Aim of the study: The aim of this trial was to compare overall survival (OS), disease-free survival (DFS), and toxicity of two adjuvant regimens in triple negative patients with Iranian ethnicity.

Material and methods: In a phase II trial, patients with previously untreated triple negative breast cancer were randomly assigned by using docetaxel 70 mg/m² and carboplatin AUC = 7 every three weeks with granulocyte colony-stimulating factor for five courses (arm A) or doxorubicin hydrochloride 60 mg/m² and cyclophosphamide 600 mg/m² every three weeks with G-CSF for four courses followed by docetaxel 70 mg/m² and carboplatin AUC = 7 every three weeks with G-CSF for four courses (arm B).

Results: A total of 119 patients were randomly enrolled in our study (60 patients in Arm A and 59 patients in Arm B) between 2011 and 2016. The mean follow-up was 40 months at the time of treatment analysis. The 2-year and 5-year DFS rates for Arm A were 92.7% vs. 85% and for Arm B were 82.6% vs. 64.4%. The 2-year and 5-year OS rates for Arm A were 96.5% vs. 91.7% and for Arm B were 90.5% vs. 81.3%. There was a significant correlation for DFS and OS in the two arms. There was no significant difference between adverse events with the two regimens.

Conclusions: In our research, less progression was found with Arm A compared to Arm B. Adding of anthracyclines such as doxorubicin hydrochloride did not increase OS and DFS in triple negative breast cancer (TNBC) patients.

Key words: breast cancer, triple negative, carboplatin, docetaxel.
The range of participants’ recruitment was from Feb 2011 to Jul 2011. During Aug 2011 to Sep 2016 the patients referred to the Breast Cancer Research Centre, Tehran University of Medical Sciences, Tehran, Iran. All patients gave written, informed consent before enrolment. Figure 1 shows the consort flow chart, which details the number of participants. The patients were divided into two groups: 60 patients in Arm A, treated with adjuvant docetaxel 70 mg/m² and carboplatin AUC = 7 every three weeks with granulocyte colony-stimulating factor (G-CSF) for six courses; and 59 patients in Arm B, treated with adjuvant doxorubicin hydrochloride 60 mg/m² and cyclophosphamide 600 mg/m² every three weeks with G-CSF for 4 courses followed by docetaxel 70 mg/m² and carboplatin AUC = 7 every three weeks with G-CSF for four courses. The mutations of BRCA 1 and 2 were not checked due to their high price, and all patients did R0 resection. The OS was defined as the time from randomisation to death, irrespective of cause, and disease-free survival (DFS) was defined as the time from randomisation to local or distant relapse or death.

Criteria

Inclusion criteria: The female patients with age > 20 years, the tumour must have been determined to be HER2-negative (IHC1+ or IHC2+ and fluorescence in situ hybridisation [FISH]-negative); the tumour must have been determined to be hormone receptor-negative (ER- and PR-negative).

Exclusion criteria: T4 tumours including inflammatory BC; definitive clinical or radiologic evidence of metastatic disease; required imaging studies (computed tomography [CT] scan and bone scan) must have been performed within 90 days prior to randomisation; any previous history of ipsilateral invasive BC or ipsilateral DCIS; history of non-breast malignancies (except for in situ cancers treated only by local excision and basal cell and squamous cell carcinomas of the skin) within 5 years prior to randomisation; active or history of cardiac disease, patients known to be human immunodeficiency virus (HIV) positive, hepatitis B or hepatitis C with abnormal liver function tests; history of hospitalisation in the past 12 months for diabetes; and pregnancy or lactation at the time of study entry.

Statistical analyses

The analysis was done using SPSS 19 software (IBM, SPSS Inc., Chicago, IL, USA). The categorical and continuous data were analysed using χ² and t-test, respectively. Outcomes for this study were OS, DFS, and toxicity. Comparison between OS and DFS for the two arms was checked by GraphPad Prism 5 software and the log-rank test was used to compare the Kaplan-Meier curves for OS and DFS. Also, Cox’s proportional hazard regression analysis was used to check the effects of various parameters on the primary analysis. A p-value < 0.05 was considered to be statistically significant.

