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A Pilot Study of MUC-1/CEA/TRICOM Poxviral-Based Vaccine in Patients with Metastatic Breast and Ovarian Cancer

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Abstract

Purpose—PANVAC is a recombinant poxviral vaccine that contains transgenes for MUC-1, CEA and 3 T-cell costimulatory molecules. This study was conducted to obtain preliminary evidence of clinical response in metastatic breast and ovarian cancer patients.

Experimental design—Twenty-six patients were enrolled and given monthly vaccinations. Clinical and immune outcomes were evaluated.

Results—These patients were heavily pretreated, with 21 of 26 patients having ≥ 3 prior chemotherapy regimens. Side effects were largely limited to mild injection-site reactions. *Breast cancer*: For the 12 patients enrolled, median time to progression was 2.5 months (1 – 37+) and median overall survival was 13.7 months. Four patients had stable disease. One patient had a complete response by RECIST and remains on study ≥ 37 months, with a significant drop in serum IL-6 and IL-8 by day 71. Another patient with metastatic disease confined to the mediastinum had a 17% reduction in mediastinal mass and was on study for 10 months. Patients with stable or responding disease had fewer prior therapies and lower tumor marker levels than patients with no evidence of response. *Ovarian cancer*: Median time to progression for patients (n=14) was 2 months (1 – 6) and median overall survival was 15.0 months. Updated data are presented here for one patient treated with this vaccine in a previous trial, with a time to progression of 38 months.

Conclusions—Some patients who had limited tumor burden with minimal prior chemotherapy appeared to benefit from the vaccine. Further studies to confirm these results are warranted.

Keywords

Therapeutic Vaccine; Immune Response; Immuno-Oncology

Introduction

Tumor-associated antigens (TAAs) are either weakly immunogenic or functionally nonimmunogenic. We have previously shown in preclinical models (1) that one strategy to break immune tolerance is to introduce TAAs to the immune system in the context of poxviral vectors. We report here the results of a study with PANVAC, a recombinant poxviral vaccine expressing the TAAs carcinoembryonic antigen (CEA) and mucin-1 (MUC-1), along with 3 costimulatory molecules. A previous safety study in patients with metastatic carcinoma (n = 25) showed evidence of clinical benefit in some patients with breast or ovarian cancer (2). This is a follow-up study consisting of 2 cohorts with either metastatic breast cancer or metastatic ovarian cancer, with clinical outcome as the primary endpoint.

The large poxviral genome allows for the insertion of multiple genes, including genes coding for multiple antigens or immunostimulatory molecules which, following vaccination, can be expressed within host cells (3). PANVAC is a recombinant poxviral vaccine platform that contains transgenes for the TAAs MUC-1 and CEA. MUC-1 is a transmembrane glycoprotein that is overexpressed in underglycosylated form in many human carcinoma cells (4, 5), and the cytoplasmic domain of MUC-1 has been shown to play a crucial role in carcinogenesis (6, 7). CEA is another glycoprotein overexpressed on the majority of adenocarcinomas. Its selective overexpression also makes it a potential target for immunotherapy (8, 9). PANVAC also includes transgenes for a triad of human T-cell costimulatory molecules designated TRICOM, composed of B7.1, intercellular adhesion molecule (ICAM)-1, and lymphocyte function-associated antigen (LFA)-3. Expression of costimulatory molecules in antigen-presenting cells (APCs) has been shown to generate a stronger immune response and higher numbers of TAA-specific T cells (1, 10-12). PANVAC is given in a diversified prime-boost fashion, which has been shown (13-15) to be superior to continuous use of a single vector. The first vaccination with recombinant vaccinia (PANVAC-V) elicits a strong initial immune response, but its continuous use is limited by the production of host-induced neutralizing antibodies to vaccinia. Subsequent vaccination with recombinant fowlpox (PANVAC-F), however, does not provoke neutralizing antibody production, since fowlpox is replication-deficient in humans and cannot produce late viral proteins, including coat proteins (14). Granulocyte-macrophage colony-stimulating factor (GM-CSF) is also given as a vaccine adjunct to enhance the migration and maturation of APCs, as previously described in several preclinical and clinical studies (14, 16).

