TBCRC 019: A Phase II Trial of Nanoparticle Albumin-Bound Paclitaxel with or without the Anti-Death Receptor 5 Monoclonal Antibody Tigatuzumab in Patients with Triple-Negative Breast Cancer

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Abstract

Purpose: Tigatuzumab (TIG), an agonistic anti-DR5 antibody, triggers apoptosis in DR5+ human tumor cells without cross-linking. TIG has strong in vitro/in vivo activity against basal-like breast cancer cells enhanced by chemotherapy agents. This study evaluates activity of TIG and chemotherapy in patients with metastatic triple-negative breast cancer (TNBC).

Experimental Design: Randomized 2:1 phase II trial of albumin-bound paclitaxel (nab-PAC) ± TIG in patients with TNBC stratified by prior chemotherapy. Patients received nab-PAC weekly × 3 ± TIG every other week, every 28 days. Primary objective was within-arm objective response rate (ORR). Secondary objectives were safety, progression-free survival (PFS), clinical benefit, and TIG immunogenicity. Metastatic research biopsies were required.

Results: Among 64 patients (60 treated; TIG/nab-PAC n = 39 and nab-PAC n = 21), there were 3 complete remissions (CR), 8 partial remissions (PR; 1 almost CR), 11 stable diseases (SD), and 17 progressive diseases (PD) in the nab-PAC arm. Grade 3 toxicities were 28% and 29%, respectively, with no grade 4–5. Exploratory analysis suggests an association of ROCK1 gene pathway activation with efficacy in the TIG/nab-PAC arm.

Conclusions: ORR and PFS were similar in both. Preclinical activity of TIG in basal-like breast cancer and prolonged PFS in few patients in the combination arm support further investigation of anti-DR5 agents. ROCK pathway activation merits further evaluation.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Introduction

Triple-negative breast cancer (TNBC) is defined by the absence of estrogen and progesterone receptors (ER/PR), and HER-2 amplification; further subclassification is being evaluated (1). TNBC represents 15% to 20% of all breast cancers (2–5) and is more frequent in younger patients, BRCA1 mutation carriers, and in specific ethnic groups such as African American women (6, 7). TNBC tumors are generally invasive ductal carcinomas and often have unfavorable features such as higher histologic grade, larger tumor size, and positive lymph nodes (8). The metastatic potential in TNBC is similar to that of other subtypes, but these tumors are associated with a shorter median time to relapse and death (9, 10). TNBC represents a significant clinical challenge, as there are no targeted drugs available; however, chemotherapy remains the mainstay of treatment, but important limitations still need to be overcome in the next few years if any significant clinical strides are to be made.

TRAIL, a member of the TNF superfamily of cytokines, is a type II membrane protein expressed in the majority of normal tissues and can undergo protease cleavage, resulting in a soluble form able to bind to TRAIL death receptors (DR; ref. 11). TRAIL induces...
Translational Relevance

The DR5 tumor cell receptor is a promising target for an antibody-based therapy, as it is expressed in solid tumors, including breast cancer. Activation of DR5 triggers apoptosis of tumor cells through activation of the extrinsic apoptotic pathway. Tigatuzumab is a novel agonistic humanized monoclonal antibody against DR5. In preclinical studies, the antibody demonstrated strong in vitro (cell lines) and in vivo (xenograft models) activity against basal-like breast cancer that is enhanced by chemotherapy agents, including albu- 
mun-bound paclitaxel (nab-PAC). Other types of breast cancer (hormone receptor and HER2-positive cancers) were resistant to tigatuzumab alone or in combination with chemotherapy. Consequently, a clinical trial with this antibody in combination with nab-PAC in patients with triple-negative breast cancer was conducted, with signs of efficacy in a subset of patients. A single arm with nab-PAC was included as there was no prior prospective experience with this agent in this patient population.

apoptosis of cancer cells in vitro and has potent tumor activity against tumor xenografts of various cancers in vivo via DRs (11). Although five receptors for TRAIL have been identified, only two (DR4 and DR5) are able to trigger apoptosis of tumor cells through activation of the extrinsic apoptotic pathway (caspase mediated; refs. 11–14). High expression of DR5 is frequently observed in various human cancers, including breast cancer (15–21). Our group has recently evaluated the phenotypic expression of DR5 in different subtypes of breast cancer; expression of DR5 was present in all triple-negative ductal breast cancer tested, including primary and metastatic tumors (data not shown).

