Multicenter, double-blind, placebo-controlled trial of seviprotimut-L polyvalent melanoma vaccine in patients with post-resection melanoma at high risk of recurrence

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ABSTRACT

Background Most patients with advanced melanomas relapse after checkpoint blockade therapy. Thus, immunotherapies are needed that can be applied safely early, in the adjuvant setting. Seviprotimut-L is a vaccine containing human melanoma antigens, plus alum. To assess the efficacy of seviprotimut-L, the Melanoma Antigen Vaccine Immunotherapy Study (MAVIS) was initiated as a three-part multicenter, double-blind, placebo-controlled phase III trial. Results from part B1 are reported here.

Methods Patients with AJCC V.7 stage IIB-III cutaneous melanoma after resection were randomized 2:1, with stage stratification (IIB/C, IIIA, IIIB/C), to seviprotimut-L 40 mcg or placebo. Recurrence-free survival (RFS) was the primary endpoint. For an hypothesized HR of 0.625, one-sided alpha of 0.10, and power 80%, target enrollment was 325 patients.

Results For randomized patients (n=347), arms were well-balanced, and treatment-emergent adverse events were similar for seviprotimut-L and placebo. For the primary intent-to-treat endpoint of RFS, the estimated HR was 0.881 (95% CI: 0.629 to 1.233), with stratified logrank p=0.46. However, estimated HRs were not uniform over the stage randomized strata, with HRs (95% CIs) for stages IIB/IIC, IIIA, IIIB/IIIC of 0.67 (95% CI: 0.37 to 1.19), 0.72 (95% CI: 0.35 to 1.50), and 1.19 (95% CI: 0.72 to 1.97), respectively. In the stage IIB/IIC stratum, the effect on RFS was greatest for patients <60 years old (HR=0.324 (95% CI: 0.121 to 0.864)) and those with ulcerated primary melanomas (HR=0.493 (95% CI: 0.255 to 0.952)).

Conclusions Seviprotimut-L is very well tolerated. Exploratory efficacy model estimation supports further study in stage IIB/IIC patients, especially younger patients and those with ulcerated melanomas.

Trial registration number NCT01546571.

INTRODUCTION

Patients with resected stage IIB, IIC, or III melanoma are at high risk for recurrence and death after surgery. The only adjuvant therapy approved for stage IIB-IIC melanoma is high dose interferon, which prolongs recurrence-free survival (RFS) but has little or no proven impact on overall survival.[1] Current National Comprehensive Cancer Network (NCCN) guidelines do not recommend interferon therapy for stage IIB-IIC melanoma.[2] For stage III melanoma, adjuvant therapies approved by the United States Food and Drug Administration (U.S. FDA) include blockade of Programmed cell Death protein 1 (PD-1) with nivolumab or pembrolizumab, blockade of cytotoxic T-lymphocyte-Associated Protein 4 (CTLA-4) with ipilimumab, and inhibition of v-raf murine sarcoma viral oncogene homolog B1 (BRAF) and mitogen-activated protein kinase kinase (MEK) with dabrafenib and trametinib, respectively. However, for those with BRAF-wild type melanomas and

low volume lymph node metastases, the risk: benefit ratio may not favor treatment with checkpoint blockade therapy. In addition, a significant portion of patients still recur despite approved adjuvant therapies. Thus, there is a need for effective systemic adjuvant therapy for patients with stage IIB-IIC melanoma and for subsets of patients with stage III melanoma.

Cancer vaccines offer promise to prevent melanoma recurrence by enhancing melanoma-specific immune responses. However, no cancer vaccine has yet proven effective for prolonging RFS or survival after melanoma resection. Seviprotimut-L (formerly POL-103A) was developed based on data from a prototype polyvalent melanoma vaccine comprised of shed antigens from four melanoma cell lines, administered with alum or interleukin (IL)-2 liposomes. That prototype vaccine contained multiple shared melanoma antigens, including MAGE-A3, MelanA, gp100, tyrosinase, melanocortin 1 receptor and tyrosinase-related protein 2[3 4] and it induced antibody responses that were associated with improved clinical outcome.[5] It also induced CD8 T cell responses to peptides restricted by Class I Major Histocompatability Complex (MHC) molecules.[3 6] Over 600 patients were enrolled on trials testing versions of the prototype vaccine, with no safety concerns. In a small double-blind, placebo-controlled trial of a version of the prototype vaccine in patients with resected high-risk stage III melanoma (n=38), RFS of the melanoma vaccine-treated patients was over twice as long as that of placebo vaccine-treated patients (p=0.03).[7]

The polyvalent vaccine has been reformulated as seviprotimut-L, prepared from antigens shed by three human melanoma cell lines administered with alum. A three-part, Phase III clinical trial program, termed Melanoma Antigen Vaccine Immunotherapy Study (MAVIS) was initiated. The trial program was originally designed to include two separate clinical trials: Part A to select between two dose levels of seviprotimut-L based on safety and immunogenicity, and Part B to evaluate safety and efficacy of the selected dose. A dose of 40 mcg was selected in Part A based on safety and induction of serum antibody to melanoma antigens. In the course of conducting Part B (in 2016), the sponsor decided to split Part B into two parts—Part B1 to identify a signal of clinical efficacy, prior to initiating Part B2 which would be the definitive evaluation of clinical efficacy. Thus, Part B1 of MAVIS was a multicenter, double-blind, placebo-controlled trial to assess the efficacy of seviprotimut-L at that dose, with the primary endpoint of RFS in patients at high risk of recurrence after definitive surgical resection. The present manuscript reports the final clinical outcome data from Part B1 of the MAVIS trial.

