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ORIGINAL REPORT

Phase III Comparison of Vitespen, an Autologous Tumor-Derived Heat Shock Protein gp96 Peptide Complex Vaccine, With Physician's Choice of Treatment for Stage IV Melanoma: The C-100-21 Study Group

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ABSTRACT

Purpose
To assess the antitumor activity of vitespen (autologous, tumor- derived heat shock protein gp96 peptide complexes) by determining whether patients with stage IV melanoma treated with vitespen experienced longer overall survival than patients treated with physician's choice.

Patients and Methods
Patients (N = 322) were randomly assigned 2:1 to receive vitespen or physician's choice (PC) of a treatment containing one or more of the following: dacarbazine, temozolomide, interleukin-2, or complete tumor resection. This open-label trial was conducted at 71 centers worldwide. Patients were monitored for safety and overall survival.

Results
Therapy with vitespen is devoid of significant toxicity. Patients randomly assigned to the vitespen arm received variable number of injections (range, 0 to 87; median, 6) in part because of the autologous nature of vitespen therapy. Intention-to-treat analysis showed that overall survival in the vitespen arm is statistically indistinguishable from that in the PC arm. Exploratory landmark analyses show that patients in the M1a and M1b substages receiving a larger number of vitespen immunizations survived longer than those receiving fewer such treatments. Such difference was not detected for substage M1c patients.

Conclusion
These results are consistent with the immunologic mechanism of action of vitespen, indicating delayed onset of clinical activity after exposure to the vaccine. The results suggest patients with M1a and M1b disease who are able to receive 10 or more doses of vitespen as the candidate population for a confirmatory study.

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INTRODUCTION

Stage IV melanoma has a dismal prognosis and there is no consensus for a standard treatment. Dacarbazine (DTIC), and interleukin-2 (IL-2) are licensed agents for stage IV melanoma in the United States; however, other agents such as the DTIC precursor temozolomide, interferon-α, and complete surgical resection, where feasible, are often used alone or in combination among other approaches.[1] These treatments are ineffective for the vast majority of patients. Melanoma has historically attracted the attention of immunologists because of the belief that it is particularly immunogenic. That belief has fuelled a range of immunotherapies: haptenated autologous cells,[2] allogeneic cells,[3] gangliosides,[4] cancer testes antigens,[5,6] differentiation antigens,[7,8] altered differentiation antigens,[9] or heat shock protein (HSP)-peptide complexes.[10] Various antigens (peptides, proteins), adjuvants, immune modulators (anti-CTLA4 antibody)[11,12] and means of delivery (dendritic cells [DCs], DNA) have been used. Adoptive immunotherapy with unmodified or engineered T cells of single or mixed specificities has been used.[13,14] Most immunologic approaches elicit a degree of serological or T-cell activity,[3,5-7,9] and most of them suggest a degree of clinical activity. A correlation between immune responses and clinical activity has been elusive, partly because of the paucity of robust clinical activity. Several approaches have been tested without evidence of benefit in randomized phase III trials in patients with stage IV melanoma.[15-18]

We present here the results of a randomized phase III trial in patients with stage IV melanoma comparing vaccination with the HSP gp96 peptide complexes derived from autologous tumors (vitespen, formerly known as Oncophage [Antigenics Inc, New York, NY]) versus physician's choice of treatment, including IL-2 and/or DTIC/temozolomide and/or tumor resection. The scientific basis for the treatment has been described elsewhere.[19] Briefly, purified preparations of gp96 (and other HSPs) from tumors are noncovalent complexes of HSPs and peptides. The peptides are derived from self- and tumor-specific proteins expressed in tumors. Immunization with gp96 peptide complexes leads to their uptake by the skin DCs through CD91 (an HSP receptor), representation of the gp96-chaperoned peptides by the DCs on MHC molecules, and stimulation of cognate T cells. Therapy of tumor-bearing mice with gp96 peptide complexes is highly effective for micrometastatic disease, and less so for progressively growing tumors.[20] Phase I/II trials with this approach in human melanoma, renal carcinoma, and colon carcinoma have demonstrated hints of clinical activity.[10,21,22] The present study builds on those trials and was designed to seek evidence of superior clinical efficacy of vitespen compared with the physician's choice (PC; in the absence of a well-defined standard of care).

