Docetaxel Alone or in Combination With a Therapeutic Cancer Vaccine (PANVAC) in Patients With Metastatic Breast Cancer: A Randomized Clinical Trial

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**IMPORTANCE** Previous phase 1 and 2 trials of PANVAC, a poxviral-based cancer vaccine, have suggested clinical efficacy in some patients with breast, ovarian, and colorectal cancer and have shown evidence of immunologic activity. Preclinical data have shown that docetaxel can modify tumor phenotype, making tumor cells more amenable to T cell–mediated killing.

**OBJECTIVE** The goal of this study was to determine if the treatment combination of docetaxel and PANVAC improves clinical outcomes in patients with metastatic breast cancer compared with docetaxel treatment alone.

**DESIGN, SETTING, AND PARTICIPANTS** Between May 2006 and February 2012, this open-label, phase 2 randomized clinical trial enrolled 48 patients with metastatic breast cancer of all subtypes, without limitation on other lines of previous therapy, to receive treatment with either docetaxel with PANVAC (arm A) or docetaxel alone (arm B). Final clinical data were collected on September 16, 2013. All patients were treated at either the National Cancer Institute or the Department of Breast Medical Oncology, MD Anderson Cancer Center.

**MAIN OUTCOMES AND MEASURES** The primary end point was progression-free survival (PFS), using a phase 2.5 statistical design, with the intent of identifying a trend toward benefit (defined as 1-sided \( P \leq 0.10 \)) to guide a larger trial design. Secondary end points included safety and immunologic correlative studies.

**RESULTS** Forty-eight participants were enrolled: 25 were randomized to the combination treatment arm A, and 23 to arm B. No patient remained in the study at the time of the final analysis. Patient and tumor characteristics were well matched. Analysis of adverse events in both treatment arms demonstrated very little difference between the 2 groups. In the combination treatment arm (arm A), statistically significant increases were noted in the frequency of grades 1 and 2 edema (\( P = .02 \), likely related to greater median number of docetaxel cycles) and injection-site reactions (\( P < .001 \)). In the final data analysis, median PFS was 7.9 months in arm A vs 3.9 months in arm B (hazard ratio, 0.65 [95% CI, 0.34-1.14]; \( P = .09 \)).

**CONCLUSIONS AND RELEVANCE** The results suggest that the combination of PANVAC with docetaxel in metastatic breast cancer may provide a clinical benefit. This study was hypothesis generating and provides both rationale and statistical assumptions for a larger definitive randomized study.

**TRIAL REGISTRATION** clinicaltrials.gov Identifier: NCT00179309


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Breast cancer is expected to cause approximately 40,000 deaths in 2015. Despite improvements in treatment, an overwhelming majority of patients diagnosed with metastatic breast cancer will die as a result of their disease. Standard chemotherapy agents are capable of shrinking tumors, but treatment usually needs to be stopped owing to tumor progression or toxic effects. At that point, the likelihood of response with each subsequent therapy decreases. Our group and others have previously demonstrated safety, immunoreactivity, and potential clinical activity of a recombinant poxviral-vector therapeutic cancer vaccine, designated PANVAC (CEA-MUC-1-TRICOM), which consists of a priming dose with recombinant vaccinia vector and subsequent doses with recombinant fowlpox vector. Each vector encodes the transgenes for CEA and MUC-1 as well as transgenes for 3 human costimulatory molecules (B7.1, ICAM-1, and LFA-3).

Docetaxel is a commonly used agent in treating patients with metastatic breast cancer. Our group has previously reported preclinical data indicating a synergistic effect of docetaxel with vaccine. Docetaxel was shown to be capable of altering human and murine carcinoma cell phenotypes, making them more amenable to T cell–mediated killing. In addition, a previous clinical trial demonstrated that docetaxel (with glucocorticoid coadministration) in combination with vaccine did not cause decreased immune responses in patients compared with vaccine alone.