METHODS

Part B1 of the MAVIS trial was a prospective double-blind, randomized, placebo-controlled phase III clinical trial. The investigators and subjects were blinded as to treatment assignment; the placebo injections appeared identical to the seviprotimut-L injections.

Participant eligibility

Participants 18–80 years of age were eligible if they had histologically confirmed AJCC Stage IIB, IIC, or III cutaneous melanoma (AJCC V.7) rendered clinically free of disease by surgery within 90 days. Patients with positive sentinel nodes were required to have a complete lymphadenectomy, except for patients on the Multicenter Selective Lymphadenectomy Trial II (MSLT-II, NCT00297895)[8] who were randomized in that study to the observation arm. Also required were: Eastern Cooperative Oncology Group (ECOG) performance status 0–1, ability and willingness to give informed consent, normal organ function, and absence of major autoimmune disorders. Participants were required either to have known BRAF V600 mutation status or archival tissue available to test for BRAF mutation status. Participants were excluded for pregnancy, prior melanoma treatment other than surgery, regional radiation therapy or adjuvant interferon alfa-2b within 1 week of starting treatment, concurrent cancer therapy, concurrent immune therapy, chronic use of systemic corticosteroids, prior splenectomy, known HIV positivity, allergy to alum, or autoimmune disorders requiring therapy or with visceral involvement, or another malignancy within the prior 5 years (except adequately treated in situ squamous cell carcinoma, basal cell carcinoma, stage I or IIA melanoma, or carcinoma in situ of the cervix).

Vaccine components and treatment regimen

Seviprotimut-L is a polyvalent suspension melanoma cancer vaccine that includes multiple melanoma antigens shed from three human melanoma cell lines as the active pharmaceutical ingredient (API). These three lines are SFHM2, SFHM4, and SFHM8 and have previously been referred to as M20, M14, and SK-Mel-28, respectively: M20 was established from a metastatic melanoma[9]; M14 was established from an amelanotic metastatic lesion of a 33-year-old man and is BRAF-mutant (V600E)[10]; and SK-Mel-28 was originally established from an axillary lymph node of a 51-year-old man and is BRAF-mutant (V600E), with wild-type Neuroblastoma RAS viral oncolog gene homolog (NRAS).[11–13] Additional information about each has been reported previously.[14] For vaccine production, the material shed into serum-free culture medium by the cells was collected, pooled, concentrated, treated with non-ionic detergent Nonidet P-40 to disaggregate antigens, and ultracentrifuged to remove particulate matter. The supernatant was filter-sterilized, adjusted to the appropriate protein concentration, and bound to alum as an adjuvant. Seviprotimut-L is thus partially purified, being made of soluble shed antigens, to exclude the bulk of unrelated cellular material that is present in the cell cytoplasm or nucleus. The Drug Product was a nominal 0.8 mL suspension containing 0.05 mg/mL API with 1 mg/mL aluminum in aluminum hydroxide suspensions in 4 mM phosphate-buffered saline and 0.9%

sodium chloride, at pH 6.8. The Drug Product was packaged in Type I glass vials with Teflon-coated bromobutyl stoppers and was stored at 2°C–8°C (refrigerated) until use. The vaccine was used as is and required no reconstitution after warming to room temperature. The doses tested in Part A had included 40 mcg and 100 mcg, where 40 mcg protein had been the dose in the small randomied trial of a similar vaccine preparation.[7]

Study design, power analysis, and objectives

Part B1 of MAVIS was a two-arm, multicenter, randomized, double-blind, placebo-controlled trial with a goal of randomizing at least 325 patients in a 2:1 ratio for probability of assignment to the experimental arm. The primary endpoint was RFS. Secondary objectives included: (a) to assess the efficacy of treatment with seviprotimut-L compared with placebo with respect to overall survival (OS); and (b) to verify the safety and tolerability of seviprotimut-L at the dose selected for Part B. As Part B1 was intended to identify a signal of clinical efficacy prior to embarking on the definitive Part B2, the p value was set at 0.2, and hence fewer events/patients were required. For assessment of RFS, the study was powered with hypothesized HR of 0.625, one-sided alpha of 0.10, and power 80%, with target assessment after 126 RFS events, based on the possibility of a delayed effect due to immunotherapy.[15]

Eligible patients received either seviprotimut-L at the dose determined from Part A (40 µg) or placebo. Patients were stratified at randomization according to their stage of disease at enrollment (IIB/IIC vs IIIA vs IIIB/IIIC) to increase the likelihood of intended distribution between the study arms. Patients were treated every 2 weeks×4, then monthly×4, then every 3 months through month 24 (ie, weeks 0, 2, 4, 6, 8, then months 3, 4, 5, 6, 9, 12, 15, 18, 21, 24). Each subject was treated unless one of the following occurred: development of recurrent disease that did not meet the criteria for continued dosing, death, subject withdrawal, or study termination, whichever came first. Patients were followed for recurrence and survival. They were not routinely unblinded following recurrence or the end of treatment except to allow the subject to pursue other treatment options.

Toxicity and dose-limiting toxicities

Safety was assessed throughout the study. Adverse events (AEs) were reported from randomization through 30 days after the last study drug administration and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events V.4. Relatedness of AEs to study drug was reported using WHO definitions: Probable, Possible, and Unlikely.

An adverse event or suspected adverse reaction was considered 'serious' (SAE) if, in the view of either the investigator or sponsor, it resulted in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial

disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Other events would be considered serious when, based on 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.

Clinical outcome

The date of recurrence was defined as the earliest date a recurrence was suspected, with biopsy confirmation, except in rare cases where biopsies were not clinically appropriate (eg, brain metastases). Recurrence events included appearance of locoregional metastasis, distant metastasis, or a new primary melanoma. Since the validation of recurrence primarily relied on biopsies, there was no central reading of CT scans to confirm recurrence.