Results

A total of 119 patients were randomly enrolled to two arms (60 patients in arm A and 59 patients in arm B). The baseline characteristics of patients in the two arms are shown in Table 1. The mean follow-up was 40 months at the time of treatment analysis. There were no significant differences between the two arms regarding the start of intervention. In the patients with lymph node involve-
ment, a minimum of three and maximum of 15 lymph nodes were involved.

The two-year OS rate was 96.5% vs. 90.5%, and also the mean OS was 20.7 months vs. 21.1 months (arm A vs. arm B), and there was no significant difference between the two arms (hazard ratio [HR] 2.56, 95% CI: 0.58–11.30; \( p = 0.21 \)) (Fig. 2). In addition, the 5-year rate and mean OS were 91.7% vs. 81.3% and 34.4 vs. 36.4 months (arm A vs. arm B); there was no significant difference between the two arms (HR 2.09, 95% CI: 0.67–6.52; \( p = 0.20 \)), whereas, the 5-year rate and the mean DFS was 85% vs. 64.4% and 32.6 vs. 32 months (arm A vs. arm B); there was significant difference between the two arms (HR 2.31, 95% CI: 1.13–4.73; \( p = 0.028 \)).

Cox’s proportional hazard regression analysis was used to evaluate the effects of various parameters on the primary analysis. There were no unfavourable predictors for OS (Table 2), but age and menopausal status were unfavourable predictors for DFS (Table 3).

### Table 1. The correlation between variables in two arms

| Variables                  | Arm A* (n = 60) | Arm B** (n = 59) | P-value |
|---------------------------|----------------|----------------|---------|
| Age, year                 |                |                |         |
| Mean ± SD                 | 45.7 ±13.7     | 44.1 ±10       | 0.490   |
| Range                     | 24–45          | 21–72          |         |
| < 50          | 41 (68.3)      | 40 (67.8)      | 0.553   |
| Menopausal status         |                |                |         |
| Premenopausal            | 41 (68.3)      | 39 (66.1)      | 0.474   |
| Postmenopausal           | 19 (31.7)      | 20 (33.9)      |         |
| Laterality                |                |                |         |
| Right                     | 25 (41.7)      | 28 (47.5)      | 0.326   |
| Left                      | 35 (58.3)      | 31 (52.5)      |         |
| Tumour size, cm          |                |                |         |
| < 2                       | 20 (33.3)      | 12 (20.3)      | 0.135   |
| 2–5                       | 34 (56.7)      | 35 (59.3)      |         |
| > 5                       | 6 (10)         | 12 (20.3)      |         |
| Lymph node invasion       |                |                |         |
| Yes                       | 24 (40)        | 33 (55.9)      | 0.060   |
| No                        | 36 (60)        | 26 (44.1)      |         |
| Vascular invasion         |                |                |         |
| Yes                       | 12 (20)        | 10 (16.9)      | 0.424   |
| No                        | 48 (80)        | 49 (83.1)      |         |
| Perineural invasion       |                |                |         |
| Yes                       | 5 (8.3)        | 4 (6.8)        | 0.511   |
| No                        | 55 (91.7)      | 55 (93.2)      |         |
| Stage                     |                |                |         |
| I                         | 12 (20)        | 10 (16.9)      | 0.102   |
| II                        | 41 (68.3)      | 33 (55.9)      |         |
| III                       | 7 (11.7)       | 16 (27.1)      |         |
| Histological Grade        |                |                |         |
| I                         | 8 (13.3)       | 5 (8.5)        | 0.584   |
| II                        | 23 (38.3)      | 27 (45.8)      |         |
| III                       | 29 (48.3)      | 27 (45.8)      |         |
| Margin involvement        |                |                |         |
| Yes                       | 4 (6.7)        | 5 (8.5)        | 0.489   |
| No                        | 56 (93.3)      | 54 (91.5)      |         |
| Radiotherapy              |                |                |         |
| Yes                       | 55 (91.7)      | 49 (83.1)      | 0.127   |
| No                        | 5 (8.3)        | 10 (16.9)      |         |
| Type of pathology         |                |                |         |
| IDC                       | 52 (86.7)      | 55 (93.2)      | 0.187   |
| ILC                       | 0              | 1 (1.7)        |         |
| MC                        | 8 (13.3)       | 3 (5.1)        |         |
| Ki-67, %                  |                |                |         |
| Mean ± SD                 | 42.2 ±28.4     | 42.8 ±25       | 0.918   |
| ≤ 20                      | 19 (31.7)      | 14 (23.7)      | 0.223   |