As single agents, therapeutic cancer vaccines have often failed to affect short-term end points, such as median progression-free survival (PFS), despite improvement in long-term outcomes, such as overall survival (OS). This pattern has also been seen with other immunotherapeutic agents, such as ipilimumab. A tumor growth rate kinetics model created with clinical trial data may help to explain the improvement in OS without effect on PFS. Clinical trial data using effective therapeutic cancer vaccines appears to induce slowing of the tumor growth rate over time. This process may take months to occur. We have hypothesized that the lag between initial vaccination and eventual slowing of tumor growth rate may explain the lack of improvement in PFS in previous vaccine monotherapy trials despite improvement in OS. If that hypothesis is correct, then combining therapeutic cancer vaccines with standard-of-care agents that can induce an improvement in PFS and not negatively affect the immune response may provide adequate time for the vaccine effect to occur, resulting in improved PFS for the combination treatment over the standard agent alone.

**Methods**

**Patient Eligibility**

Participants were required to have radiographically evaluable metastatic breast cancer that had not been treated with docetaxel in the metastatic setting. There was no limitation on prior lines of systemic therapy or subtype of breast cancer. Participants were required to be at least 18 years old. They were also required to have acceptable hematologic parameters and organ function, an Eastern Cooperative Oncology Group (ECOG) performance status of 1 or lower, no other malignant conditions within the preceding 12 months, no clinically active brain metastases, and no other significant medical illnesses or autoimmune diseases. Owing to the vaccinia vector used in priming, participants with a history of prior allergy or reaction to vaccinia-based vaccination or an open skin wound were also excluded.

**Trial Design and Treatment**

The primary end point was PFS, which was evaluated using a phase 2 trial design intended to provide evidence of a trend toward a clinical benefit with the goal of informing a larger randomized clinical trial in the future. This pilot trial had an 80% power to demonstrate a trend toward benefit (defined as 1-sided P < .10), estimated to be 4.2 months with docetaxel alone and 8 months with docetaxel and PANVAC, with 24 patients per arm, a total of 48 participants. All participants provided written informed consent, and the study protocol (Supplement 1) was approved by the National Cancer Institute (NCI) and the University of Texas MD Anderson Cancer Center (MDACC) institutional review boards, and participants were enrolled at both institutions.

Participants were randomized at a ratio of 1 to 1 to receive docetaxel alone (arm B), given 3 of every 4 weeks at a dose of 35 mg/m², or in combination with PANVAC (arm A). The PANVAC priming dose (vaccinia) was given 3 weeks prior to the first cycle of docetaxel. All booster doses (fowlpox) were given on day 1 of each docetaxel cycle. Docetaxel was given on days 2, 9, and 16 of each 28-day cycle. Treatment was continued until disease progression or unacceptable toxic effects occurred. In arm A, if docetaxel treatment was discontinued for toxic effects, PANVAC could continue to be administered until disease progression occurred.

PANVAC was manufactured by Therion Biologics Corporation as part of a Cooperative Research and Development Agreement between Therion and the Laboratory of Tumor Immunology and Biology, NCI. Vaccines were provided by the Cancer Therapy Evaluation Program, NCI.

Participants were enrolled from May 2006 to February 2012. Those enrolled at NCI and assigned to arm A received low-dose granulocyte–macrophage colony–stimulating factor (GM-CSF) (100 μg subcutaneously on the day of each vaccination and for 3 consecutive days thereafter, all near the vaccination site designed as a vaccine adjuvant) with PANVAC, while those in arm A at MDACC did not receive adjuvant GM-CSF with.

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**At a Glance**

- This is a randomized phase 2 study in metastatic breast cancer comparing PANVAC plus docetaxel to docetaxel alone (n = 48).
- The combination treatment of docetaxel with PANVAC may result in longer progression-free survival compared with docetaxel treatment alone (median progression-free survival, 7.9 vs 3.9 months; hazard ratio, 0.65 [95% CI, 0.34-1.14]; 1-sided P = .09).
- Injection-site reactions and the development of edema were significantly greater in the combination treatment arm.
- Patients in both arms developed measurable immunity to the immunizing antigens (not statistically significant).
PANVAC owing to issues of cost and preclinical and preliminary clinical data indicating that there was no clear benefit from the addition of GM-CSF in poxviral TRICOM (triat of costimulatory molecules) vector vaccines.\(^{13}\)

Participants with HER2-positive disease who had previously progressed under treatment with trastuzumab were allowed to continue trastuzumab therapy while participating in this study. Progression was defined by RECIST criteria 1.0 (Response Evaluation Criteria in Solid Tumors).\(^{19}\) Imaging studies (computed tomographic scans of the chest, abdomen, and pelvis and bone scans) were performed at baseline, 3 months in the study, and then every 2 months thereafter. For participants with evaluable disease only at baseline, progression was defined by new lesions or measurable growth of existing, previously unmeasurable lesions. At progression, participants assigned to arm B (docetaxel alone) had the option to receive vaccine alone if their clinical status met all eligibility criteria.

