*Cancer Biology and Signal Transduction*

Molecular Cancer Therapeutics

# Triple-Negative Breast Cancer Patients Treated at MD Anderson Cancer Center in Phase I Trials: Improved Outcomes with Combination Chemotherapy and Targeted Agents

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## Abstract

Patients with metastatic triple-negative breast cancer (TNBC) have poor treatment outcomes. We reviewed the electronic records of consecutive patients with metastatic TNBC treated in phase I clinic at MD Anderson Cancer Center (Houston, TX) between Augu st 2005 and May 2012. One hundred and six patients received at least 1 phase I trial. Twelve of 98 evaluable patients (12%) had either complete response (CR; $n = 1$), partial response (PR; $n = 7$), or stable disease ≥6 months (SD; $n = 4$). Patients treated on matched therapy ($n = 16$) compared with those on nonmatched therapy ($n = 90$) had improved SD ≥ 6 months/PR/CR (33% vs. 8%; $P = 0.018$) and longer progression-free survival (PFS; median, 6.4 vs. 1.9 months; $P = 0.001$). Eleven of 57 evaluable patients (19%) treated with combination chemotherapy and targeted therapy had SD ≥ 6 months/PR/CR versus 1 of 41 evaluable patients (2%) treated on other phase I trials ($P = 0.013$), and longer PFS (3.0 vs. 1.6 months; $P < 0.0001$). Patients with molecular alterations in the PI3K/AKT/mTOR pathway treated on matched therapy ($n = 16$) had improved PFS compared with those with and without molecular alterations treated on nonmatched therapy ($n = 27$; 6.4 vs. 3.2 months; $P = 0.036$). On multivariate analysis, improved PFS was associated with treatment with combined chemotherapy and targeted agents ($P = 0.0002$), ≤2 metastatic sites ($P = 0.003$), therapy with PI3K/AKT/mTOR inhibitors for those with cognate pathway abnormalities ($P = 0.018$), and treatment with antiangiogenic agents ($P = 0.023$). In summary, combinations of chemotherapy and angiogenesis and/or PI3K/AKT/mTOR inhibitors demonstrated improved outcomes in patients with metastatic TNBC. *Mol Cancer Ther; 13(12); 3175–84. ©2014 AACR.*

## Introduction

Patients with metastatic triple-negative breast cancer (TNBC), comprising 12% to 17% of breast cancers, have poor outcomes due to the aggressive nature of the disease associated with a high proliferation index (1). The majority of these patients do not respond well to conventional systemic therapy (2) and to date, there have been no clear targets identified for effective treatment. Patients with TNBC have a median overall survival (OS) of 6 months from the time of initial diagnosis with metastatic disease versus 20 months for those patients with hormone receptor–positive and/or Her2-positive metastatic breast cancer (2). While impressive gains have been realized in the outcomes of metastatic Her2-positive and hormone receptor–positive breast cancer, TNBC remains an unmet need.

Recent studies have identified molecular subtypes within TNBC that have increased our understanding of the biologic heterogeneity of the disease and have suggested further therapeutic potential targets for evaluation (3). However, in this era of personalized medicine (4), there have been no trials of targeted agents demonstrating significant benefit for patients with any subtype of TNBC (5). A phase III trial of iniparib failed to show benefit in unselected patients with TNBC (6). A subset analysis, as well as early stage PARP inhibitor trials suggest that patients with *BRCA1*- or *BRCA2*-mutant TNBC may

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benefit, contrary to those with *BRCA1* or *2* normal metastatic TNBC (7). Neoadjuvant studies with mTOR inhibitors in TNBC have also failed to show benefit (8).

We analyzed the clinical, pathologic and molecular characteristics, and treatment outcomes of patients with metastatic TNBC treated at the Clinical Center for Targeted Therapy (phase I clinic) at The University of Texas MD Anderson Cancer Center (MDACC, Houston, TX). Our objectives were to analyze associations between treatments and outcomes, including SD $\geq$ 6 months/PR (partial response)/CR (complete response), in patients with metastatic TNBC and to identify potential biomarkers of clinical benefit. Herein, we report our experience with these patients.

## Materials and Methods

### Patients

We retrospectively reviewed the medical records of consecutive patients with advanced or metastatic TNBC, who were treated on at least one phase I clinical trial at the Clinical Center for Targeted Therapy at MDACC between August 2005 and May 2012. All patients provided written informed consent before enrollment on a clinical trial, and all clinical trials were approved by the MDACC Institutional Review Board.

### Treatment

Patients were enrolled in a clinical trial after their clinical, laboratory, and pathologic data were reviewed. The assignment of a patient to a clinical trial varied over time based on the availability of the protocol, eligibility criteria, molecular profile of tumor tissue, insurance coverage, and preference of the patient or the choice of the physician.

### Definition of "matched" therapy

A phase I clinical trial was considered to be "matched" to a patient if at least one drug in the regimen was known to inhibit the functional activity of one of the molecular alterations in the tumor tissue of the patient at low nmol/L concentrations. Patients with actionable molecular alterations were preferably treated on "matched" therapy when available, if they met the eligibility criteria and were willing to comply with study requirements. If patients did not have molecular alterations or were not tested, they were considered to be treated on "nonmatched" therapies.

