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Three Phase II Cytokine Working Group Trials of gp100 (210M) Peptide Plus High-Dose Interleukin-2 in Patients With HLA-A2–Positive Advanced Melanoma

Jeffrey A. Sosman, Carole Carrillo, Walter J. Urbá, Lawrence Flaherty, Michael B. Atkins, Joseph I. Clark, Janet Dutcher, Kim A. Margolin, James Mier, Jarod Gollob, John M. Kirkwood, David J. Panka, Nancy A. Crosby, Kevin O’Boyle, Bonnie LaFleur, and Marc S. Ernstoff

Vanderbilt University Medical Center, Nashville, TN; Earle A. Chiles Research Institute, Portland, OR; Wayne State University, Detroit, MI; Beth Israel Deaconess Medical Center, Boston, MA; Loyola University Medical Center, Maywood, IL; Our Lady of Mercy Medical Center, Bronx, NY; City of Hope Medical Center, Duarte, CA; University of Pittsburgh Medical Center, Pittsburgh, PA; and Dartmouth Hitchcock Medical Center, Lebanon, NH.

Abstract

Purpose—High-dose interleukin-2 (IL-2) induces responses in 15% to 20% of patients with advanced melanoma; 5% to 8% are durable complete responses (CRs). The HLA-A2–restricted, modified gp100 peptide (210M) induces T-cell immunity in vivo and has little antitumor activity but, combined with high-dose IL-2, reportedly has a 42% (13 of 31 patients) response rate (RR).

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Corresponding author: Jeffrey A. Sosman, MD, Vanderbilt-Ingram Cancer Center Vanderbilt, University Medical Center, Section of Hematology/Oncology, 777 Preston Research Bldg, Nashville, TN 37232-6307; jeff.sosman@vanderbilt.edu..

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AUTHOR CONTRIBUTIONS **Conception and design:** Jeffrey A. Sosman

Provision of study materials or patients: Jeffrey A. Sosman, Walter J. Urbá, Lawrence Flaherty, Michael B. Atkins, Joseph I. Clark, Janet Dutcher, Kim A. Margolin, James Mier, Jarod Gollob, John M. Kirkwood, David J. Panka, Nancy A. Crosby, Kevin O’Boyle, Marc S. Ernstoff

Collection and assembly of data: Jeffrey A. Sosman, Carole Carrillo, David J. Panka

Data analysis and interpretation: Jeffrey A. Sosman, Bonnie LaFleur

Manuscript writing: Jeffrey A. Sosman, Walter J. Urbá, Michael B. Atkins, Kim A. Margolin, Bonnie LaFleur, David J. Panka, Marc S. Ernstoff

Final approval of manuscript: Jeffrey A. Sosman, Carole Carrillo, Walter J. Urbá, Lawrence Flaherty, Joseph I. Clark, Janet Dutcher, Kim A. Margolin, James Mier, Jarod Gollob, David J. Panka, Nancy A. Crosby, Kevin O’Boyle, Bonnie LaFleur, Marc S. Ernstoff

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We evaluated 210M with one of three different IL-2 schedules to determine whether a basis exists for a phase III trial.

Patients and Methods—In three separate phase II trials, patients with melanoma received 210M subcutaneously during weeks 1, 4, 7, and 10 and standard high-dose IL-2 during weeks 1 and 3 (trial 1), weeks 7 and 9 (trial 2), or weeks 1, 4, 7, and 10 (trial 3). Immune assays were performed on peripheral-blood mononuclear cells collected before and after treatment.

Results—From 1998 to 2003, 131 patients with HLA-A2–positive were enrolled. With 60-month median follow-up time, the overall RR for 121 assessable patients was 16.5% (95% CI, 10% to 26%); the RRs were 23.8% in trial 1 (42 patients), 12.5% in trial 2 (40 patients), and 12.8% in trial 3 (39 patients). There were 11 CRs (9%) and nine partial responses (7%), with 11 patients (9%) progression free at 30 months. Immune studies including assays of CD3- ζ expression and numbers of CD4⁺/CD25⁺/FoxP3⁺ regulatory T cells, CD15⁺/CD11b⁺/CD14[−] immature myeloid-derived cells, and CD8⁺gp100 tetramer-positive cells in the blood did not correlate with clinical benefit.

