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A First-in-Human Phase I Study of INVAC-1, an Optimized Human Telomerase DNA Vaccine in Patients with Advanced Solid Tumors

Luis Teixeira[1], Jacques Medioni[2], Julie Garibal[3], Olivier Adotevi[4], Ludovic Doucet[1], Marie-Agnès Dragon Durey[5], Zineb Ghrieb[1], Jean-Jacques Kiladjian[1], Mara Brizard[2], Caroline Laheurte[4], Maria Wehbe[3], Elodie Pliquet[3], Marie Escande[3], Rémy Defrance[3], Stephane Culine[1], Stephane Oudard[2], Simon Wain-Hobson[3], Valérie Doppler[3], Thierry Huet[3], and Pierre Langlade-Demoyen[3]

ABSTRACT

Purpose: Human telomerase reverse transcriptase (hTERT) is highly expressed in >85% of human tumors and is thus considered as a good tumor-associated antigen candidate for vaccine development. We conducted a phase I study to investigate the safety, tolerability, clinical response, and immunogenicity of INVAC-1, a DNA plasmid encoding a modified hTERT protein in patients with relapsed or refractory solid tumors.

Patients and Methods: INVAC-1 was either administered by intradermal route followed by electroporation or by Tropis, a needle-free injection system. Safety and tolerability were monitored by clinical and laboratory assessments. Progression-free survival and overall survival were reported using Kaplan–Meier survival analysis. Immunogenicity was studied by ELISpot, Luminex, and Flow Cytometry.

Results: Twenty-six patients were treated with INVAC-1 administered at three dose levels (100, 400, and 800 μg). Vaccination was well tolerated and no dose-limiting toxicity was reported. One treatment-related grade 3 SAE was reported. Fifty-eight percent of patients experienced disease stabilization. PFS was 2.7 months, median OS was 15 months, and 1-year survival was reached for 65% of patients. INVAC-1 vaccination stimulated specific anti-hTERT CD4 T-cell response as well as cytotoxic CD8 T-cell response. No evidence of peripheral vaccine-induced immunosuppression was observed.

Conclusions: INVAC-1 vaccination was safe, well tolerated, and immunogenic when administered intradermally at the three tested doses in patients with relapsed or refractory cancers. Disease stabilization was observed for the majority of patients (58%) during the treatment period and beyond.

See related commentary by Slingluff Jr, p. 529

Introduction

Human telomerase reverse transcriptase (hTERT), the catalytic subunit of the telomerase complex, is highly expressed in more than 85% of human tumors from diverse phenotypes and associated with poor prognosis (1–6), with little or no expression in normal somatic cells (7). hTERT expression and activity are associated with maintenance of telomere length and play a role in the unlimited proliferative capacity of cancer cells. Telomerase overexpression in tumor cells results from promotor mutations, chromosomal rearrangements, or increased MYC gene expression, all of which are part of the oncogenic process. Although telomere elongation is the major function of hTERT, it also impacts cell proliferation, resistance to apoptosis, and invasion. Novel molecular functions including transcriptional regulation and metabolic reprogramming have been attributed to hTERT, making this protein a central regulator of many of cancer hallmarks (8). Targeting hTERT, either with inhibitors or through immunotherapy may therefore have the potential to eliminate tumor cells including cancer stem cells as numerous studies have shown that cancer stem or stem-like cells also overexpress hTERT (9–11). In patients with cancer, it was shown that hTERT overexpression led to presentation of hTERT peptides at the surface of tumor cells, thus triggering a natural anti-hTERT immune response by breaking self-tolerance (12).

Therapeutic hTERT vaccines have raised great hope for cancer immunotherapy because of their potential to stimulate tumor cell killing by enhancing the activity of telomerase-specific CD8 T cells (13–15). Different vaccination strategies were developed including cellular approaches, such as autologous antigen-presenting cells (APC - dendritic cells or B lymphocytes) loaded with hTERT peptides or transduced with hTERT mRNA (16). Several studies were also performed to develop hTERT peptide vaccines containing motifs that either bind to class I or II molecules (17–20). Findings from early clinical trials of hTERT peptide vaccines in patients with cancer showed immunologic responses in >50% of the patients tested with promising clinical outcomes and favorable toxicity profile (16, 21).

INVAC-1 is a DNA plasmid encoding a modified, enzymatically inactive, hTERT protein (22). The N-terminal part of the protein, which includes the nucleolar localization sequence, was replaced by an

[1]Breast Diseases Unit, HIPI INSERM U976, AP-HP, Saint Louis Hospital, Paris University, Paris, France. [2]Center for Early Clinical Trials, Medical Oncology Department (CEPEC), Georges Pompidou Hospital, Paris Descartes University, Paris, France. [3]Invectys, Paris BioPark, Paris, France. [4]University of Bourgogne Franche-Comté, INSERM, EFS BFC, UMR1098, Biomonitoring Platform, Besançon, France. [5]Immunobiology Laboratory, Georges Pompidou Hospital, Paris, France.

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L. Teixeira and J. Medioni contributed equally to this article.

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Corresponding Author: Julie Garibal, Invectys, Paris Biopark, 12, rue Jean Antoine de Baïf, Paris 75013, France. Phone: 33-1-81-80-05-27; Fax: 33-1-81-80-05-30; E-mail: julie.garibal@invectys.com

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Translational Relevance

INVAC-1 is a DNA plasmid encoding a modified human telomerase reverse transcriptase (hTERT) protein devoid of catalytic activity. hTERT can be regarded as a universal tumor-associated antigen for anticancer therapies as it is highly expressed in more than 85% of human tumors, with little or no expression in normal somatic cells. Given intradermally at increasing doses in association with electroporation or by Tropis, a needle-free injection system, INVAC-1 was well tolerated in patients with advanced solid tumors. The majority of treated patients displayed prolonged disease stabilization, allowing treatment duration for up to 9 months. Moreover, INVAC-1 triggered anti-hTERT specific CD4 and CD8 T-cell responses. Given its safety profile, encouraging clinical response and immunogenicity, there is a strong rationale for evaluating INVAC-1 as a monotherapy or in combination with other therapies including checkpoint inhibitors, preferably in patients with adequate immune function. A phase II clinical trial using INVAC-1 is ongoing.

ubiquitin moiety to force its degradation and enhance peptide presentation, thus improving hTERT-specific immune responses induction. These modifications were introduced to make INVAC-1 a safe, potent, and unique immunotherapy drug potentially able to induce both helper CD4 and cytotoxic CD8 T-cell responses for a better antitumor immune response (23–25).

Primary pharmacodynamics, safety pharmacology, and toxicology studies, including biodistribution and local tolerance in animal models, showed that INVAC-1 was enzymatically inactive, immunogenically safe, and well tolerated. In murine models, we demonstrated that INVAC-1 was able to induce hTERT-specific cellular immune responses with $CD4^+$ Th1 effector and memory $CD8^+$ T cells. In a HLA-A2 spontaneous mouse sarcoma Sarc-T2r model, INVAC-1 vaccination was able to slow tumor growth and increased survival rate by 50% in tumor-bearing mice (22).

