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A phase II trial of biweekly vinorelbine and oxaliplatin in second- or third-line metastatic triple-negative breast cancer

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Keywords: chemotherapy, metastatic breast cancer, oxaliplatin, triple-negative, vinorelbine

Abbreviations: TNBC, triple-negative breast cancer; mTNBC, metastatic triple-negative breast cancer; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; ORR, overall response rate; MBC, metastatic breast cancer; ECOG, Eastern Cooperative Oncology Group; TTP, time to progression; SD, stable disease; CBR, rate of clinical benefit; CR, complete response; PR, partial response; CI, confidence interval; HR, hazard ratio; AE, adverse events; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; ANC, absolute neutrophil count; ULN, upper limit of normal; IV, intravenously.

Patients with metastatic triple-negative breast cancer (mTNBC) typically have a poor prognosis. The purpose of this study was to prospectively evaluate the efficacy and toxicity of biweekly combination of vinorelbine and oxaliplatin (NVBOX) in second- or third-line setting for mTNBC. Eligible patients were female with 18–70 y old, and had mTNBC that had progressed after 1or 2 prior chemotherapy regimens in the metastatic setting. NVBOX was given biweekly every 4 week for a maximum of 6 cycles. The primary endpoint was progression-free survival (PFS). Forty-4 patients were recruited. All patients had been exposed to anthracyclines and/or taxanes; 56.8% of patients were cis/carbo-platin pretreated. Among the 38 evaluable patients, overall response rate was 31.6% and 7 lasted $\geq$ 6 months. The median PFS and overall survival (OS) were 4.3 (95% CI, 3.6–5.0) months and 12.6 (95% CI, 8.1–17.0) months, respectively. PFS and OS was significantly shorter in patients with interval from diagnosis to recurrence $\leq$ 1 y and time to progression (TTP) of 1–2 previous regimens before recruitment $\leq$ 3 months. For 34 patients who were treated in second line setting, prior platinum was a factor significantly compromising the PFS of NVBOX. Grade 3/4 hematologic toxicities included neutropenia (70.5%), thrombocytopenia (27.3%) and anemia (15.9%). The most frequent grade 3/4 non-hematologic toxicities were constipation/abdominal distension (20.5%) and nausea/vomiting (13.6%). We conclude that biweekly NVBOX regimen is effective with a good safety profile in the second- or third-line mTNBC, which warrants further investigation in a phase III study. This trial was registered with www.clinicaltrials.gov (no. NCT01528826).

Introduction

Triple-negative breast cancer (TNBC), approximately 12–20% of all breast cancer, has an aggressive clinical course, and was associated with distant recurrence peaking at the first 3 y[1,2] and early visceral metastasis.[3] The treatment of metastatic TNBC (mTNBC) is especially challenging as the tumors lack recognized therapeutic molecular biology targets, such as estrogen receptor (ER), progesterone receptor (PgR) and gene amplification of human epidermal growth factor receptor (HER2).[4,5] The median distant disease-free interval for TNBC subtype was 18 month while the median survival for mTNBC ranged from 6$\sim$13.3 months.[6,7]

Cytotoxic chemotherapy remains the mainstay of treatment for mTNBC as currently there are no specific targeted or biologic agents available. Whether BRCA-associated or sporadic TNBC, the molecular characteristics are consistent with aberrant DNA repair and genome-wide instability,[8,9] supporting the use of DNA-damaging agents such as platinum, which have been or are tested in several trials.[10-19] Standard of care included antracyclines, taxanes, and cis/carbo-platin-containing regimen as first-line therapy with overall response rate (ORR) approximated 30% and progression-free survival (PFS) of 3 months as single agent treatment had resulted in less optimal outcome in patients with mTNBC. Moreover, patients with pretreated mTNBC experienced a particular dismal progressive course. Eighty-seven

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percent patients went on to receive second-line therapy with a median duration of 9 weeks, and 55% received third-line therapy with a median duration only 4 weeks.[7] There is no standard of care for pretreated mTNBC. Thus, evaluation of newer combination as second-or third-line regimen for patients with mTNBC is urgently needed. Oxaliplatin, active in cisplatin- and carboplatin-resistant cell lines, may be worth exploring for cis/carbo-platin pretreated patients.[20] An ORR of 21% had been reported for oxaliplatin as single agent and between 7.5 to 35% as combination regimen in patients with pretreated metastatic breast cancer (MBC).[21] Oxaliplatin was usually combined with fluorouracil, gemcitabine or vinorelbine, considering its additive and/or synergistic activity with these cyctotoxic agents.[20,22,23] Vinorelbine, a vinca alkaloid derivative, interferes with tubulin assembly during mitosis and is active as single agent or in combination in MBC, with response rates of 36–50%.[24-26]