Conclusion—The results again demonstrate efficacy of high-dose IL-2 in advanced melanoma but did not demonstrate the promising clinical activity reported with vaccine and high-dose IL-2 in any of three phase II trials.

INTRODUCTION

The median survival time for patients with advanced melanoma remains dismal at 6 to 10 months.¹ Chemotherapy with dacarbazine has a response rate (RR) of only 5% to 20%, with most responses being short lived. Chemotherapy combined with biologic agents provides no survival benefit.¹⁻³ In a report on 270 patients with melanoma, high-dose bolus interleukin-2 (IL-2) therapy produced a 16% objective RR with long-lasting responses in select patients (5% to 8%); thus, the US Food and Drug Administration approved high-dose IL-2 in patients with melanoma. Previous attempts to enhance high-dose IL-2 efficacy in patients with melanoma have been largely unsuccessful.⁴⁻⁹

A number of immunogenic peptides derived from tissue (lineage)-restricted antigens, cancer-testis antigens, and rarely mutated cancer-specific antigens have been identified in melanoma, and many have the ability to induce antimelanoma T-cell responses.¹⁰⁻¹⁶ A peptide derived from gp100 (a lineage-restricted antigen) has been synthesized in a mutated (heteroclitic) form for the purpose of enhancing HLA-A2.1 binding. This peptide, gp100:209-217(210M) binds with a higher affinity to HLA-A2 than the native peptide and induces better T-cell stimulation in vitro and in vivo.¹⁷ Initial clinical trials at the National Cancer Institute (NCI) with 210M demonstrated induction in peripheral blood of peptide- and tumor-specific cytotoxic T-lymphocyte responses in 10 of 11 patients with HLA-A2.1–positive melanoma, although no definitive antitumor activity was observed.^{18,19} However, clinical experience combining 210M followed by IL-2 (high-dose bolus) demonstrated tumor responses in 13 (42%) of 31 patients.¹⁸ Patients were treated with different schedules of peptide vaccines and IL-2. In responding patients receiving IL-2 early in their course of treatment, circulating tumor-specific T cells were not generated, questioning their role as a biomarker of clinical activity.

We evaluated different schedules of peptide vaccine plus high-dose IL-2 in three separate, single-arm, and single-stage phase II trials to define which schedule was most promising. The three schedules were chosen based on our discussion with the investigators at the NCI-Surgery Branch and the similarity to the regimens used by them, as previously reported.¹⁸ Our primary intent was to determine whether more extensive testing of any of the schedules was justified.

PATIENTS AND METHODS

Patient Eligibility

Patients with advanced melanoma were HLA-A2 positive by serology, flow cytometry, or molecular techniques (HLA-A2.1). Patients had an Eastern Cooperative Oncology Group performance status of 0 to 1 and adequate organ function for high-dose IL-2 therapy. Normal exercise stress test and forced expiratory volume in 1 second more than 2.0 L or 75% predicted were required. Patients with brain metastases or prior IL-2 therapy were ineligible. The protocol was approved by the institutional review boards at each of the participating centers, and written informed consent was required.

Therapeutic Agents

gp100:209-217(210M) peptide—gp100:209-217(210M) (NSC 683472) is an HLA-A2 restricted 9-mer (IMDQVPFSV) peptide from gp100 provided by Cancer Therapy Evaluation Program (CTEP) under Investigational New Drug Application BB6123 in vials containing 1 mg/mL of sterile water for injection with 0.1 N hydrogen chloride to adjust the pH.

Montanide ISA-51 adjuvant—Montanide ISA-51 (NSC 675756) is an oil-based adjuvant similar to incomplete Freund's. The product is manufactured by Seppic, Inc (Fairfield, NJ) and was provided by CTEP.