Our previous study showed the safety of PANVAC in advanced solid tumors and evidence of clinical benefit in a subset of patients (17). Results of the study reported here provide further insight into clinical efficacy and immune response, and confirm the safety and tolerability of this vaccine regimen.

Patients and Methods

Patient selection and trial design

Twenty-six patients with metastatic breast or ovarian cancer who had progressive disease following standard chemotherapy, or were not candidates for standard chemotherapy, were enrolled in this trial approved by the National Cancer Institute (NCI) Institutional Review Board and conducted at the NCI. The study was designed as a pilot trial to evaluate clinical outcomes with the use of PANVAC vaccine in metastatic breast and ovarian cancer patients, with immunologic response as the secondary endpoint. Patients were required to have an Eastern Cooperative Oncology Group performance status of 0 or 1 and adequate hematologic, hepatic, and renal function. In addition, patients were not enrolled if there was

evidence of immunosuppression (*i.e.*, reactive HIV test, diagnosis of altered immune function, prior splenectomy, or concurrent steroid use). Prior vaccinia exposure (*i.e.*, smallpox vaccination) was allowed but not required.

Exclusion criteria included known allergy to eggs; history of or for active skin disorders such as eczema, extensive psoriasis, varicella zoster, impetigo, or burns; history of seizures; serious intercurrent illness; noncutaneous malignant process; and close contact with immunocompromised individuals, individuals with the above-mentioned skin conditions, or children under 3 years of age. All patients gave written informed consent in accordance with federal, state, and institutional guidelines.

Vaccine formulation and treatment plan

Both of the viral vaccines used were manufactured as part of a Cooperative Research and Development Agreement between Therion Biologics and the Laboratory of Tumor Immunology and Biology, NCI. Vaccines were provided by the Cancer Therapy Evaluation Program, NCI. PANVAC-V [recombinant vaccinia-CEA(6D)/MUC-1(L93)/TRICOM; NSC #727026] was prepared from virus derived from the Wyeth (New York City Board of Health) strain of vaccinia, selected for its favorable toxicity profile. PANVAC-F [recombinant fowlpox-CEA(6D)/MUC-1(L93)/TRICOM; NSC #727027] was constructed by inserting the identical transgenes into the replication-defective fowlpox virus. All patients received the same dose and schedule of vaccine. The priming vaccine consisted of 2×10^8 pfu of PANVAC-V administered s.c. The boosting vaccine was given on or about days 15, 29, and 43, then every 28 days while on study. Sargramostim (GM-CSF) 100 μ g s.c. was given the day of each vaccine and for the following 3 consecutive days. A sterile, nonadherent dressing (*i.e.*, Telfa) was used to cover the site.

Patients were seen at least monthly while on study, at which time complete interval histories, physical examinations, blood chemistries, hemograms, and serum tumor markers were obtained. All patients were evaluated for toxicity by the NCI Common Toxicity Criteria version 3 and the previously published vaccinia toxicity grading scale (18). Patients had their first restaging exam at approximately day 71, with subsequent restaging exams approximately every 56 days thereafter.

Collection of peripheral blood mononuclear cells

Apheresis was done on selected patients before vaccination and again around day 71. Mononuclear cells (5×10^8 to 2×10^9) were obtained, processed and frozen as previously described.(2)

ELISPOT assay

Measurement of CD8 immune responses in HLA-A2⁺ patients was conducted by ELISPOT assay using a modification of the procedure previously described (19). The assay was performed using K562/A*0201 as APCs (20). ELISPOT measures the frequency of T cells releasing IFN- γ in response to a CEA peptide (CAP1-6D), a MUC-1 peptide, an HIV gag peptide, and a flu peptide (2) in pre- and post-vaccination peripheral blood mononuclear cells (PBMCs). A positive response was scored as a ≥ 2 -fold increase in IFN- γ -secreting cells.

Detection of cytokines

Sera from patients pre- and post-vaccination were screened for IL-8, IL-6 and TNF- α using a multiple cytokine/chemokine kit (Meso Scale Discovery, Gaithersburg, MD).