Tigatuzumab (TIG) is the humanized version of the agonistic anti-DR5 murine monoclonal antibody TRA-8 (21–23). It is composed of the complementarity-determining region of the murine antibody and the variable region framework and constant regions of human immunoglobulin IgG1 mAb58’CL (21). TIG is able to trigger apoptosis in DR5-positive human tumor cells without the aid of crosslinking (21, 22). In preclinical studies, the antibody has demonstrated strong in vitro and in vivo activity against basal-like breast cancer cells that is enhanced by chemo-
therapy agents like paclitaxel and albumin-bound paclitaxel (nab-
PAC; refs. 22–25).

A phase I, dose-escalation study of TIG in patients with relapsed or refractory carcinomas was conducted to determine the MTD, pharmacokinetics, immunogenicity, and safety (26). Seventeen patients were enrolled in four cohorts (1, 2, 4, and 8 mg/kg). TIG was well tolerated with no infusion reactions or grade 3–4 toxicity; the MTD was not reached. Plasma half-life was 6 to 10 days, and no anti-TIG responses were detected. Seven patients had stable disease (SD), with the duration of response ranging from 81 to 798 days. Phase II studies in other solid tumors using TIG in combination with chemotherapy demonstrated the safety of the combination (27).

Thus, based on the preclinical data showing the remarkable sensitivity of basal-like breast cancer to TIG in combination with nab-PAC and the safety of TIG as single agent and in combination with chemotherapy, we conducted a randomized, phase II clinical trial, of nab-PAC with or without TIG in patients with TNBC.

Materials and Methods

Patients

Patients older than 18 years of age with histologically con-

firmed metastatic TNBC were enrolled. A tumor was considered triple negative if HER-2-neu was negative (0 or 1+ staining by IHC), and the ER and PR were negative (<1% of the tumor cells by IHC). There was no restriction as to the number of prior chemotherapy regimens for metastatic disease but patients had to have prior exposure to anthracyclines and taxanes in the neoadjuvant or adjuvant settings. Patients with prior chemotherapy for metastatic disease and patients who have received prior therapy with taxanes for metastatic disease (paclitaxel or docetaxel) were eligible. All patients had to have measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST Version 1.1), an ECOG ≤ 2, and adequate organ and bone marrow function (Supplementary Material).

 Patients previously treated with nab-PAC or with active central nervous involvement were excluded.

Study design and treatment schedule

This study was a randomized (2:1) phase II multicenter trial of nab-PAC with or without TIG in patients with metastatic TNBC. The trial was conducted through the Translational Breast Cancer Research Consortium (TBRCR); 13 sites activated the study. A treatment cycle was defined as 4 weeks. Patients received intravenous nab-PAC on days 1, 8, and 15 (100 mg/m²) at 28-days interval with or without TIG Intravenously on days 1 and 15 of every cycle (10 mg/kg loading dose followed by 5 mg/kg every other week). Response to therapy was assessed every two cycles (every 8 weeks). Treatment continued without interruption in patients with a complete response (CR) or partial response (PR) or SD until progressive disease (PD) or unacceptable toxicity. Patients with tumor progression on the nab-PAC arm were allowed to rollover to the TIG/nab-PAC arm. All patients gave informed consent to participate in the study, which was approved by local Institutional Review Boards and conducted in accordance with the ethical principles of the Declaration of Helsinki, Inter-
national Conference on Harmonization Guideline E6 for Good Clinical Practice and applicable local regulatory requirements.

