Annals of Oncology Advance Access published August 25, 2011

original article

Annals of Oncology
doi:10.1093/annonc/mdr396

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

A. Kotsakis[1], E.-K. Vetsika[2], S. Christou[1], D. Hatzidaki[1], N. Vardakis[1], D. Aggouraki[2], G. Konsolakis[2], V. Georgoulias[1,2], Ch. Christophyllakis[3], P. Cordopatis[4], K. Kosmatopoulos[5] & D. Mavroudis[1,2*]

[1]Department of Medical Oncology, University Hospital of Heraklion, Crete; [2]Laboratory of Tumor Cell Biology, School of Medicine, University of Crete, Crete; [3]First Department of Medical Oncology, ''IASO'' General Hospital of Athens, Athens; [4]Department of Pharmacy, University of Patras, Patras, Greece; [5]Vaxon Biotech, Paris, France

Received 25 January 2011; revised 31 May 2011; accepted 15 June 2011

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 $TERT_{572}$ 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

Correspondence to: Prof. D. Mavroudis, Department of Medical Oncology, University Hospital of Heraklion, PO Box 1352, 711 10 Heraklion, Crete, Greece. Tel: +30-28-10-392-783; Fax: +30-28-10-392-857; E-mail: georgsec@med.uoc.gr

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 $TERT_{572}$ 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 $TERT_{572}$ 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 $TERT_{572Y}$ (YLFFYRKSV) and the native $TERT_{572}$ (RLFFYRKSV) 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 $TERT_{572}$ 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 $TERT_{572}$ 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 $TERT_{572}$ 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/2×10^5 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).

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)
≥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.

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 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	CR = 1
	SD = 9	SD = 7, PD = 2
	PD = 3	Discontinued
	NE = 3	SD = 1, PD = 2
PD ($n = 39$)	SD = 20	PR = 1, SD = 10, PD = 9
	PD = 16	Discontinued
	NE = 3	PD = 3

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

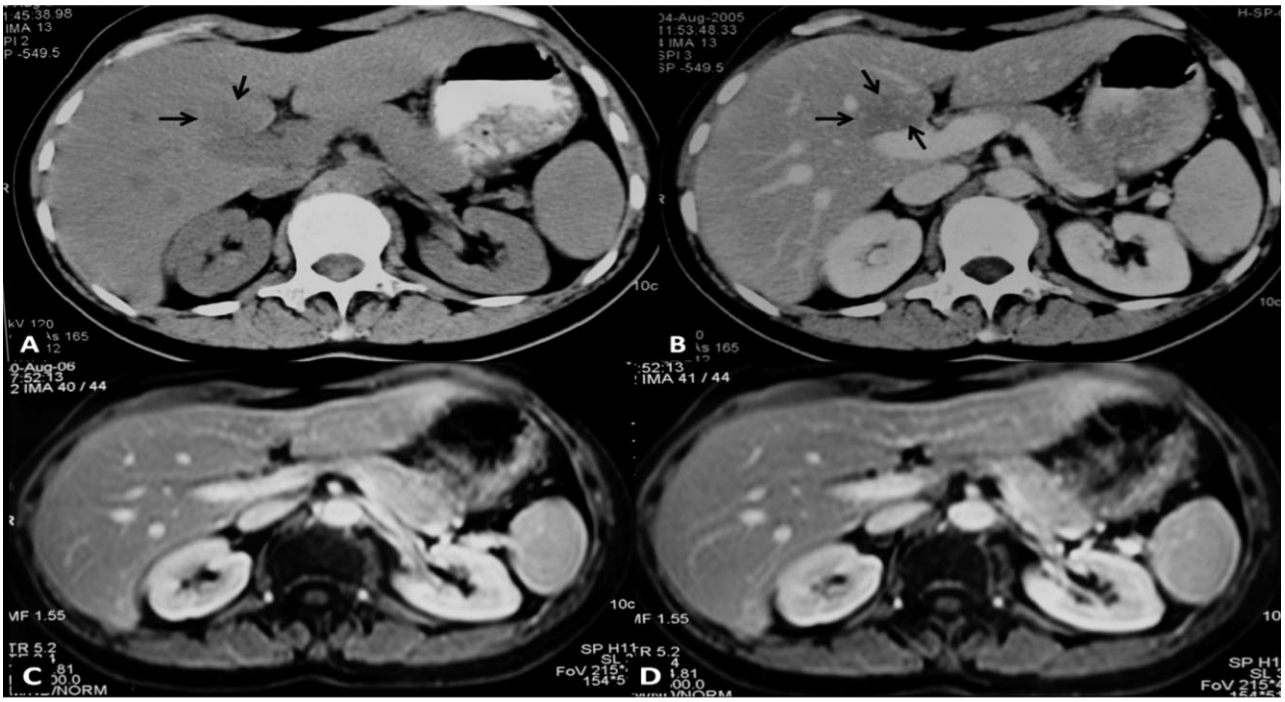

Figure 1. Breast cancer patient with liver metastasis. The pre-vaccination plain (A) and contrast-enhanced (B, portal phase of I.V. contrast administration) axial computed tomography images show the hypodense lesion anterior to the portal vein (arrows). The post-treatment (1 year later) contrast enhanced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