PATIENTS AND METHODS

Patients and Study Design

Between January 2002 and September 2004, 322 adult patients with stage IV melanoma were randomly assigned at 71 centers in the Untied States (n = 163, 50.6%), Ukraine/Russia (n = 70, 21.7%), Europe (n = 69, 21.4%), and Australia (n = 20, 6.2%). Eligibility criteria included (a) stage IV melanoma, with expected resectability of some/all lesions to obtain at least 7 g of cancer; (b) no prior therapy for stage IV melanoma; (c) no therapy with IL-2 and/or DTIC/temozolomide within 12 months before study entry; (d) Eastern Cooperative Oncology Group (ECOG) performance score of 0 or 1; (e) adequate cardiac function, with New York Heart Association class II or less; (f) normal WBC and platelet counts; and (g) bilirubin no more than 1.5 mg/dL, AST no more than 2.5× the upper limit of normal, and adequate renal function, with serum creatinine no more than 1.5 mg/dL.

Patients were excluded if they had brain metastases, mucosal or ocular melanomas, immunodeficiency, prior splenectomy, uncontrolled infection or serious intercurrent medical illnesses, or other malignancies treated within the last 5 years, with the exception of in situ cervical carcinoma or nonmelanoma skin cancer. Women of childbearing potential required a negative pregnancy test result before entry and agreed to use an effective method of contraception while receiving treatment. All patients gave written informed consent to participate in the study. The study was conducted under International Conference on Harmonisation/WHO Good Clinical Practice and was approved by the respective institutions' institutional review boards or ethics committees.

Vaccine Preparation, Quality Control, and Administration

Macroscopically non-necrotic tumor tissue obtained from the operating room or the pathologist, was shipped on dry ice to the Antigenics facilities and processed under current good manufacturing practices conditions.[23] Vitespen preparations were dispensed into individually identified, 25-μg aliquots and shipped on dry ice to the pharmacy, where they were stored at −80°C. Each aliquot of vitespen was removed from the freezer and was thawed between the fingers immediately before subcutaneous injection to the patient. The first four injections were administered weekly, and subsequent injections were administered every other week. The sites of injection were rotated among the upper and lower extremities. Patients were monitored for toxicity, including complete clinical evaluation and blood tests including differential blood counts, serum chemistry, and autoimmune reactions (antimicrosomal, antithyroid antibodies, antinuclear antibodies, and rheumatoid factor).

Treatments in the PC Arm

Patients randomly assigned to receive PC could receive any regimen containing a minimum cumulative threshold dose of IL-2 (60 million U/m^2) and/or DTIC (1,000 mg/m^2)/temozolomide (600 mg/m^2) and/or complete tumor resection with or without additional therapy, and/or any therapy licensed for the treatment of cancer. Patients who underwent incomplete resection were required to receive additional treatment, with a minimal cumulative threshold dose of IL-2 and/or DTIC/temozolomide. In both arms, therapeutic radiation at sites of pre-existing disease was allowed, but prophylactic radiation was not. This design was chosen as no treatment alone or in combination, has demonstrated an overall survival (OS) benefit for patients with stage IV melanoma in randomized trials. Thus, the heterogeneity of treatments in the PC arm was not expected to reduce the likelihood of demonstrating an OS benefit if present in the vitespen arm, and ensures applicability to common practice for treating stage IV melanoma.

Statistical Methods

The primary end point of OS was analyzed using a one-sided log-rank test with a type 1 error of .05. Analyses were conducted in both intention-to-treat (ITT) and treated patient populations. In the vaccine arm, patients had to receive at least one vaccination to be included in the treated patient analyses. For patients in the PC arm, only those who received treatment(s) per protocol were included in the treated patient analyses. Nominal *P* values, where indicated, refer to *P* values where no adjustment has been made for multiple comparisons; these were calculated purely to detect potential trends. All *P* values stated, including nominal values, are two-sided. Kaplan-Meier (KM) plots were generated and median OS times estimated from the KM plots. Hazard ratios (HRs; with the 95% CI) are reported based on a Cox regression model including ECOG status and American Joint Committee on Cancer (AJCC) stage, in case of OS. The PC arm was used as the denominator to construct the HR. The statistical program SAS 8.2 was used for the analyses (SAS Institute, Cary, NC).

RESULTS

Patient Flow and Disposition, and Preparation of Individualized Vitespen

A total of 450 patients were screened for accrual (Fig 1). There were 128 (28.4% of total screened) screening failures, mostly a result of brain metastases (n = 29, 22.6% of failures), nonstage IV melanoma (n = 27, 21.1%), unwillingness to undergo tumor resection (n = 21, 16.4%), prior treatment for stage IV melanoma (n = 16, 12.5%), and unwillingness to provide consent (n = 13, 10%). A total of 322 patients were randomly assigned, 215 (66.7%) to the vitespen arm, and 107 (33.3%) to the PC arm. The groups were balanced at baseline with respect to age, sex, performance status, stage of disease, and complete resection (Table 1). Tumors (range, 1 to 42 g; mean, 17.8 g) were obtained from skin lesions, nodes, lung, or visceral sites. On average, 56 μg of vitespen was obtained per gram of tumor. Vitespen could be prepared for only 133 (61.9%) of the 215 patients randomly assigned to the vaccine arm.