### Immune Assays

Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque density gradient separation, washed 3 times, and preserved in 90% heat-inactivated human AB serum and 10% dimethyl sulfoxide in liquid nitrogen at a concentration of \(1 \times 10^7\) cells/mL until assayed. Serum samples were collected in serum separator tubes, spun down, and stored at \(-80^\circ\)C. The analysis of the frequency of PBMC immune cell subsets in patients before therapy (baseline) and at the time of restaging (after cycle 3 of docetaxel treatment) was assessed by multiparametric flow cytometry. One vial of cryopreserved PBMCs per time point was defrosted, counted, and \(1 \times 10^6\) cells immediately stained to identify CD8\(^+\) T lymphocytes, CD4\(^+\) T lymphocytes, and T regulatory lymphocytes (Tregs) using the following antibodies: anti-CD8-PE-Cy7 (clone RPA-T8, BD Biosciences), anti-CD4-alexa fluor 700 (clone RPA-T4, BioLegend), anti-CD25-APC-Cy7 (clone M-A251, BD Biosciences), anti-CD127-FITC (clone A019D5, BioLegend), and anti-FoxP3-PE-Cy7 (clone PCH101 (eBioscience)).

Antigen-specific responses were analyzed by intracellular cytokine staining following a period of in vitro stimulation (IVS) with overlapping 15-mer peptide pools encoding the tumor-associated antigens (TAAs) CEA, MUC-1, and brachyury. The TAA peptide pools were designed to contain agonist epitopes that have been previously identified\(^{20-22}\); peptide pools encoding for HLA and CEFT (a mixture of cytomegalovirus, Epstein-Barr virus, flu, and tetanus toxin) served as negative and positive controls, respectively. Peptide mixes were purchased from JPT Peptide Technologies GmbH, reconstituted in dimethyl sulfoxide, and used immediately. Cryopreserved PBMCs obtained from patients before therapy and at the time of restaging were thawed and rested overnight at \(37^\circ\)C in 5% carbon dioxide in complete media (Isocove modified Dulbecco medium [IMDM; Gibco] supplemented with 10% human AB, 2mM glutamine, 100 U/mL of penicillin, and 100 μg/mL of streptomycin).

The next day (day 0), PBMCs were seeded in 12 well plates (\(2.5 \times 10^6\) in 1 mL) and stimulated with peptide mixes (0.1 μg/mL per peptide); cultures were supplemented on days 3 and 5 with cytokines (interleukin [IL]-7 and IL-15, 10 ng/mL; PeproTech) and fresh media and rested on day 7 (with removal of cytokines and peptide). On day 11, \(1 \times 10^6\) cells were restimulated for 24 hours in 96 well plates with peptide mixes in the presence of anti-CD107a-APC (clone H4A3, BD Biosciences); brefeldin A (1 μL/mL) and monensin (0.7 μL/mL) (BD Biosciences) were added to cultures 2 hours after the start of the restimulation and incubated for the final 22 hours. The PBMCs were then stained with anti-CD4-PerCP-Cy5.5 (clone OKT4, Biolegend), anti-CD8-AF700 (clone OKT8, Ebioscience), and anti–tumor necrosis factor (TNF)–PE (clone MAb11), anti–interferon (IFN)-γ-PE-Cy7 (clone 4SB3), and anti-IL-2-BV521 (clone 5344.111) (BD Biosciences).

For all flow cytometry experiments, at least \(3 \times 10^5\) events in the live gate were acquired with a Becton-Dickinson LSR-II flow cytometer equipped with a UV, violet, blue, and red laser. Flow cytometry standard files were imported and analyzed with FlowJo software, V.9.7 for Macintosh (TreeStar). “Fluorescence minus one” controls were used for gating, and nonviable cells were excluded.

