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ORIGINAL REPORT

Vaccination of Patients With Advanced Non–Small-Cell Lung Cancer With an Optimized Cryptic Human Telomerase Reverse Transcriptase Peptide

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ABSTRACT

Purpose
To evaluate the immunological and clinical response as well as the safety of the optimized peptide telomerase reverse transcriptase p572Y ($TERT_{572Y}$) presented by HLA-A*0201 in patients with advanced non–small-cell lung cancer (NSCLC).

Patients and Methods
Twenty-two patients with advanced NSCLC and residual (n = 8) or progressive disease (PD; n = 14) following chemotherapy and/or radiotherapy received two subcutaneous injections of the optimized $TERT_{572Y}$ peptide followed by four injections of the native $TERT_{572}$ peptide administered every 3 weeks. Peptide-specific immune responses were monitored by enzyme-linked immunosorbent spot assay and/or $TERT_{572Y}$ pentamer staining.

Results
Twelve (54.5%) of 22 patients completed the vaccination program. Toxicity consisted primarily of local skin reactions. $TERT_{572}$-specific $CD8^+$ cells were detected in 16 (76.2%) of 21 patients after the second vaccination, and 10 (90.9%) of 11 patients after the sixth vaccination. Stable disease (SD) occurred in eight (36.4%) of 22 vaccinated patients, with three (13.6%) having had PD before entering the study. The median duration of SD was 11.2 months. After a median follow-up of 10.0 months, patients with early developed immunological response (n = 16) had a significantly longer time to progression and overall survival (OS) than nonresponders (n = 5; log-rank tests $P = .046$ and $P = .012$, respectively). The estimated median OS was 30.0 months (range, 2.8 to 40.0 months) and 4.1 months (range, 2.4 to 10.9 months) for responders and nonresponders, respectively.

Conclusion
$TERT_{572Y}$ peptide vaccine is well tolerated and effective in eliciting a specific T cell immunity. Immunological response is associated with prolonged survival. These results are encouraging and warrant further evaluation in a randomized study.

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INTRODUCTION

Non–small-cell lung cancer (NSCLC) represents 80% of lung cancer cases. Most patients present with stage III/IV disease and have a median survival of less than 12 months with chemotherapy and radiotherapy. Recently, biologic agents have been evaluated with promising results.[1] Although NSCLC was initially considered weakly immunogenic or nonimmunogenic, recent studies with vaccines have shown encouraging efficacy.[2-4]

Antitumor immunotherapy is mainly based on the activation of cytotoxic T lymphocytes (CTL) recognizing endogenously processed peptides derived from tumor antigens and presented at the cell surface in association with HLA class I molecules (HLA I). Dominant peptides exhibit high HLA I affinity and immunogenicity, but most vaccines targeting dominant peptides gave relatively disappointing results in clinical studies due to the presence of tolerance.[5-6]

One simple way to break tolerance to tumor antigens is to use cryptic peptides. Indeed, we and others have shown that the T cell repertoire specific for cryptic peptides partially or completely escapes tolerance mechanisms.[7-10] This suggests that cryptic peptides would be good tumor vaccines provided they are rendered immunogenic. Cryptic peptides, which have low HLA I affinity, and therefore are not immunogenic, have to be optimized by altering their

amino acid sequence to increase their HLA I affinity while maintaining their antigenic specificity, thereby transforming them into high-affinity peptides capable of stimulating a specific T cell response. We have previously developed and described such a method for optimizing cryptic peptides presented in association with HLA-A*0201.[11]

The telomerase reverse transcriptase (TERT) subunit is a promising target for cancer immunotherapy as it is overexpressed in many human tumors and, therefore, is considered a universal tumor antigen, whereas most normal human tissues do not express TERT.[12-14] TERT is overexpressed in more than 85% of NSCLC and is associated with poor prognosis.[15-20] TERT has recently been targeted in many tumors, including NSCLC.[21-23]

$TERT_{572Y}$ (Vx-001; Vaxon Biotech, Evry, France) is an HLA-A*0201–associated optimized cryptic peptide derived from TERT. $TERT_{572Y}$ was able to induce tumor immunity, but not autoimmunity in HLA-A*0201 transgenic mice.[8,24] In vitro, $TERT_{572Y}$ stimulated antitumor CTLs from both healthy donors and prostate cancer patients; CTLs killed TERT-expressing tumor cells, but not TERT-expressing normal cells.[24,25] Vx-001 has recently been tested in a phase I clinical study in 19 patients with advanced cancer. Vx-001 was safe (only grade 1/2 toxicity was observed) and immunogenic. $TERT_{572}$-specific immune response was detected in 13 of 14 assessable patients. Although there was no objective clinical response, four patients (21%) experienced stable disease (SD) for a median of 10.5 months.[26]

As part of an expanded safety, immunological, and clinical evaluation program, 22 patients with advanced NSCLC were vaccinated with Vx-001. Here we report that in those patients, the vaccine was safe and immunogenic, generating functional CTLs, which recognize the native $TERT_{572}$ peptide. More important, patients with early developed immunological response had a significantly better overall survival (OS) than those without an immunological response.