Safety Assessment

An adverse event (AE) was categorized as related to treatment if it was considered possibly, probably, or definitely related to vitespen or PC by the investigator. The most common (≥ 5%) reported related AEs in the vitespen arm included pyrexia (8.3%), fatigue (6.0%), and nausea (5.3%), and in the PC arm included nausea (10.3%), vomiting (6.5%), rigors (6.5%), and diarrhea (5.6%). In the vaccine arm, two serious AEs (at least grade 4) were considered by investigators as possibly related to vitespen. One patient was reported to have thyroid

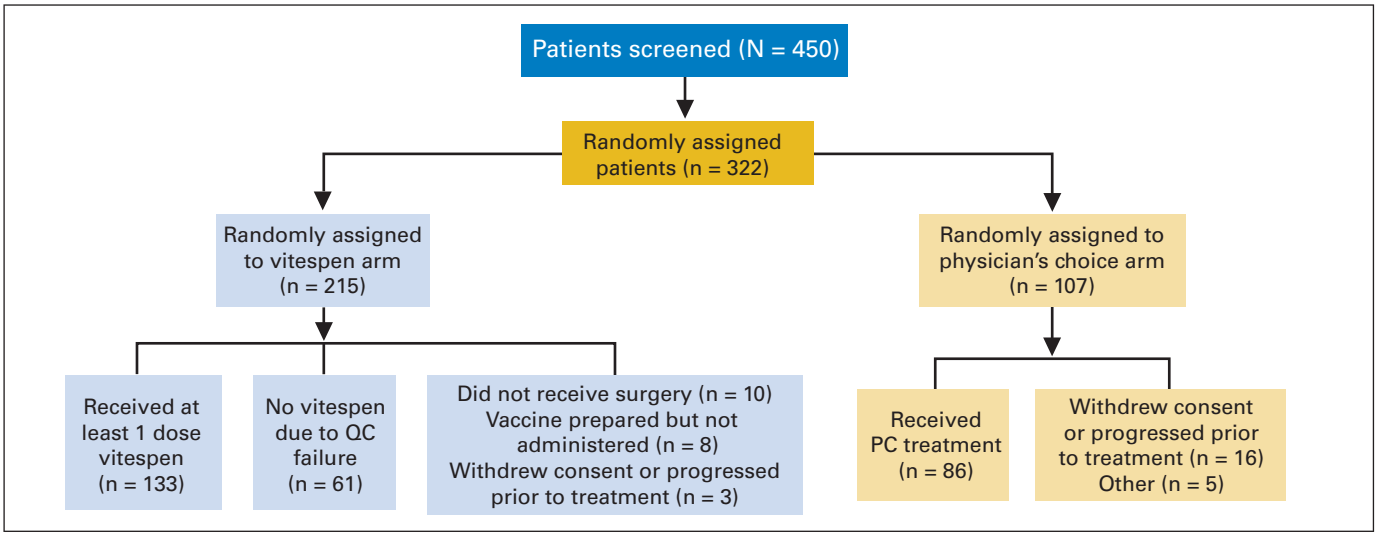

Fig 1. Overview of patient flow and disposition in this trial. PC, physician's choice; QC, quality control.

function disorder resulting from toxic dominant thyroid nodule, symptoms of which resolved with therapy and vaccine cessation. Another patient was reported to present with cellulitis 5 days after the last vaccine; the infection resolved with antibiotic therapy.

ITT Survival Analysis for All Randomly Assigned Patients

Consistent with the 2:1 randomization, 215 patients (66.7%) were randomly assigned to the vitespen arm and 107 patients (33.3%) to the PC arm. Treated patients in the vitespen arm received their first treatment a median of 41 days after random assignment, whereas the corresponding number for those in the PC arm was 9 days. Median follow-up periods for the vaccine and PC arms were 259 and 291 days, respectively. We did not see a statistically significant difference between the two arms overall (Fig 2; $P = .32$; HR = 1.16; 95% CI, 0.69 to 1.71), or when the patients are stratified into AJCC M1a (Fig 2; $P = .94$; HR = 1.03; 95% CI, 0.51 to 2.09), M1b (Fig 2; $P = .79$; HR = 0.92; 95% CI, 0.50 to 1.69)