* Arm A – docetaxel and carboplatin
** Arm B – doxorubicin hydrochloride and cyclophosphamide followed by docetaxel and carboplatin
SD – standard deviation, IDC – invasive ductal carcinoma, ILC – invasive lobular carcinoma, MC – medullary carcinoma
The comparison of adverse events for the two arms is shown in Table 4. Although thrombocytopenia was higher in arm A compared with arm B, the difference was not significant \((p > 0.05)\). Therefore, the side effects were similar in the two groups.

**Discussion**

Both arms in this study had carboplatin because we wanted to check the efficacy of adding of an anthracycline (doxorubicin) to the taxane regimen in stage I-IIITNBC patients that more patients were stages I and II (88.3% arm A and 72.9% arm B). The results showed that adding anthracycline to the chemotherapy regimen did not increase the 5-year OS (HR 2.04, 95% CI: 0.76–5.43; \(p = 0.17\)) and DFS (HR 2.31, 95% CI: 1.13–4.73; \(p = 0.028\)). Although grade 3-4 neutropaenia and cardiotoxicity was more in anthracycline-based regimen and also thrombocytopenia in the regimen without anthracycline, the differences were not significant. Patients suffering from TNBC have a poor prognosis mainly because no standard treatment is currently available [16]. Anthracyclines and taxanes are the most active and widely used chemotherapeutic agents in hormone receptor-negative patients for treating BC and those whose disease progresses while they are taking hormone therapy [17]. These agents are commonly used in the adjuvant setting, either in combination or sequentially [18]. Anthracycline use in early-stage BC has been steadily declining, especially for patients with stage I/II or...
HER2-positive disease, and the overall analysis reported that 80% of chemotherapies were anthracycline-based in these patients from 2000 to 2010. Then, the use of anthracycline-based regimens reduced 20% in stage I/II and 6% in stage III patients, while non-anthracycline regimens increased from 5% to 35% [19]; one study from Giordano et al. [20] confirmed these results. Multiple trials in the 1980s and 1990s demonstrated that an anthracycline-based chemotherapy regimen was associated with lower rates of BC recurrence and improved survival when compared with non-anthracycline regimens [12]. The addition of taxanes to anthracycline-based chemotherapy as adjuvant therapy decreases the risk of recurrence (4.6%) and overall mortality (3.2%) [21]. One trial [22] compared docetaxel plus cyclophosphamide (TC) with a first-generation anthracycline regimen (doxorubicin plus cyclophosphamide, or AC) and reported superior OS for the patients treated with TC. The patients treated with TC had more fever and neutropaenia (5% vs. 2.5%), but congestive heart failure developed in one patient treated with AC and none with TC. Smith et al. [23] demonstrated that anthracyclines increased the risk of clinical cardiotoxicity (5.43 fold), subclinical cardiotoxicity (6.25 fold), any cardiotoxicity (2.27 fold), and the risk of cardiac death (4.94 fold) compared with non-anthracycline regimens. The trial by Chen et al. [24] reported that patients with neoadjuvant treatment of TNBC or HER2-positive with docetaxel, anthracycline, and cyclophosphamide (TEC) had a higher rate of neutropaenia and leukopaenia. TEC treatment had a better survival outcome and a trend of higher complete response rate compared with TC in this trial setting, especially in TNBC subtype, which deserves further validation. On univariate analysis [25], patients who had received prior adjuvant chemotherapy with anthracyclines had a significantly lower probability of response than patients who did not: 43% vs. 58% (p = 0.02). The patients who did not receive adjuvant chemotherapy had a longer survival time than the patients previously treated