PATIENTS AND METHODS

Patients

All patients enrolled onto the trial had unresectable stage III-IV; histologically or cytologically confirmed NSCLC; and were previously treated with chemotherapy and/or radiotherapy, with radiological evidence of residual or progressive disease (PD). Other eligibility criteria included HLA-A*0201 expression; age older than 18 years; performance status (WHO) of 2 or less; measurable or assessable nonirradiated disease; adequate bone marrow (absolute lymphocyte count $\geq$ 1,300/dL), renal, and liver function. Patients with known immunodeficiency or autoimmune disease were excluded. No treatment with possible antitumor activity (ie, chemotherapy, radiotherapy, biologic agents, or corticosteroids) was allowed 4 weeks before or during the course of vaccination. The protocol was approved by the ethics and scientific committees of the University Hospital of Heraklion and the National Drug Administration of Greece. All patients gave written informed consent to participate in the study.

Peptide Vaccine Preparation

The Vx-001 vaccine consisted of optimized $TERT_{572Y}$ (YLFFYRKSV) and native $TERT_{572}$ (RLFFYRKSV) peptides emulsified in Montanide ISA51 (Seppic Inc, Paris, France). The vaccine peptides were synthesized by the Department of Pharmacognosy's faculty at the University of Patras (Patras, Greece) using an advanced ChemTech Mod 90 automatic peptide synthesizer (Advanced ChemTech, Louisville, KY), 2-chlorotrityl chloride resin, and the Fluorenyl-methoxy-carbonyl (Fmoc)/tert-Butyl (tBu) chemistry. Coupling of each amino acid was performed with a three-fold molar excess of *N*-Fmoc-amino acid, 4.5-fold molar excess of 1-hydroxybenotriazol, and 3.3-fold molar excess of diisopropylcarbodiimide in N,*N*-Dimethylformamide (DMF) for 1.5 hours. The Fmoc deprotection was accomplished by treatment with 25% piperidine in DMF. Crude peptides were subjected to gel chromatography on Sephadex G-15 (Amersham Biosciences, Piscataway, New Jersey) using 0.2 M acetic acid as the eluent. Further purification was carried out by semipreparative high-performance liquid chromatography (HPLC) with a linear gradient from 20% to 60% of acetonitrile-water for 30 minutes at 2 mL/min flow rate. The final characterization of peptides was achieved by analytic reversed phase HPLC (RP-HPLC).

Quality assurance studies included confirmation of identity, sterility, and purity (> 95% for both peptides). No decrease in purity or concentration was observed after more than 2 years of storage at −80°C. Each peptide was prepared as a lyophilized powder (2 mg/vial) for reconstitution with 0.5 mL sterile water.

Vaccination Protocol

Patients received a total of six subcutaneous vaccinations administered every 3 weeks. Two mg of each peptide in 0.5 mL of aqueous solution were emulsified with 0.5 mL of Montanide ISA51 immediately before being injected according to the manufacturer's instructions. The optimized $TERT_{572Y}$ peptide was used for the first and second vaccinations, and the native $TERT_{572}$ peptide for the remaining four vaccinations. The rationale for this strategy was to select among T cells recruited by the optimized $TERT_{572Y}$ those with the highest specificity for the native $TERT_{572}$ presented by tumor cells. In vivo preclinical studies have indeed shown that vaccination of HLA-A*0201 transgenic HHD mice with the optimized $TERT_{572Y}$ followed by the native $TERT_{572}$ peptide induces CTL with higher avidity and stronger antitumor efficacy than serial vaccination with the optimized $TERT_{572Y}$ peptide (unpublished data). Patients without PD postvaccination received boost vaccinations with 2 mg of native $TERT_{572}$ every 3 months.

Patient Evaluation

Before study entry, all patients were assessed with history, physical examination, and CBC with differential and serum chemistry. Measurable disease was determined by standard imaging procedures. CBC was repeated weekly, and clinical examination with serum chemistry every 3 weeks during the vaccination period and every month thereafter during the follow-up.