IL-2—Commercial recombinant human IL-2 (Proleukin; Chiron, Emeryville, CA), lyophilized in vials containing 22 million U (approximately 1.3 mg), was reconstituted with 1.2 mL of sterile water for injection.

Treatment Plan

Patients were injected subcutaneously with 210M and Montanide ISA-51. Vaccine was administered on days 1, 22, 43, and 65 for all three clinical trials. High-dose IL-2 (600,000 U/kg every 8 hours) was administered up to 14 times intravenous bolus over a 5-day inpatient course, starting 1 day after vaccine. Details are provided in Figure 1.

Patients underwent tumor response assessments at week 12 by WHO criteria.²⁰ Patients with stable disease, partial response (PR), or complete response (CR) could receive another course of treatment, whereas patients with disease progression were removed from protocol. All responses were confirmed at 3 months, and the RR was based on best response any time after treatment had been initiated. Patients could receive up to three 12-week courses of IL-2 and 36 weeks of vaccine. Vaccine alone could be administered without further IL-2. Progression-free survival (PFS) was defined as the time from trial entry to the time of documented objective disease progression or death. Overall survival (OS) was defined as the time from trial entry until death.

Treatment modifications were made by withholding doses of IL-2 for life-threatening toxicities including refractory hypotension, hypoxia, anuria, acidosis, and mental status changes. Toxicity was managed based on guidelines of the Cytokine Working Group (CWG).²¹ No dose modifications were required for the vaccinations because there were no systemic or severe local skin reactions.

Immunologic Assessments

Blood for immunologic assays was collected at two time points, within 2 weeks before therapy and during week 12 of therapy. Peripheral-blood mononuclear cells (PBMCs) were isolated and cryopreserved. A single center performed the analysis on cryopreserved

PBMCs. Normal controls were run with each experiment. Studies were performed blinded to the clinical results.

Flow Cytometry Analysis

Reagents—CD3-peridinin-chlorophyll-protein complex (PerCP), CD4PerCP, CD8PerCP, CD11bPE, IgG2aPE, CD14-fluorescein isothiocyanate (FITC), gp100, and influenza (flu) HLA-A2 tetramers were conjugated to phycoerythrin (PE). CD15-PC5, CD3- ζ chain-FITC, and normal mouse IgG1-FITC were purchased from BD Pharmingen (Franklin Lakes, NJ), Beckman Coulter (Fullerton, CA), Immunotech (Boston, MA), and Santa Cruz Biotechnology (Santa Cruz, CA), respectively.

ζ -chain analysis—PBMCs were stained with CD3PerCP, washed, permeabilized, stained for CD3- ζ chain-FITC or an isotype control from Caltag Laboratories (San Francisco, CA), and then analyzed. The isotype control set the boundary of zeta positivity.

Surface staining of PBMC with CD14-FITC, CD11b-PE, CD15-PC5, and CD15-isotype control was at 4°C, and staining of CD8PerCP, gp100 PE, Flu PE, and CD15-isotype control was at room temperature. Cells were washed and then analyzed on FC500 cytometer (Beckman Coulter).

Lymphocytes were first stained using CD4-FITC and CD25-APC (eBioscience, San Diego, CA). After fixation and permeabilization, FoxP3-PE was added. Cells were washed and fixed with 1% formaldehyde in phosphatebuffered saline. For analysis, CD4⁺ and CD25⁺ cells were gated and analyzed for FoxP3. Isotype controls for all antibodies were included.

Statistical Analysis

The primary end point of the trial was RR including both PRs and CRs. The design defined a minimum 30% RR (12 of 40 patients; 95% CI, 16.3% to 43.7%) as demonstrating promise for proceeding to a phase III trial. The recommendation of a 30% RR in this specific population of trials (rather than powered to demonstrate superiority to a historical control response) was based on recommendations from a number of investigators from NCI-CTEP and the CWG. The RR would assist in estimating the number of patients required for a phase III trial with adequate power. The trial was not designed to definitively prove that the peptide vaccine combined with high-dose IL-2 was or was not an improvement compared with IL-2 alone. CIs computed in the article are based on the binomial distribution.

