

Clinical outcome of patients with various advanced cancer types vaccinated with an optimized cryptic human telomerase reverse transcriptase (TERT) peptide: results of an expanded phase II study

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Background: TERT (telomerase reverse transcriptase) plays a critical role in tumor cell growth and survival. In an expanded phase II study, we evaluated the immunological and clinical responses to the TERT-targeting Vx-001 vaccine in patients with advanced solid tumors.

Methods: HLA-A*0201-positive patients received two subcutaneous injections of the optimized TERT_{572Y} peptide followed by four injections of the native TERT₅₇₂ peptide, every 3 weeks. Peptide-specific immune responses were evaluated by enzyme-linked immunosorbent spot at baseline, and after the second and the sixth vaccinations.

Results: Fifty-five patients were enrolled and 34 (62%) completed the six vaccinations. A TERT-specific T-cell immune response was observed in 55% and 70% of patients after the second and the sixth vaccinations, respectively. The disease control rate (DCR) was 36% [95% confidence interval (CI) 24% to 49%], including one complete and one partial response. Immunologically responding patients had a better clinical outcome than nonresponders [DCR: 44% versus 14% ($P = 0.047$); progression-free survival (PFS): 5.2 versus 2.2 months ($P = 0.0001$) and overall survival: 20 versus 10 months ($P = 0.041$)]. Multivariate analysis revealed that the immunological response was an independent variable associated with increased PFS (hazard ratio = 3.35; 95% CI 1.7–6.7).

Conclusion: Vx-001 vaccine was well tolerated and induced a TERT-specific immunological response, which was significantly correlated with improved clinical outcome.

Key words: cancer, cryptic, immunotherapy, telomerase, vaccine

introduction

During the past years, several tumor-associated antigens (TAA), expressed on tumor cells, have been described [1]. Antigenic peptides from these TAA are recognized by the immune system, which responds by developing peptide-specific cytotoxic T cells (CTLs) [2]. However, among the numerous characterized TAAs, the vast majority of them are not broadly expressed or even involved in tumor cell proliferation and survival. Consequently, the therapeutic strategies targeting TAAs, which are not involved in tumor cell growth, could result in the selection of aggressive clones that do not express these specific antigens [3–5].

The human telomerase reverse transcriptase (TERT) represents an 'ideal' target for cancer immunotherapy since it is considered a 'universal' tumor antigen [6]. Indeed, TERT is overexpressed in >85% of all human cancers [6] and its activity has been shown in cancer stem cells [7]. Moreover, it plays a critical role in tumor cell growth and survival and is associated with poor prognosis [8–13]. Inhibition of TERT in tumors has shown encouraging antitumor *in vitro* [14–16] and *in vivo* effects [17, 18].

Antitumor immunotherapy represents an attractive and promising therapeutic approach based on the activation of CTLs through the recognition of endogenously processed TAA-derived peptides expressed on the tumor cell surface in association with human leukocyte antigen (HLA) class I molecules. TAA-derived peptides can be either dominant or cryptic [19]. Dominant peptides demonstrate a high HLA affinity and are frequently

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presented by tumor cells, whereas cryptic peptides have a low HLA affinity and therefore are rarely presented by tumor cells. So far, the majority of the tested vaccines consisted of dominant peptides and most of them have shown low efficacy [20–26]. The immune tolerance already developed against the TAA-derived dominant peptides seems to be a possible explanation for these negative results [19–25]. Preclinical studies have shown that immunization with tumor-derived cryptic peptides could circumvent the immune tolerance to tumor antigens [20–22, 24], providing their immunogenicity is previously enhanced. Cryptic peptides are considered nonimmunogenic since they have low HLA class I affinity; however, by altering specific amino acids in their sequence, the ‘optimized’ peptides acquire a high HLA class I affinity while maintaining their antigenic specificity and become capable of stimulating a specific T-cell response [23, 27].