Proliferation assay

CD4⁺ cells were isolated by column (Miltenyi Biotec, Auburn, CA) and used at a concentration of 1×10^6 /mL in 96-well flat-bottom plates. CD4⁺ cells were mixed with irradiated (30 Gy), unfractionated PBMCs in the presence of various concentrations of antigens. Irradiated PBMCs at a concentration of 6×10^6 /mL were used as APCs. CEA protein was used as antigen at a concentration of 10 µg/mL. PHA (2 µg/mL) and myoglobin (10 µg/mL) (Sigma-Aldrich, St. Louis, MO) were used as controls. Cultures were incubated at 37°C at 5% CO₂. The cell proliferation in cultures was estimated by [³H]thymidine (1 µCi [0.037 MBq] per well) (Perkin-Elmer, Boston, MA) incorporation pulsed on day 6 and quantified 18 h later using a liquid scintillation counter (Wallac, Gaithersburg, MD). All experiments were performed in triplicate. All results are expressed as stimulation index (SI), expressing the ratio of mean counts per minute (cpm ± SD) in stimulated vs. unstimulated control cultures. An SI > 3 was interpreted as representing a significant positive response.

Flow cytometry analysis

Cryopreserved PBMCs were analyzed by 3-color flow cytometry for phenotypic characterization of regulatory T-cells (Tregs) as described by Yokokawa, et al. (21) Cells were stained with the following combination of antibodies: PerCP-Cy5.5-conjugated anti-CD4 and phycoerythrin (PE)-conjugated anti-CD25 (all from BD Pharmingen, San Diego, CA). FoxP3 intracellular staining was then performed on the cells stained with anti-CD4 and anti-CD25. Cells were fixed and permeabilized using a fix/perm kit (eBioscience, San Diego, CA) according to the manufacturer's instructions, and were labeled with FITC-conjugated anti-FoxP3 antibody (236A/E7 clone) or its isotype control antibody (eBioscience). Flow cytometry was performed on a Becton Dickinson LSRII (BD Biosciences); 1×10^5 cells were acquired and data were analyzed using FACSDiva software (BD Biosciences). To determine the percentage of Tregs, lymphocytes were gated by plotting forward vs. side scatter, followed by gating on the CD4⁺ population. Then, CD25^{high} and FoxP3⁺ populations were gated. The CD25^{high} population was separated from CD25^{low} cells on the basis of the level of CD25 expression in CD4⁺ T cells, as previously described (21).

Results

Patients' baseline characteristics are outlined in Table 1A. Median potential follow-up from date of enrollment was 51.2 months for breast and ovarian cancer patients combined (55.9 for ovarian and 46.9 for breast). Treatment was well tolerated, with no grade 3 or 4 toxicities attributable to vaccine or GM-CSF. The majority (85%) of grade 1 and 2 toxicities were injection-site reactions such as erythema, edema, and induration, which generally resolved spontaneously after a few days (Table 1B). One patient had a baseline grade 1 anemia, which transiently progressed to grade 2 before returning to baseline without any intervention while on study.

Breast cancer patients

The study enrolled 12 patients with a median age of 60 (range 32 – 75). Most of the patients (75%) had received at least 3 prior chemotherapy regimens, and 50% had received their last chemotherapy within 3 months prior to enrollment on study.

Clinical outcomes—A summary of clinical responses in the breast cancer patients is outlined in Table 2. Median time to progression for the 12 patients enrolled was 2.5 months (range 1 – 37+ months). The median overall survival for this group was 13.7 months (range 2.7 – 42.9), with one patient still alive on study at 37 months (supplemental Fig. 1). Five

patients entered the trial with elevated CEA levels, and 2 of those had a decrease in serum CEA levels (patient 8 decreased by 38% and patient 12 decreased by 15%).

