generated using the tetramer assay.

We will use the raw ELISPOT counts in a repeated measures analysis to estimate changes in the mean counts as a function of treatment group. This exploratory analysis will include the triplicate counts at baseline, one month into treatment, and three months into treatment. It will allow us to determine whether effects occur at one month or three. We will use a Poisson model for the counts. We will also perform the analysis on a logarithmic scale in order to test for multiplicative treatment effects.