The development of immune responses following therapy to a given TAA were calculated from intracellular cytokine staining experiments as follows: We first calculated the absolute number of CD4\(^+\) or CD8\(^+\) lymphocytes that produced IFN-γ, TNF, or IL-2 at the end of the IVS following stimulation with CEA, MUC-1, brachyury, or the negative control 15-mer peptides, per \(1 \times 10^6\) cells plated at the start of the IVS. Next, background values (ie, any signal obtained from the IVS with the negative control peptide pool HLA) were subtracted from the signal obtained with the CEA, MUC-1, or brachyury 15-mer peptide pools, and then pretherapy values were subtracted from posttherapy values (\(\text{post-TAA} - \text{post-HLA}\)) – \(\text{pre-TAA} - \text{pre-HLA}\). An antigen-specific immune response to a given TAA was scored as positive if a patient had more than 250 CD4\(^+\) or CD8\(^+\) T lymphocytes that produced IFN-γ, IL-2, or TNF at the end of the IVS per \(1 \times 10^6\) cells plated at the start of the IVS (following subtraction of any background and pretherapy signal).

The reproducibility of the IVS assay to measure antigen-specific immune responses has been tested by repeatedly assessing immune responders and nonresponders in independent experiments with similar results obtained in each assay. In addition, when the number of samples was large, requiring samples to be run in batches, internal controls were always included, and importantly, the pre-PBMC and post-PBMC values from a given patient were always run simultaneously to reduce assay variability.

Assays for the presence of soluble CD27 (sCD27) in serum samples were as described in detail previously.\(^{23}\)

### Statistical Methods

Analysis of the primary end point was performed with all eligible, randomized participants included, with the probability of PFS as a function of time estimated by the Kaplan-Meier method and the arms compared using a log-rank test. Secondary objectives included comparison of OS, specific T-cell responses to the target antigens of the vaccine, and other evi-
Figure 1. Patient Enrollment Flowchart

- 51 Assessed for eligibility
  - 48 Eligible and randomized
    - 25 Arm A: Docetaxel + PANVAC
    - 23 Arm B: Docetaxel alone
  - 3 Ineligible
  - 1 Withdrawal of consent
  - 25 Evaluable for primary end point
  - 23 Evaluable for primary end point
  - 2 Withdrawal of consent
  - 3 Allergy or toxic reaction to docetaxel

### Results

#### Patient Baseline Characteristics

Forty-eight participants were enrolled in the study between May 2006 and February 2012, 25 in arm A (combination of vaccine and docetaxel) and 23 in arm B (docetaxel alone) (Figure 1). Final clinical data were collected on September 16, 2013. There was similar heterogeneity with regard to prior cytotoxic chemotherapy for treatment of metastatic disease between arms (arm A, a median of 2 regimens, range 0-7; arm B, median of 1 regimen, range 0-8). Eight (32%) of 25 patients in arm A and 8 (35%) of 23 in arm B had received 3 or more lines of treatment prior to enrollment (Table 1). There was no statistical difference in any baseline characteristic ($P > .20$ for all comparisons, Table 1). The patient population for the study was heterogeneous by current standards of breast cancer clinical trial design in terms of biologic characteristics and prior treatments, but there were no statistically significant differences in baseline characteristics between groups ($P > .20$ for all comparisons, Table 1).

#### Safety

There were no differences in toxic effects between arm A and arm B, with the exception of higher rates of injection site reactions and edema in arm A (Table 2). The incidence of edema corresponded to the number of cycles of docetaxel received, which was higher in arm A (median number of cycles was 5; range, 0-17) than in arm B (median number of cycles was 3; range, 1-15) owing to prolonged PFS. There were no grade 4 toxic effects in either arm. Dose reductions and delays were similar between the arms. In arm A, 9 patients had dose reductions; 13 had dose delays; and 14 had dose delay or reduction. In arm B, 7 patients had dose reductions; 11 had dose delays; and 13 had dose delays or reductions. The likelihood of toxic effect causing delay or reduction in dose was more likely in patients who received multiple cycles, related to the cumulative toxicity of docetaxel.
Clinical Outcomes

The study was powered to detect a trend toward improvement in PFS. The median PFS in arm A was 7.9 months compared with 3.9 months in arm B (1-sided \( P = .09; \) HR, 0.65 [95% CI, 0.34-1.14]), indicating a trend toward improvement in the combination arm (Figure 2). The overall confirmed partial response rate in arm A was 16% compared with 13% in arm B. There was a notably higher confirmed partial response rate at NCI (24%) than at MDACC (4%). The median number of vaccines administered in arm A was 8 (range, 2-20). Only 4 patients were eligible and opted to cross over to vaccine after disease progression with docetaxel alone (arm B). The PFS in arm B was measured to the date of first progression, and further clinical outcome data on the patients who crossed over is not reported owing to the small sample size.