Study end points

The primary efficacy end point was objective response rate (ORR) based on RECIST 1.1 criteria. Secondary efficacy end points were progression-free survival (PFS), duration of response, clinical benefit ratio (CBR), and safety of the combination. The ORR was defined as the proportion of patients who achieved best overall response of confirmed CRs and PRs. PFS was defined as the time from the date of initial treatment to the date of the first objective documentation of PD or death. The duration of response was defined as the time from the date of the first documentation of CR or PR to the date of the first documentation of PD. CBR for this protocol was defined as the percentage of patients who have achieved CR, and PR and SD for >4 cycles. Treatment-emergent adverse events were collected and reported from the time of the first dose administration of the study drugs to 30 days after the last dose administration. Toxicities were graded
Tumor sample processing

Deidentified fresh-frozen tumor tissue biopsy specimens were obtained from the University of Alabama at Birmingham’s Comprehensive Cancer Center Tissue Procurement Shared Facility (Birmingham, AL). The specimens were macro-dissected by a board-certified pathologist at the Tissue Procurement Shared Facility to enrich for tumor cell content and remove adjacent normal tissue. The dissected specimens were weighed, transferred to a 15-mL conical tube containing ceramic beads, and RLT Buffer (Qiagen) plus 1% BME was added so that the tube contained 35 μL of buffer for each milligram of tissue. The conical tubes containing tissue, ceramic beads, and buffer were agitated in a MP Biomedicals FastPrep machine at 6.5 meters per second for 90 seconds to homogenize the tissue. The homogenized tissue was stored at −80°C. Total RNA was extracted from 350 μL of tissue homogenate (equivalent to 10 mg of tissue) using the Norgen Animal Tissue RNA Purification Kit (Norgen Biotek Corporation). Cell lysate was treated with Proteinase K before it was applied to the column and on-column DNase treatment was performed according to the manufacturer’s instructions. Total RNA was eluted from the columns and quantified using the Qubit RNA Assay Kit and the Qubit 2.0 fluorometer (Invitrogen). RNA-seq libraries for each sample were constructed from 250 ng total RNA using the polyA selection and transposase-based nonstranded library construction (TruSeq RNA-seq) described previously (28). RNA-seq libraries were barcoded during PCR using Nextera barcode primers according to the manufacturer (Epicentre). The RNA-seq libraries were quantified using the Qubit dsDNA HS Assay Kit and the Qubit 2.0 fluorometer (Invitrogen) and four barcoded libraries were pooled in equimolar quantities for sequencing. The pooled libraries were sequenced on an Illumina HiSeq 2000 sequencing machine using paired-end 50 bp reads and a 6-bp index read, and we obtained at least 50 million read pairs from each library. TopHat v1.4.1 (29) was used with the options -r 100 -mate-std-dev 75 to align 50 million RNA-seq read pairs, and used GENCODE version 9 (30) as a transcript reference. Gene expression values (Fragments Per Kilobase of transcript per Million reads; FPKM) were calculated for each GENCODE transcript using Cufflinks 1.3.0 with the -u option (31).

Statistical analysis

There were no prior data on ORR of nab-PAC in this patient population although a trial of nab-PAC in patients with therapy-resistant tumors had a 14% ORR in the TNBC patient subgroup (32). Therefore, the sample size calculation was based on estimation of ORR. With an accrual of 40 patients to the TIG/nab-PAC regimen, the ORR estimation would have a standard error of less than 7.5% if one assumes the ORR is between 20% and 35%; the estimated two-sided 95% confidence intervals (CI) would be 21.2% to 51.7% for an ORR of 35% with Blythe-Still-Casella Exact Method, and 9.4% to 34.4% if the ORRs were 20%. In the single-agent arm with 20 patients, the ORR would have a standard error of 8.9%; two-sided 95% CI would be 7.1% to 41.1% for an ORR of 20% using the same method.