To examine immune cell populations and clinical efficacy (CR, PR, or PFS > 12 months), repeated measures analysis of variance methods were used for paired (pre- and post-treatment) data within each subject. These methods take the paired nature of the data into account, resulting in a test statistic that is equivalent to a paired *t* test. The data are assumed to be normally distributed because the immune cells are measured as percentage of cells expressing the phenotype. The data are interval data (eg, bounded by 0 to 100), and not discrete samples from Poisson, Bernoulli, or binomial distributions. These tests were used to compare data both within response groups (before and after treatment) and between response groups (pretreatment and post-treatment responders and nonresponders). We tested time (before and after treatment), response, and the time by response interaction in these models. We used the log-rank test statistic to examine the difference in survival between the cohorts. Rather than show all of the pair-wise CIs, we chose to show them within response group differences along with the actual data. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Patients With Melanoma

A total of 131 patients were enrolled from September 1998 to November 2003 at eight institutions. Clinical data were available for 121 patients. One patient did not have melanoma, and nine patients were lost to follow-up and excluded from the analysis. The characteristics of the 121 patients are listed in Table 1. More than 65% of patients had M1c disease. All three cohorts were similar in patient characteristics, except that trial 2 enrolled fewer patients who had received adjuvant interferon and more patients with lactate dehydrogenase (LDH) elevations. Neither of these differences was statistically significant.

Treatment Administration and Toxicities

Toxicity of high-dose IL-2 was primarily assessed by reduction in the number of IL-2 doses administered. Toxicity was not affected by the 210M peptide administration.^{21,22} Vascular leak syndrome and mental status changes were the primary reasons for withholding doses of IL-2. Three patients died of progressive melanoma during the initial 12 weeks of the study. Fourteen patients did not receive IL-2 therapy (12 patients on trial 2), many as a result of a decline in performance status from disease progression during the initial weeks of vaccination, which prevented IL-2 administration. These patients were included in the analysis. Of patients who received one dose of IL-2, those on trial 3 received the most doses, with a median of 35 of 56 possible doses (range, eight to 51 doses) for the 12 weeks. Patients receiving one dose of IL-2 in both trials 1 and 2 received a median of 20 of 28 IL-2 doses. The number of doses ranged from nine to 27 in trial 2 and from 11 to 27 in trial 1. The only direct toxicities caused by the peptide vaccine were local swelling and discomfort. No episodes of uveitis, thyroiditis, or other autoimmune sequelae were recorded, and vitiligo was observed in some responders but not systematically collected.²³ The number of patients who received additional 12-week courses of vaccine and/or IL-2 is presented in Table 2.

Tumor Responses, Durability of Responses, OS, and PFS

With a median follow-up time of 60 months (range, 30 to 74 months), there were 20 clinical responses (16%) among 121 assessable patients (Table 3). Trial 1 had the highest RR of 23.8% (10 of 42 patients), trial 2 had an RR of 12.5% (five of 40 patients), and trial 3 had an RR of 12.8% (five of 39 patients). None of the schedules (trials) reached the 30% RR targeted in the trial. Overall, nine of the responses (7%) were PRs, and 11 (9%) were CRs. Nine of the 11 patients who achieved CR remain progression free (PF) at 30+ to 73+ months (30+, 30+, 38+, 40+, 42+, 46+, 46+, 46+, and 73+ months), whereas two patients experienced progression at 17 and 51 months. Only one of the nine patients who achieved PR remains PF at 30+ months. Five of the patients with PR experienced disease progression in less than 12 months.

The median PFS time was 3 months (time of initial tumor evaluation) for each of the three trials (log-rank test for difference in PFS between trials, $P = .165$; Appendix Fig A1, online only). Eleven patients (9%) remain PF with more than 30 months follow-up. Patients who are PF include seven patients in trial 1, three in trial 2, and one in trial 3.