Since the overall result demonstrated a trend toward benefit with vaccine, we undertook an analysis to determine if this association would remain if clinical and immune response parameters were included in a Cox model. The following parameters were all considered for evaluation: time since last chemotherapy, pretreatment sCD27, CD4/Treg ratio, Tregs, age at time of study enrollment, age at diagnosis, ECOG status, sex, estrogen receptor (ER)/progesterone receptor (PR) status, HER2 status, time from diagnosis to enrollment, time from diagnosis to metastases, and metastatic disease at diagnosis. By univariate analyses, time since last chemotherapy (<30 vs ≥30 days; \( P = .02 \), CD4/Treg ratio (<0.20 vs >0.20, unadjusted \( P = .02 \); adjusted \( P = .048 \), Tregs (<20 vs ≥20, unadjusted \( P = .02 \); adjusted \( P = .06 \)), and ER/PR status (positive vs negative, \( P < .001 \)) were considered for evaluation in a Cox model along with treatment arm to determine the association of treatment arm with PFS after accounting for potential prognostic factors. After backward selection, we found that the combination treatment (HR, 0.60; 95% CI, 0.30-1.19; 2-tailed \( P = .14 \); 1-tailed \( P = .07 \)) retained its trend toward association with PFS after adjusting for higher Tregs (HR, 0.30; 95% CI, 0.13-0.72; 2-tailed \( P = .007 \)) and for patients having ER+/PR− status (HR, 0.11; 95% CI, 0.04-0.27; 2-tailed \( P < .001 \)).

Effect of Time Since Last Chemotherapy

Patients treated at MDACC had a shorter time since last chemotherapy (median, 34.0 days; range, 15-198 days) than patients treated at NCI (median, 192.0 days; range, 25-2800 days; \( P < .001 \)). The median (range) time since last chemotherapy in the docetaxel alone arm (arm B) was 199.5 (25-2800) days for patients enrolled at NCI vs 43.0 (15-198) days at MDACC (\( P = .07 \)). For the combination treatment arm (arm A), the median (range) time since last chemotherapy was 192 (27-1607) days for patients enrolled at NCI vs 30 (20-175) days at MDACC (\( P = .003 \)). There was a trend toward improvement in median PFS in arm A for patients with more than 30 days since prior chemotherapy (n = 13) compared with those patients with less than 30 days since last chemotherapy (n = 6) (9.4 months vs 5.8 months), although the trend was not statistically significant (\( P = .12 \)). Our group has previously reported that sCD27 plays a role in T-cell activation and that levels of sCD27 in serum from healthy donors are greater than levels in serum from patients with prostate cancer. Herein we report that sCD27 levels in serum are also lower in patients with breast cancer than in healthy donors (\( P < .001 \)) (Figure 3A). Moreover, the pretreatment levels of sCD27 were somewhat lower in patients treated at MDACC than in those treated at NCI (6.4 vs 19.2 U/mL; \( P = .08 \); Figure 3A). This may be due to the differences between the 2 centers in the patients’ time since last chemotherapy prior to entering this trial.