Patients were randomized in the trial as 2:1 ratio and stratified by patients’ prior chemotherapy. All randomly assigned patients were included in the intent-to-treat efficacy analysis and safety analysis. Descriptive analysis for patients demographic and clinical characteristics such as means, medians, and ranges were used to describe continuous variables. Frequency and proportion were used to describe categorical variables. The Fisher exact test was used to examine two portions in 2 by 2 contingency table. Survival distributions for PFS were estimated using the Kaplan–Meier method and were compared with long-rank tests stratified by stratification factors. Two-sided 95% CIs for the median survival time were constructed using a nonparametric method. (33)

A modified Gehan’s two-stage design was used in the trial (34) to minimize exposure to ineffective therapy, at least one patient in the first 11 patients enrolled per arm had to have a CR or PR in order to complete enrollment in that arm. A safety interim analysis was scheduled to be done after the first 6 patients enrolled in the TIG/nab-PAC arm (Supplementary Material).

RNA-seq Gene Expression Analysis of Tumor Biopsy Tissue: DESeq2 (35) was used to analyze gene count data to identify genes whose expression was significantly associated with response to therapy. The DESeq2 nbinomLRT function was used to identify genes that were significantly differentially expressed between two classes: Class 1 contained patients who achieved CR or PR, Class 2 contained patients who had SD or PD. We also identified genes that were significantly associated with response criteria when response was represented as a quantitative variable ranging from CR (1) to PR (2) to SD (3) to PD (4). The significant genes (FDR < 0.05) were filtered to identify genes whose maximum FPKM expression value across samples was greater than or equal to 1.

Results

Patients

Sixty-four patients were enrolled; 42 in the TIG/nab-PAC arm and 22 in the nab-PAC arm (Table 1). All patients gave signed informed consent, and 60 patients received at least one cycle of therapy. In the TIG/nab-PAC arm, the median age for the patients was 51 years (range, 32–72). 33% were African American. 33% had no prior chemotherapy in the metastatic setting, and the median number of prior therapy regimens was 2 (range, 0–5). The nab-PAC arm had similar characteristics; in those patients the median age was 51 years (range, 34–75), 27% were African American, 32% had no prior chemotherapy in the metastatic setting, and the median number of prior chemotherapy regimens was 1 (range, 0–4). All patients had an ECOG of ≤2.
Thus, proportionally more patients in the combination arm had progression in the brain without progression of systemic metastasis (liver, lung, bone).

Efficacy

Of the 42 patients in the TIG/nab-PAC arm, 39 received at least one cycle of therapy and were eligible for evaluation of response (3 patients had PD before initiation of therapy); of the 22 patients in the nab-PAC arm, 21 patients were treated and were eligible for evaluation of response (1 patient had PD before initiation of therapy). At least one PR was seen in the first 11 patients treated in each arm and accrual continued to completion. Eleven patients progressed before the first protocol-specified evaluation of response.

In the TIG/nab-PAC arm, there were 3 CRs, 8 PRs (1 patient had a near CR with 96% reduction in the index lesions) with an ORR of 28% (95% exact CI, 14.9%–45.0%). The median PFS for the patients enrolled in the TIG/nab-PAC arm was 2.8 months (95% CI, 1.9–3.6; Table 2 and Fig. 1A) and 3.8 months in patients with objective response (95% CI, 2.8–19.7). Sixteen of the 39 patients (41%) in the TIG/nab-PAC arm achieved clinical benefit. There were 5 patients in the TIG/nab-PAC arm with long PFS, including 1 near CR (460 days), and 1 SD (334 days). Four of the 11 patients who achieved CR or PR in the TIG/nab-PAC arm had progression in the brain but no systemic progression.