Patient 24 was a 32-year-old diagnosed with poorly differentiated invasive ductal carcinoma of the breast, ER⁺/PR⁺ and Her2/neu⁺ by IHC (+3). She underwent lumpectomy and radiation and was found to have liver metastasis soon after treatment. She underwent bilateral oophorectomy and then received 6 cycles of docetaxel followed by partial hepatectomy, with complete pathologic response. She received trastuzumab concurrently with chemotherapy. After liver metastectomy she received letrozole for one year, which was discontinued due to progressive disease. She was then enrolled on a Her2/neu peptide vaccine for 6 months and completed all planned 6-monthly cycles. When she enrolled on our study she had progressive but minimal disease in the hilar and mediastinal lymph nodes and was 12 months removed from her only prior chemotherapy regimen. She had a confirmed CR by RECIST, with complete resolution in mediastinal/hilar lymph nodes after 18 months of vaccine (Fig. 1). Trastuzumab was continued during the study and she remains on study 37 months after enrollment.

Patient 1 was a 49-year-old with a history of hormone receptor-positive early-stage breast cancer treated with modified radical mastectomy and adjuvant chemotherapy and tamoxifen. She relapsed with bone metastases 9 years after completing adjuvant endocrine treatment. She subsequently underwent oophorectomy and then received exemestane for almost a year before bone metastases progressed. She was started on gemcitabine and docetaxel chemotherapy, but it was poorly tolerated and treatment was discontinued. She was then enrolled on the study reported here with bone and lymph node metastases and had a 20% reduction in internal mammary lymphadenopathy. She came off study after 6 months due to disease progression and went on to receive chemotherapy for a short time, followed by palliative radiation to a brain metastasis. Eventually she entered hospice and died 3 years after coming off study.

Patient 16 was a 66-year-old with invasive ductal carcinoma of the breast showing absent expression of estrogen receptor, progesterone receptor, and Her2/neu (triple negative). She was initially treated with neoadjuvant chemotherapy (4 cycles of docetaxel and epirubicin), followed by surgery and further adjuvant chemotherapy (3 cycles of docetaxel and carboplatin) and radiation. Disease recurred one year later with mediastinal lymphadenopathy, and was treated with capecitabine for a total of 6 months, with complete radiographic remission of lymphadenopathy. She was enrolled on study when mediastinal lymphadenopathy recurred 5 months after her last capecitabine dose. She had a 17% reduction in mediastinal lymphadenopathy after 8 cycles of vaccine and remained on study for 10 months.

Five of the 12 patients had either SD for ≥ 4 months or evidence of tumor regression. Those patients had a mean of 1.8 prior chemotherapies, while those with no evidence of response had a mean of 4 prior chemotherapies. Moreover, the mean serum CEA on study was 2.9 for those with progression-free survival for ≥ 4 months and 53.7 for those with PD within 3 months, while mean serum CA27.29 was 22.0 vs. 165.7 for these groups, respectively.

Immune responses—Table 3 outlines immune responses in breast cancer patients as measured by various assays. Five breast cancer patients were HLA-A2⁺, but only 2 of these (patients 1 and 24, who were on study for 6 and 37+ months, respectively) had sufficient PBMC samples for the ELISPOT assay. While ELISPOT without in vitro stimulation did not show any increase in CEA- or MUC-1-specific T-cell response in patients 1 and 24, 1 of 3 patients had substantial increases (>7 X) in both CEA and MUC-1 T-cells following 1 cycle (72 hours) of in vitro stimulation. Furthermore, the CD4 functional/proliferation assay

showed a > 3-fold increase in the 2 patients. Patient 1 had a 4-fold increase and patient 24 had an 8-fold increase from baseline. They also showed a significant drop in the cytokines and chemokines produced mainly by tumor cells, such as IL-8, IL-6, and TNF- α , corresponding with clinical response. Conversely, patient 8 had a significant rise in cytokine levels, corresponding with a rapidly progressive clinical course.

Sufficient PBMCs were available from 6 breast cancer patients to measure the number of CD4⁺CD25^{high}FoxP3⁺ Tregs as the percent of CD4⁺ cells and the ratio of T effector cells to Tregs as detailed in Patients and Methods. We have previously reported (21) that the percent of Tregs per CD4⁺ cells in healthy donors is $3.28 \pm 1.16\%$ (range, 1.46 – 5.25). As seen in Table 4, 3 of the 6 patients (patients 1, 13, and 23) showed an increase in the T effector:Treg ratio post-*vs.* pre-vaccination.