Immune Assays

Sufficient PBMCs were available from patients in the docetaxel alone arm (arm B; n = 15) and the docetaxel plus vaccine arm (arm A; n = 16) to analyze antigen-specific immune responses before vs during therapy (at first restaging, approximately day 85 from start of docetaxel regimen) using intracellular cytokine staining. Eleven (69%) of 16 patients in the docetaxel plus vaccine arm developed T-cell responses to 15-mer peptide pools of the TAAs CEA, MUC-1, and/or brachyury during treatment, while 8 (53%) of 15 patients in the docetaxel alone arm developed T-cell responses to these TAAs during treatment. In analyses of immune responses to the 2 transgenes (CEA and MUC-1) in the PANVAC vaccine, 6 (40%) of 15

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study Armb</th>
<th>Docetaxel and PANVAC (n = 25)</th>
<th>Docetaxel Alone (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), y</td>
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<td>55 (33-72)</td>
<td>52 (34-76)</td>
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<td>At study enrollment</td>
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<td>48 (31-61)</td>
<td>45 (26-74)</td>
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<td>ECOG performance status</td>
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<td>11 (48)</td>
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<tr>
<td>1 (8 (32))</td>
<td>12 (52)</td>
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<td>Receptor status</td>
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<td>7 (30)</td>
</tr>
<tr>
<td>ER+/PR− patients</td>
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<td>3 (12)</td>
<td>3 (13)</td>
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<tr>
<td>HER2+ patients</td>
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<td>25 (100)</td>
<td>22 (96)</td>
</tr>
<tr>
<td>Female patients</td>
<td></td>
<td>13 (52)</td>
<td>12 (52)</td>
</tr>
<tr>
<td>Patients enrolled at NCI</td>
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<td>1 (0-8)</td>
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<tr>
<td>Prior chemotherapy regimens for metastatic disease, median (range), No.</td>
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<td>68 (19-1583)</td>
<td>45 (10-2760)</td>
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<tr>
<td>Days since prior chemotherapy, median (range), No.</td>
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<td>4 (17)</td>
<td>6 (29)</td>
</tr>
<tr>
<td>&lt;30 d since last chemo</td>
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<td>20 (83)</td>
<td>15 (71)</td>
</tr>
<tr>
<td>Days since diagnosis, median (range), No.</td>
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<td>1834 (279-6236)</td>
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<tr>
<td>Days from diagnosis to metastasis, median (range), No.</td>
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<td>1133 (0-4916)</td>
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<tr>
<td>Patients with metastatic disease at diagnosis</td>
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<td>2 (8)</td>
<td>3 (13)</td>
</tr>
</tbody>
</table>

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; NCI, National Cancer Institute; PR, progesterone receptor.

a Unless otherwise indicated, data are number (percentage) of study participants.

b All baseline characteristics were compared between arms, and there were no statistical differences (\( P > .20 \) for all comparisons).
Table 2. Adverse Events Occurring Among Study Participantsa

<table>
<thead>
<tr>
<th>Study Arm</th>
<th>Docetaxel and PANVAC (n = 25)</th>
<th>Docetaxel Alone (n = 23)</th>
<th>P Valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gr 1</td>
<td>Gr 2</td>
<td>Gr 3</td>
</tr>
<tr>
<td>Hematologic</td>
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<td></td>
<td></td>
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<tr>
<td>Anemia</td>
<td>8 (32)</td>
<td>6 (24)</td>
<td>1 (4)</td>
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<tr>
<td>Elevated PTT</td>
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<td>0</td>
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<td>Neutropenia</td>
<td>2 (8)</td>
<td>6 (24)</td>
<td>3 (12)</td>
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<td>Thrombocytopenia</td>
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<td>0</td>
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<td>3 (12)</td>
</tr>
<tr>
<td>Lymphocytopenia</td>
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<td>3 (12)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Infections</td>
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<td>0</td>
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<tr>
<td>Nervous system</td>
<td>10 (40)</td>
<td>5 (20)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Eye</td>
<td>9 (36)</td>
<td>1 (4)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
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<td></td>
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<td>N/V/D/C</td>
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<td>6 (24)</td>
<td>2 (8)</td>
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<td>1 (4)</td>
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<td>0</td>
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<tr>
<td>Liver transaminase abnormality</td>
<td>5 (20)</td>
<td>2 (8)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>General</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever, chills, flu-like symptoms</td>
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<td>1 (4)</td>
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<tr>
<td>Edema</td>
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<td>Fatigue</td>
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<tr>
<td>Injection site reaction</td>
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<td>14 (56)</td>
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</table>

Abbreviations: Gr, adverse event grade; NS, not significant; N/V/D/C, nausea, vomiting, diarrhea, and/or constipation; PTT, prothrombin time; URTI, upper respiratory tract infection.

b There was no grade 4 or 5 adverse event seen in the study. There was no grade 3 or greater adverse event attributed to the vaccine seen in the study.

c P values were calculated by the Cochran-Armitage test for trend.

