Open-label randomized clinical trial of standard neoadjuvant chemotherapy with paclitaxel followed by FEC versus the combination of paclitaxel and everolimus followed by FEC in women with triple receptor-negative breast cancer†

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Background: Everolimus synergistically enhances taxane-induced cytotoxicity in breast cancer cells in vitro and in vivo in addition to demonstrating a direct antiproliferative activity. We aim to determine pharmacodynamics changes and response of adding everolimus to standard neoadjuvant chemotherapy in triple-negative breast cancer (TNBC).

Patients and methods: Phase II study in patients with primary TNBC randomized to T-FEC (paclitaxel 80 mg/m² i.v. weekly for 12 weeks, followed by 5-fluorouracil 500 mg/m², epirubicin 100 mg/m², and cyclophosphamide 500 mg/m² every 3 weeks for four cycles) versus TR-FEC (paclitaxel 80 mg/m² i.v. and everolimus 30 mg PO weekly for 12 weeks, followed by FEC). Tumor samples were collected to assess molecular changes in the PI3K/AKT/mTOR pathway, at baseline, 48 h, 12 weeks, and at surgery by reverse phase protein arrays (RPPA). Clinical end points included 12-week clinical response rate (12-week RR), pathological complete response (pCR), and toxicity.

Results: Sixty-two patients were registered, and 50 were randomized, 27 received T-FEC, and 23 received TR-FEC. Median age was 48 (range 31–75). There was downregulation of the mTOR pathway at 48 h in the TR-FEC arm. Twelve-week RR by ultrasound were 29.6% versus 47.8%, (P = 0.075), and pCR were 25.9% versus 30.4% (P = 0.76) for T-FEC and TR-FEC, respectively. Tumor downregulation at 48 h did not correlate with 12-week RR in the TR-FEC group (P = 0.58). Main NCI grade 3/4 toxicities included anemia, neutropenia, rash/desquamation, and vomiting in both arms. There was one case of grade 3 pneumonitis in the TR-FEC arm. No grade 3/4 stomatitis occurred.

Conclusion: The addition of everolimus to paclitaxel was well tolerated. Everolimus downregulated mTOR signaling but downregulation of mTOR at 48 h did not correlate with 12-week RR in the TR-FEC group.

Clinical trial number: NCT00499603.

Key words: triple negative breast cancer, neoadjuvant systemic therapy, PI3K pathway inhibition

introduction

Triple-negative breast cancers (TNBC) lack expression of estrogen receptor, progesterone receptor, and HER2, comprising ~12%–17% of breast cancers [1]. Patients with TNBCs have relatively poor outcome and are not eligible for endocrine or anti-HER2 therapies [1]. Pathologic complete response (pCR) after neoadjuvant chemotherapy (NCT) is known as an early surrogate for survival in TNBC [2]. Patients with TNBC attain pCR rates of 30%–40%; however, patients with residual disease are at greater risk of relapse [3]. The PI3K/AKT/mTOR pathway is downstream of most of the common growth factor tyrosine kinase receptors in cancer. It plays a key role in cell growth, protein translation,
autophagy, metabolism, and survival [4]. Pathway defects/upregulation occur frequently in TNBC [5]. Inhibition of components of the pathway has been proposed to synergize with or overcome resistance to chemotherapy [6]. Selective inhibition of PI3K/AKT/mTOR activity sensitizes breast cancer cell lines to apoptosis induced by chemotherapeutic agents including paclitaxel [7].

The mammalian target of rapamycin (mTOR), an ubiquitous protein kinase downstream of PI3K is implicated in cell growth control and proliferation. Rapamycin potentiates the cytotoxicity of paclitaxel in cell lines with PI3K/PTEN/AKT aberrations, suggesting that combination therapy may be effective in these tumors [7]. The oral mTOR inhibitor everolimus (Novartis, East Hanover, NJ) synergistically enhances taxane-induced cytotoxicity in breast cancer in vitro and in vivo in addition to having direct antiproliferative activity [7, 8].

The purpose of this study was to determine the molecular changes of the mTOR pathway in patients with TNBC when adding an mTOR inhibitor to standard NCT. Secondary end points included response rates at 12 and 24 weeks, pCR, and safety.

methods

This was a phase II, randomized trial conducted at MD Anderson Cancer Center (MDACC). Eligible patients had confirmed clinical stage IIA–IIB TNBC, measurable disease, and adequate organ function. Patients who had received mTOR inhibitors; had other invasive malignancies within the previous 5 years; impairment of gastrointestinal function or pre-existing peripheral neuropathy >grade 1 were excluded. All patients signed informed consent approved by MDACC institutional review board.