### Evaluation

Assessments, including history, physical examination, and laboratory evaluations, were performed as specified in each protocol, typically before the initiation of therapy and, at a minimum, at the beginning of each new treatment cycle. Response was assessed using computed tomography scans and/or magnetic resonance imaging at baseline before treatment initiation and then every two cycles or as specified by protocol. All radiographs were read in the Department of Radiology at MDACC and reviewed in the Department of Investigational Cancer Therapeutics tumor measurement clinic. Responses were categorized per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 (9) and were reported as best response during the whole evaluation period. Patients enrolled in phase I trials continued treatment until disease progression, unacceptable toxicity, withdrawal of consent, or at the discretion of the treating physician.

For the purpose of this analysis, electronic patient records were reviewed for medical history, laboratory results, molecular profiling data, and treatment outcome. Baseline characteristics collected were age, gender, tumor histology, Eastern Cooperative Oncology Group (ECOG) performance status, number of metastatic sites, serum albumin and lactate dehydrogenase (LDH) levels, prior systemic therapies for metastatic disease, best response to investigational phase I therapy based on RECIST, and date of death or date of last follow-up. The first phase I therapy received by the patient in our clinic was considered for this analysis.

The Royal Marsden Hospital score (RMH score; refs. 10, 11) and the MDACC score (12) were used to evaluate the prognostic status of the patients. The RMH score classified patients according to three variables: LDH normal (0) versus LDH > upper limit of normal (ULN; +1); albumin $\geq$3.5 g/dL (0) versus albumin < 3.5 g/dL (+1), and number of metastatic sites of disease $\leq$2 (0) versus metastatic sites of disease $\geq$3 (+1). The MDACC score included two additional variables namely, ECOG performance status < 1 (0) versus ECOG performance status $\geq$ 1 (+1), and nongastrointestinal tumor type (0) versus gastrointestinal tumor type (1).

### Molecular profiling studies

"Hotspot" mutation analyses were carried out in selected patients where tissue was available. These were done on archival formalin-fixed, paraffin-embedded tissue blocks or material from fine-needle aspiration biopsy obtained from diagnostic and/or therapeutic procedures. Pathology was centrally reviewed at MDACC. All testing was carried out in a Clinical Laboratory Improvement Amendment (CLIA)-certified Molecular Diagnostic Laboratory within the Division of Pathology and Laboratory Medicine at MDACC. DNA was extracted from microdissected, paraffin-embedded tumor sections and further studied using a PCR-based DNA sequencing method for *PIK3CA* mutations in codons (c) 532 to c554 of exon 9 (helical domain) and c1011 to 1062 of exon 20 (kinase domain), which included the mutation hotspot region of the *PIK3CA* proto-oncogene by Sanger sequencing after amplification of 276 and 198 base pair amplicons, respectively, using primers designed by the MD Anderson Molecular Diagnostic Laboratory. After January 2011, the assay used was mass spectrometric detection (Sequenom MassARRAY) to screen for the mutational hot spots in

exon 1 (Q60K, R88Q, E110K, and K111N), exon 4 (N345K), exon 6 (S405S), exon 7 (E418K, C420R, E453K), exon 9 [P539R, E542 (bases 1 and 2), E545 (all 3 bases), and Q546 (base 1 and 2], exon 18 (F909L), and exon 20 [Y1021 (base 1 and 2), T1025 (base 1), M1043I, M1043V, A1046V, H1047Y, H1047R, H1047L, G1049R]. The mutations identified during the initial screening were confirmed by Sanger sequencing assay. The lower limit of detection is approximately 10%. Archival samples were tested for PTEN expression by immunohistochemistry (IHC). PTEN immunostaining was performed in the MD Anderson IHC CLIA laboratory with the following antibody: PTEN (Dako #M3627, 1:100, 15 minutes). Samples with complete loss of PTEN staining were considered as PTEN loss. In addition, whenever possible, mutation analyses for *BRAF* (exon 15: codons 595–600), *KRAS* and *NRAS* (exon 2: codons 12, 13, and 61), *KIT* (exons 9, 11, 13, and 17) and *GNAQ* (exon 5), and *TP53* (exons 4–9) were carried out using PCR-based DNA sequencing mutation, as previously described (13).

In addition, tissues from 9 patients were submitted to CLIA-certified laboratory where next-generation sequencing (NGS) of 3,320 exons of 182 cancer-related genes and the introns of 14 genes frequently rearranged in cancer was performed (Foundation Medicine, Cambridge, MA).

### Statistical analysis

Statistical analysis was verified by our statistician (J. Jack Lee). Patient characteristics were summarized using descriptive statistics. The Fisher exact test was used to determine associations between categorical variables and responses (SD $\geq$ 6 months/PR/CR). Multivariable logistic regression was used to identify predictors of response. Progression-free survival (PFS) was defined as the time interval from the start of therapy to the first observation of disease progression or death, whichever occurred first. For PFS, patients were censored at the time of their last follow-up date if they were progression-free. Overall survival (OS) was measured from the date of starting treatment on the first phase I therapy until death from any cause or last follow-up. Patients were censored at the time of their last follow-up if they were alive. PFS and OS were estimated using the Kaplan–Meier method (14), and the survival function between groups was compared using a two-sided log-rank test. The multivariable Cox proportional hazards regression model was used to examine risk factors related to PFS and OS after adjusting for other factors (15).