Although the study was not designed for statistical comparison of the two treatment arms, the control arm (single-agent nab-PAC) had similar overall efficacy as combination therapy with an ORR of 38% (95% CI, exact 18%–61.1%), no CRs, and 8 PRs. Clinical benefit was noted in 11 patients (52%) enrolled in the nab-PAC arm (Table 2). The median PFS for patients enrolled in the nab-PAC arm was 3.7 months (95% CI, 2.3–5.7; Fig. 1A), and long-term PFS occurred in 1 patient (1004+ days). Two additional patients had PFS for 224 and 220 days. Thus, proportionally more patients in the combination arm experienced prolonged clinical benefit [5 out of 39 (13%) vs. 1 out of 21 (5%) patients]. No objective responders in the nab-PAC arm had progression in the brain without progression of index lesions. Only 8 patients crossover to the TIG/nab-PAC after progression in the nab-PAC arm; no responses were seen in those patients.

Patient demographics and efficacy

We examined the effect of patient demographics and prior therapy on the whole patient population since outcomes were similar in the two arms (Table 3). Chemotherapy naïve patients had an increased ORR [53% (95% CI, exact 31%–76.3%) vs. 22% (95% CI, exact 10.5%–40.1%), respectively] and decreased PD rate (26 vs. 51%, respectively). PFS was not significantly greater (3.6 vs. 2.5 months; Fig. 1B) while the median duration of the response was 137 days (range, 84–1,025+ days) and 174 days (range, 111–1,004+ days), respectively. Among the 19 patients who were chemotherapy naïve in the metastatic setting, 53% had objective response, 68% had clinical benefit, and PFS of 3.6 months (95% CI, 2.8–5.6) compared with 22%, 34%, and 2.5 months (95% CI, 1.9–3.7) for those patients that received prior chemotherapy in the metastatic setting. We found no differences in efficacy for other factors, including race (white vs. black), age (less than or greater than 50), tumor behavior (less than or greater than 2 years between primary tumor and relapse), or superficial extent (breast, soft tissue, lymph nodes) versus systemic metastasis (liver, lung, bone).

Safety

Thirty-nine patients in the TIG/nab-PAC arm and 21 in the nab-PAC arm received at least one cycle of therapy and were eligible for toxicity evaluation (Table 4). No adverse or serious adverse events (AE/SAE) related with the research agent were seen in the first 6 patients treated in the TIG/nab-PAC arm and accrual continued to completion.

Therapy in both arms was well tolerated; the majority of the AEs were grade 1–2 with very few grade 3 events and no grade 4/5 toxicity. There were no AEs or SAEs associated with TIG infusions. The most common AEs observed in at least 10% of all patients enrolled in the trial deemed by the investigators to be possibly related with the protocol therapy were fatigue (54%), alopecia (49%), peripheral sensory neuropathy (44%), anemia (41%), neutropenia (38%), nausea (23%), thrombocytopenia (10%), anorexia (10%), diarrhea (10%), and vomiting (10%). As expected, due to the use of nab-PAC, the most frequent grade 3 AEs were neutropenia (15%), fatigue (10%), anemia (2%), and peripheral sensory neuropathy (2%). The addition of TIG did not change the safety profile nab-PAC. The most frequent AE seen in the TIG/nab-PAC arm, excluding alopecia, was fatigue, whereas the most frequently seen in the nab-PAC arm was peripheral sensory neuropathy.