At the time of this analysis, there were 18 patients (15%) who remained alive for 30+ to 70+ months. The median OS time of the entire group was similar in each trial (range, 13.7 to 15.4 months) or slightly under 15 months combined (log-rank test for difference in OS between trials, $P = .723$; Appendix Fig A2, online only).

Immunologic Studies and Clinical Correlations

Paired samples from peripheral blood with more than 80% viability were obtained before therapy and on week 12 in only 53 of the 121 patients because of early disease progression, poor compliance, and poor viability in some specimens. The characteristics of these patients are presented in Table 4. Expression of the TCR- ζ chain, an indicator of T-cell signaling dysfunction; CD4⁺/CD25⁺/FoxP3⁺, an indicator of regulatory T cells (Tregs); CD11b⁺/CD15⁺/CD14⁻, an indicator of myeloid-derived suppressor cells (MDSCs); and percentage of CD8⁺ tetramers positive for gp100 peptide and flu were assayed.²⁴⁻²⁹ Among the 53 patients, 10 had responses, including six CRs and four PRs. Furthermore, 13 patients had PFS 12 months (most with response). As shown in Figure 2, with means, CIs, and statistical findings (in legend), there was no difference in the percentage of PBMCs before and after treatment for any of the immune populations (CD3- ζ chain, CD4⁺/CD25⁺/FoxP3⁺, or CD11b⁺/CD15⁺/CD14⁻) in patients with or without clinical responses and patients with or without 12 months of PFS. Additionally, mean fluorescent intensity for each marker was not different (data not shown). Some patients had impressive increases in their CD8⁺ gp100 tetramers but without a correlation with either clinical response or 12-month PFS.

DISCUSSION

In advanced melanoma, high-dose IL-2 has an RR of 16%, with a 5-year PFS rate of more than 5% (14 of 270 patients).³ In 1998, Rosenberg et al¹⁸ reported on 31 patients with melanoma receiving both 210M and high-dose IL-2, with an RR of 42% (13 of 31 patients). We report the clinical results of three separate phase II trials with 210M peptide vaccine and high-dose IL-2 against melanoma. None of the three trials reached the target clinical activity (30% RR) to proceed to an adequately powered phase III trial. Why do our results differ from the NCI trial?¹⁸ Patient characteristics such as performance status, LDH level, prior chemotherapy, and disease organ sites may have significantly differed in the two studies and are not available for the NCI-SB trial.³⁰ The NCI-SB has shown that certain characteristics are associated with responses to IL-2, such as subcutaneous or cutaneous disease.³¹ The CWG administered smaller doses of IL-2 (600,000 U/kg in CWG study v 720,000 U/kg in NCI-SB study), but the number of doses administered was greater in the CWG trial, with 20 doses over a 3-week period versus 13 to 14 doses for the NCI-SB. This more than compensated for the difference per dose. Finally, patients who travel to the NCI are a self-selected group of patients, which could impact on patient characteristics.

The overall RR of 16.5% was similar to HD IL-2 alone.³ Ten patients remain PF with 30 months of follow-up, which is similar to numbers previously reported (8.2% in this study v 5.1% historical). This remarkable benefit did not seem to be improved by the addition of an immunologically active peptide vaccine. Patients with prior adjuvant interferon alfa treatment had an RR similar to patients who had not received interferon alfa (20% v 14.2%, respectively; $P = .7303$). A number of the patients, mostly in trial 2, were removed from treatment before receiving IL-2 as a result of early disease progression. These patients were included in this analysis, but this may not be true in reports of therapies requiring a delay (cell harvest and expansion) before initiation of treatment. Attempts to combine the 210M peptide with a low-dose IL-2 regimen in patients with metastatic melanoma showed a lack of clinical activity.³²

Unfortunately, our attempts to define immune correlates of response were unsuccessful in the 53 patients for whom pre- and post-treatment specimens were available. Ten of these patients had objective clinical responses. The populations of immune cells assessed were chosen because all four populations play a potential role in an antitumor immune response. The gp100-specific CD8 T cells represent the vaccine-induced effector T cells, whereas the

CD4⁺/CD25⁺/FoxP3⁺ Tregs suppress effective T-cell responses by effector T cells, and the MDSCs (CD11b⁺/CD15⁺/CD14⁻) represent a population that also suppresses T-cell responses. Finally, low CD3- ζ expression is a potential marker of ineffective T-cell function with poor signaling through its T-cell receptor. One might hypothesize that modulation of these populations may be necessary for effective clinical responses.²⁴⁻²⁹ There was insufficient power to detect differences between responders and nonresponders in any single trial.