The following covariates were included in the analyses: age ($\leq$60 vs. >60), histology (invasive ductal carcinoma vs. noninvasive ductal carcinoma), number of prior therapies in metastatic setting (<3 vs. $\geq$3), history of thromboembolism (yes vs. no), metastatic sites ($\leq$2 vs. >2), ECOG performance status (0 vs. $\geq$1), LDH levels ($\leq$618 vs. >618 IU/L), albumin levels (<3.5 vs. $\geq$3.5 g/dL), RMH score ($\leq$1 vs. >1), MDACC score ($\leq$2 vs. >2), phase I therapy (combination that included chemotherapeutic and targeted agent vs. chemotherapeutic or targeted agent only), use of PI3K pathway inhibitors (yes vs. no), use of antiangiogenic agents (yes vs. no), and type of phase I therapy (matched vs. nonmatched therapy). All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. Waterfall plot analysis was used to graph individual patients' best response on protocol treatments. Statistical analyses were carried out using SPSS (version 19.0; SPSS).

## Results

### Baseline patient characteristics

A total of 106 consecutive patients (105 female and 1 male) with advanced or metastatic TNBC, who were treated on at least one phase I clinical trial were included in the analysis. Baseline characteristics are presented in Table 1. The median age was 51 years (range, 27–81 years). The median number of previous systemic anticancer treatments in the metastatic setting was 2 (range, 0–10) and the median number of metastatic sites present at the time of phase I trial initiation was 3 (range, 1–8).

### Molecular testing and next-generation sequencing

Molecular testing was not done in all patients due to limited tissue availability. Molecular testing data were available in 47 patients, including 9 with NGS analysis. Molecular alterations in the PI3K/AKT/mTOR pathway were noted in 21 of 43 patients (49%) tested, including PTEN loss by IHC ($n = 8/30$ tested), *PIK3CA* mutation ($n = 7/40$ tested), *PTEN* mutation ($n = 3/12$ tested), *PTEN* deletion ($n = 2/12$ tested), *PIK3R1* mutation ($n = 2/9$ tested), and *NF2* mutation ($n = 1/9$ tested). These patients had at least one gene in this pathway evaluated.

Molecular evaluation of the 9 patients with NGS profiling of their tumors demonstrated a median of 3 (range, 0–6) alterations per patient with 4 of 9 having $\geq$5 molecular alterations, including *TP53* mutation ($n = 8$), *MYC* amplification ($n = 4$), *PIK3R1* mutation ($n = 2$), *FGFR2*, *MCL1*, and *CCND1* amplification ($n = 2$ each), mutations in *NF2, PTEN, KDM6A*, and *RB1* ($n = 1$ each), amplifications in *FGFR1*, *PIK3CA*, *CDK8*, *MAP2K2*, and *KRAS* ($n = 1$ each), and *PTEN* deletion ($n = 1$).

Other molecular alterations seen in these patients were *NRAS* mutation in 1 of 24 and *TP53* mutation in 10 of 13. Thirty-five patients assessed for *KRAS* mutation, 30 for *BRAF* mutation, 21 for *c-KIT* mutation, 12 for *GNAQ* mutation, and 32 for *EGFR* mutation were all negative for alterations.

### Treatment

All 106 patients were treated on at least 1 phase I clinical trial, including chemotherapy only ($n = 8$), combination chemotherapy and targeted therapy ($n = 62$), single-agent targeted therapy ($n = 16$), and targeted therapy with 2 or more agents ($n = 20$; Table 2 and Supplementary Table S1). Nineteen patients (18%) patients received treatment on

**Table 1.** Baseline patient characteristics of 106 patients with metastatic TNBC

| Variable | Group | Chemo + targeted agents ($n = 63$) | % | Others[d] ($n = 43$) | % | Total ($n = 106$) | % |
|---|---|---|---|---|---|---|---|
| Age | ≤60 years | 49 | 78 | 31 | 72 | 80 | 75 |
| | >60 years | 14 | 22 | 12 | 28 | 26 | 25 |
| Sex | Female | 63 | 100 | 42 | 98 | 105 | 99 |
| | Male | 0 | 0 | 1 | 2 | 1 | 1 |
| Ethnicity | White | 50 | 79 | 35 | 81 | 85 | 80 |
| | African-American | 9 | 14 | 2 | 5 | 11 | 10 |
| | Hispanic | 3 | 5 | 6 | 14 | 9 | 8 |
| | Asian | 1 | 2 | 0 | 0 | 1 | 1 |
| Histology | Invasive ductal carcinoma | 44 | 70 | 39 | 91 | 83 | 78 |
| | Metaplastic | 15 | 24 | 2 | 5 | 17 | 16 |
| | Inflammatory | 3 | 5 | 1 | 2 | 4 | 4 |
| | Other[c] | 1 | 2 | 1 | 2 | 2 | 2 |
| Metastatic sites | ≤2 | 27 | 43 | 19 | 44 | 46 | 43 |
| | >2 | 36 | 57 | 24 | 56 | 60 | 57 |
| ECOG PS | 0 | 4 | 6 | 9 | 21 | 13 | 12 |
| | 1 | 58 | 92 | 33 | 77 | 91 | 86 |
| | 2 | 1 | 2 | 1 | 2 | 2 | 2 |
| Serum LDH | ≤618 U/L | 33 | 52 | 21 | 49 | 54 | 51 |
| | >618 U/L | 30 | 48 | 22 | 51 | 52 | 49 |
| Serum albumin | <3.5 g/dL | 10 | 16 | 2 | 5 | 12 | 11 |
| | ≥3.5 g/dL | 53 | 84 | 41 | 95 | 94 | 89 |
| RMH score[a] | ≤1 (low risk) | 42 | 67 | 29 | 67 | 71 | 67 |
| | >1 (high risk) | 21 | 33 | 14 | 33 | 35 | 33 |
| MDACC score[b] | ≤2 (low risk) | 42 | 67 | 30 | 70 | 72 | 68 |
| | >2 (high risk) | 21 | 33 | 13 | 30 | 34 | 32 |
| History of thromboembolism | No | 59 | 94 | 38 | 88 | 97 | 92 |
| | Yes | 4 | 6 | 5 | 12 | 9 | 8 |
| Prior therapies in | <3 | 29 | 46 | 16 | 37 | 45 | 42 |
| Metastatic setting | ≥3 | 34 | 54 | 27 | 63 | 61 | 58 |