On the basis of these encouraging preclinical results, we conducted a first-in-human (FIH) study, 2-centre, phase I, open-label, 3 + 3 escalation design and multiple dose study examining the safety and tolerability of INVAC-1 administered at three dose levels (100, 400, and 800 μg) in 26 patients with relapsed or solid refractory tumors. INVAC-1 was administered either by intradermal (ID) injection followed by electroporation (EP) using Cliniporator2 (20 patients) or by Tropis Needle Free Injection System (NFIS; 6 patients), which was shown to effectively deliver peptide, DNA and RNA vaccines (26, 27). After validation of NFIS device by bridging pharmacodynamics and local biodistribution studies in naïve dogs in comparison with EP (data not shown), 6 additional patients were enrolled in this phase I study to explore the safety, tolerability, and pharmacodynamics properties of INVAC-1 administered with NFIS/Tropis.

We show here that INVAC-1 vaccination was safe and well tolerated when administered ID (either with EP or by Tropis) at the three tested doses in patients with relapsed or refractory cancers. INVAC-1 elicited both hTERT-specific CD4 and CD8 T-cell responses with no vaccine-induced immunosuppression. Patients with overall survival (OS) >1 year showed a significantly higher hTERT immune response after INVAC-1 vaccination compared to patients with OS <1 year. Finally, although this difference was non-significant, estimated median OS in INVAC-1 immune responders was 17.4 months versus 7 months for nonresponders.

Patients and Methods

INVAC-1 plasmid

INVAC-1 is a covalently closed, circular, and supercoiled double-stranded DNA plasmid of 7,120 bp encoding a modified, enzymatically inactive hTERT protein (22). The modified hTERT sequence was synthesized by GeneCust and subcloned into the NTC8685-eRNA41H-HindIII-XbaI vector backbone designed by Nature Technology Corporation. INVAC-1 plasmid was amplified through an antibiotic-free selection procedure in NTC4862 *E. coli* cells (DH5α attλ::$P_{5/6\ 6/6}$-RNA-IN-SacB, catR). GLP and GMP batches of INVAC-1 were manufactured by Eurogentec at a final concentration of 2 mg/mL in D-PBS.

INVAC-1 administration

INVAC-1 was administered to the first 20 patients by intradermal injection combined with EP using the Cliniporator2 following local anesthesia at the site(s) of injection. In parallel, Tropis NFIS from PharmaJet Inc. was evaluated in 6 additional patients. Tropis injection system has obtained the CE Mark clearance in Europe and is currently validated in several clinical trials in the United States as an investigational device.

Study design and vaccination schedule

This study was a FIH, phase I, open-label, classical 3 + 3 escalation design investigating multiple dosing of INVAC-1 as a single agent in patients with relapsed or refractory solid tumors presenting progressive disease (#NCT02301754). An extension cohort of 11 patients was opened at the maximum tested dose to confirm safety and pharmacodynamics data in a larger set of patients. Six additional patients were also treated at the maximum tested dose using Tropis device. The trial was performed at two study centers in France (Hôpital Saint Louis and Hôpital Européen Georges Pompidou) from November 2014 to June 2018.

Three INVAC-1 dose levels (100, 400, and 800 μg) were evaluated in sequential cohorts of patients. All patients had a diagnosis of advanced or metastatic solid tumors relapsed or refractory to standard treatment (progressive disease).

INVAC-1 was prepared at fixed dose of 100, 400, or 800 μg. Depending on the dose level, single (100 μg) or multiple sites (400 and 800 μg) were injected (arms and thighs, except the left arm in case of EP).

Each patient was scheduled to receive INVAC-1 for 3 sequential cycles (day one of 28-day cycle) unless motivated treatment interruption. Doses were escalated in the absence of dose-limiting toxicity (DLT) during the first two cycles of treatment. However, in case of good tolerance and provided a positive opinion of the Data Safety Monitoring Board, treatment could be prolonged for a maximum duration of 6 additional cycles.

Inclusion criteria

Patients aged >18 years with a diagnosis of advanced or metastatic solid tumors relapsed or refractory to standard treatment, with life expectancy >4 months and Eastern Cooperative Oncology Group (ECOG) performance status ≤1 were enrolled. Patients needed to have healthy skin surface (for ID administration of INVAC-1 associated with EP) and adequate bone marrow, renal, liver, and cardiac functions.

Safety monitoring and clinical study procedures

Adverse events (AE) meeting the following definition occurring in the first two cycles of treatment (up to 28 days after second dose)

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should be classified as DLT: grade 4 neutropenia lasting >7 days, febrile neutropenia (defined as neutropenia ≥grade 3), neutropenic infection, grade ≥3 thrombocytopenia with bleeding, grade 4 thrombocytopenia lasting >3 days, grade 4 anemia, grade ≥3 hemolysis, or any grade ≥3 nonhematologic toxicities. The MTD was the dose at which no more than one patient out of six experienced DLT.

AEs were documented and recorded at each visit using the NCI's Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 and coded at the time of the analysis using the Medical Dictionary for Regulatory Activity (MedDRA) version 18.1. Patients were followed for AEs for 28 days after the last treatment administration or until all drug-related toxicities had resolved, whichever the latest.

Tumor imaging was obtained through CT scanning every 8 weeks during INVAC-1 treatment. Tumor response was assessed using RECIST (version 1.1) adapted to immune response (28, 29). Best overall response was defined as the best tumor response (complete response, partial response, stable disease, or progressive disease) observed and confirmed after a second observation not less than 4 weeks apart.

Plasma inflammation markers (TNFα, IFNγ, IL17, IL8, IL6, IL1β) were analyzed using multiple cytokine analysis (Luminex). Antinuclear (ANA), anti-DNA, and anti-thyroid peroxidase antibodies were monitored.

Peripheral blood samples were collected for immune monitoring analysis before and at different time points after vaccination. T and B lymphocytes as well as natural killer (NK) cells' absolute counts were determined by flow cytometry in K2-EDTA blood samples. Peripheral blood mononuclear cells (PBMC) were purified by Ficoll–Hypaque density gradient and freshly used for further analysis or frozen and stored in liquid nitrogen.

IFNγ ELISpot

To measure specific CD4 T-cell responses induced after vaccination, we used a mixture of 8 highly promiscuous HLA-DR and HLA-DP4–restricted peptides derived from hTERT (30, 31). Patients were HLA-typed and anti-hTERT CD8 T cells responses were evaluated in HLA-matched patients by using mixtures of hTERT-derived peptides restricted either to HLA-A2 (4 peptides), HLA-B7 (4 peptides), HLA-A24 (3 peptides), or HLA-A3 (1 peptide) according to the haplotype of patients (32). All hTERT-derived peptides were purchased from JPT.

CD4 and CD8 T-cell immune responses were analyzed by ELISpot IFNγ assay. Briefly, PBMCs were cultured for 9 days with hTERT-derived peptide mixtures (5 μg/mL) in the presence of IL7 (5 ng/mL, Peprotech) and IL2 (20 UI/mL, Peprotech) in RMPI medium supplemented with 5% human serum (SH-PBM06, EFS-Besançon; refs. 30, 33). Cells were then harvested, washed, numerated, and seeded in ELISpot 96-well microplates (10^5 cells/well – triplicates) and stimulated again with peptide mixtures for an additional 15 hours. IFNγ-secreting cells [i.e., spot-forming cells (SFC)] were revealed according to the manufacturer's instructions (Diaclone) and counted using the ELISpot ImmunoSpot, S6 Ultra-V Analyser (Cellular Technology Ltd.). The number of specific T cells, expressed as SFC per 1×10^5 cells, was calculated after subtracting unstimulated control values. PMA (100 ng/mL) and ionomycin (10 ng/mL) were used as positive control. Responses were considered as positive when: at least 10 IFNγ SFCs were counted and a 2-fold or greater increase above background levels was measured (34). For patients with preexisting CD4 T hTERT-specific immune response, increase >1.5-fold baseline value was considered as relevant.