Recurrence assessments were to be based on cross-sectional imaging every 6 months. Serum lactate dehydrogenase (LDH) was also monitored every 3 months, and LDH elevations ≥1.5×upper limit of normal were followed up with radiological scans, provided there were no other obvious medical reasons for the elevated LDH.

Statistical methods

The primary analysis outcome is RFS time, computed as the number of days from the date of randomization to the earliest of recurrence or death. Patients without recurrence or death were censored at the date last assessed for recurrence. All analyses are based on the intent-to-treat (ITT) principle. The primary analysis is the stratified logrank test, with the stage at randomization as stratifier. HR estimates and their 95% CIs are from proportional hazard regression models using stage at randomization as stratifier as applicable. Sensitivity and exploratory analyses included effect modification assessment for the existence of heterogeneity of RFS effect estimated across subsets defined by baseline attributes and is based on a proportional hazard regression with terms for baseline attribute, arm, and one or more terms as necessary for the interaction between baseline attribute and arm. In effect modification assessments, an interaction p value in the neighborhood of 0.1 or less is regarded as evidence of effect modification (this interaction p value is to be interpreted as a measure of the contribution of interaction to the fit of the model). Graphs show the Kaplan-Meier estimators of the time-to-event distributions. Beyond the primary analysis, all analyses are presented as exploratory with arm effect estimates and 95% CIs.

RESULTS
Patients

Three hundred and forty-seven patients at 65 centers in the USA and Canada were randomized January 2015 through August 2016. Arms were well-balanced (table 1). About one-third of patients had a BRAF mutation. Only 5% had had any prior melanoma therapy other than surgery, and these were primarily radiotherapy. None had

Table 1 Enrollment, demographics, and clinical features

	Seviprotimut-L	Placebo
N	230	117
Age: median (range)	58 (18, 80)	56 (26, 80)
Sex: % female	42	44
Race: % white	99	100
Prior melanoma therapy (%)		
Any	5.2	4.3
Radiotherapy	4.3	4.3
Tumor location (%)		
Extremities	35	37
Head and neck	26	21
Trunk	37	41
Other	2.6	0.9
Ethnicity: % Hispanic or Latino	3	3.4
ECOG performance status 0 (%)	86	86
Completed lymphadenectomy (%)	64	64
Median Breslow depth (mm)	2.8	3
T staging (%)		
T0 or TX	4.8	5.1
T1–T2a	29	27
T2b–T4a	48	55
T4b	18	17
N staging (%)		
N0	32	32
N1a	36	30
N1b–N2b	19	23
N2c/N3	13	15
Summary AJCC stage (V.7, %)		
IIB/IIC	32	32
IIIA	30	29
IIIB/IIIC	38	39
BRAF mutant* (%)	35	33

*BRAF mutation status known for 93%, 96%, and 94% of patients receiving vaccine, placebo, or total. Values shown above are percentages of all.

prior immunotherapy, small molecule targeted agents, antibody therapy, or anti-angiogenic therapy. Patients on the MSLT-II trial were allowed to enroll only after amendment 3. Only one, on the observation arm, is documented to be enrolled also on MSLT-II. The data in this manuscript were collected after datalock in January 2020, and findings were provided to the coauthors in April 2020, after which analyses and statistical review led to the present manuscript.

Adverse events and safety assessment
Over 95% of patients on both study arms had one or more treatment-emergent AE's, and 71% had one or more treatment-related AEs (TRAEs), all of which are detailed in online supplemental table 1. The vast majority were grade 1, with fewer grade 2 TRAEs and very few grade 3 TRAEs. The TRAEs observed in 3% or more of patients overall, listed in table 2, included local symptoms at the injection sites (erythema, pruritus, pain, swelling, bruising, induration, and rash) and systemic symptoms (fatigue, headache, nausea, pruritus, myalgia, rash, influenza-like illness symptoms, and diarrhea). The incidence of those AEs and associated AE categories were similar between the two study arms. The only AE with slightly higher incidence in the vaccine arm was injection site pruritus (20% vs 12%). There were no grade 4–5 TRAEs and no treatment-related SAEs (table 3). Only one patient discontinued therapy for a TRAE: a macular rash on the vaccine arm. Sixty-two per cent completed all 24 months of therapy; 32% discontinued early for disease progression (table 3). Evidence of autoimmune disease in the eyes and skin was assessed every 6 months. Evidence of ocular autoimmune disease was reported only in one participant at month 12 on the vaccine arm and in none on the placebo arm. Evidence of autoimmune disease in the skin was observed in small numbers on both vaccine and placebo arms, with no evidence of a treatment-related effect (online supplemental table 2).

RFS
The primary analysis in this report is based on data with 149 RFS events at the data cut-off date. For this primary analysis, the estimated HR is 0.881 (95% CI: 0.629 to 1.233), with p=0.460. Kaplan-Meier estimates are shown in figure 1A. Thus, the trial failed to meet the planned primary statistical criterion. However, there was notable quantitative heterogeneity in HR estimates across stage-specific randomization strata. For stages IIB/IIC (n=111), IIIA (n=103), IIIB/IIIC (n=133), respectively, these HR estimates (and 95% CIs) are 0.666 (95% CI: 0.373 to 1.189), 0.721 (95% CI: 0.347 to 1.496), and 1.191 (95% CI: 0.720 to 1.970). Figure 1B shows the Kaplan-Meier estimates by stage and arm. The sites of metastasis were defined as locoregional or distant for 137 patients, including 47, 24, and 66 for stages IIB/IIC, IIIA, IIIB/C, respectively: of these, relapses were distant in 26 (55%), 10 (42%), and 37 (56%), respectively (data not shown). Patients were stratified and randomized for enrollment on this trial based on AJCC V.7 staging. AJCC V.8 classifies stage IIB and IIC patients the same as V.7; so, analyses of this subset are the same using AJCC V.8. There were changes for AJCC V.8 in substaging stage III patients, but reanalysis of outcomes in stage III patients is inconsistent with the randomization stratification. The finding of a potentially clinically meaningful stage IIB/IIC point estimate and the consistent and distinct separation between arms for stage IIB/IIC as seen in the Kaplan-Meier estimates (figure 1B) led to further exploration of the stage IIB/IIC patients with the intent to inform planning the Part B2 phase III trial. Additional factors explored as potential effect modifiers within the stage IIB/IIC group included age, sex, tumor location, thickness, ulceration, BRAF mutation status, and LDH. Suggestion of the existence of