Table 1. Patient Characteristics

	Randomly Assigned Patients (2:1)					
	Vitespen (n = 215)		Physician's Choice (n = 107)		Overall (N = 322)	
Characteristic	No.	%	No.	%	No.	%
Age, years						
Mean	55.6		54.7		55.3	
Standard deviation	13.63		12.87		13.37	
Median	55.0		56.0		55.0	
Range	19.0-87.0		21.0-79.0		19.0-87.0	
Sex						
Male	124	57.7	66	61.7	190	59.0
Female	91	42.3	41	38.3	132	41.0
Race						
White	211	98.1	106	99.1	317	98.4
Hispanic	4	1.9	0	0	4	1.2
Other	0	0	1	0.9	1	0.3
ECOG score						
0	152	70.7	76	71.0	228	70.8
1	63	29.3	31	29.0	94	29.2
AJCC staging						
M1a	45	20.9	25	23.4	70	21.7
M1b	52	24.2	21	19.6	73	22.7
M1c	118	54.9	61	57.0	179	55.6
Complete resection						
Yes	34	15.8	18	16.8	52	16.1
No	181	84.2	89	83.2	270	83.9

Abbreviations: ECOG, Eastern Cooperative Oncology Group; AJCC, American Joint Committee on Cancer.

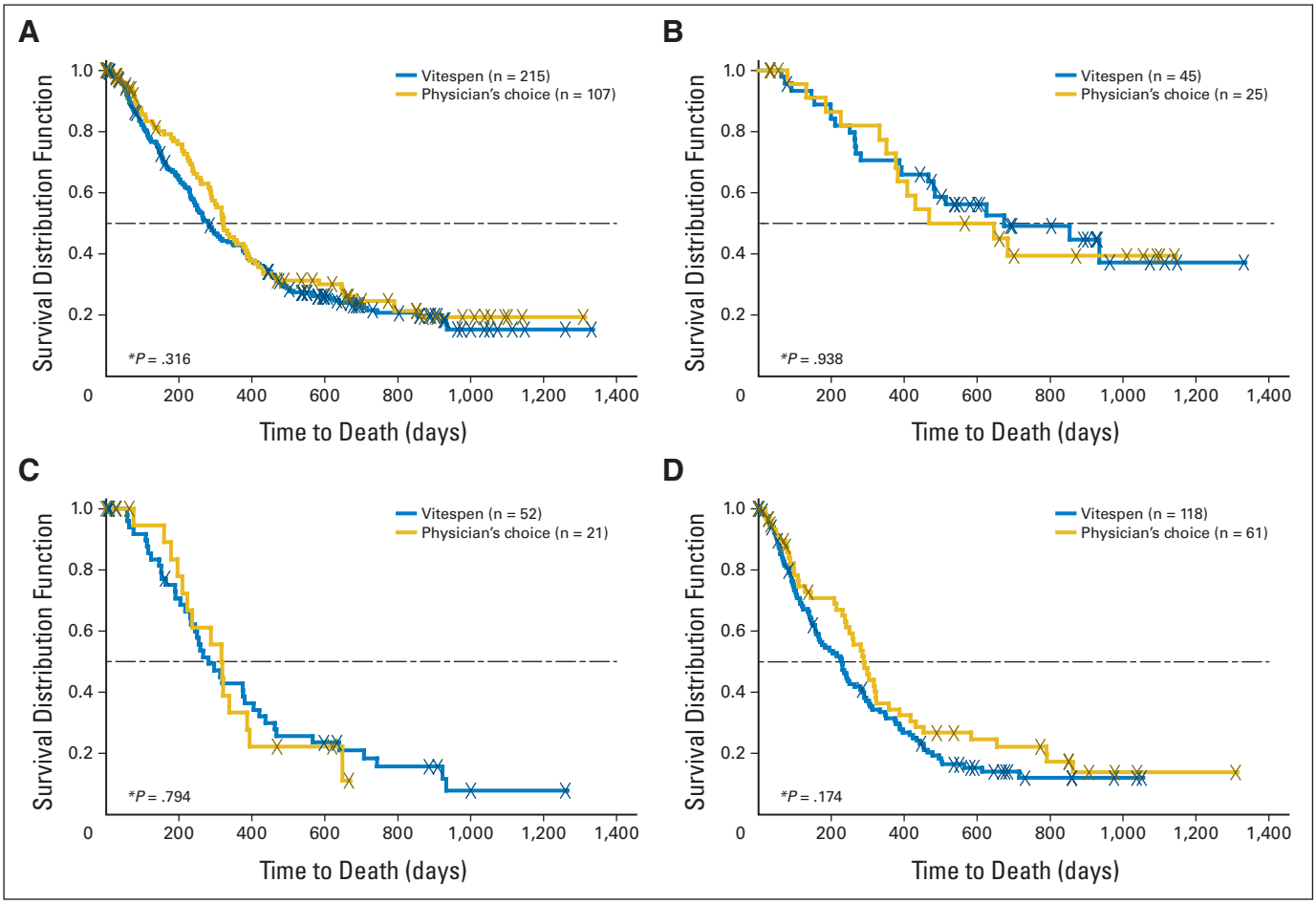

Fig 2. Intention-to-treat analysis of survival for all patients and for patients stratified by substage. (A) Overall, (B) M1a, (C) M1b, and (D) M1c. The number of patients in each arm and a two-sided *P* value are indicated. The horizontal line in each panel reflects the point of median survival.

and M1c substages (Fig 2; $P = .17$; HR = 1.28; 95% CI, 0.89 to 1.84).

Analysis of All Treated Patients

Of the 107 patients randomly assigned to the PC arm, 21 (20.2%) were unable to receive the PC treatment, mostly as a result of consent withdrawal or disease progression before treatment. Similarly, of the 215 patients randomly assigned to the vaccine arm, 82 (38.1%) were unable to receive vaccine treatment. Most (61 of 82) did not receive vaccine treatment because vaccine could not be prepared for them because of technical difficulties; others did not receive vaccine because they did not undergo surgery, had vaccine prepared but not administered, withdrew consent, or experienced disease progression before initiation of treatment. Of the 215 patients