Cytokine analysis

Cytokine production in response to hTERT peptides stimulation in ELISpot assay was measured using Bio-Plex Pro Human Cytokine 17-plex assay (Bio-Rad) and Luminex technology (Bio-Rad). ELISpot culture supernatants were analyzed in duplicates for secretion of the following cytokines: G-CSF, GM-CSF, IL1β, IL2, IL4, IL5, IL6, IL7, IL8, IL10, IL12 (p70), IL13, IL17A, MCP-1 (MCAF), MIP-1β, and TNFα according to the manufacturer's instructions.

Flow cytometry analysis

PBMCs were stained for viability and incubated with antibodies (Supplementary Table S1). For monocytic myeloid-derived suppressor cells (M-MDSC) and immune checkpoint molecule staining, cells were then washed and immediately analyzed on a Canto II Flow Cytometer (Becton Dickinson). For Treg staining, cells were washed, fixed/permeabilized with eBioscience FoxP3 staining buffer set according to the manufacturer's instructions (eBiosciences, #00-5523-00), and stained with FoxP3 antibody. After one wash, cells were analyzed on a Canto II Flow Cytometer (Becton Dickinson).

Study approval

The study protocol, all amendments, and informed consents were approved by the French Competent Authority and by one of the French independent ethics committee (IEC), the Comité de Protection des Personnes. The study was conducted in accordance with the protocol, applicable local regulatory requirements and laws, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Patients (Council for International Organizations of Medical Sciences 2002), Guidelines for Good Clinical Practice [International Conference on Harmonization 1996 (ICH GCP)], and the Declaration of Helsinki (World Medical Association 2008). All participants provided written informed consent upon enrolment in the study.

Statistical analysis

Statistical analysis of clinical parameters was performed using GraphPad Prism software. The time-to-event endpoints were described using Kaplan–Meier methods measured from the date of first dosing (35). Primary set analysis and safety analysis were performed on the modified intention-to-treat population.

Statistical analysis of immune monitoring parameters was performed using GraphPad Prism software with either Wilcoxon nonparametric matched paired signed rank test or Mann–Whitney nonparametric unpaired *U* test.

Results

Study population

A total of 26 patients (out of 41 patients screened) identified as disease progressors were included and received at least two cycles of INVAC-1. Three patients were treated with INVAC-1/EP at each dose level of the escalation phase (100, 400, and 800 μg) and 11 patients were treated in the 800 μg EP extension cohort (Supplementary Fig. S1). Six additional patients were treated at 800 μg with Tropis NFIS. Diagnoses were mainly infiltrating ductal breast carcinoma triple negative (5 patients), endometrial adenocarcinoma (4 patients), and metastatic colon cancer (3 patients; Supplementary Table S2).

Of the 26 patients treated, 8 patients (31%) received only two cycles and therefore discontinued before cycle 3 for objective disease progression or health status requiring treatment discontinuation. The other 18 patients (69%) received at least the scheduled three cycles of

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Table 1. Demographic characteristics of enrolled patients.

	INVAC-1 Dose level				
Characteristics	**EP 100 μg** ***n*** **= 3**	**EP 400 μg** ***n*** **= 3**	**EP 800 μg** ***n*** **= 14**	**Tropis 800 μg** ***n*** **= 6**	**Overall (*N* = 26)**
Gender					
Female *n* (%)	2 (66.7)	2 (66.7)	7 (50.0)	4 (66.7)	15 (57.7)
Male *n* (%)	1 (33.3)	1 (33.3)	7 (50.0)	2 (33.3)	11 (42.3)
Age (years)					
Mean (SD)	59.0 (10.1)	57.3 (14.4)	57.5 (13.6)	52.5 (13.3)	56.5 (12.7)
Med (range)	57 (50–70)	49 (49–74)	60 (31–74)	52 (34–67)	58 (31–74)
Disease duration (months)					
Mean (SD)	59.4 (53.8)	29.7 (17.1)	58.8 (47.2)	71.4 (88.3)	58.4 (55.8)
Median (range)	34.5 (22.5–121.1)	37.5 (10.1–41.6)	42.5 (9.3–162.4)	33.9 (15.3–244)	39.0 (9.3–244)
Tumor with distant metastasis					
1 Line	1 (33.3)	0 (0.0)	1 (7.1)	3 (50.0)	5 (19.2)
2 Lines	0 (0.0)	1 (33.3)	4 (28.6)	1 (16.7)	6 (23.0)
≥3 Lines	2 (66.7)	(66.7)	9 (64.2)	2 (33.3)	15 (57.7)
ECOG score					
0	3	1	9	1	14
1	0	2	5	5	12

treatment. As a result of an apparent benefit of the treatment (based on stabilization of target lesions evaluated by CT scan), 11 of them (42%) received additional cycles (up to 9 cycles for 2 patients).

At the start of the treatment, all patients had progressive disease and median disease duration since diagnosis was 39 months (range, 9.3–244 months). All patients had received at least one line of chemotherapy treatment prior to enrolment in the study and the majority (57.7%) had received more than 3 treatment lines (**Table 1**).

Safety

Increasing INVAC-1 doses did not increase study treatment–related AEs, suggesting there was no more deleterious effect at 100 μg than at 800 μg. Moreover, there were no more treatment-related AEs when patients received more than one cycle of treatment (12 AE) than after a single cycle (13 AE), suggesting that there was no cumulative adverse effect of INVAC-1 (Supplementary Table S3).

No death was reported within 28 days after the last dose of study treatment whatever the number of cycles the patient received (up to 9 cycles). The most common study treatment-related AEs were fatigue (16 AEs occurring in 8 patients) and AEs related to EP (17 AEs: burns, scars, skin erosion, pruritus, erythema). All patients recovered from these EP-related AEs without sequelae and continued with treatment cycles. In the Tropis cohort, no reaction at injection site was reported (**Table 2**). Eleven grade 3 AEs were reported for 5 patients but only one was considered as related to the study treatment (lymph node abscess). This event, occurred after cycle 4, was declared as serious and led to treatment discontinuation. It resolved without treatment after the end of patient participation in the study (**Table 2**; Supplementary Table S3). Five SAEs were reported. Only the lymph node abscess was considered as related to study treatment.

ECG analysis did not show any conduction abnormality or QT prolongation that would be considered as related to the study treatment. There were no clinically relevant morphological ECG changes from baseline throughout the study. Vital signs mean values reported during the study were all normal.

There were no clinically significant changes in laboratory parameters over time except for CRP, gamma GT, and ALP, mainly due to one or few patients with high to very high value related to their disease

Table 2. Number of patients reporting the most frequent AE by grade regardless of causality (AE reported for at least 10% of patients).

	Grade 1	**Grade 2**	**Grade 3**	**Overall**
Injection site reaction	13[a]	4[a]	0	17[a]
Gastrointestinal disorders				
(Diarrhea, constipation, pain, vomiting...)	15	4	0	19
General disorders				
Fatigue	8	8	0	16
Blood and lymphatic system disorders				
Lymphopenia and lymphocyte count decrease	9	3	0	12
Anemia	4	2	1	7
Respiratory disorders				
Cough	6	1	0	7
Metabolic disorders				
AESI: Hyperglycemia	5	1	0	6

Note: Patient may have experienced the same AE several times and may have encountered different AEs. Grade is the maximum reported per patient for the AE.
[a]Only occurred in EP cohort.