Fifty patients were randomly assigned, 1:1 to T-FEC (paclitaxel 80 mg/m² i.v. weekly for 12 weeks, followed by 5-fluorouracil 500 mg/m², epirubicin 100 mg/m², and cyclophosphamide 500 mg/m² every 3 weeks for four cycles) versus TR-FEC [paclitaxel 80 mg/m² i.v. and everolimus 30 mg orally (p.o.) weekly for 12 weeks, followed by FEC]. Tumor measurements were obtained by ultrasound at baseline, 12 weeks, and before surgery. All patients underwent surgery and adjuvant radiotherapy if indicated. Chemotherapy dose reductions were permitted as per protocol. Everolimus was interrupted for grade 2 nonhematological toxicity or grade 2 thromboctopenia and reintroduced at the initial dose after recovery to grade ≤1. If grade 2 pneumonitis, grade 3 nonhematologic toxicity, thrombocytopenia, or neutropenia occurred, treatment was interrupted and then resumed at 15 mg p.o. weekly after recovery to grade <1. Treatment was discontinued for any grade 4 toxicity, if treatment was interrupted for >21 days, or if toxicity reoccurred after dose reduction.

All patients underwent imaging at presentation, and before FEC to establish clinical response after paclitaxel with/without everolimus. Tumor responses were assessed based on regional ultrasound examination. A decrease in the size of the product of the two largest dimensions ≥50% was considered a partial response. Complete disappearance of the primary tumor and normalization of the lymph nodes was considered a complete clinical response. Complete disappearance of the invasive carcinoma in the breast and axillary nodes at surgery was considered a pCR. Progression of disease was defined as ≥30% increase in the size of the primary tumor and/or lymph nodes on physical exam and/or ultrasound. Toxicities were graded according

Figure 1. Patient enrollment and disposition.

Patients registered: 62
Screen failures: 9
Consent withdrawal: 2
Off due to therapy interruption after first dose: 1

Patients randomly assigned (1:1) to 24 weeks of T-FEC or TR-FEC
n=50

T-FEC
n=27

FNA biopsy (n=27)
48 hrs post-therapy (n=27)
12 weeks post-therapy (n=21)

Completed treatment
(n=23, 85.2%)
Discontinue treatment
(n=4, 14.8%)
Disease progression (n=3)

Surgery
residual tissue collection (n=12)

Included in analysis
(n=27)

TR-FEC
n=23

FNA biopsy (n=23)
48 hrs post-therapy (n=23)
12 weeks post-therapy (n=18)

Completed treatment
(n=20, 87.0%)
Discontinue treatment
(n=3, 13.0%)
Disease progression (n=1)

Surgery
residual tissue collection (n=11)

Included in analysis
(n=23)
to the NCI Expanded Common Toxicity Adverse Event (AE) Criteria, version 3.0 and assessed weekly in the first four cycles, then on day 1 of each cycle until up to 4 weeks after the last cycle.

Fine-needle aspirations (FNAs) were obtained pretreatment, at 48 h, and at week 12. Residual tissues were collected at time of surgery. Biopsies were evaluated by reverse phase protein arrays (RPPA). RPPA was carried out as previously described [9].

Patients were randomly assigned 1:1 to receive T-FEC or TR-FEC. Randomization was carried out using a balanced block design stratified by disease stage (II and IIIA, IIIB, IIIC) and menopausal status. Primary end point was defined as the proportion of patients with inhibition of the mTOR signaling pathway 48 h after the start of treatment. Secondary efficacy end points between the two arms were estimated with the corresponding 95% posterior credible intervals compared with a χ² or Fisher’s exact tests. Toxicity frequency tables were constructed and compared with χ² or Fisher’s exact tests.