randomly assigned to the vaccine arm, only 133 patients (61.8%) received one or more doses of vaccine. The treated patient population was also balanced with respect to the baseline demographic and disease criteria shown in Table 1 for the ITT population (data not shown). A comparative survival analysis of 133 vitespen- and 86 PC-treated patients showed similar patterns of survival to those seen in the ITT populations (Fig A1, online only; $P = .25$; HR = 1.29; 95% CI, 0.86 to 1.96). The patterns remain similar when analyzed by AJCC substage (Fig A1).

Exploratory Landmark Analyses

In experiments in animal models using autologous tumor-derived gp96, multiple immunizations (typically four) have been required for therapeutic effect.[20,24] Theoretical grounds for continued vacci-treatment of cancer patients have also been proposed.[25] One approach to address the effect of different numbers of vacci-treatments on survival may be to compare the survival curves of patients who received increasing numbers of vacci-treatments. This inevitably carries the bias that patients who live longer receive a larger number of vacci-treatments regardless of any effects of vacci-treatments. Hence, an exploratory landmark analysis, where this specific bias is eliminated, was initiated.

We aimed to explore any differences in OS among patients who received one or more (1+), or 10 or more (10+) immunizations with vitespen (Fig 3). For this comparison, all patients in the analysis, including those in the PC arm, should have lived long enough to potentially receive at least 10 immunizations. This time period was calculated as 150 days postrandom assignment (median of 41 days to first immunization + 21 days for three additional weekly immunizations + 84 days for the remaining six immunizations). Therefore, patients who died within 150 days of random assignment were excluded from both arms, and the survival of patients who received 1+