The main limitation in the immune testing is the amount of intrasubject variation inherent in the assays. Using pre- and post-treatment paired samples is generally an analytic approach for data with this type of variation. The results have limitations because the trials are not randomized and each cohort was relatively small. This set of phase II trials cannot definitively establish whether or not vaccine adds to the benefit of IL-2, but we did not reach the 30% RR that was targeted a priori.

Powering this type of study effectively is difficult. Serum LDH levels were elevated in only 18 of those patients tested and do not explain the variability of the groups. A great need exists to learn whether any biomarker might predict clinical outcome.³² Our results should not deter others from pursuing biomarkers that can predict response to immunotherapy. Functional studies were not feasible because of the number of cells collected and concern for loss of function with processing.³³ The timing of the sample collections may be critical, and although samples were all obtained at week 12, the time after IL-2 dosing was variable. Two different approaches might have made the immune assays more informative. Most important is the need to examine the tumor site, not the peripheral blood, for immunologic changes. Changes in the blood may not reflect changes in tumor. Also, assays that include T-cell function may have been more useful, such as enzyme-linked immunosorbent spot assays for cytokine release or flow cytometry for intracellular cytokine expression.

Given our results, the combination of 210M peptide vaccine and high-dose IL-2 does not seem to represent a significant advance. This does not substitute for a phase III trial of high-dose IL-2 with or without peptide vaccination, which is ongoing and will accrue 160 patients. On the basis of our results (trial 1), this phase III trial could be underpowered to detect a difference. We must find better treatments for patients with melanoma. Peptide vaccines alone are likely not going to be the answer. Advances in immunization methods, understanding mechanisms of escape from immunotherapy, and improved assays for immune activation have all taken place since this trial.²⁴⁻²⁹ Presently, attempts to overcome host immune suppression induced by tumor is a major target of tumor immunology. Attempts include elimination of Tregs, induction of the differentiation of MDSCs, and utilization of ex vivo autologous tumor-specific T cells (adoptive cellular therapy) with nonmyeloablative chemotherapy and cell infusion with high-dose IL-2.³³⁻³⁷ These approaches are under investigation and will hopefully fulfill their promise to improve therapy of patients with advanced melanoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

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®).

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Fig 1.

Treatment schema (initial 12-week course). gp100 (210M) was administered in incomplete Freund adjuvant every 3 weeks (weeks 1, 4, 7, and 10) for all three clinical trials. High-dose interleukin-2 (IL-2; 600,000 U/kg every 8 hours) was administered up to 14 times intravenous bolus over 5 days, starting the day after vaccine, on weeks 1 and 3 for trial 1, on weeks 7 and 9 for trial 2, and on weeks 1, 4, 7, and 10 for trial 3 (maximum).

Fig 2.