Vx-001 consists of the HLA-A*0201-associated optimized cryptic peptide TERT_{572Y} and its native TERT₅₇₂ counterpart. TERT_{572Y} induced tumor immunity but not autoimmunity in HLA-A*0201 transgenic mice [21]. *In vitro*, TERT_{572Y} stimulated antitumor CTLs, from both healthy donors and prostate cancer patients, were able to kill TERT-expressing tumor but not normal cells [28, 29]. It has been previously reported that almost 90% of patients vaccinated with the Vx-001 mounted a TERT-specific immune response [27]. Furthermore, vaccination of non-small-cell lung cancer (NSCLC) patients with Vx-001 resulted in a significantly improved overall survival (OS) of immunologically responding patients compared with nonresponders [18].

As part of an expanded safety, immunological and clinical evaluation phase II program, 55 patients with various advanced solid tumors other than NSCLC were vaccinated with Vx-001. The findings of the current report confirm our previous observations and show that the vaccine is safe and immunogenic, capable of generating functional CTLs, which recognize the native TERT₅₇₂ peptide. More importantly, immune responders had a significantly better clinical outcome compared with nonresponders.

patients and methods

patients

Patients with histologically or cytologically confirmed advanced solid tumors were enrolled. Additional eligibility criteria included clinical and radiological evidence of stable disease (SD) or progressive disease (PD) to prior treatment; prior therapy with at least one ‘standard’ chemotherapy regimen and/or hormone therapy and/or radiotherapy when indicated; age >18 years; performance status (PS) of zero to two (Eastern Cooperative Oncology Group); HLA-A*0201 expression; measurable or assessable disease; adequate bone marrow, renal and liver function. Patients with known immunodeficiency, autoimmune disease, treatment with possible antitumor activity or corticosteroids within 4 weeks before or during the study were excluded. The protocol was approved by the Ethics and Scientific Committees of the participating centers and the National Drug Administration (EOF) of Greece. All enrolled patients gave their written informed consent.

peptide vaccine preparation and vaccination protocol

The Vx-001 vaccine consisted of the HLA class I-restricted optimized TERT_{572Y} (YLFFYRKS_V) and the native TERT₅₇₂ (RLFFYRKS_V) peptides.

The peptides were synthesized in the Department of Pharmacognosy, Faculty of Pharmacy, University of Patras, Greece; the peptide synthesis procedure as well as its physicochemical characteristics has been previously described [18]. Each peptide was prepared as a lyophilized powder (2 milligrams per vial).

Patients received six subcutaneous injections administered every 3 weeks. Two milligrams of each peptide in 0.5 ml of sterile water was emulsified with 0.5 ml of Montanide ISA51 (Seppic Inc., Paris, France) immediately before being injected. The optimized TERT_{572Y} peptide was used for the first and second injections and the native TERT₅₇₂ peptide for the following four injections. The rationale for this strategy was to select among the stimulated by the optimized TERT_{572Y} T cells those with the highest specificity for the native TERT₅₇₂ presented by tumor cells. After a protocol amendment, patients without evidence of PD after the sixth vaccination were allowed to receive boost vaccinations with native TERT₅₇₂ peptide every 3 months until disease progression, consent withdrawal or unacceptable toxicity.

patients’ evaluation

Before study entry, all patients were assessed with history, physical examination, complete blood cell count (CBC) and serum chemistry. Measurable disease was determined by standard imaging techniques (ultrasound, computed tomography scans, magnetic resonance imaging). CBC, serum chemistry and clinical examination were repeated in 3 weeks schedule during the vaccination period and monthly during the follow-up.

Response was evaluated clinically and by imaging studies (using the standard RECIST criteria [30]) after the third and the sixth vaccination and every 3 months thereafter. Progression-free survival (PFS) was determined by the interval from the date of first injection to the date that PD was documented, consent withdrawn or death. Correspondingly, OS was measured from the date of first injection to the date of death or consent withdrawal.