Table 2 Treatment-related adverse events (AEs) observed in 3% or more of patients

Category	AE	N=230 Seviprotimut-L				N=117 Placebo				N=347 Total				# (%) any grade	% any grade Seviprotimut-L	% any grade Placebo
		G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4	G1–G3	G1–G3	G1–G3
	****													245 (71)		
General and administration site	Any	130	14			68	5			198	19			217 (63)	63	62
Skin/subcutaneous tissue	Any	27	6	3		17	4			44	10	3		57 (16)	16	18
Nervous system	Any	19	2	1		16				35	2	1		38 (11)	10	14
Gastrointestinal	Any	23	1			9	1			32	2			34 (10)	10	9
Musculoskeletal/connective tissue	Any	16	4			11	1			27	5			32 (9)	9	10
General and administration site	Injection site erythema	72	1			48				120	1			121 (35)	32	41
General and administration site	Fatigue	34	8			23	3			57	11			68 (20)	18	22
General and administration site	Injection site pruritus	44	2			13	1			57	3			60 (17)	20	12
General and administration site	Injection site pain	29	2			12	1			41	3			44 (13)	13	11
Nervous system	Headache	13	2	1		12				25	2	1		28 (8)	7	10
General and administration site	Injection site swelling	15				6				21				21 (6)	7	5
Gastrointestinal	Nausea	13	1			4	1			17	2			19 (5)	6	4
General and administration site	Injection site bruising	12				6				18				18 (5)	5	5
Skin/subcutaneous tissue	Pruritus	11	2			5				16	2			18 (5)	6	4
Musculoskeletal/connective tissue	Myalgia	9				4	1			13	1			14 (4)	4	4
Skin/subcutaneous tissue	Rash	7		1		4	2			11	2	1		14 (4)	3	5
General and administration site	Influenza-like illness	6	3			3				9	3			12 (3)	4	3
General and administration site	Injection site induration	9				3				12				12 (3)	4	3
Gastrointestinal	Diarrhea	5				6				11				11 (3)	2	5
General and administration site	Injection site rash	8				3				11				11 (3)	3	3

Table 3 Summary of adverse events (AEs) and protocol discontinuation

	Seviprotimut-L	Placebo	Total
N	230	117	347
AEs	96%	97%	96%
Grade 3 AEs	12%	9%	11%
Grade 4–5 AEs	0%	0%	0%
Treatment-related AEs (TRAEs)	70%	73%	71%
Treatment-related serious AEs	0%	0%	0%
AEs leading to d/c study drug*	0.9%	0.9%	0.9%
TRAEs leading to d/c study drug	0.4%	0%	0.3%
Completed 24 months of treatment	61.3%	63.2%	62.0%
D/C early for progressive disease	31.7%	31.6%	31.7%
D/C early for withdrawal of consent	3.9%	1.7%	3.2%
D/C early for TRAE	0.4%	0%	0.3%
Lost to follow-up, pregnancy, other cancer	2.6%	3.4%	2.9%
Total	100%	100%	100%

*AEs leading to discontinuation (d/c) of study drug included one TRAE (macular rash) on the vaccine arm, and development of adenocarcinoma of the colon on the vaccine arm and squamous cell carcinoma of the larynx on the placebo arm.

an effect modification was found for age as <60 versus ≥60 years and for the presence of ulceration, with interaction p values of 0.0581 and 0.1136, respectively. The findings are illustrated for age in figure 1C,D for the ITT data set and stage IIB/C group, respectively.

Findings regarding effect modification by the presence of ulceration and age for the stage IIB/C stratum are illustrated in figure 2. For this stratum, the HR for ulcerated melanomas was 0.493 (95% CI: 0.255 to 0.952), as shown in figure 2A. The interactions between age (≤60 and >60 years) and ulceration for the stage IIB/IIC stratum are further detailed in figure 2B,C. For those under age 60 with ulcerated melanomas, specifically, the HR was 0.213 (95% CI: 0.065 to 0.702) favoring the vaccine arm.

Overall survival

OS was a secondary endpoint. With 41 deaths, there is reduced sensitivity for detecting treatment effects on OS; however, by ITT analysis, the estimated HR favors the vaccine arm (HR=0.64 (95% CI: 0.34 to 1.18), figure 3A). Also, the ranking across disease stages for OS is similar to the quantitative relationships in the RFS analyses. Analysis of subgroups based on planned stratification by stage (figure 3B) replicated the RFS trend, with evidence of a strong HR effect in Stage IIB/IIC patients (n=111, HR 0.338 (95% CI: 0.117 to 0.975), figure 3B,D). Also, the

survival HR for the 191 patients <60 years old was 0.412 (95% CI: 0.167 to 1.014, figure 3C,D), while for the 156 patients ≥60 years old, the HR was 0.91 (95% CI: 0.38 to 2.17, figure 3C,D). For stage IIB/IIC stratum, the survival HR for the 52 patients <60 years old was 0.216 (95% CI: 0.039 to 1.19), and for the 59 patients ≥60 years old, the HR was 0.49 (95% CI: 0.12 to 1.98, figure 3E). The survival HR for 160 patients with ulcerated primary melanomas was 0.51 (95% CI: 0.22 to 1.16), and for the stage IIB/IIC stratum, the survival HR for 84 patients with ulcerated melanomas was 0.345 (95% CI: 0.10 to 1.14; not shown).