Abbreviation: PS, performance status.

[a]RMH score classified patients according to three variables: LDH normal (0) versus LDH > ULN (+1); albumin ≥3.5 g/dL (0) versus albumin <3.5 g/dL (+1) and number of metastatic sites of disease ≤2 (0) versus metastatic sites of disease ≥3 (+1).

[b]MDACC score included two additional variables to that of RMH score, namely, ECOG performance status <1 (0) versus ECOG performance status ≥1 (+1), and nongastrointestinal tumor type (0) versus gastrointestinal tumor type (1).

[c]One patient with adenoid cystic breast carcinoma and another with lobular carcinoma.

[d]Includes patients treated with either chemotherapeutic agents or targeted agents only.

more than 1 phase trial. Fourteen patients (13%) received 2 trials, 4 (4%) received 3 trials, and 1 received 5 trials. Sixty-three of 106 patients (59%) received a phase I trial with combination chemotherapy and targeted therapy. Of the 106 patients, 16 (15%) were treated on matched therapies. Thirty-nine of 106 patients (37%) received protocols containing drugs targeting the PI3K/AKT/mTOR pathway [37/39 (95%) included mTOR inhibitors, 1 (3%) a PI3K inhibitor, and 1 (3%) a combination of FGFR and AKT inhibitors]. Thirty-eight of 106 patients (36%) received protocols containing an antiangiogenic agent [34/38 (89%) bevacizumab, 2/38 (5%) pazopanib-based therapy, 1/38(3%) sorafenib-based therapy, and 1/38 (3%) anti-HIF1 therapy].

## Response to phase I clinical trial

Out of 106 patients who initiated phase I therapy, 98 were evaluable for response. Response assessments were not carried out in 8 patients (5 withdrew consent and 3 stopped therapy in less than a month because of toxicity). Twelve of 98 patients (12%) had SD ≥ 6 months/PR/CR, including 1 CR, 7 PRs, and 4 SD ≥ 6 months (Fig. 1). Eleven of 57 evaluable patients (19%) who received a combination of chemotherapy and targeted agent(s) had SD ≥ 6 months/PR/CR versus 1 of 41 evaluable patients (2%) who received either targeted agents alone or chemotherapy alone ($P$ value = 0.013). Five of 15 evaluable patients (33%) treated on matched therapies had SD ≥ 6 months/PR/CR compared with 7

**Table 2.** Therapies received in the phase I clinical trials program

| Phase I therapy | No. of patients | Evaluable, *n* | CR, *n* | PR, *n* | SD ≥ 6 months, *n* | SD ≥ 6 months/PR/CR *n* (%) | Median PFS, months (95% CI) |
|---|---|---|---|---|---|---|---|
| Chemotherapy alone[a] | 8 (8) | 7 | 0 | 0 | 0 | 0/7 (0) | 2.1 (0.9–3.3)[c] |
| Chemotherapy and targeted agent[b] | 63 (59) | 57 | 1 | 6 | 4 | 11/57 (19) | 3.0 (1.9–4.1)[c] |
| Single agent targeted drug | 15 (14) | 15 | 0 | 0 | 0 | 0/15 (0) | 1.1 (0.7–1.4)[c] |
| ≥2 targeted agents | 20 (19) | 19 | 0 | 1 | 0 | 1/19 (5) | 1.9 (1.4–2.4)[c] |
| Total | 106 (100) | 98 | 1 | 7 | 4 | 12/98 (12) | 2.1 (1.5–2.6) |

[a]One or more agents.
[b]One or more of each.
[c]$P < 0.0001$ for comparison of PFS by log-rank test across all 4 groups.

of 83 (8%) patients who were treated on nonmatched therapy ($P = 0.018$). Among the 12 patients who had SD ≥ 6 months/PR/CR (Table 3), 5 received the same combination of chemotherapy (liposomal doxorubicin), antiangiogenic agent (bevacizumab), and mTOR inhibitor (temsirolimus). Three of these 5 patients had metaplastic histology (16–18). Eleven of 12 patients with SD ≥ 6 months/PR/CR received a combination of chemotherapy and targeted agent(s) while one received a combination of two targeted agents.