Peripheral blood mononuclear cells (PBMCs) were collected at predefined time points and frozen at –80°C until used. Immunological responses were examined at baseline, after the second and sixth vaccinations and after each boost vaccination thereafter by using a human interferon gamma (IFN-γ) enzyme-linked immunosorbent spot (ELISpot) assay (polyvinylidenedifluoride-Enzymatic kit; Diaclone, Besançon, France) as previously described in detail [17]. Spots were counted using the automated image analysis system BioreaderAxio Imager M1 and the KS ELISpot software (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). Six wells were tested for each group (unstimulated, peptide stimulated and ConA stimulated) in three independent experiments. The ELISpot assay was positive when there was (i) difference of >10 spots between and (ii) statistically significant difference between peptide-stimulated and unstimulated cultures. TERT reactive cells were calculated in ELISpot-positive assays according to the formula: number of spots in the peptide-stimulated group minus number of spots in the unstimulated group. Results are estimated as the number of TERT-reactive cells/2 × 10⁵ PBMCs.

statistical analysis

Since this was an expanded evaluation program for the safety, immunological and clinical outcome of patients with advanced solid tumors vaccinated with Vx-001, no formal sample size calculation was carried out. The reported results herein refer to the cumulative experience of using Vx-001 in various solid tumors other than NSCLC. The primary end point of the study was to assess the safety (early and late toxicity), while the evaluation of the immune response, the disease control rate [DCR; complete response (CR) or partial response (PR) or SD], PFS and OS were secondary end points. The potential correlation between immune response and clinical outcome was also included in the proposed data analysis.

A patient was characterized as 'immune responder' if the number of spots, which correspond to the specific IFN- γ -producing T cells as assessed by the ELISpot, was significantly increased compared with the background (nonstimulated cultures) after the second and/or the sixth vaccination. The frequencies of TERT-specific cells detected by ELISpot at predefined time points (see above) were compared by using the *t*-test. The association of immune response with time-to-event end points was analyzed using the log-rank test. The Kaplan–Meier method was used to plot the corresponding PFS and OS curves [31] and a univariate Cox regression analysis [31], with hazard ratios (HRs) and 95% confidence intervals, was carried out to explore the association between each potential prognostic factor with them. Prognostic factors with significant univariate associations were then included in a multivariate Cox proportional hazards regression model [31], with a stepwise procedure (unconditional backward procedure) evaluating their independent prognostic value on PFS and OS. All tests were considered significant when the resulting *P* value was ≤ 0.05 .

results

patients and vaccine administration

Fifty-five patients with advanced solid tumors, other than NSCLC, enrolled onto the expanded evaluation program between January 2005 and July 2007. Patients' characteristics are presented in Table 1. The patients' median age was 55 years. Thirty (55%) patients had previously received two or more chemotherapy regimens. Thirty-nine (71%) and 16 (29%) patients had a documented PD or SD as best response to prior treatment, respectively.

All patients received at least the first two vaccinations, whereas 34 (62%) completed the six vaccinations. Twenty-one (38%) patients discontinued treatment before the sixth vaccination because of PD (14 patients after the third, five patients after the fourth and two patients after the fifth vaccination). Eight (15%) patients continued to receive boost vaccinations and six of them for >2 years (supplemental Table S1, available at *Annals of Oncology* online).

toxicity

The early adverse events (EAEs) were mild (grade 1) and occurred in 29 (52%) patients. The most common EAE was grade 1 local skin reaction ($n = 15$; 27%). Other grade 1 EAEs possibly related to vaccination included asthenia (7%), anemia (13%) and nausea (4%). One patient with extensive metastatic liver lesions experienced grade 3 transaminases elevation. No symptoms or laboratory findings suggesting late toxicity or an autoimmune syndrome were observed. Similarly, the booster vaccinations for up to 2 years were also proved safe with minimal toxicity.

response to treatment

A CR was documented in one (1.8%) patient, a PR in another one (1.8%) and SD in 18 (33%) patients (DCR = 36%; 95% CI 24% to 49%). All objective responses and SDs were confirmed by an external independent radiologist. The DCR was 56% for patients with SD at enrollment into the study (one CR and eight SDs) and 28% for those with PD at the same time point (one PR and 10 SDs) ($P = 0.05$; 95% CI 14% to 42%) (Table 2). A woman with metastatic hormone-resistant breast cancer, who