DISCUSSION

Seviprotimut-L treatment was very well tolerated, with no SAEs and without a clear signal for increases in TRAEs compared with placebo injections. Part B1 of the MAVIS trial was designed to obtain preliminary data on clinical impact of seviprotimut-L, at 40 µg/dose, as measured by RFS. The study was powered to detect a HR of 0.625, with one-sided alpha of 0.10, and power 80%. The target of 126 RFS events was met. The HR for RFS for the ITT data set was 0.88 (95% CI: 0.63 to 1.23); so, these data do not support benefit of seviprotimut-L for the whole patient data set. However, a planned randomization stratification by stage identified a subset, representing 32% of the study data set (stage IIB/IIC), for whom there was a trend toward longer RFS with vaccine. Further, subgroup analyses by age and ulceration suggest benefit for patients under age 60 and, for the stage IIB/C stratum, those with ulcerated primary melanomas. Analysis of impact on OS was limited by the smaller number of events, but the HR of 0.64 is promising. For the Stage IIB/IIC subset, OS was longer with seviprotimut-L, with HR 0.338 (95% CI: 0.117 to 0.975). Also, OS trended longer for patients with stage IIB/IIC melanoma under age 60 (HR 0.216). In the recently reported S1404 study evaluating adjuvant pembrolizumab versus standard of care (ipilimumab or high-dose interferon) for patients with resected stage III-IV melanoma, significant improvement in RFS did not translate to a significant impact on OS.[16] Thus, the promising early RFS and OS data for stage IIB/C patients in the MAVIS trial are particularly promising. These data support selection of stage IIB/C patients for the definitive final part B2 of the MAVIS phase III trial to test seviprotimut-L, with stratification by age and ulceration.