### Response assessment in patients with evidence of PI3K/AKT/mTOR pathway activation

Of 43 patients evaluated for alterations in the PI3K/AKT/mTOR pathway, 21 (49%) demonstrated at least one alteration (including mutations in *PIK3CA*, *PIK3R1*, *PTEN*, *NF2*, deletion in *PTEN*, *PIK3CA* amplification, and PTEN loss on IHC). Sixteen of these 21 patients received protocols with at least one drug targeting the PI3K/AKT/mTOR pathway and 15 were evaluable for response. Five of 15 evaluable patients (33%) treated with matched therapy had SD ≥ 6 months/PR/CR, versus 6 of the 25 evaluable patients (20%) treated on nonmatched therapy ($P = 0.716$).

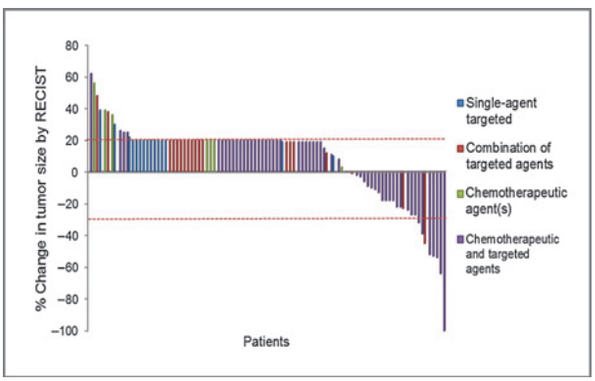


Figure 1. Waterfall plot. Best response by RECIST of 98 evaluable patients with TNBC by treatment received in the phase I clinical trials program. Patients with clinical progression or with new metastasis were graphed as 20% progression. Dotted horizontal line at −30% and 20% indicates border for partial response and progression, respectively.

### Factors predicting response to treatment

Factors that were associated with improved response (SD ≥ 6 months/PR/CR) on univariate analysis included: ≤2 metastatic sites ($P = 0.004$), treatment that included both chemotherapeutic and targeted agent(s) ($P = 0.013$), treatment with a matched therapy (0.018), and, <3 prior therapies in metastatic setting ($P = 0.032$; Supplementary Table S2). On multivariate analysis, independent factors that predicted greater response (SD ≥ 6 months/PR/CR) were ≤2 metastatic sites ($P = 0.017$) and combination therapies that included chemotherapeutic and targeted agents ($P = 0.028$; Table 4).

### PFS on the first phase I trial and prognostic factors

The median PFS of 106 patients treated on a first phase I trial was 2.1 months [95% confidence internal (CI): 1.6–2.6 months]. The median PFS was significantly longer for 16 patients treated on matched therapy (6.4 months) versus 1.9 months for the 90 patients treated on nonmatched therapy ($P = 0.001$). Of 43 patients tested for molecular alterations in the PI3K/AKT/mTOR signaling pathway, the median PFS was significantly longer for 16 patients with molecular alterations in PI3K pathway treated on matched therapy (6.4 months) versus 3.2 months for 27 patients with or without molecular alterations treated on nonmatched therapy ($P = 0.036$).

Baseline characteristics associated with longer PFS on univariate analysis (Supplementary Table S2) included RMH score ≤1 ($P = 0.009$), noninvasive ductal carcinoma histology ($P = 0.010$), ≤2 metastatic sites ($P = 0.014$), and MDACC score ≤2 ($P = 0.017$). Improved PFS was associated with combinations treatment with chemotherapy and targeted agents ($P < 0.0001$), PI3K/AKT/mTOR pathway inhibitors ($P < 0.0001$; regardless of molecular alterations), therapies that included antiangiogenic agents ($P < 0.0001$), and matched therapies ($P = 0.002$). Combination treatment with chemotherapy and targeted agents