Table 1. Patients' characteristics

Patient characteristics ($n = 55$)	
Sex, No. (%)	
Male	37 (67)
Female	18 (33)
Age, years	
Median	57
Minimum–maximum	31–84
ECOG performance status, No. (%)	
0	34 (62)
1	19 (34)
2	2 (4)
Type of neoplasm, No. (%)	
Breast cancer	11 (20)
Colorectal cancer	3 (5)
Ovarian cancer	1 (2)
Head and neck	2 (4)
Hepatocellular carcinoma	2 (4)
Melanoma	7 (13)
Prostate cancer	11 (20)
Kidney cancer	7 (13)
Pancreatic cancer	3 (5)
Cholangiocarcinoma	6 (11)
Other	2 (4)
Prior treatment, No. (%)	
First line	24 (43.6)
\geq Second line	30 (54.6)
None	1 (1.8)
Stage of disease at enrolment, No. (%)	
Stage III	5 (9)
Stage IV	50 (91)
Status of disease at enrolment, No. (%)	
SD	16 (29)
PD	39 (71)

ECOG, Eastern Cooperative Oncology Group; PD, disease progression; SD, stable disease.

had previously received chemotherapy and hormonal therapy, entered into the study with SD and demonstrated CR of the hepatic lesions after the sixth vaccination; moreover, after nine boost vaccinations, the patient remains in CR without radiological evidence of disease for 36 months (Figure 1). Another patient with an advanced hepatocellular carcinoma with extensive involvement of locoregional lymph nodes, obstructive jaundice and clinical signs of hepatic failure (ascites, portal hypertension, spiders and hepatosplenomegaly) refused any other treatment and was enrolled on the Vx-001 vaccination protocol, after biliary stenting. Tumor shrinkage and disappearance of all signs of hepatic insufficiency were documented after the sixth vaccination; the patient continued with 12 boost vaccinations, every 3 months, for 41 months before a clinical relapse was documented (Figure 2).

After a median follow-up period of 37 months (range, 2–52), the median PFS for the entire group of patients was 4 months (range, 0.9–51.8). The median PFS for patients entering the study with SD and PD was 7 months (range, 1.5–41.5) and 4 months (range, 0.9–51.8), respectively ($P = 0.038$). In six out of the eight patients who received boost vaccinations, the PFS was

>6 months from the time of the first booster vaccination and in three patients >3 years. The median OS for all patients was 19 months (range, 2–52) and the 1-year survival was 66%. There was no difference in terms of median OS for patients enrolled with SD versus PD [20 months (range, 2.2–44.8) versus 15 months (range, 1.7–51.8), respectively; $P = 0.116$].

clinical outcome and immunological response

The developed immune response after TERT vaccination, as well as the primary tumor localization, the pre-vaccination disease status and the clinical outcome are shown in supplemental Table S2 (available at *Annals of Oncology* online). Blood samples for monitoring the immune response were available in 53 (96%) patients after the second vaccination and in all patients who completed the six vaccinations ($n = 34$). Patients who developed an immunological response any time

during vaccination had a significantly higher PFS (5.2 months; range, 0.9–51.8) compared with those who failed to develop any (2.2 months, range, 1.4–6.5; $P = 0.0001$; supplemental Figure S1A, available at *Annals of Oncology* online). The positive correlation of the development of immunological response with higher PFS was independent on disease status at study entry (SD: 7.2 versus 1.4 months; $P = 0.008$ and PD: 4.0 versus 2.0 months; $P = 0.020$) (supplemental Table S3, available at *Annals of Oncology* online). Similarly, the immunological response was associated with a significantly higher OS (20 months; range, 3.8–51.8 versus 10.5 months; range, 1.7–30; $P = 0.041$) (supplemental Figure S1B, available at *Annals of Oncology* online). The difference in the median OS of patients entering the study with SD or PD, although was numerically higher for immune responders compared with nonresponders, could not reach statistical significance (supplemental Table S3, available at *Annals of Oncology* online). Finally, the DCR was also higher in immune responders (44.0% versus 14%; $P = 0.047$).