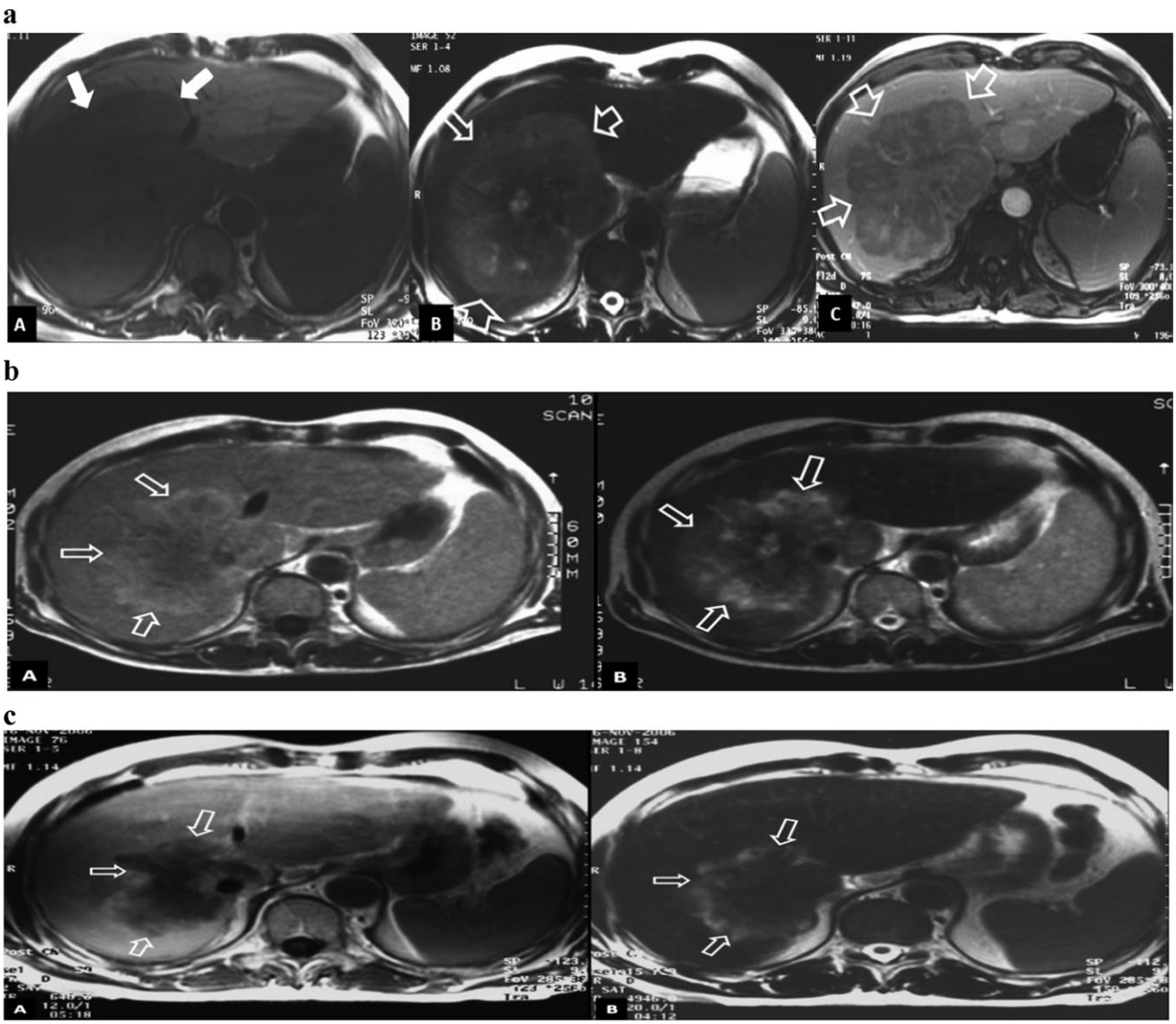

Figure 2. Hepatocellular carcinoma patient. (a) Pre-vaccination imaging studies. The T1-w (A), T2-w (B) and contrast enhanced T1-w (C) axial magnetic resonance (MR) images show the initial size of the hepatic neoplasm (arrows). (b) The 6-month imaging follow-up study shows reduced size of the lesion 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 ($TERT_{572}$) to overcome the development of immune tolerance [21, 24]. The present study confirmed our previous observations that Vx-001 can induce a $TERT_{572}$-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 ($n = 55$)					
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 ($n = 55$)					
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 ≤ 0.05).