Response to treatment was evaluated after the third and sixth vaccinations and every three months thereafter or sooner if clinically indicated. Response to treatment was scored as complete response (CR), partial response (PR), SD, and PD using the standard Response Evaluation Criteria in Solid Tumors Group criteria.[27] Radiological responses and SD findings were confirmed by an independent panel of radiologists. Time to progression (TTP) was determined by the time from the first treatment administration to the first date that disease progression was objectively documented. OS was measured from the date of study entry to the date of death. Follow-up time was measured from the day of first treatment administration to last contact or death. Immune responses were examined before the first injection, after the second and sixth injections, and after each boost vaccination for continuing patients. Peripheral blood mononuclear cells (PBMCs) were collected at each time point and frozen at −80°C until used.

Peptides

Class I–restricted peptides used for laboratory studies included $TERT_{572Y}$ (YLFFYRKSV) and $TERT_{572}$ (RLFFYRKSV), produced by Epytop (Nimes, France).

Enzyme-Linked Immunosorbent Spot Assay

The human interferon gamma (IFN-γ) enzyme-linked immunosorbent spot (ELIspot) polyvinylidene difluoride-Enzymatic kit (Diaclone, Besançon, France) was used according to the manufacturer's recommendations. Nitrocellulose 96-well plates were coated with human IFN-γ–specific capture monoclonal antibody (mAb) overnight at 37°C, and 2×10^5–thawed PBMCs were distributed in each well and stimulated with 10 μmol/L of $TERT_{572}$ native peptide for 18 hours. Concanavalin A (5 μg/mL) and unstimulated PBMCs served as positive and negative controls, respectively. Plates were incubated for 18 hours at 37°C in 5% CO_2, washed, and then incubated with biotinylated anti-IFN-γ detection mAb and then with alkaline phosphatase-conjugated streptavidin. Spots were developed by adding peroxidase substrates and

counted using the automated image analysis system Bioreader 2000 (Bio-Sys, Karben, Germany). Six wells were tested for each group, and the standard deviation of replicates was 23% ± 14% of means in all groups for all tested samples. Eighty-eight samples were tested, and the background (unstimulated cultures) was 36 ± 23 spots/2 × 10^5 PBMCs. Statistical analysis for positivity was done using the *t* test. ELIspot assay was considered positive when there was (1) a difference of more than 10 spots between unstimulated and $TERT_{572}$-stimulated cultures and (2) a statistically significant difference between unstimulated and $TERT_{572}$-stimulated cultures. $TERT_{572}$ reactive cells were calculated in ELIspot-positive assays according to the formula: number of spots in the $TERT_{572}$-stimulated group − number of spots in the control group. Results are presented as the number of $TERT_{572}$ reactive cells per 10^5 $CD8^+$ cells calculated according to the formula: number of $TERT_{572}$ reactive cells × percentage of CD8 cells (measured by double CD3/CD8 immunofluorescence staining).

$TERT_{572Y}$ Pentamer Staining

The 10^6-thawed unstimulated PBMCs were incubated with phycoerythrin-conjugated HLA-A*0201/$TERT_{572Y}$ or the control phycoerythrin-conjugated HLA-A*0201/human immunodeficiency virus p76 ($HIVgag_{76}$) pentamer (Proimmune Ltd, Oxford, United Kingdom) for 30 minutes at room temperature, and then with allophycocyanin-conjugated anti-CD8 and fluorescein-conjugated anti-CD3 (BD Pharmingen, Mississauga, Canada) mAbs for 30 minutes at 4°C. Stained cells were analyzed by flow cytometry (FACSCalibur; BD Biosciences, Mountain View, CA). The frequency of $TERT_{572Y}$ pentamer–positive cells was calculated according to the formula: (number of $TERT_{572Y}$-pentamer stained CD8 cells − number of $HIVgag_{76}$-pentamer–stained CD8 cells)/10^5 CD8 cells.

Statistical Analysis

The frequencies of $TERT_{572}$-reactive CD8 cells detected by ELIspot before and after vaccination were compared using the *t* test. The probability of survival was estimated using the Kaplan-Meier method and tested for differences by the log-rank test. All tests were considered significant when the resulting $P = \leq .05$.