Fourteen patients (25%) had immune reactivity against TERT before vaccinations. In order to investigate the effect of this preexisting TERT-specific immune reactivity on patients' clinical outcome, this particular group of patients was studied separately. Supplemental Table S5 (available at *Annals of Oncology* online) clearly indicates that the preexisting immune reactivity had no impact on either PFS ($P = 0.67$) or OS ($P = 0.26$). However, it is of interest that patients who had remaining immune reactivity after two or/and six vaccine administrations had a significantly higher OS compared with those who lost it ($P = 0.02$). Nevertheless, there was no significant difference in terms of PFS ($P = 0.09$; supplemental Table S4, available at *Annals of Oncology* online).

Table 2. Clinical response of patients after the second and sixth vaccinations

Status pre-vaccination	After the second vaccination	After the sixth vaccination
SD ($n = 16$)	PR = 1 SD = 9 PD = 3 NE = 3	CR = 1 SD = 7, PD = 2 Discontinued SD = 1, PD = 2
PD ($n = 39$)	SD = 20 PD = 16 NE = 3	PR = 1, SD = 10, PD = 9 Discontinued PD = 3

CR, complete response; NE, not evaluable; PD, disease progression; PR, partial response; SD, stable disease.

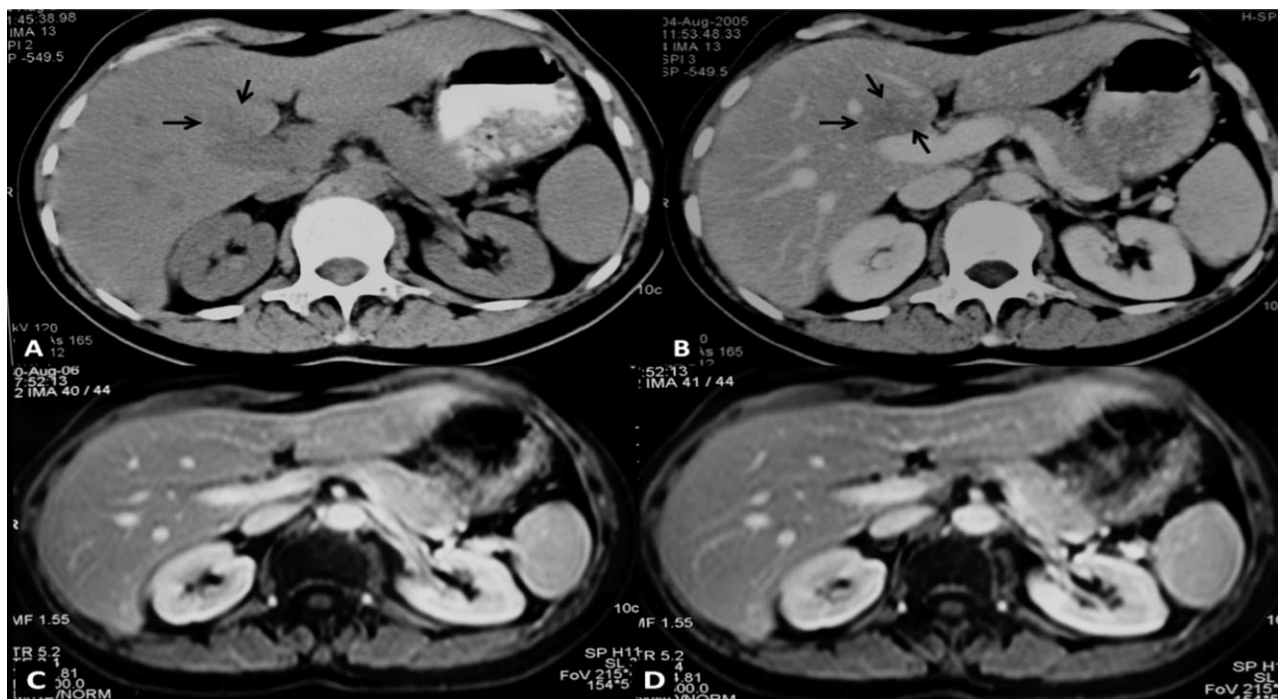


Figure 1. Breast cancer patient with liver metastasis. The pre-vaccination plain (A) and contrast-enhanced (B, portal phase of I.V. contrast administration) axial computed tomography images show the hypodense lesion anterior to the portal vein (arrows). The post-treatment (1 year later) contrast enhanced T1-w magnetic resonance consecutive images at the same anatomical levels (C, D) show normal appearing hepatic parenchyma.