The safety and efficacy of oxaliplatin and vinorelbine combination chemotherapy have been tested in breast cancer,[27] lung cancer,[28-30] and malignant pleural mesothelioma.[31] Recently, a phase I/II study selected 4 different dose levels of vinorelbine and oxaliplatin (NVBOX) for phase I study in MBC and no dose-limiting toxicities occurred even at the highest level (vinorelbine 30 mg/m^2 and oxaliplatin 90 mg/m^2 every 2 weeks). Then this dosage and schedule of NVBOX as first-line treatment was recommended to the phase II study including 44 MBC patients and was found well tolerated and highly active with ORR of 59%, PFS of 9.2 months and overall survival (OS) of 18.6 months.[32] In another phase II study [27] with 3-week schedule, the patients received an equivalent of 8.7 mg/m^2/week of vinorelbine and 43.3 mg/m^2/week of oxaliplatin, whereas in the biweekly schedule study, the dose of vinorelbine was almost double (15 mg/m^2/week). Considering these data and dose-effect relationship, our phase II open-label, non-randomized, single center study (NCT 01528826) was conducted to evaluate the efficacy and tolerability of biweekly NVBOX, as second- or third-line therapy in patients with mTNBC. Preliminary results had been reported at the 36th San Antonio Breast Cancer Conference (P3–13–06).

Results

Patients

Between Dec 2011 and Nov 2012, 44 patients with invasive ductal carcinoma were recruited. Patient characteristics are listed in **Table 1**.The median age was 47 y (range: 28–70). Thirty-9 (88.6%) patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 1. Seventeen (38.6%) patients developed recurrence within one year from initial diagnosis of breast cancer. Seventeen (38.6%) patients had $\geq$ 3 metastatic organ sites and visceral involvement was noted in 34 (77.3%) patients. All patients had been exposed to anthracyclines and/or taxanes-containing regimens, among whom 25 (56.8%) were pretreated with cis/carbo-platin for MBC. Thirty-4 (77.3%) patients had 1 previous regimen for metastatic disease and 38.6% with time to progression (TTP) of 1–2 previous regimens before recruitment $\leq$ 3 months.

Efficacy

The median number of treatment cycles was 3 (range: 1–6 cycles). The outcomes of the patients treated with NVBOX as salvage chemotherapy in mTNBC was presented in **Table 2**. The ORR was 31.6% (1 complete response, 11 partial responses) and 10 achieved stable diseases (SD, 7 lasting more than 6 months) in 38 evaluable patients (2 patients without measurable disease, 4 withdrew the consent before first evaluation). The rate of clinical benefit (CBR, complete response [CR] + partial response [PR] + SD $\geq$ 6 months) was 50.0% (19 of 38 patients). After a median follow-up of 12.8 months, the median PFS and OS of the intent-to-treat population were 4.3 (95% confidence interval [CI], 3.6–5.0) months and 12.6 (95% CI, 8.1–17.0) months, respectively. No significant differences between the second-line and third-line were found in all terms of efficacy.

Predictors of outcome

Age, menopausal status, interval from diagnosis of breast cancer to recurrence, extent of disease, prior cis/carbo-platin for MBC, and TTP of 1–2 previous regimens before recruitment $\leq$ 3 months, were included in the prognostic factors model. PFS and OS was significantly shorter in patients with interval from diagnosis of breast cancer to recurrence $\leq$ 1 y (Hazard ratio [HR] = 2.10; 95% CI, 1.05–4.21; P = 0.037 and HR = 5.45; 95% CI, 2.08–14.32; $P < 0.001$) and TTP of 1–2 previous regimens before recruitment $\leq$ 3 months (HR = 3.39; 95% CI, 1.66–6.89; $P < 0.001$ and HR = 4.09; 95% CI, 1.73–9.68; $P < 0.001$)(**Fig. 1**). Multivariate analysis showed that TTP of 1–2 previous regimens before recruitment $\leq$ 3 months was the only independent predictor for PFS, while interval from diagnosis of breast cancer to recurrence was the only independent factor to influence OS (**Table 3**). For 34 patients who received NVBOX as second line treatment, prior 1st-line platinum treatment was a factor significantly compromising the PFS of NVBOX (HR = 2.29; 95% CI, 1.03–5.10; P = 0.043).