**Table 3.** Profile of patients with SD $\geq$ 6 months/PR/CR ($n = 12$)

| Case no. | Histopathology | Age, years | No. of metastatic sites | Molecular alterations | ECOG PS | RMH Score | MDACC Score | Prior therapies in metastatic setting | Phase I therapy | PFS on phase I therapy (months) | Best response (RECIST %) | OS from phase I therapy (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Invasive ductal carcinoma | 46 | 1 | TP53[a] (splice site 559 + 1 G>A) | 1 | 0 | 1 | 1 | PI3K and AKT inhibitor | 2.5 | PR (−45) | 4.9 |
| 2 | Metaplastic | 60 | 2 | PIK3CA (H1047R) | 1 | 0 | 1 | 1 | DAT | 11.7 | PR (−64) | 24.3 |
| 3 | Metaplastic | 52 | 4 | NF2[a] (p. K159fs*16) TP53[a] (p. F109fs*39) | 0 | 1 | 1 | 0 | DAT | 19.1 | CR (−100) | 48.3 |
| 4 | Invasive ductal carcinoma | 51 | 2 | NA | 0 | 0 | 0 | 3 | Dasatinib, gemcitabine | 4.2 | PR (−54) | 5.3 |
| 5 | Invasive ductal carcinoma | 65 | 1 | TP53[a] (p.R273H) CDK8 amp[a] MYC amp[a] | 1 | 0 | 1 | 1 | Aurorakinase inhibitor, taxol | 8.0 | PR (−52) | 16.2 |
| 6 | Invasive ductal carcinoma | 56 | 1 | NA | 1 | 1 | 2 | 0 | DAT | 9.3 | PR (−39) | 10.7 |
| 7 | Metaplastic | 70 | 1 | PTEN loss (IHC) | 1 | 1 | 2 | 0 | DAT | 11.0 | PR (−32) | 11.4 |
| 8 | Invasive ductal carcinoma | 37 | 1 | NA | 1 | 1 | 2 | 4 | Carboplatin, etoposide, IGFR inhibitor | 6.1 | SD (−24) | 13.6 |
| 9 | Invasive ductal carcinoma | 72 | 3 | PIK3CA (H1047R) | 1 | 2 | 3 | 0 | DAT | 6.4 | SD (+8) | 6.4 |
| 10 | Adenoid cystic | 47 | 1 | NA | 1 | 0 | 1 | 1 | HAI abraxane, IV gemcitabine, IV avastin | 7.5 | SD (−18) | 11.8 |
| 11 | Invasive ductal carcinoma | 57 | 1 | PTEN mutation (D92G) | 1 | 0 | 1 | 3 | DAT | 7.8 | SD (−18) | 9.6 |
| 12 | Invasive ductal carcinoma | 48 | 2 | PTEN loss (IHC) | 1 | 1 | 2 | 0 | mTOR inhibitor, taxol | 3.0 | PR (−53) | 10.7 |

Abbreviations: DAT, liposomal doxorubicin, bevacizumab, and temsirolimus; HAI, hepatic arterial infusion; IV, intravenous; NA, not available; PS, performance status.
[a]NGS data.

**Table 4.** Summary of multivariate analysis for response, PFS, and OS

| Variable | Estimated effect | *P* |
|---|---|---|
| **Response (SD ≥ 6 months/PR/CR)** | **OR[a] (95% CI)** | |
| Metastatic sites ≤2 (vs. >2) | 10.62 (1.52–74.09) | 0.017 |
| Chemotherapeutic and targeted agents (vs. chemotherapeutic or targeted agent only) | 27.02 (1.43–511.4) | 0.028 |
| **PFS** | **HR[b] (95% CI)** | |
| Metastatic sites ≤2 (vs. >2) | 0.44 (0.26–0.75) | 0.003 |
| Chemotherapeutic and targeted agents (vs. chemotherapeutic or targeted agent only) | 0.38 (0.22–0.633) | 0.0002 |
| PI3K pathway inhibitors, yes (vs. no) | 0.49 (0.27–0.88) | 0.018 |
| Antiangiogenic agents, yes (vs. no) | 0.52 (0.29–0.91) | 0.023 |
| **OS** | **HR[b] (95% CI)** | |
| MDACC score ≤2 (vs. >2) | 0.25 (0.15–0.41) | <0.0001 |

[a]OR > 1 is associated with higher response.
[b]HR < 1 is associated with longer PFS or OS.

demonstrated improved PFS (3.0 months; 95% CI: 1.5–4.4 months) when compared with other therapies (either chemotherapy or targeted therapies alone; 1.6, 95% CI: 0.9–2.3 months; $P < 0.0001$; Supplementary Table S2; Fig. 2). On multivariate analysis (Table 4), factors which were predictive of improved PFS were therapies containing chemotherapeutic and targeted agents ($P = 0.0002$), ≤2 metastatic sites ($P = 0.003$), use of PI3K/AKT/mTOR pathway inhibitors ($P = 0.018$), and combinations that included an antiangiogenic agent ($P = 0.023$).

## Overall survival on the first phase I trial and prognostic factors

The median OS of the 106 patients with TNBC starting from the beginning of a phase I trial was 7.7 months (95% CI: 6.3–9.0 months). Factors that were associated with