RESULTS

Patients and Vaccine Administration

The characteristics of the 22 patients enrolled onto the trial from February 1, 2003, to July 31, 2006, are presented in Table 1. All patients had received at least one prior chemotherapy regimen for the treatment of advanced/metastatic disease. Different numbers and types of chemotherapy regimens had been previously used. At the time of enrollment, 14 patients (63.6%) and eight patients (36.4%) presented PD and SD, respectively, after the completion of the last chemotherapy regimen. Twelve patients (54.5%) have completed the vaccination protocol, and 10 patients (45.4%) were withdrawn after the second (patients 20 and 22), third (patients 4, 6, 8, 9, and 21), fourth (patients 12 and 17), and fifth (patient 18) vaccinations because of rapid disease progression (Tables 1 and 2). Four (patients 1, 2, 5, and 16) with SD lasting more than 3 months after the sixth vaccine administration received boost vaccinations with the native $TERT_{572}$ peptide every 3 months. The median follow-up period for the whole group of patients was 10.0 months (range, 2.4 to 40.0 months).

Toxicity

Sixteen patients (72.7%) developed grade 1 toxicity. The most common adverse events were local skin reaction (n = 8; 36.4%),

Table 1. Characteristics of Patients With NSCLC Enrolled Onto the Trial (N = 22)

Patient No.	Age (years)	Sex	Histology	Previous Treatment	Response to Previous Treatment	Time Elapsed From Previous Treatment (months)	Stage	Status Before Vaccination	PS	No. of Vaccinations
1	55	F	LCC	First-line CT/RT	PR	1	III	SD	1	6, 3*
2	48	M	LCC	First-line CT/RT	PR	3	III	SD	0	6, 4+*
3	56	M	AD	Second-line CT	SD	3	IV	SD	1	6
4	61	M	SCC	First-line CT	PR, 6 months	6	IV	PD	0	3
5	73	F	AD	First-line CT	SD, 5 months	5	IV	PD	0	6, 3+*
6	56	M	AD	Fifth-line CT	PD	1	IV	PD	2	3
7	61	M	AD	First-line CT/RT	SD	5	III	SD	0	6
8	65	M	SCC	Second-line CT	PR, 6 months	7	IV	PD	0	3
9	55	M	LCC	Third-line CT/RT	PD	1	IV	PD	0	3
10	48	M	AD	Second-line CT/RT	SD	7	IV	SD	0	6
11	60	M	AD	Second-line CT	PD	1	IV	PD	1	6
12	60	M	SCC	Sixth-line CT	PD	2	IV	PD	1	4
13	46	M	AD	Third-line CT	PD	1	IV	PD	0	6
14	73	M	Poor differentiated	Second-line CT/RT	PR, 10 months	10	IV	PD	0	6
15	47	M	SCC	First-line CT	SD	1	IV	SD	0	6
16	60	M	SCC	First-line CT/RT	PR, 11 months	11	III	PD	0	6, 4+*
17	58	M	Poor differentiated	First-line CT/RT	SD, 4 months	7	IV	PD	0	4
18	75	M	SCC	First-line CT/RT	SD	2	III	SD	0	5
19	55	F	AD	First-line CT/RT	PR	2	III	SD	0	6
20	55	F	AD	First-line CT	PD	1	IV	PD	1	2
21	67	M	Poor differentiated	First-line CT	SD, 5 months	6	IV	PD	1	3
22	52	M	Poor differentiated	First-line CT	PD	3	IV	PD	2	2

Abbreviations: NSCLC, non–small-cell lung cancer; PS, performance status; F, female; LCC, large-cell carcinoma; M, male; CT, chemotherapy; RT, radiotherapy; PR, partial response; SD, stable disease; AD, adenocarcinoma; SCC, squamous cell carcinoma; PD, progressive disease.

*Four patients with stable disease lasting more than 3 months after the sixth vaccine administration received boost vaccinations with the native $TERT_{572}$ peptide every 3 months.

Table 2. Immunomonitoring and Clinical Outcome of Patients Vaccinated With Vx-001