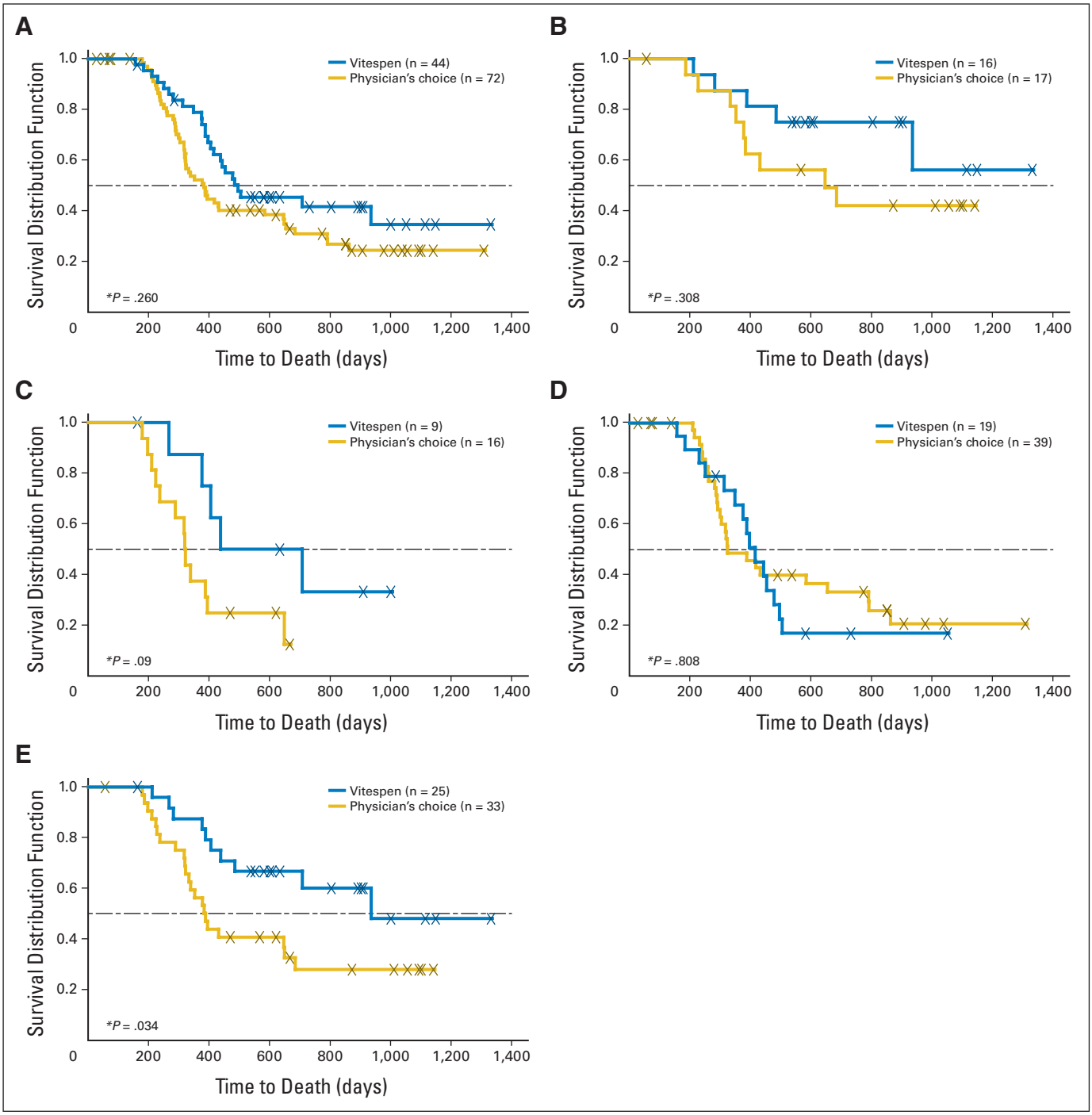

Fig 3. Kaplan-Meier plots based on landmark analyses of survival of patients who received 10+ doses of vitespen, or physician's choice (overall and by substage). (A) Overall, (B) M1a, (C) M1b, (D) M1c, and (E) M1a + M1b. Patients who died within 150 days and those lost to follow-up or who withdrew consent were removed from both arms. The number of patients in each arm and a two-sided nominal *P* value are indicated. The horizontal line reflects the point of median survival.

or 10+ immunizations was compared with patients in the PC arm. The data for 1+ immunized patients are essentially identical to those shown in Figure A1. In contrast, the KM plots for 10+ immunized patients show a clear separation of the two arms in favor of the vitespen-treated patients for all patients as well as M1a (nominal $P = .31$; HR = 0.56; 95% CI, 0.18 to 1.72) and M1b (nominal $P = .09$; HR = 0.39; 95% CI, 0.13 to 1.20), but not M1c (nominal $P = .81$; HR = 1.08; 95% CI, 0.57 to 2.08) patients (Fig 3). Combined data from M1a + M1b patients show a clinically significant benefit of vitespen over PC in this subset of patients (nominal $P = .03$; HR = 0.45; 95% CI, 0.21 to 0.96).

Such an unplanned subset analysis carries obvious risks.[26] Although the most obvious bias, that patients who live longer are able to receive more vaccines (rather than the converse), was addressed

through exclusion from both arms, of patients who lived less than 150 days postrandom assignment, other biases through unknown or unmeasured factors may bias the analyses shown in Figure 3. Hence, a more complete analysis, looking for trends in OS as a function of increasing number of vacci-treatments (from 1+ to 10+), was undertaken. Figure 4 shows the HRs (with CIs) between the two arms for all patients as well as for patients stratified by substage, as a function of the number of vacci-treatments. With increasing number of immunizations, the HR shifts to the left (in favor of vaccine) in all patients, as well as in M1a and M1b, but not M1c, substages. The initial benefit of vitespen is most evident in the M1a substage, but the benefits of additional immunizations are most evident in the M1b substage. Combined data from M1a + M1b substages show the benefit of both trends, such that, among M1a + M1b patients who received 10+ immunizations, the benefit of vitespen is clinically significant and meaningful (nominal $P = .03$) and the limits of the CI for the HR (0.45; 95% CI, 0.21 to 0.96) exclude unity.