Figure 2. Progression-Free Survival in the 2 Treatment Arms

The combination treatment group underwent a median of 5 treatment cycles over 7.9 months; the docetaxel group, 3 cycles over 3.9 months (1-sided P = .09, which met the predefined statistical definition of P ≤ .10); hazard ratio, 0.65 (95% CI, 0.34-1.14). Median potential follow-up was 42.8 months.

In patients treated at MDACC, 3 (60%) of 5 in the docetaxel alone arm and 2 (33%) in the combination arm developed responses vs 9 (56%) of 16 in the combination arm (eTable and eFigure in Supplement 2).
arm developed CEA and/or MUC-1 responses. In the NCI-treated patients, 3 (30%) of 10 in the docetaxel alone arm vs 7 (70%) of 10 in the combination arm developed CEA and/or MUC-1 responses. Thus, owing to the small number of patients in each cohort, these results are descriptive only.

There was also no statistical correlation seen between the generation of TAA-specific immune responses in PBMCs and time to progression in either arm. There are several possible reasons for these findings. No differences in arms A and B were seen at baseline in CD4+, CD8+, or Tregs. The CD8+/Treg ratios increased similarly in both arms A and B after 3 cycles of chemotherapy. Both CD4− T cells and Tregs decreased in both arms after treatment. Because of a greater decrease in Tregs vs CD4+ T cells, there was an increase in the CD4+/Treg ratios in both arms. However, after 3 cycles of chemotherapy, there was a clear trend in a greater CD4+/Treg increase in the combination treatment arm (32 vs 82, \( P < .001 \)) vs the docetaxel alone arm (43 vs 102, \( P = .01 \)) (Figure 3B).

### Discussion

This trial was designed to show a trend in improved PFS in the combination docetaxel plus vaccine treatment arm vs the docetaxel alone arm. The trend of improved PFS in the combination arm (7.9 vs 3.9 months; Figure 2) thus informs a potential larger multicenter randomized trial. The trial reported herein was a dual-center study conducted at NCI and MDACC. Both centers entered approximately the same number of patients in each arm.

Some interesting observations can be made concerning patient baseline characteristics. Patients in both arms treated at MDACC had a shorter interval since last chemotherapy (median of 34 days vs a median of 192 days at NCI). And for those patients in the combination arm who received chemotherapy greater than 1 month prior to entering this trial, there was a trend toward longer PFS (median PFS, 9.4 months in arm A vs 5.8 months in arm B), although the trend did not reach statistical significance (\( P = .12 \)). This observation could be owing to at least 2 non–mutually exclusive factors: (1) patients at MDACC had more advanced or progressive disease and thus required a subsequent therapy sooner, and/or (2) the short interval since the last chemotherapy reduced the capacity of the patient to respond to vaccine therapy. This is supported by the sCD27 results seen in Figure 3A, in which patients enrolled at MDACC had a statistically significant lower pretreatment level of serum sCD27 than patients enrolled at NCI (6.4 vs 19.2 U/mL; \( P = .08 \)). Our research group has previously shown sCD27 to be a stimulator of effector T cells, and serum sCD27 levels in patients with prostate cancer were lower than those in healthy participants. The findings reported herein also support earlier findings using another vaccine (rF-CEA-TRICOM) in patients with advanced carcinoma; in that study there was a direct correlation between the length of time since last chemotherapy and immune response to vaccine.

Another factor in the interpretation of the results of the trial reported was that patients at NCI received GM-CSF along with vaccine while patients at MDACC did not. In both preclinical and
clinical studies, GM-CSF has been associated with both enhancing and suppressing immune responses. These prior findings may be related to the dose and schedule of GM-CSF used and the vaccine with which it was combined. A small phase 2 study attempted to answer the question of the use of GM-CSF in combination with a similar vaccine platform, ie, PROSTVAC, in patients with prostate cancer, but the results were inconclusive. Subsequently, an international phase 3 study of PROSTVAC vaccine in 1200 patients with asymptomatic prostate cancer has recently completed accrual (NCT01322490). In that trial, patients (n = 400 per arm) received PROSTVAC vaccine, PROSTVAC vaccine plus GM-CSF, or placebo.