Toxicity

Toxicity profile of the combination was acceptable and manageable. The most common AEs were presented in **Table 4**. The most common grade 3/4 hematologic toxicities were neutropenia (70.5%), thrombocytopenia (27.3%) and anemia (15.9%). Four (9.1%) patients experienced febrile neutropenia. The most frequent grade 3/4 non-hematologic toxicities were constipation/abdominal distension (20.5%) and nausea/vomiting (13.6%). Two patients developed grade 3 peripheral sensory neurotoxicity. Dose adjustment due to adverse events (AEs) occurred in 14 patients (31.8%). Median total dose intensity for vinorelbine and oxaliplatin was 0.90 and 0.88, respectively. There were no treatment-related deaths.

Discussion

Our report is the first prospective phase II study evaluating the safety and activity of a third-generation platinum (other than cis/carbo-platin) based regimen as second or third-line treatment for

Table 1. Patient characteristics at baseline

Characteristics	No. (%)
Patients enrolled	44
Age, years	
Median (Range)	47 (28–70)
≤ 40	12 (27.3)
> 40	32 (72.7)
ECOG performance status	
0	5 (11.4)
1	39 (88.6)
Menopausal status	
Pre- or perimenopause	20 (45.5)
Postmenopause	24 (54.5)
Interval from diagnosis of breast cancer to recurrence	
≤ 1 year	17 (38.6)
> 1 year	27 (61.4)
Metastatic sites	
Lymph nodes	23 (52.3)
Liver	13 (29.5)
Lung	26 (59.1)
Bone	14 (31.8)
Pleural effusion	2 (4.5)
Local recurrence	16 (36.4)
Contralateral breast	2 (4.5)
Brain	3 (6.8)
Type of metastasis	
Non-visceral	10 (22.7)
Visceral	34 (77.3)
No. of metastatic organ sites	
1	9 (20.5)
2	18 (40.9)
≥ 3	17 (38.6)
Previous regimens for MBC	
1	34 (77.3)
2	10 (22.7)
Cis/carbo-platin pretreated for MBC	
Yes	25 (56.8)
No	19 (43.2)
TTP of 1–2 previous regimens before recruitment ≤ 3 months	
Yes	17 (38.6)
No	27 (61.4)

Abbreviation: ECOG, Eastern Cooperative Oncology Group; No., number; MBC, metastatic breast cancer; TTP, time to progression.

mTNBC. The CBR was 50.0%, with 31.6% of patients achieving responses, with median PFS of 4.3 months, and median OS of 12.6 months. While direct inter-trial comparisons of response rates and PFS may not be possible due to large patient heterogeneity, our results with biweekly NVBOX are nevertheless promising, particularly in view of the high proportion (56.8%) of cis/carbo-platin pretreatment seen in the study. Given the limited effective treatment options available for pretreated patients with mTNBC, these results are encouraging, with the testing of oxaliplatin impact in larger size phase III trial and earlier disease stages the next step to consider.

Platinum single agent in this population was of poor efficacy. In a randomized phase II BALI-1 trial,[33] the control group, cisplatin had only modest activity (ORR 6%) as second-line treatment in sporadic mTNBC patients. Similarly, in another phase II study (TBCRC009), single agent cisplatin 75 mg/m^2 or carboplatin AUC = 6 every 21 d in second-line setting was tested with the ORR 20%.[14] For patients with more extensive, rapidly progressive, or symptomatic disease, many oncologists prefer combination therapies because, even if the advantage in terms of survival has not been demonstrated to date, they seem to offer better results in terms of response rate, PFS and CBR that could be translated to better control of symptoms. However, even with platinum-based combination chemotherapy GC (gemcitabine plus carboplatin), the PFS and OS for the second- or third-line mTNBC population (n = 222) were only 2.9 and 9.1 months, respectively.[11]