Effects of gp100 (210M) plus high-dose interleukin-2 (IL-2) on immune populations. Peripheral-blood mononuclear cells were obtained before treatment and during week 12 of treatment and assayed by flow cytometry. Responding patients are defined in the Results. Dot plots for data are shown with the mean pre- and post-treatment difference (horizontal line) and 95% CI (vertical line) for both responding and nonresponding patients. Left-column responders are based on PFS, and right-column responders are based on objective response. The results are based on percentage of mononuclear cells. (A) Percentage of CD11b⁺/CD15⁺/CD14⁻ pre versus post-treatment expression in responders versus nonresponders by progression-free survival (PFS; $P = .5052$) or by objective responses ($P = .4269$). (B) Percentage of CD4⁺/CD25⁺/FoxP3⁺ pre versus post-treatment levels in responders versus nonresponders by PFS ($P = .6844$) or by objective responses ($P = .6340$). (C) Percentage of CD3-ζ pre versus post-treatment levels of expression in responders versus nonresponders by PFS ($P = .7522$) or by objective responses ($P = .1445$). (D) Percentage of CD8⁺ T cells expressing gp100-209 tetramers as the log of percentage of CD8⁺ T cells. There is no difference between pre versus post-treatment levels of gp100 tetramer-positive cells in responders versus nonresponders by PFS ($P = .7839$) or by objective responses ($P = .1502$). (E) CD8⁺ T cells expressing influenza tetramers as the log of percentage of CD8⁺ T cells. There is no difference between pre- and post-treatment levels of flu-positive cells in any of the cohorts.

Table 1

Patient Characteristics

Characteristic	Trial 1 (n = 42)		Trial 2 (n = 40)		Trial 3 (n = 39)		All Patients (N = 121)	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Sex								
Male	24		25		24		72	
Female	18		15		15		49	
Age, years								
Median	52		52		48		50	
Range	21-76		20-72		20-69		20-76	
ECOG PS								
0	34		35		29		99	
1	8		5		10		22	
LDH								
Elevated	10	31	13	72	9	47	34	46
Normal	21	66	5	28	10	53	40	54
Unknown	11		22		20		53	
AJCC staging								
M1a	5	11	2	5	6	15	13	11
M1b	6	14	10	25	11	28	27	22
M1c	31	74	28	70	22	56	81	67
Prior therapy								
Interferon alfa	15	36	9	23	16	41	40	34
Chemotherapy	5	12	5	12.5	5	13	15	13

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase; AJCC, American Joint Committee on Cancer.

Table 2

Subsequent Vaccine and IL-2 Therapy in Courses 2 and 3

Course of Treatment	No. of Patients		
	Trial 1 (n = 42)	Trial 2 (n = 40)	Trial 3 (n = 39)
Second course of vaccine	17	9	9
Second course of IL-2	17	7	4
Third course of vaccine	15	5	1
Third course of IL-2	8	3	0

NOTE. Number of patients receiving therapy in subsequent 12-week courses. Vaccine could be administered without IL-2.

Abbreviation: IL-2, interleukin-2.

Table 3

HD IL-2 Plus gp100 209-2 M Peptide Vaccine Trial

Cohort	Response by WHO Criteria				
	No. of Patients Assessed	No. of CR	No. of PR	RR (%)	95% CI* (%)
Overall	121	11	9	16.5	10 to 26
Trial 1	42	6	4	23.8	12 to 40
Trial 2	40	4	1	12.5	4 to 27
Trial 3	39	1	4	12.8	4 to 27
HD IL-2 database	270	17	26	15.9	12 to 21
NCI-surgery branch trial	31	13 [†]		41.9	26 to 59

Abbreviations: HD IL-2, high-dose interleukin-2; CR, complete response; PR, partial response; RR, response rate; NCI, National Cancer Institute.

*
CIs based on the binomial distribution.

[†]
CR + PR.

Table 4

Patient Characteristics of Subset Tested for Immune Parameters

Characteristic	Patients With Immune Tests (n = 53)		Patients Not Immune Tested (n = 68)		All Patients (N = 121)	
	No.	%	No.	%	No.	%
Sex						
Male	31		41		72	
Female	22		27		49	
Age, years						
Median		50		50		50
Range		21-69		20-76		20-76
ECOG PS						
0	43		56		99	
1	10		12		22	
Trial						
Trial 1	21	39	21	31	42	
Trial 2	13	25	27	40	40	33
Trial 3	19	36	20	29	39	32
AJCC staging						
M1a	4	8	9	13	13	11
M1b	15	28	12	18	27	22
M1c	34	64	47	69	81	67
Prior therapy						
Interferon alfa	21	40	21	31	42	34
Chemotherapy	5	9	10	15	15	13

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