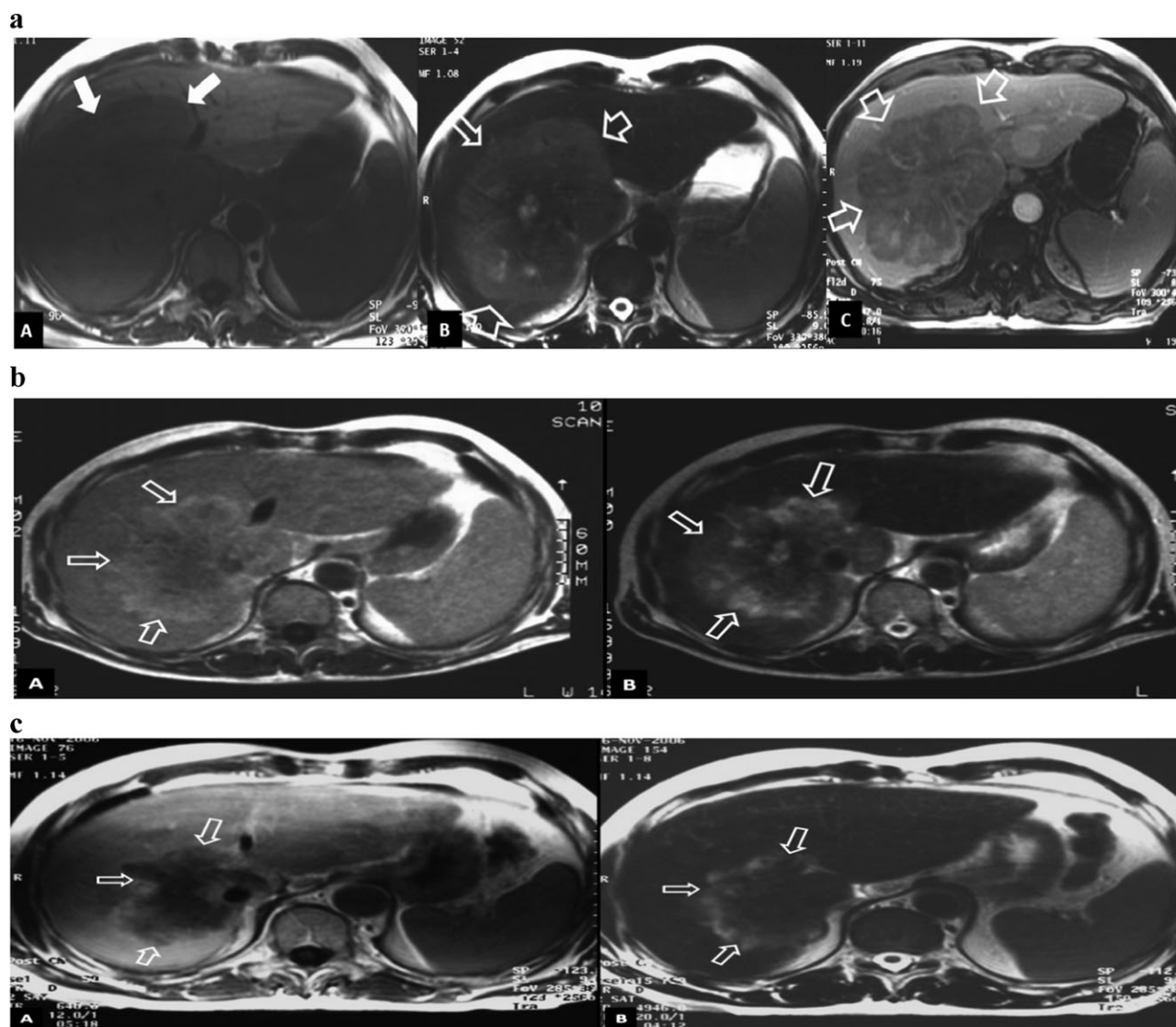


Figure 2. Hepatocellular carcinoma patient. (a) Pre-vaccination imaging studies. The T1-w (A), T2-w (B) and contrast enhanced T1-w (C) axial magnetic resonance (MR) images show the initial size of the hepatic neoplasm (arrows). (b) The 6-month imaging follow-up study shows reduced size of the lesion on both the T1-w (A) and T2-w (B) axial MR images (arrows). (c) The 10-month imaging follow-up study shows a further reduction of the size of the lesion on both the T1-w (A) and T2-w (B) axial MR images (arrows).