The seviprotimut-L vaccine contains soluble antigens shed from three human melanoma cell lines, in a preparation that is readily scaled up for large patient populations. The preparation contains multiple defined antigens shared among melanomas from many patients, including melanocytic differentiation proteins and cancer-testis antigens.[3 6] There is evidence that such proteins can serve as relevant tumor-rejection antigens: vaccination with a single shared melanocytic antigen improved progression-free survival of patients with melanoma when added to systemic interleukin-2 therapy in a randomized trial,[17] and vaccination with a mixture of peptides from shared
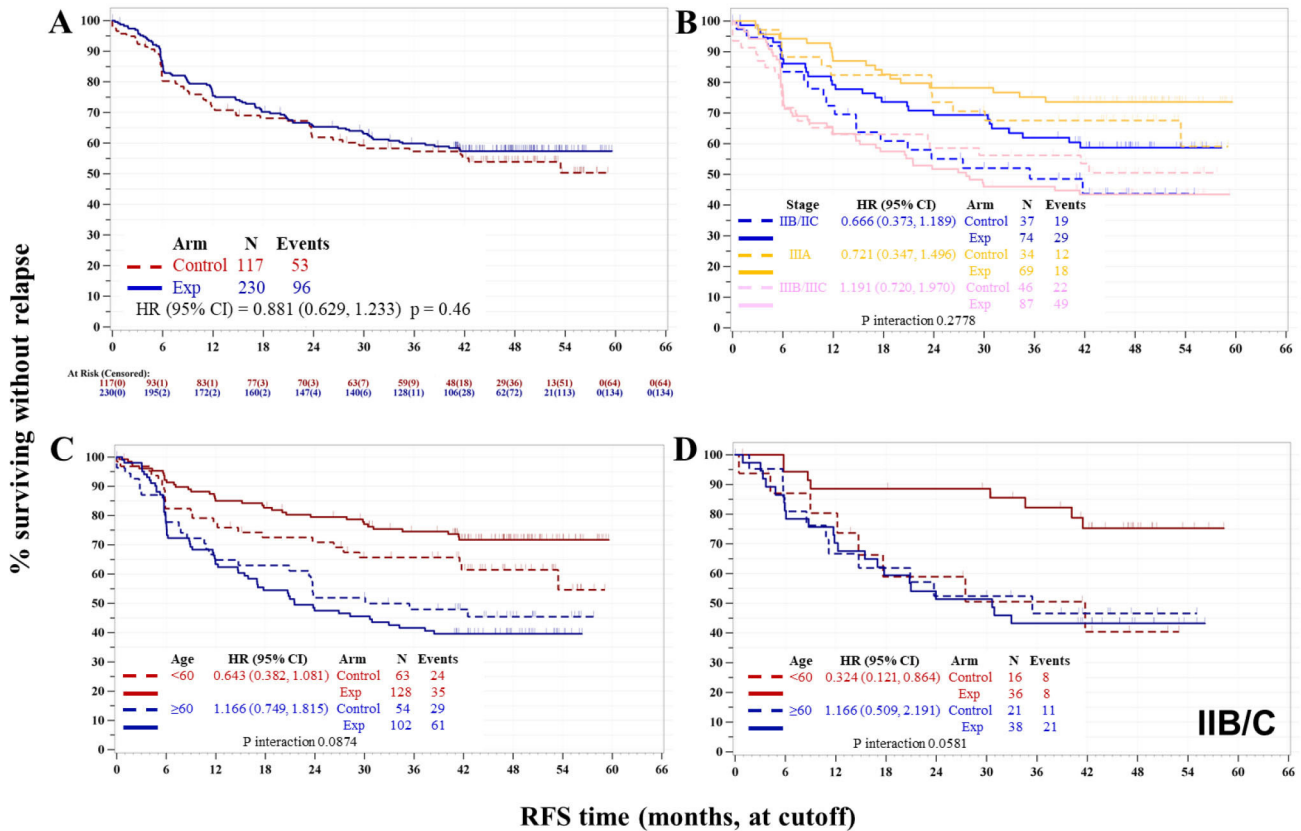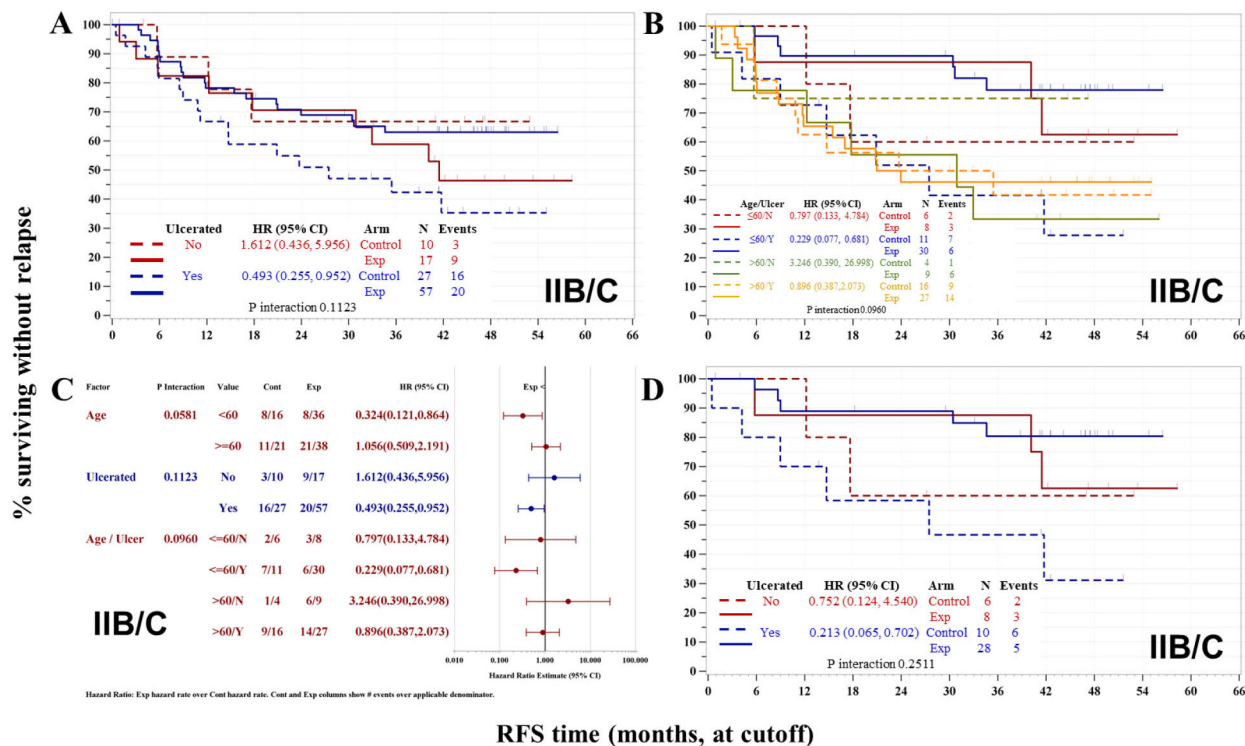
Figure 1 Recurrence-free survival (RFS) by arm, stage, and age. Kaplan-Meier estimates of RFS are plotted, with analysis results in the legend and risk set and censoring accumulation below the time axis. The designation '(cut-off)' indicates possible data truncation based on designated cut-off date. (A) RFS by arm, stratified by stage, for the intent-to-treat (ITT) data set, (B) RFS by arm and stage for the ITT data set, (C) RFS by arm and age for the ITT data set, (D) RFS by arm and age for Stage IIB/C stratum. P values by logrank test. P value, HR and 95% CI are based on a univariate Cox regression model assuming proportional hazards with treatment, age, and treatment×age as factors, stratified for the randomisation stratification variable of disease stage.

melanocytic and cancer testis proteins has induced durable clinical benefit in patients with advanced melanoma.[18] Also, adoptive cell therapy targeting shared cancer-testis antigens has induced durable clinical benefit in melanoma and other cancers.[19 20] A limitation of the approach is the complexity of the antigen composition, which limits the ability to test for immune responses that are most relevant for tumor control. However, an advantage of the seviprotimut-L approach is that it is not limited to a small number of peptides or proteins and does not require selection of patients based on human leukocyte antigen (HLA) expression.

The subgroup analyses in this report are obligatory and inform directions for future study of this vaccine. In stage IIB/IIC patients, there was a trend to prolonged RFS (HR 0.666, figure 1B) and prolonged OS (HR 0.338, figure 3B) for those who received seviprotimut-L. The reasons for these different results based on stage are not clear. It is true that in prior trials with PD-1 inhibitors, and BRAF/MEK inhibition, there has been benefit across a range of stages, for stage III and IV, as evidenced by FDA approvals in these settings. Vaccines may have a greater chance of benefit in earlier stages. Patients without lymph node metastases may have more intact immune function.