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conditions. Some patients displayed mild hyperglycemia. Consequently, hyperglycemia was followed as an AE of special interest (AESI). Six patients (23.1%) experienced 16 hyperglycemia events, consisting of a mild increase of fasting glucose above the upper normal range (maximum observed value 7.9 mmol/L vs. 5.9 mmol/L; **Table 2**). None of these events was associated with marked changes in HbA1c, thus showing no evidence for a role of INVAC-1 in pre-diabetes development. All hyperglycemia events were grade 1 except one grade 2 related to Olimel infusion. All events resolved within a month. Most of the cases were considered as not related to INVAC-1 (11/16). Therefore, these hyperglycemia episodes were not considered as a safety signal.

No significant biological sign of autoimmunity was reported except for one patient who was positive for anti-TPO antibodies at baseline and displayed biological signs of hyperthyroidism after 3 cycles of treatment. Another patient, who also had anti-TPO antibodies at baseline, presented a progressive decrease of anti-TPO titers until normalization at the end of treatment (EOT). No other significant modification of circulating auto-antibodies was observed.

Plasma levels of inflammatory cytokines (TNFα, IFNγ, IL17, IL8, IL6, IL1β) were evaluated at defined time points according to study protocol (baseline, day 2, and day 8 of cycle 1 and EOT). No significant modification of IFNγ, IL17, IL8, IL6, and IL1β concentrations was observed at any time point. In contrast, a significant increase of TNFα level was observed in samples collected at EOT as compared with the other time points. No biological sign was correlated to this TNFα increase.

Absolute cell counts of B, T, CD4, and CD8 lymphocytes as well as NK cells were determined by flow cytometry every 2 weeks from baseline to EOT and remained stable in all patients throughout the study (data not shown).

Overall, the study showed that INVAC-1 administration by ID injection either with EP or by NFIS was safe and well tolerated for each dose cohort. One patient presented a grade 3 SAE that would have met DLT criteria except for occurring after cycle 4. Thus, according to protocol's definition, no DLT was observed in patients receiving escalating doses of INVAC-1. Consequently, no MTD was defined.

Best overall response

Overall, the majority of patients (58%, 15 patients) experienced disease stabilization for 1.8 to 9.9 months, including all patients in the 100 μg dose cohort, 2 patients (67%) in the 400 μg cohort, and 10 patients (50%) in the 800 μg (7 in the EP arm, 3 in the Tropis arm). The median SD duration was 2.7 months and, interestingly, 4 of 15 (26.7%) patients showed SD for more than 6 months (Supplementary Table S4). No complete regression (CR) could be observed but two

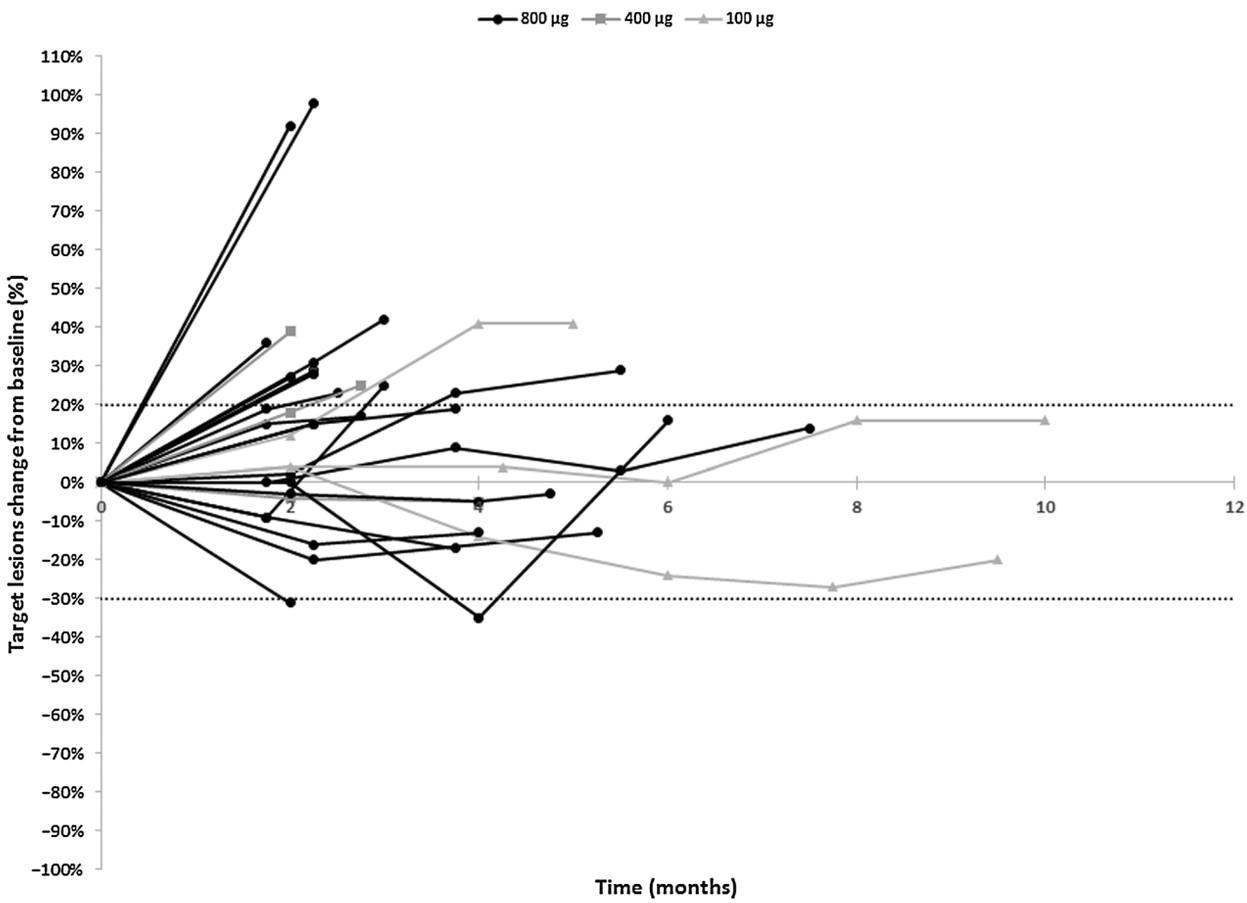

Figure 1.

Spider plot representing percentage change in tumor size from baseline in each subject by month. PD, progressive disease; SD, stable disease; PR, partial regression. Each dose cohort is represented: 100 μg (light gray line, $n = 3$), 400 μg (gray line, $n = 3$), and 800 μg EP + Tropis (black line, $n = 20$).

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patients of the 800 μg cohort presented unconfirmed partial regression (PR), one after 2 cycles of INVAC-1 (tumor size -31%) and the second after 4 cycles of treatment (tumor size -35%). However, these tumor responses could not be confirmed by the subsequent CT scan evaluation (**Fig. 1**).

The Kaplan–Meier estimated median progression-free survival was 2.7 months [95% confidence interval (CI), 1.8–3.7 months]. OS information up to 45 months was recorded across all dosage cohorts. During this observational period, 17 patients of 26 (65.4%) died from their disease. The Kaplan–Meier estimated median OS was 15 months (95% CI, 9.8–29.3). Estimated 1-year survival rate reached 65.4% (SE: 9.3; Supplementary Fig. S2). Populations were homogeneous between patients who survived to one year and those who did not and no difference in age, sex, or ECOG status were observed (Supplementary Tables S2 and S5). Interestingly, 3 patients with triple-negative breast carcinoma, enrolled after 2 or 3 lines of treatment, presented an OS >1 year (15, 25.9, and 25.5 months, respectively; Supplementary Table S2).