univariate and multivariate analysis

In the univariate analysis, the PS, the disease status after the prior treatment and the development of immunological response were significantly associated with better PFS and OS (Table 3). Multivariate analysis (Table 4) demonstrated that the development of immunological response was an independent factor associated with better PFS (HR = 3.35, 95% CI 1.7–6.7; $P = 0.001$), whereas worse PS was associated with shorter OS (HR = 3.0, 95% CI 1.5–5.8; $P = 0.001$). There was a trend for worse OS in patients who failed to respond immunologically during the vaccination (HR = 2.0, 95% CI 1.0–4.0; $P = 0.057$).

discussion

TERT comprises a potential target of therapeutic vaccination in cancer patients, given its high expression in most of the solid tumors. Since several preclinical and clinical studies have failed

to demonstrate an efficient immune response to dominant tumor antigens/peptides [26, 32], we employed a cryptic peptide (TERT₅₇₂) to overcome the development of immune tolerance [21, 24]. The present study confirmed our previous observations that Vx-001 can induce a TERT₅₇₂-specific T-cell immune response associated with higher PFS and OS. The new information provided by the current study is that Vx-001 is not only active in patients with advanced NSCLC, as already shown [18], but also in patients with other types of advanced cancer; in addition, the administration of Vx-001 showed an outstanding toxicity profile without serious EAEs or late adverse events and with no evidence of autoimmune reactions even after its administration for up to 2 years.

Measurable tumor regression is rarely achieved by most vaccines in patients with advanced solid tumors [33–35]; furthermore, an objective response (based on the RECIST criteria) is not considered an ideal end point for the evaluation

Table 3. Univariate analysis for PFS and OS

	Log-rank test	P value	Hazard ratio	P value	95% CI
PFS (<i>n</i> = 55)					
Sex (male versus female)	0.000	0.992	1.003	0.992	0.551–1.827
PS (1–2 versus 0)	5.178	0.023	1.955	0.026	1.084–3.526
Stage (IV versus IIIb)	0.767	0.381	1.676	0.388	0.519–5.412
Pre-vaccination status (PD versus SD)	4.301	0.038	1.949	0.042	1.024–3.709
Immune response at any time (no versus yes)	13.571	0.0001	3.346	0.0001	1.692–6.615
OS (<i>n</i> = 55)					
Sex (male versus female)	0.010	0.919	1.037	0.919	0.520–2.066
PS (1–2 versus 0)	11.843	0.001	3.002	0.001	1.559–5.783
Stage (IV versus IIIb)	0.997	0.318	2.037	0.328	0.489–8.482
Pre-vaccination status (PD versus SD)	2.470	0.116	1.813	0.121	0.854–3.851
Immune response at any time (no versus yes)	4.160	0.041	2.074	0.046	1.013–4.245

CI, confidence interval; OS, overall survival; PFS, progression-free survival; PD, disease progression; PS, performance status; SD, stable disease. Bold values indicate statistically significant correlations ($P \leq 0.05$).

Table 4. Multivariate analysis for PFS and OS

	Hazard ratio	P value	95% CI
PFS (<i>n</i> = 55)			
PS (1–2 versus 0)	1.668	0.099	0.907–3.065
Pre-vaccination status (PD versus SD)	1.513	0.233	0.766–2.990
Immune response at any time (no versus yes)	3.346	0.001	1.692–6.615
OS (<i>n</i> = 55)			
PS (1–2 versus 0)	2.970	0.001	1.536–5.743
Pre-vaccination status (PD versus SD)	1.283	0.538	0.580–2.837
Immune response at any time (no versus yes)	2.020	0.057	0.980–4.164