It has been hypothesized that for melanoma to establish metastases in regional nodes, the primary tumor must first induce immune dysregulation in those nodes.[21] Regardless, these findings support limiting the design of the future part B2 trial to patients with stage IIB/IIC melanoma, who are those for whom no systemic adjuvant therapy is currently recommended.

Other subset findings are suggestions of benefit with seviprotimut-L for patients younger than age 60 and for those with ulcerated primary melanomas. In particular, for stage IIB/IIC patients, RFS was more favorable with seviprotimut-L for age <60 (HR 0.324, figure 1D) and for ulcerated melanomas (HR 0.493, figure 2A). The rationale for improved outcomes in younger patients is supported by prior experience. Older individuals develop immune senescence that decreases immune responsiveness,[22] and they have lower immune response rates to peptide vaccines for melanoma.[23] Thus, the present study supports evaluating the clinical impact of seviprotimut-L in patients under age 60 in the subsequent MAVIS clinical trial. Interestingly, PD-1 blockade has been reported to be more effective in patients over age 60,[24] suggesting the possible benefit of combining vaccine and PD-1 blockade specifically in older patients. The improved outcome in

Figure 2 Recurrence-free survival (RFS) by arm, age, and ulceration for stage IIB/IIC stratum. Kaplan-Meier estimates of RFS are plotted for stage IIB/IIC patients (A) by arm and ulceration, and (B) by arm, age and ulceration. Shown in (C) is a forest plot for stage IIB/IIC patients as a function of age, ulceration, and both. In (D) is the Kaplan-Meier estimate for RFS for stage IIB/IIC patients under age 60, by arm and ulceration. P values by logrank test.

patients with ulcerated primary melanomas is consistent with prior reports of improved outcome with another cancer vaccine and with interferon therapy for patients with ulcerated melanomas.[25 26] The finding in this trial is also promising and may guide future trial design as well.

Several prior randomized clinical trials of cancer vaccines have been disappointing, including the Canvaxin trials of an allogeneic whole melanoma cell vaccine administered with Bacillus Calmette-Guérin adjuvant.[27 28] The definitive phase III trials of that vaccine were performed in patients with stage III and stage IV melanoma, which were negative. The lack of an efficacy signal for stage III patients in the present trial is consistent with that finding. Also, a randomized trial of an autologous heat-shock protein vaccine vitespen in patients with stage IV melanoma, also revealed no impact on overall survival.[29] Thus, the promise of cancer vaccines for melanoma remains to be realized. On the other hand, a vaccine approach has been approved in hormone-refractory prostate cancer,[30] and a phase III trial of a peptide vaccine plus high-dose IL-2 significantly prolonged progression-free survival of patients with advanced melanoma, compared with IL-2 alone.[31] The data from Part B1 of the MAVIS trial do not meet the target impact on RFS for the whole ITT population, but promising trend to longer overall survival for the ITT population (HR 0.64) support continued investigation of this approach. A goal of the present study was to assess for evidence of clinical benefit to guide a definitive Part B2 randomized phase III trial. Failure of prior trials may also have related to the study population. The present study has

identified subsets who for whom there is promising preliminary evidence of efficacy of this vaccine approach, which will thus guide the follow-up definitive trial.

Limitations of the present study include its modest sample size and the negative findings overall for impact on RFS. Also, the composition of the vaccine, being derived from cell-associated proteins, is not conducive to laboratory correlates to assess immune response to defined antigens. The present B1 trial did not include analyses of immune responses; so, there are no data on the strength of the immune responses or their association with clinical outcome. BRAF mutation status has been associated with clinical outcome and response to checkpoint blockade therapy, as well as BRAF/MEK inhibitor therapy. However, during this trial, it was not routine to assess BRAF mutation status for resected stage II-III melanomas; yet, the arms were well-matched for BRAF mutation status overall in those for whom it was known (table 1). There may be value in stratifying also for BRAF status in subsequent vaccine trials.