Ovarian cancer patients—The study enrolled 14 patients with a median age of 55 (39 – 73). Twelve of 14 had received ≥ 3 chemotherapy regimens (Table 5). Median time to progression was 2 months (range 1 – 6 months). Median overall survival was 15.0 months (range 1.5 – 57+), with one patient still alive (supplemental Fig. 1). Two patients (patients 3 and 6) had declines in CA-125 levels of 18% and 11%, respectively. There was no significant decline in CEA.

Patient 19 was a 50-year-old diagnosed with stage III-C endometrioid-type ovarian adenocarcinoma 10 years prior to enrollment on study. She was initially treated with debulking surgery, followed by 8 cycles of carboplatin and taxol. Disease recurred 8 months later and was treated with another debulking surgery, followed by 8 cycles of liposomal doxorubicin. Her second recurrence occurred 2 years later, when she had another debulking surgery and autologous stem cell transplant, with a conditioning regimen of melphalan, cyclophosphamide, and paclitaxel. She had a second infusion of autologous stem cells shortly after the first one, conditioned by mitoxantrone and melphalan. She remained in remission for 3 years, then received 4 cycles of imatinib on a clinical trial. Upon progression, she was enrolled onto another clinical trial with sorafenib and bevacizumab and remained on study for 21 months. She was enrolled on our study when a CT scan of her abdomen and pelvis showed retroperitoneal disease (largest lesion, 3.6 cm). She remained stable for 4.5 months before disease progressed. She went on to receive palliative radiation and then surgical resection of a pelvic mass. She then had cisplatin and gemcitabine for 4 cycles. This had to be discontinued for cytopenias. She died 3 years after going off study.

We also report here further follow-up from a patient previously reported on the previous study of PANVAC (2). She is a 53-year-old with ovarian cancer originally diagnosed as stage III-A one year prior to her enrollment (patient 25 on that study). The treatment on that study was identical to the study reported here. She had undergone debulking followed by 6 cycles of carboplatin and paclitaxel. A second-look operation demonstrated microscopic residual disease, for which she received 4 cycles of intraperitoneal cisplatin. She enrolled on the previous study 4 months later with no radiographic evidence of disease and normal levels of CA-125 (3 U/mL). She was taken off study after 38 months when slowly rising CA-125 levels suggested recurrent disease. CT scan showed no evidence of recurrent disease; however, a small focus of uptake in the pelvis on PET scan suggested possible recurrence. When she went off study, her CA-125 was 15.4 U/mL, which was within normal range.

Immune response—Two HLA-A2⁺ ovarian cancer patients on study for 2 months were evaluable by ELISPOT assay, which revealed no significant changes in either CEA or MUC-1 without *in vitro* stimulation however with *in vitro* stimulation with HLA-A2 restricted CEA and MUC-1 peptides for 72 hours, 1 of 2 patients (pt 14) had an increase in

CEA specific T-cells of 2.7 fold. Sufficient PBMCs were available from 8 patients for Treg evaluation (Table 4). Three patients (patients 7, 10, and 14) had increases in T effector:Treg ratios.

Discussion

This follow-up study was designed to evaluate the clinical outcome of therapy with PANVAC vaccine in metastatic breast and ovarian cancer patients. The first safety study consisted of 25 patients with metastatic GI, lung, breast, and ovarian malignancies (2).

In the study reported here, some patients who had limited tumor burden and whose immune system was not compromised by multiple rounds of prior chemotherapy appeared to benefit from the vaccine. Patient 24, with metastatic breast cancer, had her last chemotherapy 12 months prior to enrollment on study, with minimal hilar and mediastinal lymphadenopathy at the time of her first vaccine. She had a 50% reduction in the diameter of her lymph nodes after 10 months of vaccine, followed by complete radiographic resolution of disease by 18 months, and remains on study after 37 months with no sign of progression. While anecdotal findings must not be over interpreted, it is interesting to note that this patient, who has had a prolonged CR, also had the best CD4 response (Table 4). Due to limited pre-vaccination sample, a comparative analysis of CD8 immune response following in vitro stimulation could not be performed. At baseline, all patients tested had no evidence of CEA specific T-cells following IVS (lower limit of detection 1 in 200,000 PBMC). However, it is interesting to note that by day 71 following vaccine, she had 5 times that number of CEA specific T-cells. Another patient from the previous study (2) had advanced ovarian cancer, with no radiographic evidence of disease at the time of enrollment and a normal CA-125 level. She remained on study for 38 months before eventually progressing.