In this study, interval from diagnosis of breast cancer to recurrence ≤ 1 y and TTP of 1–2 previous regimens before recruitment ≤ 3 months were identified as significant unfavorable factors for PFS and OS of previously treated mTNBC patients. Multivariate analysis also revealed that they were independent predictor for OS and PFS, respectively. Our study is the first to show the role of TTP of 1–2 previous regimens before recruitment ≤ 3 months in predicting the significantly poor prognosis (median PFS only 2.1 months and OS 4.0 months). A short TTP of the second- and/or first-line therapy (≤ 3 months) might suggest the tumor be primarily or secondarily resistant to the cytotoxic agents, indicating primarily resistance to NVBOX that

Table 2. Summary of efficacy

Efficacy	Total population (N = 44) No. (%)	Second-line (N = 34) No. (%)	Third-line (N = 10)* No. (%)
Progression-free survival, months			
Median (95% CI)	4.3 (3.6 to 5.0)	4.1 (2.1 to 6.2)	4.4 (4.0 to 4.8)
Overall survival, months			
Median (95% CI)	12.6 (8.1 to 17.0)	10.0 (4.6 to 15.5)	14.1 (12.8 to 15.5)
Best overall response	38 evaluable patients	29 evaluable patients	9 evaluable patients
Complete response	1 (2.6)	1 (3.4)	0
Partial response	11 (28.9)	8 (27.6)	3 (33.3)
Stable disease	10 (26.3)	6 (20.7)	4 (44.4)
Stable disease for ≥6 months	7 (18.4)	5 (17.2)	2 (22.2)
Progressive disease	16 (42.1)	14 (48.3)	2 (22.2)
Overall response rate	12 (31.6)	9 (31.0)	3 (33.3)
Clinical benefit rate	19 (50.0)	14 (48.3)	5 (55.5)

*No significant differences between the second-line and third-line in all terms.