Table 4. Multivariate analysis for PFS and OS

	Hazard ratio	*P* value	95% CI
PFS ($n = 55$)			
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 ($n = 55$)			
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 ≤ 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 $TERT_{572}$-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.

acknowledgements

We thank Prof. A. Karantanas for his assistance in the presentation of radiographic images.

funding

Supported by research grants from the Cretan Association for Biomedical Research (CABR). Also supported by personal grants (VG and DM) project KA 1967 from the Special Account for Research of the University of Crete (ELKE).

disclosures

KK is stockholder of Vaxon Biotech. The other co-authors declare no conflicts of interest. SC was a recipient of a clinical fellowship from Cretan Association for Biomedical Research (CABR).

references

1. Van Der Bruggen P, Zhang Y, Chaux P et al. Tumor-specific shared antigenic peptides recognized by human T cells. Immunol Rev 2002; 188: 51–64.
2. Nagorsen D, Scheibenbogen C, Marincola FM et al. Natural T cell immunity against cancer. Clin Cancer Res 2003; 9: 4296–4303.
3. Jager E, Ringhoffer M, Karbach J et al. Inverse relationship of melanocyte differentiation antigen expression in melanoma tissues and CD8+ cytotoxic-T-cell responses: evidence for immunoselection of antigen-loss variants in vivo. Int J Cancer 1996; 66: 470–476.
4. Schmollinger JC, Vonderheide RH, Hoar KM et al. Melanoma inhibitor of apoptosis protein (ML-IAP) is a target for immune-mediated tumor destruction. Proc Natl Acad Sci U S A 2003; 100: 3398–3403.
5. Wenandy L, Sorensen RB, Sengelov L et al. The immunogenicity of the hTERT540-548 peptide in cancer. Clin Cancer Res 2008; 14: 4–7.
6. Kim NW, Piatyszek MA, Prowse KR et al. Specific association of human telomerase activity with immortal cells and cancer. Science 1994; 266: 2011–2015.
7. Hiyama E, Hiyama K. Telomere and telomerase in stem cells. Br J Cancer 2007; 96: 1020–1024.
8. Kumaki F, Kawai T, Hiroi S et al. Telomerase activity and expression of human telomerase RNA component and human telomerase reverse transcriptase in lung carcinomas. Hum Pathol 2001; 32: 188–195.
9. Wu TC, Lin P, Hsu CP et al. Loss of telomerase activity may be a potential favorable prognostic marker in lung carcinomas. Lung Cancer 2003; 41: 163–169.
10. Lu C, Soria JC, Tang X et al. Prognostic factors in resected stage I non-small-cell lung cancer: a multivariate analysis of six molecular markers. J Clin Oncol 2004; 22: 4575–4583.
11. Fujita Y, Fujikane T, Fujiuchi S et al. The diagnostic and prognostic relevance of human telomerase reverse transcriptase mRNA expression detected in situ in patients with nonsmall cell lung carcinoma. Cancer 2003; 98: 1008–1013.
12. Komiya T, Kawase I, Nitta T et al. Prognostic significance of TERT expression in non-small cell lung cancer. Int J Oncol 2000; 16: 1173–1177.
13. Marchetti A, Bertacca G, Buttitta F et al. Telomerase activity as a prognostic indicator in stage I non-small cell lung cancer. Clin Cancer Res 1999; 5: 2077–2081.
14. Hahn WC, Stewart SA, Brooks MW et al. Inhibition of telomerase limits the growth of human cancer cells. Nat Med 1999; 5: 1164–1170.
15. Herbert B, Pitts AE, Baker SI et al. Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. Proc Natl Acad Sci U S A 1999; 96: 14276–14281.
16. Domchek SM, Recio A, Mick R et al. Telomerase-specific T-cell immunity in breast cancer: effect of vaccination on tumor immunosurveillance. Cancer Res 2007; 67: 10546–10555.
17. Brunsvig PF, Aamdal S, Gjertsen MK et al. Telomerase peptide vaccination: a phase I/II study in patients with non-small cell lung cancer. Cancer Immunol Immunother 2006; 55: 1553–1564.
18. Bolonaki I, Kotsakis A, Papadimitraki E et al. Vaccination of patients with advanced non-small-cell lung cancer with an optimized cryptic human telomerase reverse transcriptase peptide. J Clin Oncol 2007; 25: 2727–2734.
19. Moudgil KD, Sercarz EE. Can antitumor immune responses discriminate between self and nonself? Immunol Today 1994; 15: 353–355.
20. Cibotti R, Kanellopoulos JM, Cabaniols JP et al. Tolerance to a self-protein involves its immunodominant but does not involve its subdominant determinants. Proc Natl Acad Sci U S A 1992; 89: 416–420.
21. Gross DA, Graff-Dubois S, Opolon P et al. High vaccination efficiency of low-affinity epitopes in antitumor immunotherapy. J Clin Invest 2004; 113: 425–433.
22. Theobald M, Biggs J, Hernandez J et al. Tolerance to p53 by A2.1-restricted cytotoxic T lymphocytes. J Exp Med 1997; 185: 833–841.
23. Colella TA, Bullock TN, Russell LB et al. Self-tolerance to the murine homologue of a tyrosinase-derived melanoma antigen: implications for tumor immunotherapy. J Exp Med 2000; 191: 1221–1232.
24. Grossmann ME, Davila T, Celis T. Avoiding tolerance against prostatic antigens with subdominant peptide epitopes. J Immunother 2001; 24: 237–241.
25. O'Mahony D, Kummar S, Gutierrez ME. Non-small-cell lung cancer vaccine therapy: a concise review. J Clin Oncol 2005; 23: 9022–9028.
26. Parkhurst MR, Riley JP, Igarashi T et al. Immunization of patients with the hTERT:540-548 peptide induces peptide-reactive T lymphocytes that do not