Forty-two SAEs were reported; 4 were classified as possibly related with the protocol therapy and 38 associated with PD. The 2

<table>
<thead>
<tr>
<th>Table 1. Patient demographics</th>
<th>TIG/nab-PAC (N = 42)</th>
<th>nab-PAC (N = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race</td>
<td></td>
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</tr>
<tr>
<td>White</td>
<td>26 (63%)</td>
<td>16 (73%)</td>
</tr>
<tr>
<td>Black</td>
<td>14 (33%)</td>
<td>6 (27%)</td>
</tr>
<tr>
<td>American Indian or Alaska native</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (min–max)</td>
<td>51 (32–72)</td>
<td>50.5 (34–75)</td>
</tr>
<tr>
<td>Prior treatment in metastatic setting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No prior chemotherapy</td>
<td>14 (33%)</td>
<td>7 (32%)</td>
</tr>
<tr>
<td>Chemotherapy but no taxane</td>
<td>16 (39%)</td>
<td>10 (45%)</td>
</tr>
<tr>
<td>Chemotherapy with a taxane</td>
<td>12 (28%)</td>
<td>5 (23%)</td>
</tr>
<tr>
<td>Median of chemotherapy regimens*, n (range)</td>
<td>2 (0–5)</td>
<td>1 (0–4)</td>
</tr>
</tbody>
</table>

*Chemotherapy in the metastatic setting.

<table>
<thead>
<tr>
<th>Table 2. Efficacy data</th>
<th>TIG/nab-PAC (n = 39)</th>
<th>nab-PAC (n = 21)</th>
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</thead>
<tbody>
<tr>
<td>Best response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>3 (8%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Partial response</td>
<td>8 (21%)</td>
<td>8 (38%)</td>
</tr>
<tr>
<td>Objective response</td>
<td>11 (28%; 95% CI, 14.9%–45%)</td>
<td>8 (38%; 95% CI, 18%–61.1%)</td>
</tr>
<tr>
<td>Stable disease</td>
<td>11 (28%)</td>
<td>4 (19%)</td>
</tr>
<tr>
<td>Clinical benefit rate (&gt;4 cycles)</td>
<td>16 (41%)</td>
<td>11 (52%)</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>17 (44%)</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>Median duration of response, days (range)</td>
<td>118+ (84 to 1,025+)</td>
<td>167+ (91 to 1,004+)</td>
</tr>
<tr>
<td>Median PFS, mo</td>
<td>2.8 (95% CI, 1.9–3.6)</td>
<td>3.7 (95% CI, 2.5–5.7)</td>
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</tbody>
</table>

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biopsied, 31 (48%) were judged adequate by macro-dissection, and 28 (44%) had appropriate DNA/RNA yield for the study. Of the 28 samples, two were from patients who had PD before therapy, 20 received combination therapy (5 patients with CR/PR, 7 patients with SD, and 8 patients with PD) and 6 received single-agent nab-PAC (3 patients with PR and 3 with PD). Seventeen of the 28 patients had only a single tissue sample adequate for DNA/RNA analysis, whereas 11 of 28 had multiple adequate DNA/RNA samples. The most common biopsy sites for tissue inadequacy were nodes and soft tissue. The reason for tissue macro-dissection failure was extensive necrosis in 50% and absence of tumor cells (benign tissue) in 50% of the specimens. This 40% yield of tissue analysis in treated patients limits the genomic analysis but the 28 metastatic tissues will be extremely valuable in studies relevant to metastatic TNBC.

RNA-seq (28) was used to measure gene expression in the tumors. Each tumor was classified as belonging to one of the six Vanderbilt TNBC subtypes (36, 37). There was no significant association between subtypes and response to therapy. Expression of all genes was examined and seven were significantly associated with response in the TIG/nab-PAC (FDR < 0.05): ACTA2, DNM3, FBXO32, IFFO2, LIMK2, MYLK, and ZNF469. All seven genes were expressed at a higher level in tumors from patients who responded to the combination therapy compared with those who did not. Several of these genes are involved in apoptotic membrane blebbing through DR5/Casp-3/ROCK1 signaling (Fig. 2 and Supplementary Fig. S1 in Supplementary Data). Activation of DR5 leads to activation of caspase-3, which cleaves and activates ROCK1. ROCK1 phosphorylates and inhibits MLCP leading to unopposed MYLK phosphorylation of MLC, which catalyzes the generation of an actin–myosin contractile force that causes blebbing (38). ROCK1 also phosphorylates and activates LIMK2 which leads to the accumulation and stabilization of actin filaments, such as those composed of ACTA2, involved in constriction of the cytoskeleton and apoptotic membrane blebbing (39, 40). DNM3 is a member of the dynamin family that interacts with actin membrane processes and is responsible for constricting and releasing membrane vesicles. Thus, four of the seven genes significantly associated with response to TIG/nab-PAC are associated with the membrane blebbing process. This enrichment suggests that higher expression of this apoptotic pathway could be related to sensitivity to one or both of these drugs. Although the number of cases in the nab-PAC arm were very limited (6 patients), the expression of these seven genes was examined in tumors from those patients; these