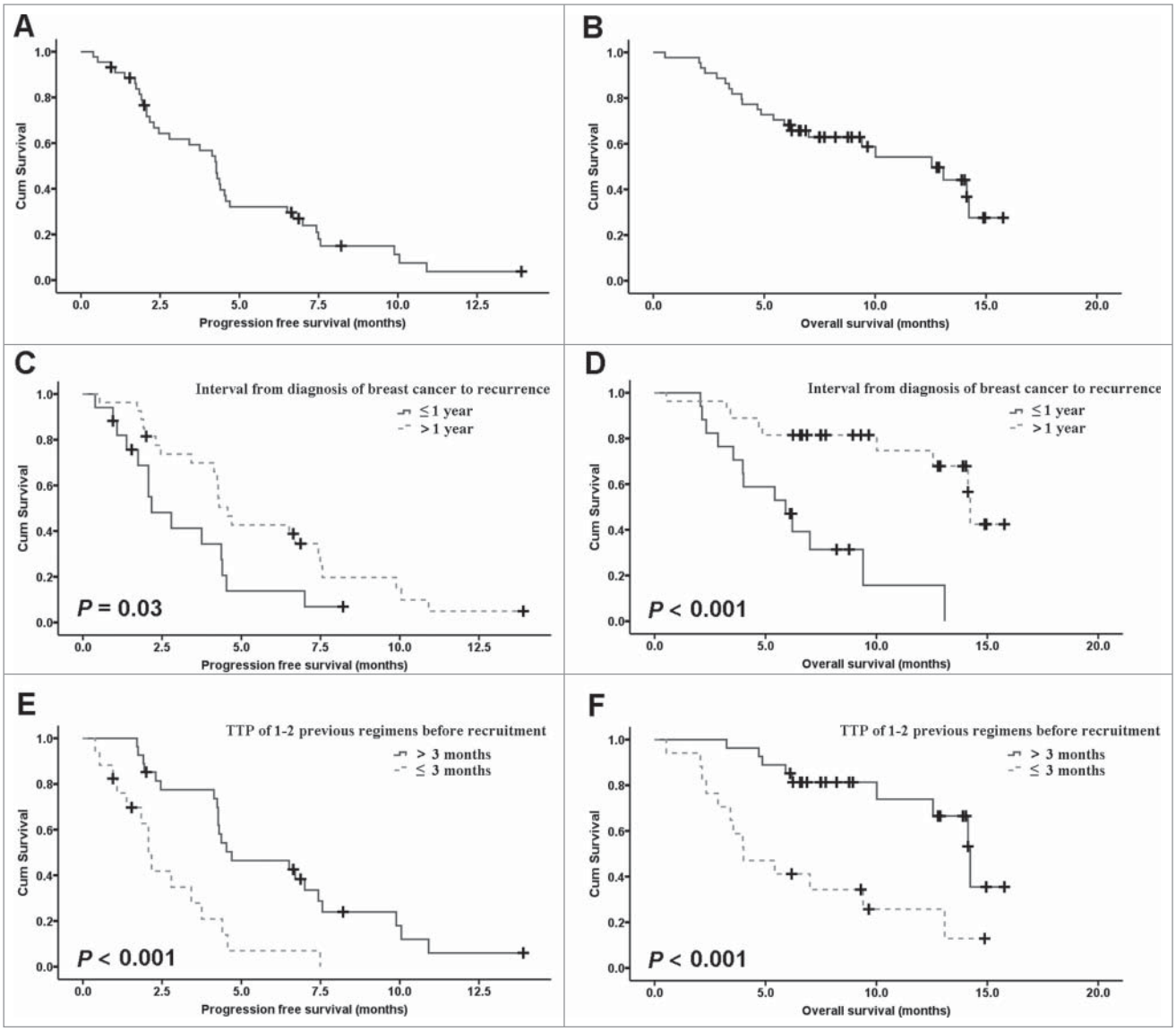

Figure 1. Kaplan-Meier estimates of progression-free survival (PFS) (**A**) and overall survival (OS) (**B**) for the ITT population. Kaplan-Meier estimates of PFS (**C**) and OS (**D**) for different interval from diagnosis of breast cancer to recurrence ($\leq$ 1 y vs. $>$ 1 year).Kaplan-Meier estimates of PFS (**C**) and OS (**D**) for different TTP of 1–2 previous regimens before recruitment ($\leq$ 3 months vs. $>$ 3 months).

resulted in shorter survival. To these patients, chemotherapy may not be able to improve survival for this group of patients. Best supportive care (BSC) might be a better option. A randomized study including patients with TTP of previous therapies $\leq$ 3 months to compare the salvage treatment and BSC is worth to be encouraged.

Prior first-line treatment with cis/carbo-platin–containing regimen was a factor significantly compromising the PFS of NVBOX. Oxaliplatin has a target mechanism of action and mechanisms of resistance different from cis/carbo-platin, thus shows little or no cross-resistance with cis/carbo-platin-resistant human breast, ovarian, cervix squamous cell carcinoma, non-small-cell lung cancer, germ cell cancer and mouse leukemia cell lines.[23] However, the non-cross resistance may not be absolute, as 1,2-diaminocyclohexane (DACH) -Pt complexes are not effective in all platinum-resistant cell lines.[34,35] A methodology of biomarkers to predict the efficacy of oxaliplatin in cis/carbo-platin pretreated patients remains to be explored.

The most frequently reported grade 3/4 AEs of NVBOX were neutropenia, thrombocytopenia and constipation/abdominal distension. Neutropenia was widespread and often grade 3/4 (70.4%), similar to triweekly NVBOX (78.6%),[27] as expected with vinorelbine considering the population's pre-treatment profile. Four patient (9.1%) experienced febrile neutropenia, the incidence of which was a bit higher than other combinations such as 5-fluorouracil/oxaliplatin (0–2%) or vinorelbine/cisplatin

Table 3. Univariate and multivariate models for progression free survival and overall survival in patients with metastatic triple negative breast cancer

		Univariate						Multivariate					
		Progression Free			Overall			Progression Free			Overall		
Factor	No.	Median(months)	95% CI	*P*	Median (months)	95% CI	*P*	HR	95% CI	*P*	HR	95%CI	*P*
Age				.59			.61						
≤ 40	12	3.8	2.5 to 5.0		13.1	-							
> 40	32	4.3	4.1 to 4.4		12.6	7.3 to 17.8							
Menopausal status				.91			.59						
Pre- or perimenopause	20	3.8	2.2 to 5.3		12.6	7.3 to 17.8							
Postmenopause	24	4.3	4.1 to 4.5		14.2	4.3 to 24.1							
Interval*				.03			<.001						<.001
≤ 1 year	17	2.2	0.8 to 3.5		5.9	3.2 to 8.6					5.45	2.08 to 14.32	
> 1 year	27	4.6	4.1 to 5.0		14.2	14.0 to 14.5							
Visceral involvement				.71			.20						
No	10	4.4	4.0 to 4.8		Not reached								
Yes	34	4.1	3.2 to 5.1		10.0	3.4 to 16.7							
No. of metastatic sites				.62			.18						
1	9	4.6	3.3 to 5.8		14.1	12.2 to 16.1							
2	18	4.3	0.0 to 8.6		9.4	4.5 to 14.3							
≥ 3	17	4.2	1.5 to 7.0		12.6	8.1 to 17.0							
Cis/carbo-platin pretreated for MBC				.11			.14						
Yes	25	2.2	1.6 to 2.8		10.0	3.1 to 16.9							
No	19	4.5	4.1 to 5.0		Not reached								
TTP of 1–2 previous regimens before recruitment ≤ 3 month				<.001			<.001			<.001			
Yes	17	2.1	1.7 to 2.5		4.0	1.5 to 6.5		3.39	1.66 to 6.89				
No	27	4.7	2.0 to 7.4		14.2	12.4 to 16.1							