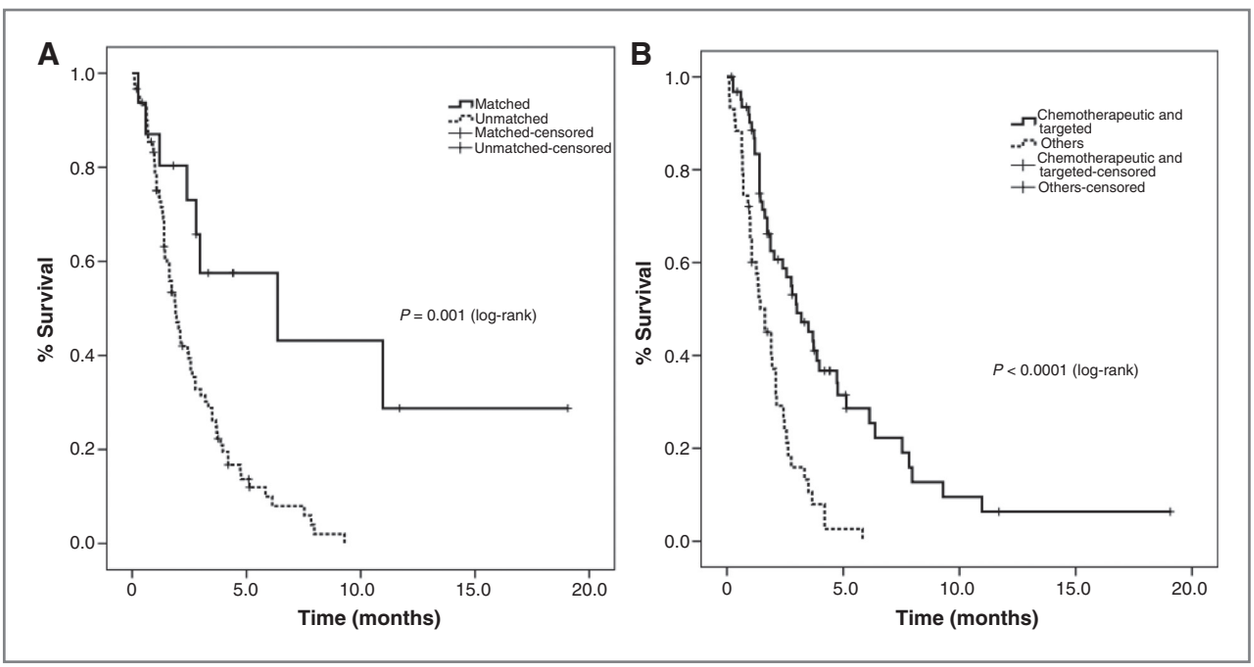


Figure 2. Kaplan–Meier estimates of PFS in 106 patients with TNBC. A, PFS of 16 patients treated on matched therapy (6.4 months) versus those treated on other therapies (1.9 months), $P < 0.001$; log-rank test. B, PFS of 63 patients treated on combination therapies that included chemotherapy and targeted agents (3.0 months) versus 43 treated on other therapies (1.6 months), $P < 0.0001$; log-rank test.

improved OS on univariate analysis (Supplementary Table S1) were MDACC score $\leq 2$ ($P < 0.0001$), RMH score $\leq 1$ ($P < 0.0001$), serum albumin $\geq 3.5$ g/dL ($P = 0.001$), $\leq 2$ metastatic sites ($P = 0.007$), serum LDH $\leq$ 618 IU/L ($P = 0.026$), less than 3 prior therapies in metastatic setting ($P = 0.026$), noninvasive ductal carcinoma histology ($P = 0.033$), and ECOG performance status of 0 ($P = 0.040$). On multivariate analysis, MDACC score $\leq 2$ was predictive for overall survival ($P < 0.0001$; Table 4).

## Discussion

Historically, patients treated on phase I studies have response rates of 4% to 10.6% (19, 20). With the evolving paradigm shift favoring personalized medicine that "matches" molecular profiles of patients' tumors with targeted therapies, response rates on phase I trials are higher. A recent study demonstrated that patients treated with molecularly matched targeted therapy compared with nonmatched therapy had improved clinical benefit (4). In general, patients with metastatic TNBC have very poor outcomes and limited treatment options. Metastatic TNBC, therefore, remains an urgent, unmet need. (21). Nevertheless, certain patients with TNBC characterized by low proliferation index, tumor with lymphocytic infiltration, and absence of central fibrosis, or, a rare type of TNBC such as adenoid cystic and secretory carcinoma with *ETV6-NTRK3* and *MYB-NFIB* fusion genes and KIT positivity may demonstrate indolent disease, excellent response to therapy, and good prognosis (22–24). The purpose of this study was to systematically analyze the clinical outcomes of 106 patients with metastatic TNBC, who were mostly referred to the phase I clinic to receive second, third, or salvage line treatments.

We demonstrated that patients with TNBC treated on phase I trials with combinations of chemotherapy and targeted agents had a significant improvement in PFS versus those patients treated on either chemotherapeutic or targeted agents only (3.0 vs. 1.6 months; $P < 0.0001$). In particular, the median PFS was significantly longer in patients who were treated on a combination that included an antiangiogenic agent (3.7 vs. 1.7 months; $P < 0.0001$) and/or a PI3K/AKT/mTOR inhibitor (3.5 vs. 1.6 months; $P < 0.0001$) than patients who did not receive these agents. On multivariate analysis (Table 4), the most significant independent predictor of longer PFS was treatment with both chemotherapy and targeted agent(s) ($P = 0.0002$). Treatment with a PI3K pathway inhibitor ($P = 0.018$) or an antiangiogenic agent ($P = 0.023$) were also significant predictors of increased PFS.