| Variables | P-value | HR (95% CI) |
|-----------|---------|-------------|
| Treatment arm, arm A vs. arm B | 0.070 | 0.444 (0.184-1.070) |
| Menopause status, pre vs. postmenopausal | 0.022 | 0.283 (0.097-0.831) |
| Age, ≥ 50 vs. < 50 years | 0.007 | 0.248 (0.091-0.679) |
| Laterality, right vs. left | 0.603 | 0.779 (0.303-2.001) |
| Tumour size, < 2 vs. 2–5 or > 5 cm | 0.456 | 0.752 (0.355-1.592) |
| LN involvement, yes vs. no | 0.320 | 0.607 (0.227-1.624) |
| Vascular invasion, yes vs. no | 0.128 | 2.706 (0.752-9.742) |
| Perineural invasion, yes vs. no | 0.566 | 0.628 (0.128-3.071) |
| Stage, I vs. II or III | 0.454 | 1.368 (0.602-3.106) |
| Grade, I vs. II or III | 0.235 | 0.640 (0.307-1.337) |
| Margin involvement, yes vs. no | 0.132 | 2.902 (0.726-11.600) |
| Radiotherapy, yes vs. no | 0.114 | 2.399 (0.810-7.106) |
| Type of pathology, DC vs. LC or MC | 0.604 | 1.190 (0.616-2.297) |
| Ki-67 status, ≤ 20 vs. > 20% | 0.144 | 2.084 (0.777-5.588) |

*HRs (hazard ratios) are presented as the risk of the right-side category (i.e. right side of vs in Parameter column) to the left-side category (i.e. left side of vs).

Table 3. Multivariate survival analysis using Cox’s regression model for affecting of variables on disease-free survival

| Variables | Arm A | Arm B | P-value |
|-----------|-------|-------|---------|
| Grade 3–4 vomiting | 1 (1.7) | 1 (1.7) | > 0.05 |
| Grade 3–4 mucositis | 1 (1.7) | 1 (1.7) | > 0.05 |
| Grade 3–4 diarrhoea | 1 (1.7) | 1 (1.7) | > 0.05 |
| Grade 3–4 neutropenia | 1 (1.7) | 1 (1.7) | > 0.05 |
| Grade 3–4 thrombocytopenia | 6 (10.2) | 2 (3.4) | > 0.05 |
| Cardioxicity | 1 (1.7) | 1 (1.7) | > 0.05 |
| Hypersensitivity reaction | 1 (1.7) | 1 (1.7) | > 0.05 |
| Peripheral neuropathy | 2 (3.4) | 1 (1.7) | > 0.05 |
| Sepsis | 0 | 1 (1.7) | > 0.05 |

Arm A – docetaxel and carboplatin, Arm B – doxorubicin hydrochloride and cyclophosphamide followed by docetaxel and carboplatin

Table 4. The adverse events for treatment regimens (two arms)
with anthracycline-based (21.1 vs. 15.8 months) adjuvant chemotherapy. Also, multivariate analysis confirmed adjuvant chemotherapy with anthracyclines to be among the strongest prognostic factors associated with both poor PFS and OS. Piccart-Gebhart et al. [26] reported that taxanes were significantly worse compared with single-agent anthracyclines in terms of PFS, but not in terms of response rates or survival. Taxane-based combinations were significantly better than anthracycline-based combinations in terms of response rates and PFS, but not in terms of survival. A taxane-based treatment regimen may be a better option than a combined taxane/anthracycline regimen for patients with advanced BC because it produces equivalent clinical outcomes and has lower toxicity compared to other similar regimens [27]. Five randomised studies compared anthracyclines (doxorubicin, epirubicin, pegylated liposomal doxorubicin) vs. other drugs, but it did not reach the statistical significance for the endpoints of response rate, time to progression, and OS, suggesting a minor role for anthracycline in the therapeutic strategy of pretreated metastatic BC patients [28–32]. Although doxorubicin has become one of the most effective chemotherapeutic agents, it was noted early on that its use was complicated by the development of heart failure [33, 34]. Multiple large cohort trials and meta-analysis studies showed that the addition of a taxane to an anthracycline-based regimen in the adjuvant setting has improved the PFS and OS in patients with early BC. According to these, the use of anthracyclines as initial chemotherapy in early BC may continue to be replaced by taxane-based and novel regimens in the future [35]. We need a prospective, more advanced trial with clearly more rigorous reporting and data monitoring (a larger group of patients).