In summary, this final report of data from part B1 of the prospective randomized multicenter MAVIS clinical trial of seviprotimut-L provides guidance useful for design of phase III part B2 of the MAVIS clinical trial program. Subgroup efficacy analyses identified three groups who may benefit from seviprotimut-L: those with AJCC (V.7) stage IIB/C melanoma, those under age 60, and those with ulcerated melanomas. These
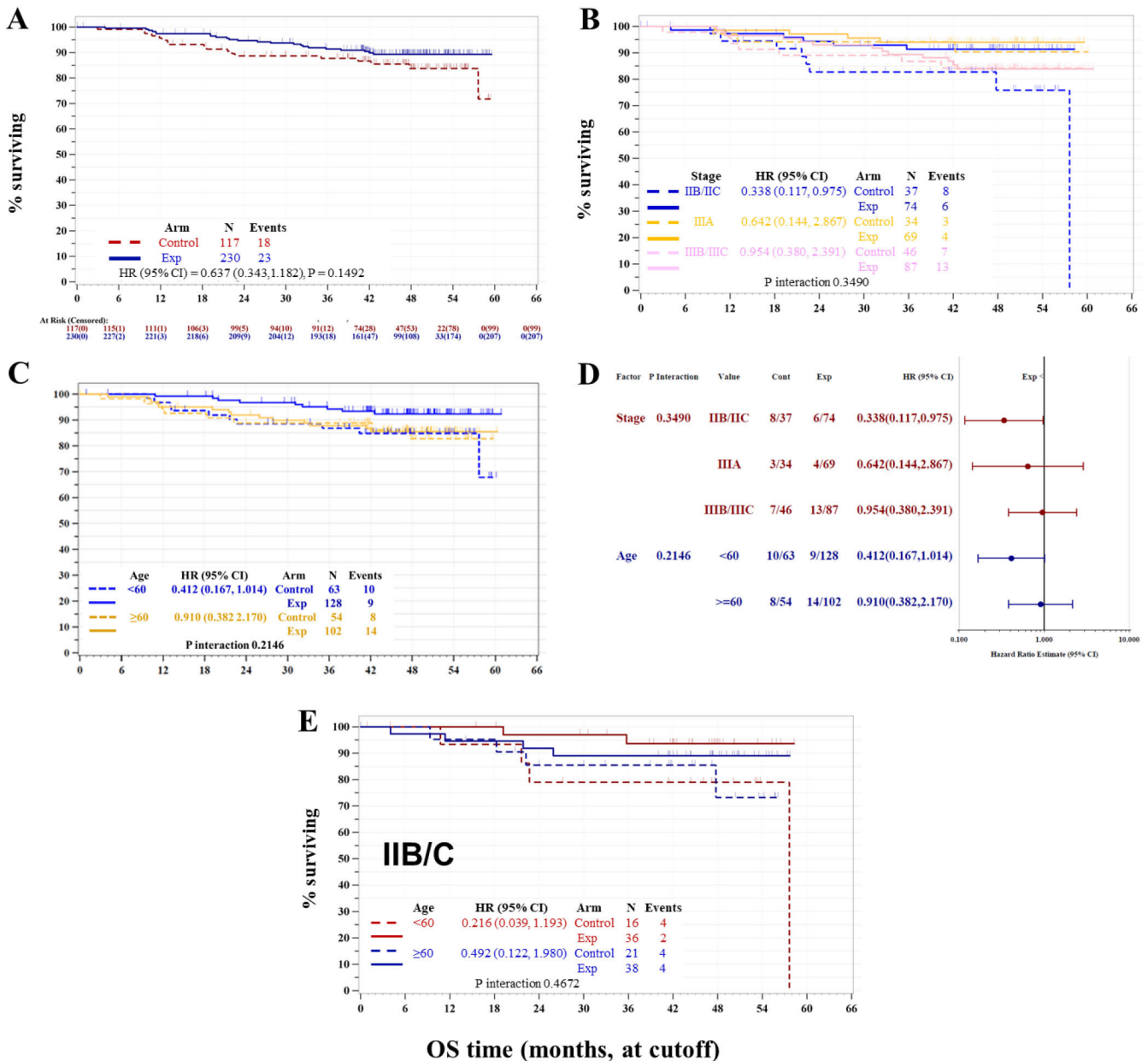
Figure 3 Survival effect modification by arm, age, and stage. Kaplan-Meier estimates of overall survival (OS) are plotted (A) for all intent-to-treat (ITT) patients stratified by stage, by arm, (B) for all ITT patients by arm and stage, and (C) for all ITT patients by arm and age. Shown in (D) is a forest plot for all ITT patients by stage and by age. In (E) is the Kaplan-Meier estimate for OS for stage IIB/IIC patients by arm and age. P values by logrank test.

data support proceeding to the definitive final part B2 of the MAVIS phase III trial to test seviprotimut-L for stage IIB/C patients, with stratification by age and ulceration. Currently available adjuvant therapies for patients with stage III melanoma include immune checkpoint blockade with antibodies to CTLA-4 or PD-1, and small molecule inhibitors of V600-mutated BRAF and MEK. However, they are not currently approved for patients with resected stage IIB/C melanoma. Clinical trials currently underway evaluating PD-1 blockade for stage IIB/IIC melanoma include Keynote 716 (NCT03553836) and Checkmate 76K (NCT04099251). A recent press release of Keynote 716 reports that adjuvant pembrolizumab met its primary endpoint for RFS for patients with stage IIB/IIC melanoma[32]; so, the FDA is likely to review of those data in the near future. However, in patients without metastatic disease, the tolerance of SAEs is less than in patients with advanced melanoma. Though PD-1 blockade is well tolerated by most patients, severe and life-threatening toxicities can occur,[33] including cardiac and neuromuscular toxicities, which have a high mortality risk,[34] as well as diabetes[35] and other endocrinopathies.[36] To our knowledge, there are no phase III BRAF/MEK inhibitor adjuvant trials in stage IIB/C melanoma. The toxicity of dabrafenib/trametinib in the COMBI-AD adjuvant melanoma trial in Stage III patients led to SAEs in 36% of patients and permanent discontinuation of study drug in 26% of patients.[37] On the other hand, vaccination

with seviprotimut-L has an advantage of very low toxicity without significant immune-related adverse events, and no significant increase in toxicity over placebo. Thus, if definitive evaluation of this vaccine therapy confirms clinical benefit in patients with stage IIB/C melanoma, the low toxicity of this approach will be a valuable option for these patients.

STATEMENT OF TRANSLATIONAL RELEVANCE

Seviprotimut-L, a vaccine containing antigens from human melanoma cells, is being evaluated for efficacy in this randomized, placebo-controlled trial for patients with AJCC v7 stage IIB-III cutaneous melanoma after resection. Treatment-emergent adverse events (AEs) were similar for seviprotimut-L and placebo patients. For the primary intent-to-treat (ITT) endpoint of RFS, the estimated HR was 0.881 (95% CI: 0.629,1.233). However, subgroup efficacy analyses identified three groups who may benefit from Seviprotimut-L: those with AJCC stage IIB/IIC melanoma, those under age 60, and those with ulcerated melanomas. These data support proceeding to the definitive final part B2 of the MAVIS phase III trial to test seviprotimut-L for stage IIB/C patients, with stratification by age and ulceration. In summary, seviprotimut-L is very well tolerated. Exploratory efficacy model estimation supports further study in stage IIB/IIC patients, especially younger patients and those with ulcerated melanomas.

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