recognize tumors endogenously expressing telomerase. Clin Cancer Res 2004; 10: 4688–4698.
27. Mavroudis D, Bolonakis I, Cornet S et al. A phase I study of the optimized cryptic peptide TERT(572y) in patients with advanced malignancies. Oncology 2006; 70: 306–314.
28. Scardino A, Gross DA, Alves P et al. HER-2/neu and hTERT cryptic epitopes as novel targets for broad spectrum tumor immunotherapy. J Immunol 2002; 168: 5900–5906.
29. Hernandez J, Garcia-Pons F, Lone YC et al. Identification of a human telomerase reverse transcriptase peptide of low affinity for HLA A2.1 that induces cytotoxic T lymphocytes and mediates lysis of tumor cells. Proc Natl Acad Sci U S A 2002; 99: 12275–12280.
30. Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000; 92: 205–216.
31. Armitage P, Berry G. Statistical Methods in Medical Research, 3rd edition. Boston, MA: Blackwell Scientific Publications 1994.
32. Ayyoub M, Migliaccio M, Guillaume P et al. Lack of tumor recognition by hTERT peptide 540-548-specific CD8(+) T cells from melanoma patients reveals inefficient antigen processing. Eur J Immunol 2001; 31: 2642–2651.
33. Rosenberg SA, Yang JC, Schwartzentruber DJ et al. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nat Med 1998; 4: 321–327.
34. Simon RM, Steinberg SM, Hamilton M et al. Clinical trial designs for the early clinical development of therapeutic cancer vaccines. J Clin Oncol 2001; 19: 1848–1854.
35. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004; 10: 909–915.
36. Buyse M, Thirion P, Carlson RW et al. Relation between tumour response to first-line chemotherapy and survival in advanced colorectal cancer: a meta-analysis. Meta-Analysis Group in Cancer. Lancet 2000; 356: 373–378.
37. Johnson JR, Williams G, Pazdur R. End points and United States Food and Drug Administration approval of oncology drugs. J Clin Oncol 2003; 21: 1404–1411.
38. Hoos A, Parmiani G, Hege K et al. A clinical development paradigm for cancer vaccines and related biologics. J Immunother 2007; 30: 1–15.
39. Wolchok JD, Hoos A, O'Day S et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009; 15: 7412–7420.
40. Colombo MP, Piconese S. Regulatory-T-cell inhibition versus depletion: the right choice in cancer immunotherapy. Nat Rev Cancer 2007; 7: 880–887.
41. Lechner MG, Liebertz DJ, Epstein AL. Characterization of cytokine-induced myeloid-derived suppressor cells from normal human peripheral blood mononuclear cells. J Immunol 2010; 185: 2273–2284.
42. Nemunaitis J, Jahan T, Ross H et al. Phase 1/2 trial of autologous tumor mixed with an allogeneic GVAX vaccine in advanced-stage non-small-cell lung cancer. Cancer Gene Ther 2006; 13: 555–562.
43. Gonzalez G, Crombet T, Torres F et al. Epidermal growth factor-based cancer vaccine for non-small-cell lung cancer therapy. Ann Oncol 2003; 14: 461–466.
44. Lonchay C, van der Bruggen P, Connerotte T et al. Correlation between tumor regression and T cell responses in melanoma patients vaccinated with a MAGE antigen. Proc Natl Acad Sci U S A 2004; 101(Suppl 2): 14631–14638.
45. Clark WH Jr., Elder DE, Guerry Dt et al. Model predicting survival in stage I melanoma based on tumor progression. J Natl Cancer Inst 1989; 81: 1893–1904.
46. Marrogi AJ, Munshi A, Merogi AJ et al. Study of tumor infiltrating lymphocytes and transforming growth factor-beta as prognostic factors in breast carcinoma. Int J Cancer 1997; 74: 492–501.