As previously reported, another patient from the prior study was a 42-year-old with stage III-C clear cell ovarian cancer. She had a substantial clinical response to PANVAC, with normalization of rapidly rising CA-125 (> 300 U/mL) and resolution of symptomatic ascites (2).

Median time to progression for patients on study was 2.5 months (breast cancer) and 2.0 months (ovarian cancer). The majority of patients had widely metastatic disease and a history of multiple lines of chemotherapy. Our findings agree with others that suggest RECIST may not be the best tool to measure clinical response in cancer vaccine studies (22-25). Many of our patients had progressed at or before their first scheduled restaging. As previously demonstrated, it takes at least a few months to mount an optimal immune response by vaccination, which is usually shorter than the interval between the initiation of immune therapy and the first restaging. Therefore, the reported progression may not truly represent the effect of vaccine therapy, but may reflect an ongoing process before immune activation (26, 27). Overall survival, rather than time to progression or tumor shrinkage, may be a more relevant endpoint in evaluating the effects of immunotherapies. Sipuleucel-T, the dendritic-cell vaccine for prostate cancer, failed to show a significant improvement in progression-free survival in advanced prostate cancer patients, but demonstrated an absolute overall survival benefit over placebo, despite a cross-over of more than 70% of patients in the placebo arm (27, 28). This result was reproduced in a larger study randomizing 512 patients with metastatic androgen-insensitive prostate cancer to either sipuleucel-T or placebo. There was a 4-month difference in median overall survival favoring the vaccine arm, with no significant delay in time to objective disease progression (24). Similar findings were reported (25) in a randomized, placebo (empty vector)-controlled, multicenter study employing another TRICOM-based vaccine in patients with metastatic castration-resistant prostate cancer. While no difference was observed in time to progression in both arms,

patients receiving PSA-TRICOM (Prostvac) vaccine had an improved median overall survival of 8.5 months and a 44% reduction in death rate *vs.* the control arm. A discordance between median time to progression *vs.* survival has also recently been reported (29) in patients with metastatic melanoma receiving anti-CTLA-4 monoclonal antibody.

Some patients in the present study continued to show very good clinical response to subsequent therapies after coming off study. There is accumulating evidence that once a cancer vaccine provokes an immune response in the host, it may initiate a prolonged dynamic process leading to marked responses to subsequent therapies (2, 22, 30, 31). This may be a function of a number of mechanisms, including tumor phenotype alteration and antigen cascade. Also, subsequent therapies (*i.e.*, chemotherapy or radiation therapy) may reduce suppressor cell populations, allowing for the enhancement of T-cell responses (22, 30, 32). However, some chemotherapies may be more likely to lead to immunologically relevant tumor-cell killing (33).

Future cancer vaccine studies should consider new endpoints and select patients with less aggressive tumors in the earlier stages of disease. Several studies have suggested that patients with more advanced disease or more heavily pretreated with chemotherapy may be less likely to mount immune responses or derive clinical benefit from therapeutic vaccines (34-37). Indeed PANVAC has been tested in patients with metastatic colorectal cancer rendered without evidence of disease surgically (*i.e.*, low disease volume) with substantial improvements in overall survival compared with concurrent controls (38). Vaccines used in the adjuvant setting in breast cancer patients with early-stage disease and high-risk features, or in ovarian cancer patients with a rise in CA-125 and no evidence of metastatic disease, may confer more clinical benefit than in patients with more advanced, chemotherapy-refractory disease. An approach that may provide improved immune responses and clinical benefit is the combination of vaccines with conventional cancer treatments such as chemotherapy, monoclonal antibodies, small-molecule targeted therapies, or radiation. An ongoing randomized study in patients with metastatic breast cancer (39) is providing preliminary evidence of patient benefit in patients receiving PANVAC vaccine plus docetaxel *vs.* docetaxel alone. In these combinations, the enhanced effect of active immunotherapy may be a result of different mechanisms, including alteration of tumor-cell phenotype and microenvironment, better antigen presentation, and inhibition of Tregs (40-45).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Translational Relevance