Pharmacodynamics

To evaluate INVAC-1 vaccination ability to elicit T-cell immunogenicity, hTERT-specific CD4 and CD8 T-cell responses were measured by IFNγ ELISpot after 9 days of prestimulation in 24 evaluable

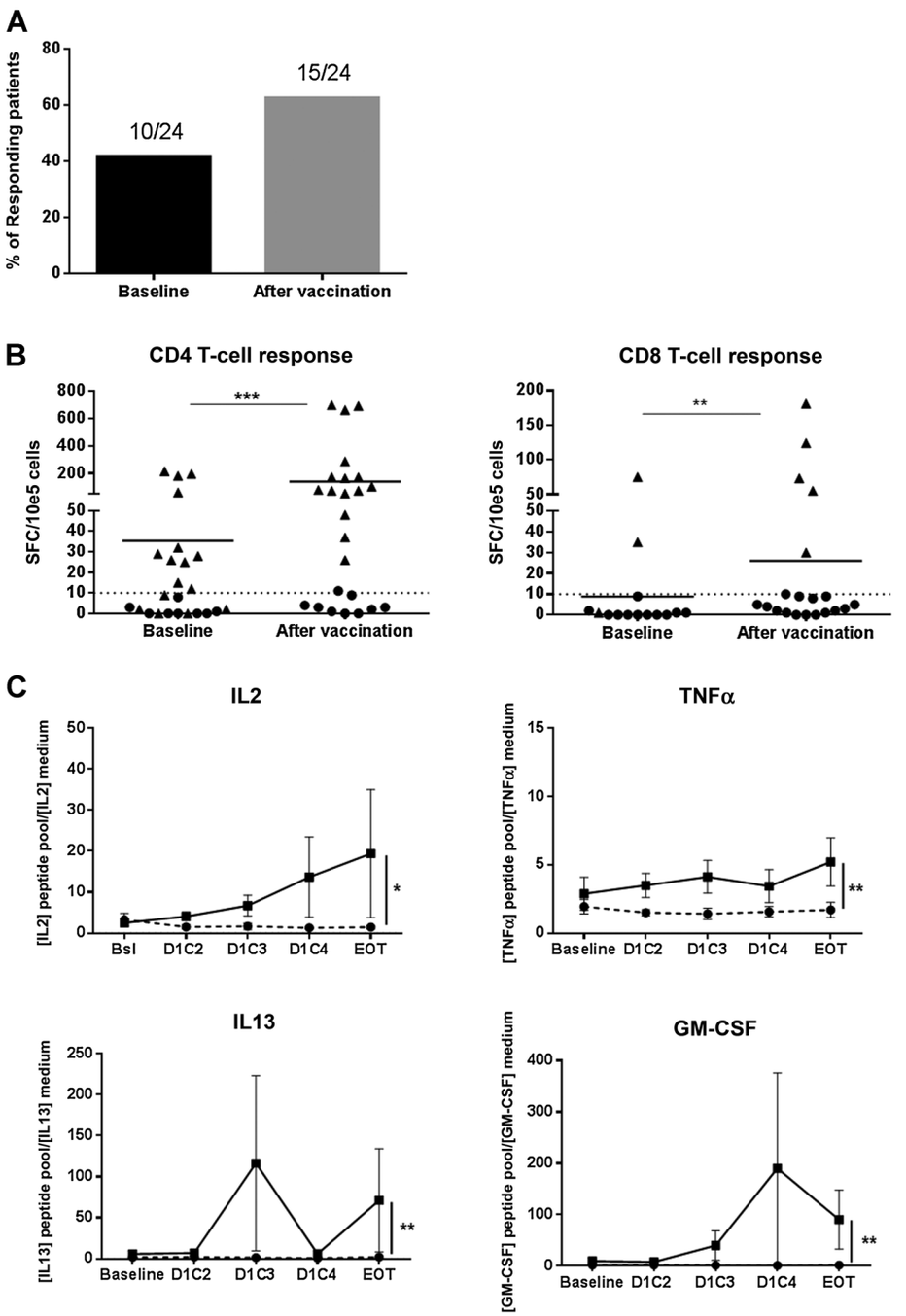

Figure 2.

Characterization of anti-hTERT immune responses in response to INVAC-1 vaccination in 24 patients for all cohorts (100, 400, and 800 μg). **A,** Frequency of CD4 responding patients at baseline (black bar) and after vaccination (gray bar). **B,** Anti-hTERT CD4 and CD8 T-cell responses at baseline and after vaccination. After vaccination, each patient's best response was shown. CD4 (left) and CD8 (right) T-cell responses are represented; line represents IFNγ SFC mean. Mean of triplicate is plotted for each responder (black triangle) and nonresponder (black circle) patient ($n = 24$). Positivity threshold (>10 SFC/10^5 cells) is represented by dotted line. ***, $P < 0.001$, Wilcoxon nonparametric matched-pairs signed rank test. **, $P < 0.01$, Wilcoxon nonparametric matched-pairs signed rank test. **C,** IL2, TNFα, IL13, and GM-CSF secretion in CD4 ELISpot responders versus nonresponders. Dotted line, CD4 nonresponders; solid line, CD4 responders. D1C2, day 1 cycle 2; D1C3, day 1 cycle 3; D1C4, day 1 cycle 4; EOT, end of treatment. For each patient, supernatant was tested in duplicate and cytokine concentration (pg/mL) was back-calculated using a 5-parameter logistic (5PL) regression curve. Ratio (cytokine in stimulated wells)/(cytokine in nonstimulated wells) was calculated for each patient and mean values ± SD (responders $n = 15$, nonresponders $n = 9$) were plotted. Statistical comparison of cytokine ratio variations according to time for CD4 responders versus nonresponders was performed using Mann–Whitney nonparametric unpaired signed rank test. *, $P < 0.01$; **, $P < 0.001$.

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patients. As no dose effect of INVAC-1 on the magnitude of immune response was observed in the different cohorts (data not shown), data from all doses were pooled. After INVAC-1 administration, we observed a specific anti-hTERT CD4 T-cell response in 15 of 24 patients (63%; $P = 0.0007$), of which 10 had spontaneous CD4 T-cell responses against hTERT at baseline (**Fig. 2A**). All patients presenting an anti-hTERT CD4 response at baseline showed an increased (8/10) or sustained (2/10) response after vaccination. Thus, INVAC-1 vaccination was able to enhance preexisting reactive T-cell clones as well as trigger *de novo* anti-hTERT CD4 immune response in 33% of responding patients (**Fig. 2B**, left). The majority of patients mounted an anti-hTERT CD4 T-cell response following the second or third vaccination. These results suggest that additional boosts (>3) are not formally required for the induction of an anti-hTERT CD4 response after vaccination (data not shown). Five patients (out of 20 evaluable, i.e., HLA-A2, A3, A24, and/or B7 patients) exhibited specific CD8 T-cell responses (**Fig. 2B**, right; $P = 0.002$). Interestingly, all vaccine-induced anti-hTERT CD8 T-cell responses were associated with concomitant anti-hTERT CD4 T-cell responses (data not shown).