Our results are consistent with previously published studies of patients with TNBC treated with a combination including an antiangiogenic agent that reported improved response (SD $\geq$ 6 months/PR/CR) in the neoadjuvant setting and prolonged PFS in patients with metastatic disease (25–28). Several studies have demonstrated that the addition of bevacizumab to neoadjuvant chemotherapy improved pathologic CR rate in early and locally resectable TNBC (25, 26). A meta-analysis of three phase III trials with first-line bevacizumab-containing combinations in patients with metastatic TNBC showed a 35% reduction in risk of disease progression and longer PFS than those treated with regimens that included a chemotherapeutic agent without an antiangiogenic agent (8.1 vs. 5.4 months; HR = 0.680; $P = 0.0002$; ref. 27). Similarly, RIBBON-2 trial demonstrated 51% reduction in risk of progression with second-line bevacizumab-containing combinations in patients with metastatic TNBC, compared with chemotherapy alone (6.0 vs. 2.7 months; HR = 0.494; $P = 0.0006$; 28). The effectiveness of therapies that target VEGF may be explained by the higher expression of intratumoral VEGF by 1.5 to 3 times in TNBC compared with non-TNBC and dysregulation of VEGF-related genes (29, 30). There are also studies, however, that failed to demonstrate benefit with adjuvant antiangiogenic therapy (31). Unfortunately, biomarkers to identify angiogenesis inhibitor responders remain speculative (32, 33) and there are no validated biomarkers to identify potential responders to antiangiogenic therapy and improvement in OS has not yet been demonstrated (34).

Treatment of advanced cancer with inhibitors of the PI3K/AKT/mTOR axis is supported by both preclinical studies (35) and early clinical data (17, 18, 36). Preclinical models have shown that the PI3K/AKT/mTOR is activated in TNBC and that blocking this pathway can induce tumor regression (1, 37). While clinical data are still limited (38) and early results are mixed, there is some indication that treatment with a PI3K/AKT/mTOR inhibitor in combination may be effective in TNBC (39).

Though limited in sample size, our molecular data were revealing of several trends. We report that 21 of 43 patients (49%) demonstrated at least one direct PI3K/AKT/mTOR pathway alteration (including mutations in *PIK3CA*, *PIK3R1*, *PTEN*, *NF2*, deletion in *PTEN*, *PIK3CA* amplification, and PTEN loss on IHC). Seven of 43 patients (16%) tested for the *PIK3CA* mutation were positive. Among 37 patients tested for either a genomic or proteomic alteration in PTEN, 8 had PTEN loss by IHC, 5 had either *PTEN* mutation or deletion, and 1 had both PTEN loss by IHC and *PTEN* mutation. Previous studies have reported on the prevalence of PI3K molecular alterations in breast cancer, including different subtypes (36, 38). Prior data suggest that 8% of patients with TNBC harbor a *PIK3CA* mutation (40). Higher rates of these alterations reported in our analysis may be explained by a higher representation of patients with metaplastic breast cancer, 17 of 106 (16%) patients in our study, a rare subtype known to harbor PI3K alterations at a higher rate than other TNBC (16, 41). In addition, the higher overall percentage of PI3K/AKT/mTOR pathway alterations in our study versus other analyses may be due to inclusion of patients with *PIK3R1* and *PTEN* mutations and PTEN loss on IHC.

NGS profiling demonstrated a high number of alterations. Four of 9 patients who underwent NGS profiling had at least 5 alterations. Previous studies have demonstrated that an increased number of molecular alterations

may be associated with more aggressive disease and worse outcomes (4). Consistent with these reports, we noted that the 3 of 9 patients in our study with NGS testing who demonstrated response (including 2 patients with PR, cases #1 and #5; and one with a CR, case #3; Table 3) had $\leq$3 molecular alterations. Of the two patients with PR, case #1 demonstrated only a *TP53* mutation, case #5 had two amplifications in addition to the TP53 mutation, and the patient with a CR (case #3) demonstrated *TP53* and *NF2* mutations.

There are several limitations to this retrospective study. Of 106 patients, only 47 (44%) had molecular profiling, including 9 with NGS. The tissue used for analysis was also not consistent among patients. That is, some of the tissue was from the primary tumor while others were from a metastatic site. Unfortunately, none included pre- and posttreatment biopsies, ideal for molecular analysis. In terms of the patient population in this study, patients with metaplastic TNBC were overrepresented; thus, our results may be less generalizable to other metastatic TNBC subtypes. Furthermore, different imaging studies such as computed tomography scans and/or magnetic resonance imaging were used to evaluate therapeutic response in these patients with TNBC. Despite these shortcomings, this is one of the largest reports to date describing outcomes in patients with metastatic TNBC treated on phase I studies including targeted therapy.

In conclusion, our study suggests that identification of molecular alterations and optimizing treatment with agents that targeted these molecular alterations (matched therapy) enhanced response (SD $\geq$ 6 months/PR/CR) and PFS in this heavily pretreated group of patients with metastatic TNBC. Furthermore, response (SD $\geq$ 6 months/PR/CR) and PFS was significantly longer in patients on a treatment that included either an antiangiogenic and/or a PI3K/AKT/mTOR inhibitor in addition to a chemotherapeutic agent. Our data showed higher frequency of molecular alterations in patients with metastatic TNBC than has previously been reported. We currently are exploring NGS in a large prospective study. The study also demonstrated that patients with metastatic TNBC treated on phase I trials have comparable overall outcomes to those patients with TNBC treated with chemotherapy. Therefore, our data support participation in phase I clinical trials with novel agents to develop new options for these patients with limited therapeutic options and poor prognosis.

## Disclosure of Potential Conflicts of Interest

P. Stephens has ownership interest (including patents) in Foundation Medicine. R. Yelensky has ownership interest (including patents) in Foundation Medicine. F. Meric-Bernstam is a consultant for Novartis and Roche and on the advisory board of Genentech. F. Janku received research funding from Novartis. No potential conflicts of interest were disclosed by the other authors.

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