With the first FDA-approved therapeutic cancer vaccine providing definitive proof-of-concept for this class of agents, a further understanding of the types of patients most likely to benefit from therapeutic cancer vaccines is crucial. This trial provides additional insight into the potential of a therapeutic vaccine to lead to improved outcomes in select patients and supports the use of vaccines in patients with low tumor burden and minimal prior chemotherapy. This has implications for both the selection of patient populations for clinical trials utilizing therapeutic cancer vaccines as single agents, and also for timing of vaccine relative to other available therapeutics. The sustained complete response in a patient who remains on study for over 3 years also underscores the potential for therapeutic vaccines to provide patient benefit without toxicity. Further studies in appropriate patient populations may provide more insight into how to maximize the potential of therapeutic vaccines as monotherapy.



Fig. 1. Patient 24 had a subcarinal lymph node mass measuring 2.8 cm (panel A) which decreased to 0.8 cm (panel B) after 10 months of vaccine, a > 50% reduction by RECIST, followed by a CR after 18 months (panel C). She remains on study 37 months after enrollment, with a durable CR.

Table 1A

Patient baseline characteristics

Baseline characteristics	No. patients/median (range)	
	Breast	Ovarian
No. of patients	12	14
Measurable disease	12	13
Age (years)	60 (32 – 75)	55 (39-73)
Performance status		
ECOG 0	2	5
ECOG 1	10	9
ER+/PR+	6	N/A
Her2/neu+	2	N/A
Prior therapy		
Prior chemo regimens	3 (0 – 8)	5 (1-9)
< 3 chemo regimens	3	2
≥ 3 chemo regimens	9	12
Radiation	8	0
Concurrent trastuzumab	2	N/A
Time since last chemo (months)	3 (1 – 19)	1.5 (1-13)
CEA level	3.5 ng/mL (0.7 – 153.6)	N/A
CA 27.29 level	167 U/mL (<12 – 276)	N/A
CA-125 level	N/A	135 U/mL (11-17,333)

ECOG = Eastern Cooperative Oncology Group; N/A, not applicable

Table 1B

Grade 2 or greater toxicities associated with PANVAC + GM-CSF

Toxicity	Grade 2	
	No.	%
Injection-site reaction	22	85
Musculoskeletal pain	2	7
Anemia	1	4

Note: No grade 3 or 4 toxicity was attributed to PANVAC or GM-CSF.

Table 2

Clinical outcomes in breast cancer patients by best response

Patient no.	Age	ER/PR	Her2/neu	No. prior chemo regimens	Time since last chemo (months)	Baseline CEA (ng/mL)	Baseline CA 27.29 (U/mL)	Best response	PFS (months)	OS (months)
8	62	ER+, PR-	Negative	5	1	153.6	-	PD	1	2.8
17	62	ER+, PR-	Negative	6	4	95.2	276	PD	1	4.9
12	57	ER-, PR-	Negative	3	4	71.4	254	PD	2	25.3
13	65	ER+, PR+	Negative	3	1	48.6	182	PD	2	14.2
21	45	ER-, PR-	Negative	3	2	1.7	78.9	PD	2	4.9
25	48	ER-, PR+	Negative	6	2	1.3	52.2	PD	2	2.7
22	51	ER+, PR+	Negative	2	19	4.2	151	PD	3	31.3
23	75	ER-, PR-	Negative	2	1	1.8	36.5	SD	4	11.2
26	65	ER-, PR-	Positive	0	N/A**	8.4	16.2	SD	5	24.7
1	49	ER+, PR-	Negative	3	2	2.7	-	SD, 20% ↓	6	42.9
16	66	ER-, PR-	Negative	3	5	0.7	23.1	SD, 17% ↓	10	13.0***
24*	32	ER+, PR+	Positive	1	12	0.7	<12.0	CR	37+	37.0+

PFS, progression-free survival; OS, overall survival; ER+, estrogen receptor positive; PR+, progesterone receptor positive; PD, progressive disease; SD, stable disease; CR, complete response.