In conclusion, less progression was found with arm A as compared to arm B. Therefore, the addition of anthracyclines such as doxorubicin hydrochloride did not increase PFS and DFS in TNBC patients. Due to the number of TNBC patients in stages I and II, we can easily omit anthracyclines in the treatment of these patients.

The authors declare no conflict of interest.

References

1. Kröger N, Achterrath W, Hegewisch-Becker S, Mross K, Zander AR. Current options in treatment of anthracycline-resistant breast cancer. Cancer Treat Rev 1999; 25: 279-91.
2. Wahba HA, El-Hadaad HA. Current approaches in treatment of triple-negative breast cancer. Cancer Biol Med 2015; 12: 106-16.
3. Bauer KR, Brown M, Cress RD Parise C.A, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer 2007; 109: 1721-8.
4. Hurvitz S, Mead M. Triple-negative breast cancer: advancements in characterization and treatment approach. Curr Opin Obstet Gynecol 2016; 28: 59-69.
5. Dent R, Trudeau M, Pritchard KI, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007; 13: 4429-34.
6. Glück S, Ross JS, Royce M, McKenna EF Jr, Perou CM, Avisar E, Wu L. TP53 genomics predict higher clinical and pathologic tumor response in operable early-stage breast cancer treated with docetaxel-capetitabine + trastuzumab. Breast Cancer Res Treat 2012; 132: 781-91.
7. Liedtke C, Mazoni C, Hess KR, et al. Response to neoadjuvant therapy an long-term survival in patients with triple-negative breast cancer. J Clin Oncol 2008; 26: 1275-81.
8. Lehmann BD, Jovanovic B, Chen X, et al. Refinement of triple-negative breast cancer molecular subtypes: implications for neoadjuvant chemotherapy selection. PLoS One 2016; 11: e0157368.
9. Portha H, Jankowski C, Cortet M, Desmoulins I, Martin E, Lorigs V, Arnould L, Coutant C. Non-metastatic triple-negative breast cancer in 2016: Definitions and management. Gynecol Obstet Fertil 2016; 44: 492-504.
10. Rampurwala MM, Rocque GB, Burkard ME. Update on adjuvant chemotherapy for early breast cancer. Breast Cancer (Auckl) 2014; 8: 125-33.
11. Yamamoto Y, Iwase H. Clinicopathological features and treatment strategy for triple-negative breast cancer. Int J Clin Oncol 2010; 15: 341-51.
12. Early Breast Cancer Trials’ Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. Lancet 2005; 365: 1687-717.
13. Moreno-Aspitia A, Perez EA. Treatment options for breast cancer resistant to anthracycline and taxane. Mayo Clin Proc 2009; 84: 533-45.
14. Joensuu H, Giglroy J. Adjuvant treatments for triple-negative breast cancers. Ann Oncol 2012; 23: vi40-5.
15. Pai VB, Nahata MC. Cardiotoxicity of chemotherapeutic agents: incidence, treatment and prevention. Drug Saf 2000; 22: 263-302.
16. Wang S, Shi Y, Yuan Z, et al. Classical CMF regimen as adjuvant chemotherapy for triple-negative breast cancer may be more effective compared with anthracycline or taxane-based regimens. Med Oncol 2012; 29: 547-53.
17. National Comprehensive Cancer Network Web site NCCN Practice Guidelinesin Oncology-v.1.2009: Breast Cancer.http://www.nccn.org/professionals/physician_gls/PDF/breast.pdf. http://www.nccn.org/professionals/physician_gls/PDF/breast.pdf, Accessed May 1, 2009.
18. Dean-Colomb W, Esteva FJ. Emerging agents in the treatment of anthracycline- and taxane-refractory metastatic breast cancer. Semin Oncol 2008; 35: 531-8quiz S40.
19. Helwick C. Anthracyline Use Steadily Declining in Early Breast Cancer Population. ASCO Post 2013: 3.
20. Giordano SH, Lin YL, Kuo YE. Hortobagyi GN, Goodwin JS. Decline in the use of anthracyclines for breast cancer. J Clin Oncol 2012; 30: 2322-9.
21. Early Breast Cancer Trials’ Collaborative Group (EBCTCG) Peto R, Davies C. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet 2012; 379: 432-44.
22. Jones SE, Savin MA, Holmes FA, et al. Phase III trial comparing doxorubicin plus cyclophosphamide with docetaxel plus cyclophosphamide as adjuvant therapy for operable breast cancer. J Clin Oncol 2006; 24: 5381-7.
23. Smith LA, Cornelius VR, Slummer CI, Levitt G, Vernill M, Canney P, Jones A. Cardiotoxicity of anthracycline agents for the treatment of cancer: systematic review and meta-analysis of randomised controlled trials. BMC Cancer 2010; 10: 337.
24. Chen X, Ye G, Zhang C, et al. Superior outcome after neoadjuvant chemotherapy with docetaxel, anthracycline, and cyclophosphamide versus docetaxel plus cyclophosphamide: results from the NATT trial in triple-negative or HER2 positive breast cancer. Breast Cancer Res Treat 2013; 142: 549-58.
25. Venturini M, Bruzzi P, Del Maistro L, et al. Effect of adjuvant chemotherapy or with anthracyclines on the activity and efficacy of first-line cyclophosphamide, epirubicin, and fluorouracil in patients with metastatic breast cancer. J Clin Oncol 1996; 14: 764-73.
26. Piccart-Gebhart MJ, Burzykowski T, Buyse M, et al. Taxanes alone or in combination with anthracyclines as first-line therapy of patients with metastatic breast cancer. J Clin Oncol 2008; 26: 1980-6.