47. Zhang L, Conejo-Garcia JR, Katsaros D et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003; 348: 203–213.
48. Galon J, Costes A, Sanchez-Cabo F et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006; 313: 1960–1964.
49. Kusmartsev SA, Li Y, Chen SH. Gr-1+ myeloid cells derived from tumor-bearing mice inhibit primary T cell activation induced through CD3/CD28 costimulation. J Immunol 2000; 165: 779–785.
50. Melani C, Chiodoni C, Forni G, Colombo MP. Myeloid cell expansion elicited by the progression of spontaneous mammary carcinomas in c-erbB-2 transgenic BALB/c mice suppresses immune reactivity. Blood 2003; 102: 2138–2145.
51. Almand B, Clark JI, Nikitina E et al. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. J Immunol 2001; 166: 678–689.
52. Pandit R, Lathers DM, Beal NM et al. CD34+ immune suppressive cells in the peripheral blood of patients with head and neck cancer. Ann Otol Rhinol Laryngol 2000; 109: 749–754.
53. Bronte V, Serafini P, Apolloni E, Zanovello P. Tumor-induced immune dysfunctions caused by myeloid suppressor cells. J Immunother 2001; 24: 431–446.
54. Gabrilovich D. Mechanisms and functional significance of tumour-induced dendritic-cell defects. Nat Rev Immunol 2004; 4: 941–952.
55. Kusmartsev S, Nagaraj S, Gabrilovich DI. Tumor-associated CD8+ T cell tolerance induced by bone marrow-derived immature myeloid cells. J Immunol 2005; 175: 4583–4592.
56. Vuk-Pavlovic S, Bulur PA, Lin Y et al. Immunosuppressive CD14+HLA-DRlow/- monocytes in prostate cancer. Prostate 2010; 70: 443–455.
57. Hoechst B, Ormandy LA, Ballmaier M et al. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. Gastroenterology 2008; 135: 234–243.
58. Mandruzzato S, Solito S, Falisi E et al. IL4Ralpha+ myeloid-derived suppressor cell expansion in cancer patients. J Immunol 2009; 182: 6562–6568.
59. Liu CY, Wang YM, Wang CL et al. Population alterations of L-arginase- and inducible nitric oxide synthase-expressed CD11b+/CD14-/CD15+/CD33+ myeloid-derived suppressor cells and CD8+ T lymphocytes in patients with advanced-stage non-small cell lung cancer. J Cancer Res Clin Oncol 2009; 136(1): 35–45.
60. Srivastava MK, Bosch JJ, Thompson JA et al. Lung cancer patients' CD4(+) T cells are activated in vitro by MHC II cell-based vaccines despite the presence of myeloid-derived suppressor cells. Cancer Immunol Immunother 2008; 57: 1493–1504.
61. Rodriguez PC, Ernstoff MS, Hernandez C et al. Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. Cancer Res 2009; 69: 1553–1560.
62. Vonderheide RH, Hahn WC, Schultze JL, Nadler LM. The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. Immunity 1999; 10: 673–679.
63. Kolquist KA, Ellisen LW, Counter CM et al. Expression of TERT in early premalignant lesions and a subset of cells in normal tissues. Nat Genet 1998; 19: 182–186.
64. Nair SK, Heiser A, Boczkowski D et al. Induction of cytotoxic T cell responses and tumor immunity against unrelated tumors using telomerase reverse transcriptase RNA transfected dendritic cells. Nat Med 2000; 6: 1011–1017.
65. Danet-Desnoyers GA, Luongo JL, Bonnet DA et al. Telomerase vaccination has no detectable effect on SCID-repopulating and colony-forming activities in the bone marrow of cancer patients. Exp Hematol 2005; 33: 1275–1280.