<table>
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<th>Table 3. Prior therapy effect on efficacy data</th>
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<tr>
<td></td>
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<tr>
<td>Complete response</td>
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<tr>
<td>Partial response</td>
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<tr>
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<tr>
<td>Progressive disease</td>
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<tr>
<td>Median duration of response, days (range)</td>
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<td>Median PFS, mo</td>
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</table>

*Initial evaluation at day 56 (two cycles of therapy).

**CR and PR and stable disease greater than four cycles of therapy.

*P < 0.0347 (Fisher exact test).
genes were not positively correlated with response to nab-PAC.

**Discussion**

This trial was undertaken based on the preclinical studies which indicated that basal-like breast cancer cells were highly sensitive to anti-DR5 (22–25), that the combination of an anti-DR5 monoclonal antibody and chemotherapy was quite effective in murine models of basal-type breast cancer in vivo (24) and that basal-type breast tumor stem cells were killed by anti-DR5 (25). Similar studies in hormone-dependent and HER2-positive breast cancer demonstrated resistance to anti-DR5 therapy (22–25).

At the time of this protocol design, it was not feasible to use platinum compounds as the chemotherapy backbone of our study in view of the expanded access program that was available for this study.

![Figure 2](image-url)

Figure 2.
Apoptotic membrane blebbing through the DR5/Casp-3/ROCK1 signaling pathway. Genes associated with response to treatment with nab-PAC and TIG are highlighted in orange.

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**Table 4. Adverse events related to protocol therapy**

<table>
<thead>
<tr>
<th>Adverse events related to protocol therapy seen in more than 10% of all patients (%)</th>
<th>Toxicity grade</th>
<th>Nab-PAC (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>33 (54)</td>
<td>14 (23)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>30 (49)</td>
<td>11 (28)</td>
</tr>
<tr>
<td>Peripheral sensory neuropathy</td>
<td>27 (44)</td>
<td>13 (33)</td>
</tr>
<tr>
<td>Anemia</td>
<td>25 (41)</td>
<td>8 (21)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>23 (38)</td>
<td>5 (13)</td>
</tr>
<tr>
<td>Nausea</td>
<td>14 (23)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>6 (10)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>6 (10)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6 (10)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>6 (10)</td>
<td>2 (5)</td>
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</tbody>
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<th>Toxicity grade</th>
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<td>Fatigue</td>
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<tr>
<td>Alopecia</td>
<td>30 (49)</td>
</tr>
<tr>
<td>Peripheral sensory neuropathy</td>
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<td>6 (10)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>6 (10)</td>
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then for the combination of carboplatin, gemcitabine, and ini-
parib for patients with newly diagnosed metastatic TNBC follow-
ing a promising phase II randomized trial of chemotherapy with/
without iniparib (41). In addition, there was no prior prospective
experience with nab-PAC in this patient population although
heavily pretreated TNBC patients appeared to have a 14% 
response rate in retrospective analysis of nab-PAC as single agent
in patients with metastatic breast cancer. Thus, we designed a
randomized phase II to obtain a reasonable measure of efficacy
with the combination arm (ORR in 40 patients with a standard
error < 7.5%). In addition, we included a single-agent nab-PAC
arm as a frame of reference for this patient population.