Patient No.	Status Before Vaccination	$TERT_{572}$-Specific Cells/10^5 CD8 Cells						Clinical Outcome (months)	Overall Survival (months)
		Prevaccination		Second Vaccination		Postvaccination			
		ELIspot	Pentamer	ELIspot	Pentamer	ELIspot	Pentamer		
1	SD	< 1	< 1	45	540	88	70	SD, 13.3*	19.7+
2	SD	< 1	< 1	58	560	80	190	SD, 17.7+*	17.7+
3	SD	< 1	150	32	250	19	330	PD	19.9+
4	PD	< 1	< 1	40	120	NA	NA	PD	17.1
5	PD	< 1	< 1	200	170	140	250	SD, 20+*	20.0+
6	PD	< 1	< 1	85	340	NA	NA	PD	3.0
7	SD	< 1	< 1	357	130	237	260	SD, 6.8+*	6.8+
8	PD	< 1	< 1	133	300	NA	NA	PD	4.3
9	PD	< 1	< 1	45	90	NA	NA	PD	5.5
10	SD	< 1	< 1	27	100	39	110	SD, 9.1+*	9.1+
11	PD	Inadequate specimen	< 1	Inadequate specimen	350	Inadequate specimen	100	PD	30.0
12	PD	Inadequate specimen	< 1	Inadequate specimen	450	NA	NA	PD	5.7
13	PD	Inadequate specimen	< 1	Inadequate specimen	700	Inadequate specimen	700	SD, 9*	40.0+
14	PD	Inadequate specimen	< 1	Inadequate specimen	350	Inadequate specimen	600	PD	21.4+
15	SD	< 1	Inadequate specimen	313	Inadequate specimen	Inadequate specimen	Inadequate specimen	SD, 7.5*	15.0+
16	PD	< 1	Inadequate specimen	220	Inadequate specimen	180	Inadequate specimen	SD, 18+*	18.0+
17	PD	< 1	< 1	< 1	< 1	NA	NA	PD	8.0
18	SD	< 1	< 1	< 1	< 1	NA	NA	PD	3.5
19	SD	< 1	< 1	< 1	< 1	< 1	< 1	PD	10.9+
20	PD	< 1	Inadequate specimen	< 1	Inadequate specimen	NA	NA	PD	2.4
21	PD	< 1	Inadequate specimen	< 1	Inadequate specimen	NA	NA	PD	3.5
22	PD	Inadequate specimen	Inadequate specimen	NA	NA	NA	NA	PD	8.7+

Abbreviations: $TERT_{572}$, XXX; ELIspot, enzyme-linked immunosorbent spot assay; SD, stable disease; PD, progressive disease; NA, not applicable (no blood sample because of PD).

*Eight of 22 vaccinated patients showed SD postvaccination, with a median duration of 11.2 months (range, 6.8 to 20.0 months).

anemia (n = 3; 13.6%), thrombocytopenia (n = 3; 13.6%), and fever (n = 3; 13.6%). One patient developed grade 2 fatigue and nausea. No patient presented moderate or severe toxicity.

Vaccine-Induced Immune Response

Vaccine-generated immune response was evaluated by detecting $TERT_{572}$-specific $CD8^+$ cells in the PBMCs by IFN-γ ELIspot assay and HLA-A*0201/$TERT_{572Y}$ pentamer staining. Monitoring of the immune response by either ELIspot or pentamer staining was performed in 21 patients after the second vaccination (13 patients were monitored with both ELIspot assay and pentamer staining), and 11 patients after the sixth vaccination (seven patients were monitored with both ELIspot assay and pentamer staining). Figure 1D shows representative results of pentamer staining. Immunomonitoring results of individual patients are presented in Table 2, and cumulative results are presented in Figures 1A and 1B. $TERT_{572}$-specific IFN-γ–producing $CD8^+$ cells were not detected in any patient before vaccination, whereas they were detected in 12 (70.6%) of 17 patients after the second vaccination and 7 (87.5%) of 8 after the sixth vaccination (postvaccination). The mean (± standard deviation) frequency of $TERT_{572}$-specific cells was less than $1/10^5$ CD8 cells prevaccination, 87 ± $112/10^5$ CD8 cells after the second vaccination ($P < .005$), and 98 ± $81/10^5$ CD8 cells postvaccination ($P < .00003$; Fig 1A). HLA-A*0201/$TERT_{572Y}$ pentamer–positive cells were detected in one (5.1%) of 17 patients before vaccination, 14 (82.4%) of 17 patients after the second vaccination, and 9 (90%) of 10 patients postvaccination. The mean (± standard deviation) frequency of pentamer-positive cells was 11 ± $37/10^5$ CD8 cells before vaccination, 261 ± $212/10^5$ CD8 cells after the second vaccination ($P < .0002$), and 261 ± $229/10^5$ CD8 cells postvaccination ($P < .01$; Fig 1B). There was a good correlation between the results of ELIspot and pentamer assays; the correlation coefficient R^2 was 0.5163 and 0.8013 after the second and sixth vaccinations, respectively. In all four patients who received boost vaccinations with the native $TERT_{572}$ peptide, the ELIspot assay confirmed that the immune response was maintained (Fig 1C). Six (37.5%) of the 16 immune responders versus two (40%) of the five immune nonresponders had SD before entering the study (Table 2).