* Patient still on study.

** Patient refused chemotherapy.

*** No further follow-up from this patient after this point.

Table 3

Immune responses in breast cancer patients

Patient no.	CD4 assay ¹	ELISPOT ² CEA	MUC-1	IL-8 ³	IL-6 ³	TNF- α ³
1	+ >4X	-	-	↓	↓	↓
8	+ >2.3X	NA	NA	↑	↑	↑
12	-	+ >7.7X	+ >9.1X	NC	NC	NC
13	- ⁴	-	-	↑	NC	↑
23	-	NA	NA	↓	NC	NC
24	+ ⁵ >8X	NA ⁶	NA ⁶	↓	↓	↓
25	NA	NA	NA	↓	NC	↑

NA = not applicable; NC = no change.

¹ > 3-fold increase in stimulation index was considered positive.

² ELISPOT was performed with *in vitro* stimulation with HLA-A2 restricted CEA and MUC-1 peptides along with HIV peptide controls (which were all negative).

³ > 2-fold increase or decrease in cytokine assays was considered positive or negative, respectively.

⁴ Patient had high CD4 function pre-vaccination.

⁵ Test turned positive on day 154.

⁶ Patient had inadequate pre-vaccine sample available for testing; however, had a positive ELISPOT response to CEA on day 71 following vaccine.

Table 4

Peripheral blood Tregs* pre- and post-vaccination

Patient	Sample	% Treg in CD4+	Ratio of Teff:Treg**
Breast cancer patients:			
1	Pre	5.3%	12.5:1
	D131	3.9%	17.8:1
8	Pre	2.4%	35.1:1
	D44	2.5%	22.5:1
12	Pre	2.6%	28.9:1
	D27	5.1%	13.1:1
13	Pre	8.2%	7.5:1
	D41	6%	11.5:1
23	Pre	3%	22.3:1
	D70	2.3%	29.3:1
24	Pre	4.2%	13.6:1
	D71	5.9%	10.4:1
Ovarian cancer patients:			
2	Pre	3.3%	21.8:1
	D71	4.2%	16.9:1
3	Pre	4.5%	16.2:1
	D28	4.0%	16.9:1
5	Pre	3.1%	21.6:1
	D70	3.7%	17.7:1
7	Pre	6.2%	9.2:1
	D76	4.2%	14.2:1
10	Pre	6.4%	9.1:1
	D77	3.4%	17.7:1
11	Pre	3.8%	17:1
	D76	4.4%	14:1
14	Pre	5.1%	13.5:1
	D71	4.0%	17.5:1
19	Pre	2.4%	23.3:1
	D69	3.1%	18.3:1

Bold font indicates decreases in Treg or increases in Teffs:Treg.

* Tregs (T regulatory cells) defined as CD4⁺CD25^{high}Foxp3⁺, percentage of CD4⁺CD25^{high}Foxp3⁺ in CD4⁺ cells.

** Ratio of Teffs (T effector cells; CD4⁺CD25⁻) to Tregs (CD4⁺CD25^{high}Foxp3⁺).

Table 5

Clinical outcomes for ovarian cancer patients

Patient no.	Age	No. prior chemo regimens	Time since last chemo (months)	Baseline CEA (ng/mL)	Baseline CA-125 (U/mL)	Best response	PFS (months)	OS (months)
20	60	7	1	15	17333	PD	1	1.5
3	52	6	1	<0.5	142	PD	1	2.4
11	70	3	1	1	1162	PD	2	11.6
15	55	7	1	1	277	PD	2	9.6
5	73	9	1	1.7	128	PD	2	35.0
6	54	7	2	1.3	117	PD	2	57.0+
14	57	1	13	0.8	115	PD	2	32.7
9	48	4	1	1.5	80	PD	2	15.0
7	45	2	3	1.2	11	PD	2	8.0
4	50	4	3	0.8	182	PD	3	41.9
18	57	5	6	<0.5	592	PD	3	8.7
10	39	5	1	1	195	SD	4	29.3
2	56	4	4	0.5	46	SD	4	16.1
19	50	8	8	1.8	114	SD	6	40.5