Supplementary File S1, available at Annals of Oncology online describes complete RPPA methodology. Paired t-test was used to examine the difference between baseline and 48-h post-treatment samples within the same arm. We defined the change (X) at 48 h for each T-FEC and TR-FEC tumor by subtracting the baseline protein expression from the 48-h post-treatment protein expression (X = 48 h – baseline). Two-sample t-test was used to determine the difference of the changes at 48 h between T-FEC and TR-FEC. mTOR downregulation was defined when at least two of eight (S6, pS6-S235/236, pS6-S240/244, pS6K-T389, and, 4EBP1, p4EBP1-T37/46, p4EBP1-S65, p4EBP1-T70) proteins decreased at least 1.5 standard deviations (SD) from baseline to 48 h. A logistic model was used to test the interaction between treatment arm and mTOR downregulation. A multivariate model was developed to test the association of treatment arm and mTOR downregulation with 12-week RR and pCR.

**results**

Sixty-two patients were registered, and 50 patients were randomized (27 to T-FEC, and 23 to TR-FEC). Nine patients failed the screening process, two patients withdrew consent and one was discontinued after the first dose due to therapy interruption for >21 days (Figure 1).

Patient and tumor characteristics are presented on Table 1. There were no differences by treatment group, 50% of patients were younger than 50 years, more than 65% were Caucasian, and had at least T2 disease.

There was no difference on protein expression at baseline between groups validating the randomization. Supplementary Table S2, available at Annals of Oncology online, lists the proteins that significantly changed from baseline to 48 h. In the T-FEC arm, there were 70 proteins with significant changes, including PI3K/AKT/mTOR-pathway components (pS6-S240/244, pS6-S235/236, pAkt0S473, and 4EBP1). When comparing the change (X) at 48 h of protein expression differences between arms (supplementary Table S3, available at Annals of Oncology online), a phosphorylation of ribosomal S6 protein, known to be downstream target of the mTOR signaling pathway, on both pS6-S240/244 (P < 0.0001) and pS6-S235/236 (P = 0.0001) showed significant differences. Interestingly pS6-S240/244 and pS6-S235/236 increased in T-FEC-treated tumors, while they decreased in TR-FEC-treated tumors (Figure 2).

Twelve-week RR by ultrasound were 29.6% versus 47.8%, (P = 0.075) for T-FEC and TR-FEC, respectively. There were no differences in response rates at 24 weeks (P = 0.27). pCR rates were seen in seven patients in each arms, (P = 0.76 one-sided) (Table 2).

mTOR downregulation (1.5SD) at 48 h did not correlate with 12-week RR in the TR-FEC group (P = 0.58), and the likelihood ratio test for interaction between treatment arm and mTOR downregulation was not significant (P = 0.7454) demonstrating no evidence of treatment effect on 12-week RR. The multivariate model showed no independent predictors of 12-week RR (supplementary Table S4, available at Annals of Oncology online). We found no associations when repeating the analysis at 1 SD or 2 SD. Similar findings appeared when correlating with pCR (supplementary Table S4, available at Annals of Oncology online).

Table 3 summarizes all grade 3/4 AEs. Main NCI grade 3/4 toxicities included anemia 4% versus 17%, neutropenia 41% versus 52%, rash/desquamation 4% versus 9%, gastrointestinal disturbances 19% versus 26%, in T-FEC and TR-FEC, respectively. There was one case of grade 3 pneumonitis in the TR-FEC arm that required everolimus dose reduction. No grade 3/4 stomatitis occurred. Majority of patients completed all treatment, 85.2% and 87% on the T-FEC and on the TR-FEC arms,
Figure 2. Plots showing mTOR-pathway regulation differences from baseline to 48-h post-treatment between T-FEC (red) and TR-FEC (blue) protein expression.
Discussion

The purpose of this study was to determine the early molecular changes of the PI3K/AKT/mTOR pathway in patients with TNBC when adding an mTOR inhibitor to standard NCT. We found that S6 phosphorylation decreased and pAKT increased in TR-FEC-treated tumors consistent with the effects of mTOR inhibition.

Our preclinical data showed that rapamycin synergistically enhanced the cytotoxicity of paclitaxel in TNBC [7], and phase II trials of mTOR inhibition with rapalogs have shown single-agent activity in metastatic breast cancer [10]. The efficacy of NCT in TNBC varies according to the molecular characteristics of the disease [11]. Furthermore, we have observed that PI3K signaling activity was present and correlated with bad outcome in patients with residual disease after NCT [12]. As expected, we documented downregulation of pS6 residues in the post-treatment tumor samples of the patients that received everolimus. In a preoperative study of 31 breast cancers, everolimus exposure decreased downstream components of mTOR signaling and pS6 staining was significantly reduced independently of proliferation ($P < 0.001$) [13]. Decreased pS6 was also seen when adding everolimus to letrozole in neoadjuvant hormone receptor-positive disease [14].