The outcome of the trial was that the combination arm had an
ORR of 28% and PFS of 2.8 months. The experience was similar in
the concurrent single arm with ORR of 38% and PFS of 3.7
months. This experience did not support moving forward with
this current combination regimen in the same population of
patients. Despite the negative overall trial findings, we did note
that the combination arm included 3 CRs and 1 near CR, whereas
no CRs occurred in the single-agent arm. In addition, propor-
tionally more patients in the combination arm experienced pro-
longed clinical benefit [5 out of 39 (13%) vs. 1 out of 21 (5%)
patients]. nab-PAC was associated with an unexpectedly high rate
of objective response in patients with TNBC, reinforcing the need
for a reference arm in our trial design; unfortunately, as with other
agents evaluated in this patient population, responses were often
not durable. A new anti-DR5 monoclonal antibody (DS8273
from Daiichi Sankyo) has shown better preclinical activity than
TIG as a single agent in combination with chemotherapy and is
now being evaluated in a phase I trial (NCT02076451).

Metastatic TNBC is an aggressive disease as illustrated in our
trial with 4 enrolled patients having progression before initiation
of therapy and 26 of 60 (43%) of treated patients had progression
before or at their initial evaluation (8 weeks). Patients with no
prior therapy for metastatic disease experienced a higher ORR and
clinical benefit rate.

Our experience with core needle biopsies for genomic studies
is informative in designing future correlative studies within
trials. First, trials should be designed for patients with access-
sible metastases and biopsies should be required (100% biop-
sies). Second, duplicate biopsies would increase the yield of
appropriate tissue samples and third, incisional biopsies on
superficial metastatic sites (chest wall, breast, and lymphatic
nodes) should be considered. Also, standard for needle biop-
sies should be considered (e.g., ≥1.0 cm in length). CTCs were
collected and the complete analysis of the data is presented in a
companion article by Paolletti and colleagues (see p. 2771).

CTCs were detected in approximately one-third of the patients.
Elevated CTCs at baseline and days 15 and 29 were prognostic,
companion article by Paoletti and colleagues (see p. 2771).

Additionally, more patients in the TIG/nab-PAC combination arm
experienced progression-free survival (PFS) of 3.7 months. This
experience did not support moving forward with this current com-
ination regimen in the same population of patients. The combina-
tion arm included 3 CRs and 1 near CR, whereas no CRs occurred
in the single-agent arm. In addition, proportionally more patients
in the combination arm experienced prolonged clinical benefit
[5 out of 39 (13%) vs. 1 out of 21 (5%) patients]. nab-PAC was associated
with an unexpectedly high rate of objective response in patients
with TNBC, reinforcing the need for a reference arm in our trial
design; unfortunately, as with other agents evaluated in this
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CTCs were detected in approximately one-third of the patients.
Elevated CTCs at baseline and days 15 and 29 were prognostic,
and reductions in CTC levels reflected response.

Finally, our genomic analysis (RNA-seq) relating to therapeutic
response was limited due to small numbers of patient tissues, with
20 samples in the combination therapy arm and six samples in the
single-agent nab-PAC arm. In the combination arm, efficacy was
significantly associated with elevated levels of seven genes, includ-
ing four of which participate in DR5-mediated ROCK1 activation
of apoptosis-associated membrane blebbing. This is an important
and interesting observation; interpretation is tempered by limited
patient samples.

In conclusion, the high degree of anti-DR5 sensitivity of basal-
like breast cancer cell lines compared with other tumor cell lines
and the prolonged PFS in a few patients in the TIG/nab-PAC
suggest that DR5-mediated therapy deserves further investigation
with novel, more efficacious, anti-DR5 agents.

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M.C. Liu reports receiving research grants from Celgene. T.A. Traina is a
consultant/advisory board member for Celgene. No potential conflicts of
interest were disclosed by the other authors.

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