Regarding polarization of the specific anti-hTERT CD4 immune response, a multiplex bead assay was used to analyze production of 17 cytokines in ELISpot culture supernatants. As a consequence, due to its capture by ELISpot plate, IFNγ was not measured. Cytokine secretion analysis was combined with IFNγ ELISpot results (according to CD4 responder/nonresponder status) and demonstrated no significant difference in production of G-CSF, IL1β, IL4, IL5, IL6, IL7, IL8, IL10, IL12 (p70), IL17A, MCP-1, and MIP-1β between CD4 responders and nonresponders. In contrast, CD4 T-cell responders showed significantly higher secretion of IL2 ($P = 0.016$) and TNFα ($P = 0.008$) compared with nonresponders. CD4 hTERT-specific responders also showed a significant higher production of GM-CSF ($P = 0.008$), cytokine known to promote DC licensing, compared with nonresponders. Finally, secretion of IL13, a Th2 cytokine known to promote B cells function and growth, was significantly higher in CD4 hTERT-specific responders ($P = 0.008$; **Fig. 2C**). As hTERT-specific CD4 T cells were shown to produce IFNγ by ELISpot and no elevation of IL4, IL5, or IL6 Th2 cytokines could be observed, these data suggest that INVAC-1 vaccination induced a mixed Th1/Th2, Th1 dominant hTERT-specific CD4 immune response as well as cytotoxic CD8 T-cell response.

Finally, phenotyping of CD4 and CD8 T-cell subsets revealed that INVAC-1 treatment did not modify CD4 or CD8 T-cell subset distribution (naïve, memory, effector memory) nor immune checkpoint molecule (PD-1, TIM3, or TIGIT) expression at their surface (Supplementary Fig. S3). To identify any immunosuppression induction, circulating tolerogenic subpopulations such as M-MDSC and Treg lymphocytes were studied on 19 and 16 evaluable patients, respectively. INVAC-1 had no effect on M-MDSCs, whereas the Treg subpopulation was found significantly lower at EOT compared with baseline [1.2% (SD: 0.3522) vs. 3.6% (SD: 1.482), $P = 0.0186$; **Fig. 3**]. Circulating M-MDSC and Treg frequencies at baseline were analyzed according to patients' responder/nonresponder status and showed no significant difference between responders and nonresponders neither for Tregs nor for M-MDSCs, suggesting that frequency of these populations in peripheral blood at baseline did not influence vaccine efficacy (data not shown).

Correlation between clinical response and anti-hTERT immune response

Interestingly, when comparing hTERT-specific CD4 T-cell responses at a fixed time point after only 2 cycles (prime/boost) of

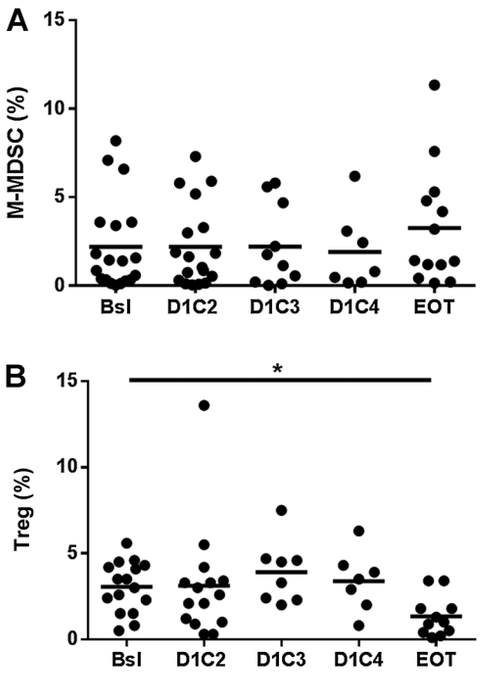

Figure 3.

Circulating immunosuppressive cell populations' characterization. **A,** Peripheral blood monocytic MDSC characterization. Raw peripheral blood proportion (%; black line represents mean for each time point). Monocytic MDSCs (M-MDSC) were defined as Lin^{-} $CD33^{+}$ $HLADR^{low/-}$ $CD14^{+}$ $CD11b^{+}$ cells. **B,** Peripheral blood Treg lymphocyte characterization. Raw peripheral blood proportion (%; black line represents mean for each time point). Tregs were defined as $CD3^{+}$ $CD4^{+}$ $FoxP3^{+}$ $CD25^{high}$ $CD127^{low}$ cells. *, $P < 0.01$, Wilcoxon nonparametric matched pairs signed rank test.

INVAC-1, patients who survived more than one year showed a significantly higher hTERT specific CD4 T-cell response than patients whose OS was less than one year ($P = 0.034$; **Fig. 4A**). Moreover *post hoc* analysis of the OS by subgroups according to the presence or absence of anti-hTERT CD4-specific T-cell response showed a non-significant difference with a longer estimated median OS for responders compared with nonresponders [17.4 (12.4–29.3) vs. 7.0 months (3.0-), $P = 0.48$; **Fig. 4B**].

Discussion

Several clinical trials have evaluated the efficacy of hTERT-related vaccines against a variety of tumors (14, 15). Peptide vaccines are immunogenic and easy to produce but are HLA-restricted and therefore cannot be used to treat all patients. In contrast, DNA vaccines are not HLA-restricted and can be presented by both MHC-I and MHC-II, thus triggering a broader immune response. Nevertheless, the cellular uptake of DNA is quite low and numerous studies focused on improving this uptake by different techniques. EP has indeed been shown to dramatically enhance DNA uptake by APC (36, 37). In this FIH phase I study in patients with advanced cancers, we evaluated INVAC-1, a DNA plasmid encoding an engineered hTERT protein modified to increase its safety and antigen presentation by APC. INVAC-1 was delivered either by intradermal injection followed by EP or by NFIS/Tropis. We previously showed in dogs that Tropis device was safe and as effective as intradermal injection followed by EP at generating T-cell immune responses against TERT (unpublished

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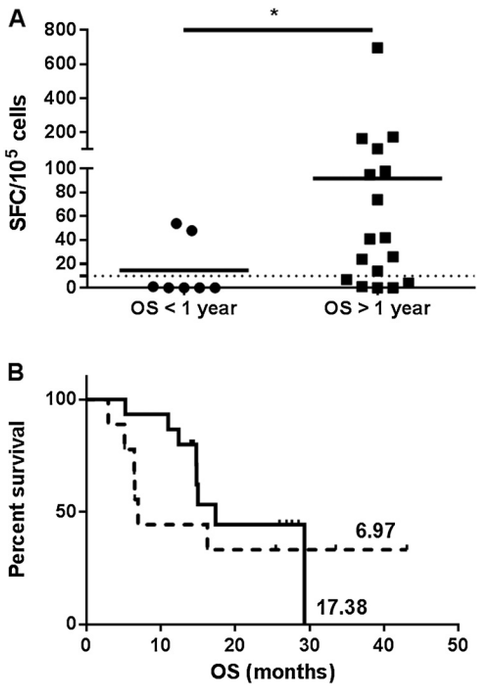

Figure 4.