DISCUSSION

Vitespen is the first, and thus far the only, autologous tumor-derived protein therapeutic vaccine tested in a randomized phase III trial to our knowledge. ITT analysis shows that the outcome of treatment with vitespen is statistically indistinguishable from treatment with PC including chemotherapy with DTIC/temozolomide and/or IL-2 and surgery. Vitespen was noted to be safe and without significant toxicity.

Questions of efficacy are inextricably linked with whether patients were adequately treated, and that in turn is linked with the number of injections of vitespen administered. Of the 215 patients randomly assigned to the vitespen arm, vitespen could not be prepared for 82 patients (62% success rate). However, even that modest success rate represents an exaggerated number, because it counts even a single vial of vitespen as a success. Animal experiments suggest a minimum threshold of four administrations of vitespen (gp96) to be necessary for protection from tumor growth.[20,24] Using this criterion as a measure of feasibility, the success rate for production of vitespen was a mere 49%. Moreover, patients in the vitespen arm who could not receive vaccine generally received PC therapy, further diluting the ability to detect a difference between both arms. In light of these handicaps in the formal ITT analysis, we find it notable that the outcome of therapy with vitespen was statistically indistinguishable from the best standard of care.

Exploratory landmark analyses, carried out to assess the impact of vitespen on patients who received multiple immunizations, show two notable phenomena: (a) among AJCC M1a and M1b substage patients, particularly among the latter, there is a clear trend toward improved survival as patients received more immunizations, from 1 to 10 or more; (b) among M1c substage patients, there is no such trend. The HR for OS in M1c patients remains steady at approximately 1.0, regardless of the number of immunizations they received. These landmark analyses are subset analyses that must be interpreted with caution because the primary analysis itself does not show significant differences between the arms. Figure 4 shows the results of approximately 40 subset analyses; the fact that one of them (10+ immunizations in M1a + M1b patients) achieves nominal statistical significance could well be a matter of chance. It is of interest to recognize, however, that the data in Figure 4 show a consistent and sustained trend through all of the analyses, and not simply a significant result in a single subset of patients; the trend is consistent with the idea that patients with less advanced disease (M1a and M1b) benefit from increasing doses of vacci-treatments, whereas patients with more advanced disease (M1c) do not. An obvious source of bias in this analysis (ie, that patients who live longer can receive more immunizations) has been eliminated by the design of the landmark analyses as described in the Results section. The lack of efficacy as a function of increasing doses of vacci-treatments in the M1c patients, in addition to being consistent with the mechanism of action of vitespen, may be viewed as a negative control for methodologic biases in the subset analyses.

Because there exists a greater collective experience with chemotherapies than with vacci-therapies, it is easy, and dangerous in our view, to judge one by the standards of the other. Appropriate