We found that 12-week RR by ultrasound after paclitaxel versus paclitaxel/everolimus treatment were 29.6% versus 47.8%, ($P = 0.075$); however, pCR rates after chemotherapy completion were 25.9% versus 30.4%, ($P = 0.76$) for T-FEC and TR-FEC, respectively. Chemotherapy resistance in human cancer is complex, therefore, adding targeted agents requires careful evaluation to balance potential toxicity with increased efficacy. In breast cancer, the use of everolimus in combination with chemotherapy is limited to early phase studies [8, 15, 16]. Everolimus was safely combined with chemotherapy and there was activity in heavily pretreated patients at doses ranging from 30 mg weekly to 5–10 mg daily. In a phase II randomized study of patients with HER2-negative disease, additional everolimus did not improve paclitaxel/bevacizumab efficacy [8, 15–17]. Only one randomized trial has assessed the efficacy of adding everolimus to NCT. GeparQuinto randomized patients with HER2-negative tumors after four cycles of epirubicin/cyclophosphamide with/without bevacizumab to weekly paclitaxel with/without everolimus if there was no clinical response. Of 1948 patients that started NCT, 403 were randomized. As expected, pCR rates were low: 3.6% in the paclitaxel and everolimus arm, and 5.6% in the paclitaxel alone arm [18]. These low responses when compared with our study can be explained by the preselected resistant disease in this portion of GeparQuinto.

We did not see a correlation of mTOR downregulation at 48 h and 12-week RR in the TR-FEC group ($P = 0.58$). Several studies have looked at predictors of rapalogs benefit in cancer. In a phase II randomized study of letrozole with/without everolimus for hormone receptor-positive breast cancer, Ki-67 correlated with clinical response, and tumors with PIK3CA exon 9 mutations were less responsive to letrozole and more sensitive to the combination [14]. However, this correlation was not reproduced in a large phase III trial in the metastatic setting [19]. Correlative work on a preoperative study in hormone receptor-positive breast cancer showed that the genes that most clearly separated before treatment responding from nonresponding tumors were those involved with protein modification and dephosphorylation, including DYNLBB2, ERBB4, PTMPN13, ULK2 and DUSP1 [20]. Analyses to study the predictive role of a PI3K/mTOR-pathway gene signature (PIK3CA-GS) and PI3KCA status to proliferation changes and pS6 levels in these trials were completed. In the randomized dataset, the PIK3CA-GS could identify those patients with the largest relative decreases in Ki67 to letrozole/everolimus compared with letrozole/placebo (interaction $P = 0.02$). However,
in the single-agent everolimus dataset, the PIK3CA-GS did not correlate with relative change in Ki67 (P = 0.37) but did correlate with relative change in pS6-S240 (P = 0.028). PIK3CA genotype was not significantly associated with any end point [21]. In a clinical trial of octreotide and everolimus for advanced neuroendocrine tumors, we found that progression-free survival correlated with pAKT-T308 in pretreatment (P = 0.0533) and on-treatment biopsies (P = 0.0102), and that patients who had a documented partial response were more likely to have pAKT-T308 increase (P = 0.0146) [22]. Cho et al. studied the potential histologic and molecular predictors of response to temsirolimus in paraffin-embedded sections of 20 patients with advanced renal cell carcinoma. They found a positive association of pS6 expression (P = 0.02) and a trend toward positive association of pAKT expression (P = 0.07) with response [23].

The incidence of adverse events was not significantly higher in patients with the combination and overall safety of the combination was good. There was one case of grade 3 pneumonitis in the TR-FEC arm and no grade 3/4 stomatitis occurred, which can be explained by the weekly dose of everolimus.

In conclusion, adding everolimus to neoadjuvant paclitaxel demonstrated downregulation of the mTOR pathway at 48 h, and showed a trend toward an improvement in the 12-week RR, demonstrated downregulation of the mTOR pathway at 48 h, and showed a trend toward an improvement in the 12-week RR, but it did not improve the pCR rate in TNBC. Extensive on-

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disclosure

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references