Correlation between clinical response and anti-hTERT immune response. **A,** Anti-hTERT CD4 T-cell response after 2 cycles of INVAC-1 (prime/boost) according to 1-year survival. Mean of triplicate is plotted for each patient with OS < 1 year (black circle, $n = 7$) and OS > 1 year (black square, $n = 17$); line represents IFNγ SFC mean. Positivity threshold (>10 SFC/10^5 cells) is represented by dotted line. *, $P < 0.05$, Mann–Whitney nonparametric unpaired signed rank test. **B,** Overall survival (45 months) according to CD4 responder versus nonresponder status. Solid line, CD4 responders; dashed line, CD4 nonresponders; median survival (months) for each group is indicated.

data). Here, we confirmed in humans that INVAC-1 ID administration with Tropis is immunogenic and able to elicit hTERT-specific CD4 and CD8 immune responses in patients. This represents a great improvement for patient care as administration by Tropis is easy to perform, reliable, and precise. Moreover, this system is cost-effective and brings increased comfort for patients with lower pain and better local tolerance (compared with EP).

CD4 and CD8 T-cell responses elicited by DNA vaccination were shown to be essential for efficient antitumoral immune responses. Although CD8 T cells are directly involved in antitumor cytotoxic responses, CD4 Th1 responses also seem to play an important role for optimizing the antitumor immune response *in vivo* (38). Although DNA vaccines are known to trigger strong CD8 T-cell responses, anti-hTERT specific CD8 T-cell responses were detected in 25% of patients in our study, whereas 63% of patients presented CD4 T-cell responses. One hypothesis to explain these results would be that peptides used to detect CD4 responses in ELISpot were based on "universal" promiscuous epitopes (30, 31, 33), whereas HLA-matched peptides used to detect CD8 T-cell responses were more specific and less immunogenic and thus could not allow CD8 T responses to be detected in as many patients as CD4 T-cell responses.

Some studies have reported that vaccination could induce immunosuppression and impaired cellular responses (39, 40). Such suppression resulted from MDSC and/or Treg induction, which express elevated levels of IL10. In this study, INVAC-1 vaccination did not modify circulating M-MDSC subpopulation frequency and even induced a significant decrease of circulating Treg subpopulation. Moreover, cytokine responses after antigen stimulation revealed no significant difference in IL10 secretion. Thus, by triggering anti-hTERT Th1-polarized CD4 T-cell response as well as CD8 cytotoxic response without immunosuppression induction, INVAC-1 demonstrated strong immunogenicity in patients enrolled in this study.

To our knowledge, despite a good induction of anti-hTERT specific T cells (>50% in the majority of the studies), all phase I clinical trials involving hTERT vaccination reported disease stabilization for less than 50% of evaluable patients (15, 16). Only two studies evaluating either a peptide-based (41) or a DC-loaded (42) based vaccine reported 72% (16/22 patients) and 67% (4/6 evaluable patients) of patients presenting stable disease after 3 months follow-up. In this phase I study conducted in advanced cancer patients all showing progressive disease at inclusion, we observed a similar induction of anti-hTERT immunity. Importantly, although no CR or confirmed PR could be observed, stabilization was achieved in 58% of patients, for up to 9.9 months. However, this better stabilization rate is balanced by a lower SD median duration (2.7 months), compared with several other studies (43, 44).

Finally, this study showed that after only two cycles of INVAC-1, specific anti-hTERT immune response was significantly higher in patients who survived to one year. When analyzing OS according to hTERT responder versus nonresponder status, our results showed a nonsignificant tendency toward prolonged OS for patients presenting a CD4 hTERT immune response compared with nonresponders (17.4 vs. 7 months) whatever their immune status at baseline (pre-existing anti-hTERT immune response or not), thereby providing encouraging data for INVAC-1 vaccine.

In conclusion, INVAC-1 was demonstrably safe and well tolerated as a single ID treatment in patients with advanced solid tumors. Despite the absence of CR or confirmed PR and the low specific CD8 T-cell response, the good induction of hTERT-specific CD4 T-cell responses and disease stabilization rate supports further clinical evaluation of INVAC-1. In this study, enrolment did not target any specific cancer and diseases were already metastasized for approximately two thirds of patients. Indeed, the enrolled patients were heavily pretreated, mainly beyond second or third line of treatment. Moreover, these patients were affected by histologic types of cancers known to be poor immunotherapy responders (except for mesothelioma). Therefore, we are of the opinion that targeting a specific indication with high telomerase expression at an earlier stage or with minimum residual disease should lead to greater clinical efficacy. A phase II study evaluating INVAC-1 in chronic lymphocytic leukemia patients with minimal residual disease is currently ongoing and further phase II studies evaluating early-stage solid tumors are to be started soon.

Disclosure of Potential Conflicts of Interest

J. Garibal, M. Wehbe, E. Pliquet, R. Defrance, and T. Huet are employees/paid consultants for Invectys. M.-A. Dragon Durey is an employee/paid consultant for Invectys and reports receiving speakers bureau honoraria from Gilead, Bristol-Myers Squibb, and Roche. S.M. Oudard reports receiving speakers bureau honoraria from Invectys. S. Wain-Hobson and P. Langlade-Demoyen are employees/paid consultants for and hold ownership interest (including patents) in Invectys. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: J. Medioni, R. Defrance, S. Oudard, S. Wain-Hobson, T. Huet, P. Langlade-Demoyen

Development of methodology: J. Medioni, R. Defrance, S. Oudard, T. Huet, P. Langlade-Demoyen

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Teixeira, J. Medioni, O. Adotevi, L. Doucet, M.-A. Dragon Durey, J.-J. Kiladjian, C. Laheurte, E. Pliquet, M. Escande, S. Culine, S. Oudard
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Teixeira, J. Medioni, J. Garibal, O. Adotevi, L. Doucet, M.-A. Dragon Durey, J.-J. Kiladjian, C. Laheurte, R. Defrance, S. Oudard, V. Doppler, T. Huet
Writing, review, and/or revision of the manuscript: L. Teixeira, J. Medioni, J. Garibal, O. Adotevi, L. Doucet, M.-A. Dragon Durey, J.-J. Kiladjian, M. Brizard, M. Wehbe, R. Defrance, S. Culine, S. Oudard, V. Doppler, T. Huet, P. Langlade-Demoyen
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.-A. Dragon Durey, Z. Ghrieb, E. Pliquet, M. Escande, T. Huet
Study supervision: L. Teixeira, Z. Ghrieb, R. Defrance, T. Huet, P. Langlade-Demoyen