Clinical Outcome

Fourteen (63.6%) of 22 patients progressed either during the vaccination (patients 4, 6, 8, 9, 12, 17, 18, 20, 21, and 22) and were withdrawn from the study or following the completion of vaccination (patients 3, 11, 13, and 19). Seven of these patients with disease progression subsequently received chemotherapy and one radiotherapy.

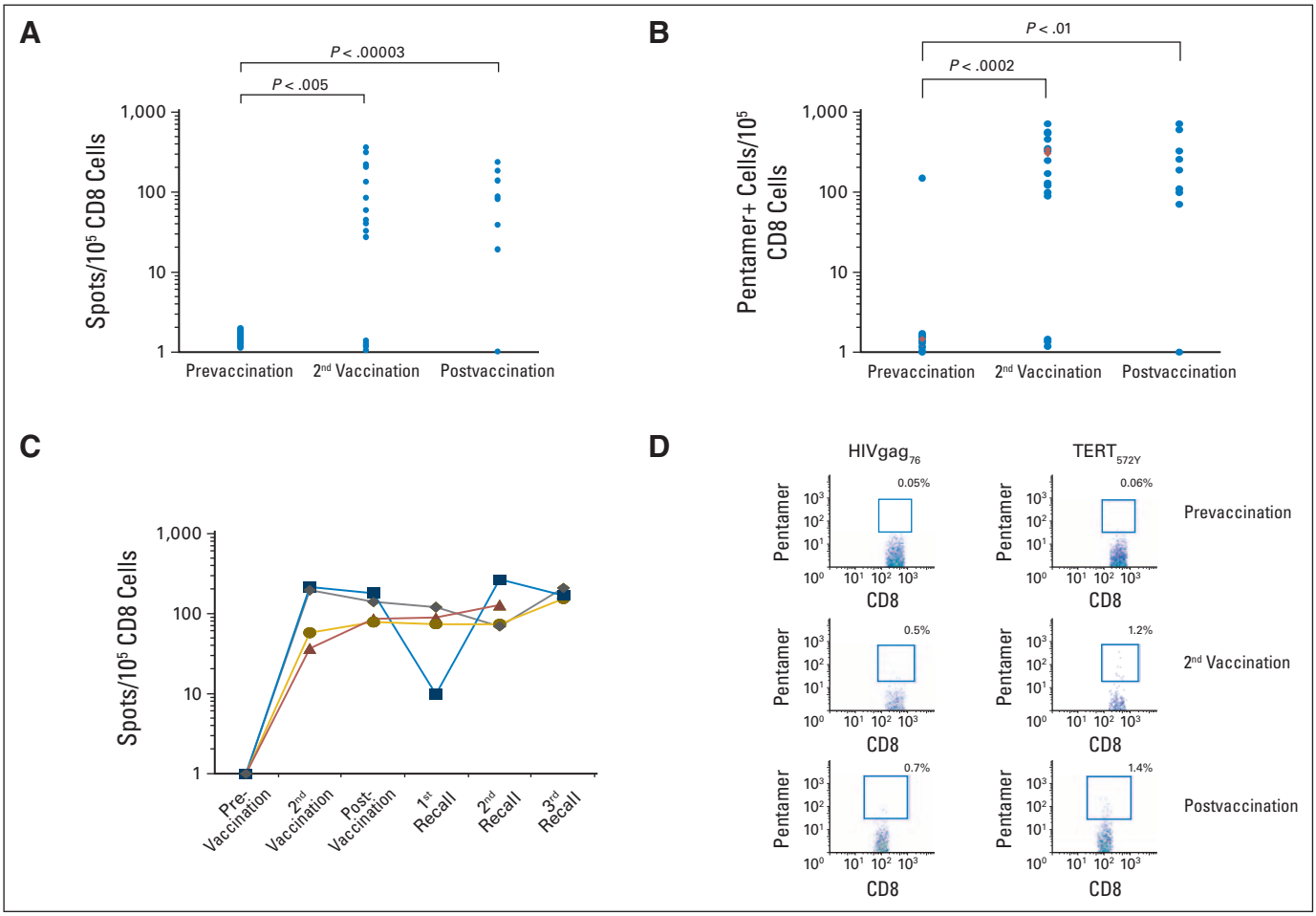

Fig 1. Immune response developed in vaccinated patients. (A, B) Immune response was evaluated by (A) enzyme-linked immunosorbent spot (ELIspot) assay (B) and pentamer staining. (C) Kinetics of the immune response in patients 1 (▲), 2 (•), 5 (◆), and 16 (■) who received boost vaccinations as detected by ELIspot assay. (D) Pentamer staining of patient 13's peripheral blood mononuclear cells.