* Interval from diagnosis of breast cancer to recurrence

(0%), but much lower than the 5-fluorouracil/vinorelbine combination (up to 33%) in later-line treatment setting.[36-39] It should be noted that in the previous phase I/II study of biweekly NVBOX, not only the grade 3/4 neutropenia (45.5%), but also the grade 3/4 constipation/abdominal distension (2.3%) were much less frequent than those in our study. The less incidence of grade 3/4 neutropenia in the previous study might be due to the recruitment of previous untreated MBC patients, while the higher frequency of constipation/abdominal distension observed in our study might be due to the fact that all patients were

Table 4. Adverse events

Description of Toxicity†	Any No. (%)	Grade 3 No. (%)	Grade 4 No. (%)
Hematologic			
Neutropenia	38 (86.4)	14 (31.8)	17 (38.6)
Thrombocytopenia	23 (52.3)	11 (25.0)	1 (2.3)
Anemia	30 (68.2)	7 (15.9)	0
Febrile neutropenia	4 (9.1)	4 (9.1)	0
Nonhematologic			
Sensory neuropathy	21 (47.7)	2 (4.5)	0
Constipation/Abdominal distension	20 (45.5)	9 (20.5)	0
Nausea/Vomiting	20 (45.5)	6 (13.6)	0
Fatigue	15 (34.1)	1 (2.3)	0
Anorexia	14 (31.8)	0	0
Increased ALT/AST*	2 (4.5)	0	0
Arthralgia	2 (4.5)	0	0
Allergic reaction	1 (2.3)	1 (2.3)	0

† Grade used was the worst recorded per patient.
* Abbreviation: ALT = alanine transaminase; AST = aspartate transaminase.

taxane-pretreated and 47.7% were cisplatin pretreated. A possible role of ethnic differences (Asian vs. Spanish) in genetic backgrounds may also contribute to the difference. Most of the constipation/abdominal distension were considered autonomic nerve damage related, but was manageable by appropriate medications and dose interruption or reduction in this study. Taking into account the prior exposure of taxane and/or cisplatin, NVBOX regimen was considered reasonably well tolerated.

Although cytotoxic chemotherapy remains the mainstay of treatment for mTNBC, chemotherapy combined with targeted agents such as PARP inhibitors,[11] EGFR inhibitors,[12,13] antiangiogenic agents[40] and Chk1 inhibitor [41] were recently investigated in the later-line setting, some of which [11,13,40] showed gains in response rate and PFS and were worthy of being further explored.

In conclusion, our study demonstrated that biweekly NVBOX regimen is effective and well-tolerated as second- or third-line treatment for patients with mTNBC. Future trials of NVBOX that focus on its interaction and role with target agents for mTNBC in the later-line setting are urgently required.

Patients and Methods

Patients

Women age between 18–70 y with histologically confirmed MBC documented as ER negative (IHC <10 %), PgR negative (IHC <10 %), and HER2 negative, were eligible. HER2 status was assessed by immunohistochemistry (IHC) and/or fluorescence in situ hybridization (FISH). HER2 negative was defined as no staining by IHC, and HER2 gene amplification by FISH was performed for those cases of 1+ and 2+ by IHC and confirmed absence of gene amplification. All patients must have progressed after 1or 2 prior chemotherapy regimens for metastatic disease. Pretreatment with vinorelbine and/or oxaliplatin was not permitted. Patients must have at least one measurable disease according to RECIST 1.1 criteria, a life expectancy of no less than 3 months, ECOG performance status $\leq$ 1 and adequate hematologic, renal, and hepatic function, as indicated by hemoglobin $\geq$ 9 g /dl, absolute neutrophil count (ANC) $\geq 1.5\times10^9$ /L, platelet count $\geq 75\times10^9$ /L, total serum bilirubin $\leq$ 1.5 $\times$ upper limit of normal (ULN), AST/ALT $\leq$ 2.5$\times$ULN ($\leq$ 5$\times$ ULN in case of liver metastases) and serum creatinine $\leq$ 1.0 $\times$ ULN (calculated creatinine clearance $\geq$ 50 mL/min). Patients who had received radiotherapy, chemotherapy, endocrine therapy, target therapy or any other investigated drugs within 4 weeks before the recruitment or has not recovered from the treatment-related toxicity(such as the severity of peripheral neuropathy> grade 1) were excluded. Patients with symptomatic central nervous system metastases or who were pregnant were ineligible.