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References

1. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer.Science 1994;266:2011–5.
2. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer.Eur J Cancer 1997;33:787–91.
3. Hiyama E, Hiyama K. Telomerase as tumor marker.Cancer Lett 2003;194:221–33.
4. Greider CW, Blackburn EH. The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity.Cell 1987;51:887–98.
5. Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC. Telomere reduction in human colorectal carcinoma and with ageing.Nature 1990;346:866–8.
6. Buseman CM, Wright WE, Shay JW. Is telomerase a viable target in cancer? Mutat Res 2012;730:90–7.
7. Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW. Telomerase activity in human germline and embryonic tissues and cells.Dev Genet 1996;18:173–9.
8. Low KC, Tergaonkar V. Telomerase: central regulator of all of the hallmarks of cancer.Trends Biochem Sci 2013;38:426–34.
9. Ho MM, Ng AV, Lam S, Hung JY. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells.Cancer Res 2007;67:4827–33.
10. Marian CO, Wright WE, Shay JW. The effects of telomerase inhibition on prostate tumor-initiating cells.Int J Cancer 2010;127:321–31.
11. Marian CO, Cho SK, McEllin BM, Maher EA, Hatanpaa KJ, Madden CJ, et al. The telomerase antagonist, imetelstat, efficiently targets glioblastoma tumor-initiating cells leading to decreased proliferation and tumor growth.Clin Cancer Res 2010;16:154–63.
12. Zanetti M, Hernandez X, Langlade-Demoyen P. Telomerase reverse transcriptase as target for anti-tumor T cell responses in humans.Springer Semin Immunopathol 2005;27:87–104.
13. Harley CB. Telomerase and cancer therapeutics.Nat Rev Cancer 2008;8:167–79.
14. Xu Y, Goldkorn A. Telomere and telomerase therapeutics in cancer.Genes 2016;7:pii:E22.
15. Zanetti M. A second chance for telomerase reverse transcriptase in anticancer immunotherapy.Nat Rev Clin Oncol 2017;14:115–28.
16. Kailashiya C, Sharma HB, Kailashiya J. Telomerase based anticancer immunotherapy and vaccines approaches.Vaccine 2017;35:5768–75.
17. Wenandy L, Sørensen RB, Sengeløv L, Svane IM, thor Straten P, Andersen MH. The immunogenicity of the hTERT540-548 peptide in cancer.Clin Cancer Res 2008;14:4–7.
18. Liao ZL, Tang XD, Lü MH, Wu YY, Cao YL, Fang DC, et al. Antitumor effect of new multiple antigen peptide based on HLA-A0201-restricted CTL epitopes of human telomerase reverse transcriptase (hTERT).Cancer Sci 2012;103:1920–8.
19. Kokhaei P, Palma M, Hansson L, Osterborg A, Mellstedt H, Choudhury A. Telomerase (hTERT 611-626) serves as a tumor antigen in B-cell chronic lymphocytic leukemia and generates spontaneously antileukemic, cytotoxic T cells.Exp Hematol 2007;35:297–304.
20. Inderberg-Suso EM, Trachsel S, Lislerud K, Rasmussen AM, Gaudernack G. Widespread CD4+ T-cell reactivity to novel hTERT epitopes following vaccination of cancer patients with a single hTERT peptide GV1001.Oncoimmunology 2012;1:670–86.
21. Kotsakis A, Papadimitraki E, Vetsika EK, Aggouraki D, Dermitzaki EK, Hatzidaki D, et al. A phase II trial evaluating the clinical and immunologic response of HLA-A2(+) non-small cell lung cancer patients vaccinated with an hTERT cryptic peptide.Lung Cancer 2014;86:59–66.
22. Thalmensi J, Pliquet E, Liard C, Escande M, Bestetti T, Julithe M, et al. Anticancer DNA vaccine based on human telomerase reverse transcriptase generates a strong and specific T cell immune response.Oncoimmunology 2016;5:e1083670.
23. Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V, Cech TR. Reverse transcriptase motifs in the catalytic subunit of telomerase.Science 1997;276:561–7.
24. Drosopoulos WC, Prasad VR. The active site residue Valine 867 in human telomerase reverse transcriptase influences nucleotide incorporation and fidelity. Nucleic Acids Res 2007;35:1155–68.
25. Poch O, Sauvaget I, Delarue M, Tordo N. Identification of four conserved motifs among the RNA-dependent polymerase encoding elements.EMBO J 1989;8:3867–74.
26. Resik S, Tejeda A, Mach O, Sein C, Molodecky N, Jarrahian C, et al. Needle-free jet injector intradermal delivery of fractional dose inactivated poliovirus vaccine: association between injection quality and immunogenicity.Vaccine 2015;33:5873–7.
27. McAllister L, Anderson J, Werth K, Cho I, Copeland K, Le Cam Bouveret N, et al. Needle-free jet injection for administration of influenza vaccine: a randomised non-inferiority trial.Lancet 2014;384:674–81.
28. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbé C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria.Clin Cancer Res 2009;15:7412–20.
29. Nishino M, Giobbie-Hurder A, Gargano M, Suda M, Ramaiya NH, Hodi FS. Developing a common language for tumor response to immunotherapy: immune-related response criteria using unidimensional measurements. Clin Cancer Res 2013;19:3936–43.
30. Godet Y, Fabre E, Dosset M, Lamuraglia M, Levionnois E, Ravel P, et al. Analysis of spontaneous tumor-specific CD4 T-cell immunity in lung cancer using promiscuous HLA-DR telomerase-derived epitopes: potential synergistic effect with chemotherapy response.Clin Cancer Res 2012;18:2943–53.
31. Laheurte C, Galaine J, Beziaud L, Dosset M, Kerzerho J, Jacquemard C, et al. Immunoprevalence and magnitude of HLA-DP4 versus HLA-DR-restricted spontaneous CD4(+) Th1 responses against telomerase in cancer patients. Oncoimmunology 2016;5:e1137416.
32. Vonderheide RH Prospects and challenges of building a cancer vaccine targeting telomerase.Biochimie 2008;90:173–80.
33. Dosset M, Godet Y, Vauchy C, Beziaud L, Lone YC, Sedlik C, et al. Universal cancer peptide-based therapeutic vaccine breaks tolerance against telomerase and eradicates established tumor.Clin Cancer Res 2012;18:6284–95.
34. Butterfield LH, Buffo MJ. Immunologic monitoring of cancer vaccine trials using the ELISPOT assay.Methods Mol Biol 2014;1102:71–82.
35. Barker C. The mean, median, and confidence intervals of the kaplan meier survival estimate – computations and applications.Am Stat 2009;63:78–80.

36. Jorritsma SHT, Gowans EJ, Grubor-Bauk B, Wijesundara DK. Delivery methods to increase cellular uptake and immunogenicity of DNA vaccines.Vaccine 2016; 34:5488–94.
37. Sardesai NY, Weiner DB. Electroporation delivery of DNA vaccines: prospects for success.Curr Opin Immunol 2011;23:421–9.
38. Ostroumov D, Fekete-Drimusz N, Saborowski M, Kühnel F, Woller N. CD4 and CD8 T lymphocyte interplay in controlling tumor growth.Cell Mol Life Sci 2018; 75:689–713.
39. Jin H, Kang Y, Zheng G, Xie Q, Xiao C, Zhang X, et al. Induction of active immune suppression by co-immunization with DNA- and protein-based vaccines.Virology 2005;337:183–91.
40. Sui Y, Hogg A, Wang Y, Frey B, Yu H, Xia Z, et al. Vaccine-induced myeloid cell population dampens protective immunity to SIV.J Clin Invest 2014;124: 2538–49.
41. Lilleby W, Gaudernack G, Brunsvig PF, Vlatkovic L, Schulz M, Mills K, et al. Phase I/II a clinical trial of a novel hTERT peptide vaccine in men with metastatic hormone-naïve prostate cancer.Cancer Immunol Immunother 2017;66:891–901.
42. Vonderheide RH, Domchek SM, Schultze JL, George DJ, Hoar KM, Chen DY, et al. Vaccination of cancer patients against telomerase induces functional antitumor CD8+ T lymphocytes.Clin Cancer Res 2004;10:828–39.
43. Berntsen A, Trepiakas R, Wenandy L, Geertsen PF, thor Straten P, Andersen MH, et al. Therapeutic dendritic cell vaccination of patients with metastatic renal cell carcinoma: a clinical phase 1/2 trial.J Immunother 2008;31:771–80.
44. Bolonaki I, Kotsakis A, Papadimitraki E, Aggouraki D, Konsolakis G, Vagia A, 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;25:2727–34.

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A First-in-Human Phase I Study of INVAC-1, an Optimized Human Telomerase DNA Vaccine in Patients with Advanced Solid Tumors

Luis Teixeira, Jacques Medioni, Julie Garibal, et al.

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