Eight (36.4%; patients 1, 2, 5, 7, 10, 13, 15, and 16) of 22 vaccinated patients showed SD postvaccination, with a median duration of 11.2 months (range, 6.8 to 20.0 months; Table 2). Of the eight patients with SD postvaccination, three (patients 5, 13, and 16) had PD and five (patients 1, 2, 7, 10, and 15) had SD before entering the study. Three (patients 1, 13, and 15, respectively) of these eight patients progressed with a TTP of 13.3, 9.0, and 7.5 months and received chemotherapy, whereas five patients (patients 2, 5, 7, 10, and 16, respectively) are still in SD with a follow-up of 17.7, 20.0, 6.8, 9.1, and 18.0 months. The median TTP for the whole group of patients was 3.8 months (range, 1.4 to 20.0 months). Ten (45.4%) of 22 vaccinated patients have died. Interestingly, 11 (91.7%) of 12 patients who completed the vaccination protocol were alive at the time of analysis, with an estimated median OS of 18.0 months (range, 5.7 to 40.0 months; Table 2). The estimated median OS time for all 22 patients was 30.6 months (95% CI, 10.9 to 48.9 months), and the 1- and 2-year OS rates were 63.3% and 56.3%, respectively.

Clinical Outcome and Immune Response

The patient characteristics according to the development of an early immune response are presented in Table 3. The correlation of clinical outcome and immune response developed after the second vaccination demonstrated that eight (50%) of 16 immune responders but none of the five nonresponders experienced long-lasting (> 6 months) disease stabilization ($P < .04$). Moreover, the overall strength of the immune response, as measured by ELIspot assay, was significantly higher in patients with SD (174 ± 134 $TERT_{572}$-specific cells/10^5 $CD8^+$ cells) than in patients with PD (34 ± 45 $TERT_{572}$-specific cells/10^5 CD8 cells; $P < .04$). In addition, the TTP and OS according to the immune response after the second vaccination demonstrated that early immune responders (n = 16) had a significantly longer TTP and OS than the immune nonresponders (n = 5; log-rank tests $P = .046$ and $P = .012$; Figs 2 and 3). Median TTP was 4.2 months (range, 1.6 to 20.0 months) and 2.3 months (range, 1.8 to 6.2 months), and OS was 30.0 months (range, 2.8 to 40.0 months) and 4.1 months (range, 2.4 to 10.9 months) for immune responders and nonresponders, respectively.

DISCUSSION

The aim of the present study was to evaluate toxicity, immune response, and clinical outcome in patients with advanced NSCLC vaccinated with the optimized cryptic $TERT_{572Y}$ peptide (Vx-001) as part of an expanded evaluation program. Our results showed that Vx-001 was safe and immunogenic in almost all vaccinated patients. No objective response was observed, but eight patients showed disease

Table 3. Characteristics of Early Immune Responders and Nonresponders

Characteristic	Early Responders (n = 16) No.	%	Early Nonresponders (n = 5) No.	%
Age, years				
Median	58		58	
Range	48-73		55-75	
Sex				
Male	14	87.5	3	60
Female	2	12.5	2	40
Histology				
Squamous cell	5	31.2	1	20
Adenocarcinoma	7	43.7	2	40
Large cell	3	18.8	0	0
Poorly differentiated	1	6.3	2	40
Stage				
III	4	25	2	40
IV	12	75	3	60
Performance status, WHO				
0	11	68.7	3	60
1	4	25	2	40
2	1	6.3	0	0
Line of treatment				
Second	7	43.7	5	100
≥ Third	9	56.3	0	0
Clinical status before vaccination				
Stable disease	6	37.5	2	40
Progressive disease	10	62.5	3	60

stabilization for 6.8 to more than 20 months. More important, patients who developed early immune response had a significantly better OS than patients who didn't (30 *v* 4.1 months; $P = .012$). This difference was observed despite the similar proportions of patients with SD at study entry between early immune responders and nonresponders.