CI, confidence interval; OS, overall survival; PFS, progression-free survival; PD, disease progression; PS, performance status; SD: stable disease. Bold values indicate statistically significant correlations ($P \leq 0.05$).

of an immunotherapy strategy, given that some patients with radiologically nonresponding tumors may also benefit from a delay in tumor progression [36, 37]. The Cancer Vaccine Trial Working Group concludes that tumor shrinkage may be a less relevant measure of vaccine efficacy in the treatment of solid tumors and recommends the duration of SD as an indicator of antitumor activity [38, 39]. Interestingly, in the present study, two objective clinical responses were documented in addition to a relatively high rate of SD (overall DCR = 36%). Moreover, the disease status (SD versus PD) at the time of study enrollment was associated with the DCR ($P = 0.050$), suggesting that patients previously responding to systemic treatment may be better candidates for the Vx-001 vaccine compared with those with PD.

In addition, among eight patients who received boost vaccinations, six experienced a long-lasting SD (>6 months from the first booster vaccination) and three of them continued

to receive boost vaccinations for >2 years, with no evidence of disease progression. Whether this observation could be attributed to the patients' better PS or to the lower tumor burden remains undetermined [40, 41]. Nevertheless, preliminary data indicate that boost vaccinations could maintain the immune responses for a long time (data not shown).

The immune response induced by the Vx-001, as detected by ELISpot, was clearly associated with a significantly better PFS and OS (supplemental Figure S1A and B, available at *Annals of Oncology* online). However, subgroup analysis showed that the immune response had a significant impact on PFS irrespectively of the patients' disease status (SD or PD) at enrollment (supplemental Table S1, available at *Annals of Oncology* online). More importantly, multivariate analysis revealed that the development of immunological response at any time during the vaccination program was an independent prognostic factor associated with higher PFS, which might be associated with the relatively high DCR achieved with the Vx-001 vaccine. Future studies including more patients may clarify whether the detection of TERT-induced CTLs could be a reliable predictive marker for patients' clinical outcome.

In one-fourth of the patients, a preexisting T-cell immune reactivity against the TERT antigen was detected; the analysis of the clinical data of these particular patients could not reveal any significant improvement of OS or PFS compared with those without pre-vaccination immune reactivity. However, patients who maintained the preexisting immune reactivity during the vaccination period had a significantly higher OS compared with those who lost it. The reason for the loss of preexisting immune reactivity, occurring in some patients, is not clear and its biological relevance and importance will be the subject of future studies.

These encouraging results should be interpreted with caution since the study population composed of a very heterogeneous

group of patients with different types of tumor and different response to prior treatment. Nevertheless, most of the patients had chemoresistant disease with poor prognosis.

Similar association between the development of immunological response and the clinical outcome has been reported in studies employing different vaccines [16, 42–44]; moreover, other studies have clearly demonstrated that the presence of specifically stimulated T cells in the tumor independently predicts better patients' clinical outcome [45–48]. However, it remains unclear why only a subset of patients responds immunologically. The immune tolerance, induced by immune-suppressive cells, has been shown to increase in cancer patients [49–55]. Indeed, regulatory T cells (Tregs), myeloid-derived suppressor cells and others have been proposed for the failure of immune system to mount an efficient response against cancer cells [56–61]; to this end, we have already initiated a study to prospectively investigate the role of various suppressive cells in the development of immune responses after vaccination with Vx-001.

Finally, the Vx-001 was well tolerated, with transient and mild skin reactions to be the most common EAEs. In addition, no clinical or laboratory signs of late toxicity or development of autoimmune were noticed [62–65].

In conclusion, Vx-001 vaccine was well tolerated and induced a TERT-specific immune response, which was associated with better clinical outcome. Further evaluation in randomized studies is required to confirm these promising results and to exclude the possibility that the better immune response is merely a reflection of immunologically healthier patients. Moreover, tumor biopsies for better investigation of tumor microenvironment and the presence of tumor infiltrating TERT₅₇₂-specific T cells are also required. Finally, future vaccination studies in patients with low tumor burden such as in the adjuvant setting would be of great interest.

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