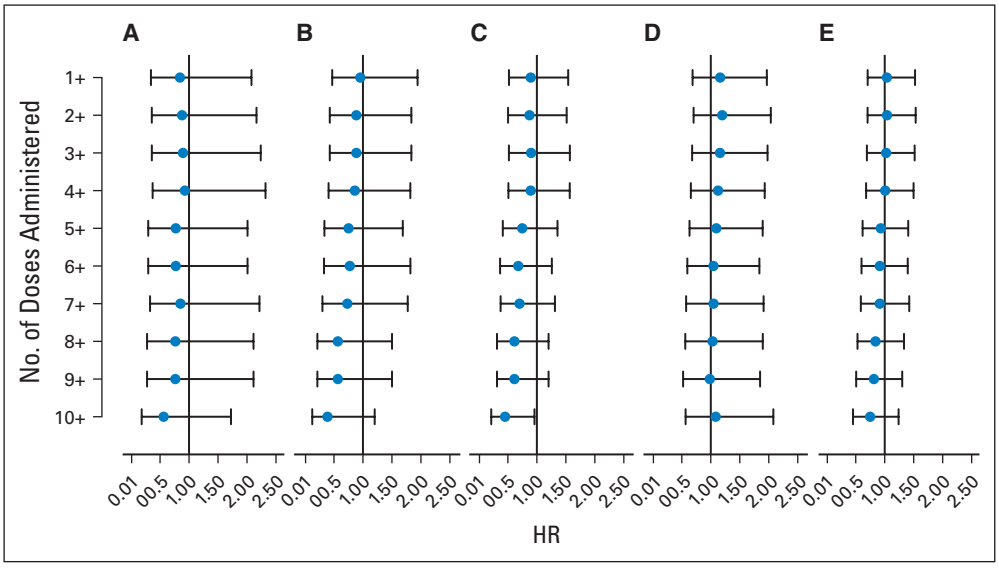

Fig 4. Analyses of hazard ratios (HRs) between vitespen (1+ to 10+ vaccinations) and physician's choice (PC) arms (overall and by substage; based on a 150-day landmark analysis as in Fig 3). (A) M1a, (B) M1b, (C) M1a + M1b, (D) M1c, and (E) Overall. Each HR is indicated by a circle situated within its 95% CI (horizontal line). Intersecting vertical lines represent HRs of 1. HRs left of unity represent benefit by vitespen, and HRs right of unit represent benefit by PC.

evaluation of the results from the two approaches requires recognition that the mechanisms of action of immunotherapy are distinct from those of most chemotherapies in two fundamental ways. First, chemotherapies act directly on cancers, whereas immunotherapy stimulates the host immune response, which must then act on the cancer. This difference has important implications; patients need to be sufficiently healthy for a sufficiently long time to benefit from immunotherapy. Such constraints apply to chemotherapies to a more limited extent. Second, because immunotherapy relies on a secondary inducible mechanism (ie, immunologic activation of the host), its activity is modulated by physiologic parameters. Because cancers in earlier stages are less likely to have acquired immune-subversive armamentarium than are cancers in later stages simply as a consequence of immunologic editing,[27] the former are more susceptible to immunotherapy. This principle does not directly apply to chemotherapies. Attention to these considerations suggests that the results of the landmark analyses presented here are consistent with the immunologic mechanisms of action of vitespen and with the natural history or biology of stage IV melanoma. Our results require formal confirmation through a randomized trial in which patients in the M1a and M1b substages are treated with a suitable number of vacci-treatments with vitespen.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

ERRATA

The November 15, 2004, article by Armanios et al entitled, "Adjuvant Chemotherapy for Resected Adenocarcinoma of the Esophagus, Gastro-Esophageal Junction, and Cardia: Phase II Trial (E8296) of the Eastern Cooperative Oncology Group" (J Clin Oncol 22:4495-4499, 2004) contained an error.

In the Introduction, the second sentence of the third paragraph indicated that a 7-month improvement in median survival was found, whereas it should have been a 9-month improvement, as follows:

"The INT0116 trial evaluated chemoradiotherapy in patients with completely resected adenocarcinoma of the stomach and found a **9-month improvement** in median survival in the treatment arm compared with the surgery-alone arm.[5]"

DOI: 10.1200/JCO.2008.18.8656

The February 20, 2008, article by Testori et al entitled, "Phase III Comparison of Vitespen, an Autologous Tumor-Derived Heat Shock Protein gp96 Peptide Complex Vaccine, With Physician's Choice of Treatment for Stage IV Melanoma: The C-100-21 Study Group" (J Clin Oncol 26:955-962, 2008) contained an error. The legend for Figure 4 did not include labels for figure parts A-E, and should have been:

Fig 4. Analyses of hazard ratios (HRs) between vitespen (1+ to 10+ vaccinations) and physician's choice (PC) arms (overall and by substage; based on a 150-day landmark analysis as in Fig 3). **(A) M1a, (B) M1b, (C) M1a + M1b, (D) M1c, and (E) Overall.** Each HR is indicated by a circle situated within its 95% CI (horizontal line). Intersecting vertical lines represent HRs of 1. HRs left of unity represent benefit by vitespen, and HRs right of unit represent benefit by PC.

The online version has been corrected in departure from the print.

DOI: 10.1200/JCO.2008.18.8664

The June 10, 2008, review article by Sell and Leffert entitled, "Liver Cancer Stem Cells" (J Clin Oncol 26:2800-2805, 2008) contained errors.

In the References section, the first author's name was inadvertently misspelled in references 91 and 92, which should have been Yang ZF, as follows:

91. **Yang ZF**, Ngai P, Ho DW, et al: Identification of local and circulating cancer stem cells in human liver cancer. Hepatology 47:919-928, 2008

92. **Yang ZF**, Ho DW, Ng MN, et al: Significance of CD90+ cancer stem cells in human liver cancer. Cancer Cell 13:153-166, 2008

DOI: 10.1200/JCO.2008.18.8672