Immune response was induced by the Vx-001 vaccine in 76% and 91% of evaluated patients after the second and sixth vaccinations, respectively, thus confirming previous results.[26] More important, Vx-001-generated CTLs recognized the native $TERT_{572}$ peptide and were

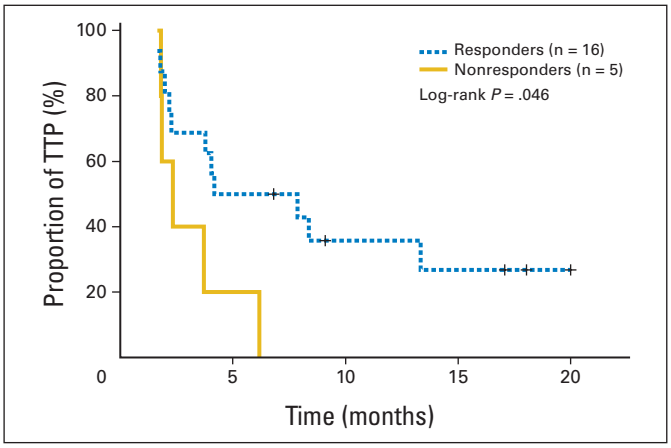

Fig 2. Time to progression for patients with early immunological response (n = 16) and nonresponders (n = 5).

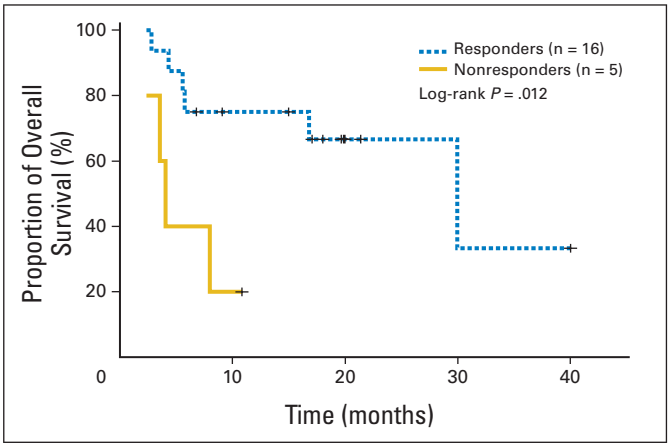

Fig 3. Survival for patients with early immunological response (n = 16) and nonresponders (n = 5).

maintained for at least 9 months in patients boosted with the native $TERT_{572}$ peptide. Compared with other vaccines tested in patients with NSCLC, Vx-001 induced an immune response in a higher proportion of vaccinated patients. Indeed, an immune response was detected in 20.5% and 54.2% of patients vaccinated with the BLP-25 and GV1001 vaccines, respectively.[2,23]

None of Vx-001–vaccinated patients showed an objective PR or CR. However, with rare exceptions, tumor regression may not be achievable by most vaccines in patients with advanced cancer.[28-30] Four CRs and one PR were observed in a total of 248 NSCLC patients treated with different vaccines.[23,31-38] Conversely, an objective response is not always required for a meaningful clinical benefit. Some patients with nonresponding tumors may benefit from prolonged delay in tumor progression.[39,40] Indeed, although no objective responses were achieved with BLP-25 vaccine,[36] the OS of vaccinated stage IIIB NSCLC patients was significantly higher compared with that of nonvaccinated patients.[2] Similarly, Vx-001-vaccinated patients didn't show any objective clinical response but presented a prolonged survival.

An interesting observation is the correlation between early immune response and clinical outcome. Early immune responding patients had a significantly better survival compared with nonresponding patients. However, this encouraging observation should be interpreted with caution because the group of nonresponders is small (five patients) and the decision to compare responders/nonresponders was taken after inspecting the data. It could be argued that the absence of an immune response was due to rapid disease progression, which could explain the early death of nonresponding patients. However, five patients withdrawn from the study due to rapid disease progression developed an early immune response, and three of them survived for 5.5, 5.7 and 17.1 months, respectively. Interestingly, a correlation between immune response and clinical outcome of vaccinated patients is rarely observed.[33,38,41] This could be due to poor quality (low avidity) of induced CTLs, high tumor burden, and the criteria used for measuring an objective response that might not be adaptable to tumor vaccines.[29] Moreover, immunotherapy may be more effective in patients with low tumor burden, such as in the adjuvant setting or following response to first-line therapy.[2,3,42]

To determine whether a vaccine improves TTP or survival, a nonvaccinated control arm is always necessary.[29] Since our study doesn't provide a controlled comparison, our findings should be interpreted with caution. However, based on these encouraging results, we are planning a multicenter controlled study to appropriately evaluate the true clinical benefit of vaccination with Vx-001 in patients with NSCLC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. 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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