In the present study, T-cell immune responses in PBMCs to the 2 transgenes (CEA and MUC-1) in the vaccine and a cascade antigen not in the vaccine (brachyury) were seen after therapy in both arms. Eleven (69%) of 16 patients developed immune responses after therapy to CEA, MUC-1, or brachyury pools of 15-mer peptides in the combination arm, and 8 (53%) of 15 patients developed these responses in the docetaxel alone arm; these results were not statistically significant. Similar responses to brachyury 15-mer peptides were seen after therapy in both arms. Although there is no clear relationship between the evaluated T cell–specific response to CEA and MUC-1 and PFS, this finding is not without precedent in the field of cancer immunotherapy. One possible explanation is that tumor-associated T cells observed in PBMCs do not necessarily reflect those at the site of the tumor. Unfortunately, it was not feasible to obtain tumor biopsy specimens in these patients with metastatic breast cancer. Another possibility is that after vaccination against target antigens (CEA and MUC-1), T cell–mediated killing results in antigen cascade, and more ideal candidate antigens are selected, resulting in expansion of a population of T cells against an unknown antigen, which was not measured in this analysis.

Because of these limitations, we explored other markers of immune activation and response as correlates of clinical benefit. Patients in the combination arm also had a greater increase in posttreatment ratio of CD4 to Tregs than patients in the docetaxel alone arm (Figure 3B). It has previously been shown that docetaxel alone has immune modulatory effects. Preclinical in vivo murine studies and in vitro studies involving murine and human T cells have shown that docetaxel can alter the phenotype of tumor cells to render them more susceptible to T-cell lysis. In the trial reported herein, this could have initiated a cascade of immune responses mediated by T cells at the tumor site.

Conclusions

We demonstrate herein the ability to safely combine vaccine therapy with a standard-of-care chemotherapy. This study was powered to detect a trend toward improvement in PFS. The results suggest that the combination therapy of PANVAC with docetaxel in metastatic breast cancer may provide a clinical benefit. The clear separation of the treatment arm curves indicates potential benefit, which meets the prespecified parameters of the trial design, but the difference was not statistically significant, likely owing to the small number of participants enrolled. The small patient population size, its heterogeneous character, and an unknown effect of GM-CSF are limitations of this trial. However, the intriguing findings in this hypothesis-generating study provide both rationale and statistical assumptions on which to base a larger, appropriately powered and designed definitive randomized clinical trial in a more uniform patient population, such as patients with ER+/PR+ tumors who have not previously received cytotoxic chemotherapy.

Invited Commentary

Chemotherapy—A Viable Partner for Cancer Immunotherapy?

Leisha A. Emens, MD, PhD

For many years, efforts to harness the power of the immune system to treat cancer were stymied by suboptimal cancer vaccine strategies based on inadequate knowledge of the host-tumor interaction. However, with newfound insights into human tumor immunology and breakthroughs in biotechnology, cancer immunotherapy is finally beginning to deliver on its promise of effective cancer treatments.

Advances in immuno-oncology began quietly several years ago, when sipuleucel-T, an autologous dendritic cell-based vaccine specific for prostatic acid phosphatase, was approved by the US Food and Drug Administration (FDA) for the treatment of metastatic prostate cancer. Next, ipilimumab, a monoclonal antibody specific for the immune checkpoint molecule CTLA-4 (cytotoxic T-lymphocyte antigen-4), was FDA approved for metastatic melanoma. Notably, although both agents improve median overall survival by about 3 to 4 months, objective clinical responses are uncommon, and neither improves progression-free survival (PFS). Most recently, long-lasting clinical responses of 10% to 50% in a broad range of solid tumors have been reported with monoclonal antibodies that target PD-1 (programmed death-1) or its ligand PD-L1. Some PD-1-specific agents are now FDA approved for metastatic melanoma (pembrolizumab and nivolumab) and advanced nonsmall-cell lung carcinoma (nivolumab). Their unprecedented clinical activity has ignited an explosion of interest in cancer immunotherapy across the oncology community. The durability of clinical responses to these agents is unique to immunotherapy and primarily reflects the induction and activation of a potent, tumor-specific memory T-cell response that mediates long-term cancer control, improving overall survival.