The study was approved by the Fudan University Cancer Hospital Ethic Committee for Clinical Investigation (approval number: 1111104–12).The study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients prior to enrollment.

Treatment

Patients were treated with vinorelbine 30 mg/m^2 intravenously (IV) and oxaliplatin 90 mg/m^2 IV on day 1 every 2 weeks until disease progression or unacceptable toxicity or up to 6 cycles. A period of 4 weeks (NVBOX twice) was considered as one treatment cycle. Administration of prophylactic G-CSF was not permitted in the study. Dose modifications were made if Grade 4 neutropenia lasted longer than 3 days, Grade 3–4 thrombocytopenia, febrile neutropenia and/or Grade 3 nonhematological toxicity (except alopecia and inadequately treated nausea/vomiting). In this case, treatment was interrupted for up to 2 weeks until resolution to Grade $\leq$ 2, and doses of vinorelbine and oxaliplatin were reduced permanently by 20% in subsequent cycles. If toxicity did not resolve after 2 weeks or dose modifications occurred more than twice, the patient was withdrawn from the study. In addition, patients who experienced any grade 4 non-hematological toxicity were withdrawn from the study. The primary endpoint was PFS. Secondary endpoints included OS, ORR, and safety.

Assessment

Pretreatment assessment included a detailed medical history, physical examination, routine laboratory tests and performance status. Laboratory evaluation included a routine blood count, biochemistry including electrolytes, renal and liver function tests, and urinalysis. AEs and concomitant medications were recorded at the end of each cycle throughout the study period until 30 d after the last dose of a study treatment was administered. Toxicity was evaluated and graded according to National Cancer Institute Common Terminology Criteria for AEs, version 4.0.

Radiographic scans (CT scan or MRI) for efficacy evaluation were conducted at baseline and every 2 treatment cycles thereafter per RECIST 1.1 guidelines. The best overall response was reported. For patients without progress at the end of treatment, radiographic assessment was performed every 2 months within the first 6 months and every 3 months thereafter until disease progress. Survival status was assessed every 3 months after disease progress.

Statistical methods

The primary objective of this study was to evaluate the NVBOX regimen as second- or third-line treatment for mTNBC. The primary endpoint was PFS. The sample size was based on testing the hypothesis that NVBOX is superior to historical data from the report of Kassam et al.[7] With a 2-sided test of survival time differences between NVBOX doublet and historical data (12 months enrollment duration, 12 months follow up duration after enrollment), a sample of 38 evaluable patients would allow detecting an increase of 2.0 months (from 2.0 to 3.4 months) in the PFS on therapy, with 90% power and 5% significance level. A total of 42 patients were to be enrolled assuming < 10% patient discontinuation rate due to noncompliance or toxicity.

PFS was defined as the interval between treatment start and documented disease progression, or death as a result of any cause in patients with no evidence of disease progression. OS was

defined as the interval between the initiation of treatment and death. CBR was defined as the percentage of patients who had a complete response, a partial response, or stable disease for at least 6 months. Safety issues including incidence and severity of AEs were also investigated.

All statistical analyses were carried out using SPSS 19.0 (SPSS, Inc..). PFS and OS were estimated and 95% confidence intervals were calculated by means of the Kaplan–Meier method. All P values and confidence intervals reported are 2-sided, and all analyses are of data for the ITT population unless otherwise noted. Univariate survival curves were generated by the Kaplan-Meier method and differences in survival among the variables were assessed by the log-rank test. Prognostic variables identified by univariate analysis, with $P < 0.1$, were analyzed in the multivariate Cox model. All tests were 2-sided and P values <0 .05 were considered statistically significant.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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