27. Zheng R, Han S, Duan C, et al. Role of taxane and anthracycline combination regimens in the management of advanced breast cancer: a meta-analysis of randomized trials. Medicine (Baltimore) 2015; 94: e803.

28. Henderson IC, Allegra JC, Woodcock T, Wolff S, Bryan S, Cartwright K, Dukart G, Henry D. Randomized clinical trial comparing mitoxantrone with doxorubicin in previously treated patients with metastatic breast cancer. J Clin Oncol 1989; 7: 560-71.

29. Bontenbal M, Andersson M, Wildiers I, et al. Doxorubicin vs epirubicin, report of a second-line randomized phase II/III study in advanced breast cancer. EORTC Breast Cancer Cooperative Group. Br J Cancer 1998; 77: 2257-63.

30. Joensuu H, Holli K, Heikkinen M, Suonio E, Aro AR, Hietanen P, Huovinen R. Combination chemotherapy versus single-agent therapy as first- and second-line treatment in metastatic breast cancer: a prospective randomized trial. J Clin Oncol 1998; 16: 3720-30.

31. Norris B, Pritchard KL, James K, et al. Phase III comparative study of vinorelbine combined with doxorubicin versus doxorubicin alone in disseminated metastatic/recurrent breast cancer: National Cancer Institute of Canada Clinical Trials Group Study MA8. J Clin Oncol 2000; 18: 2385-94.

32. Keller AM, Mennel RG, Georgoulias VA, et al. Randomized phase III trial of pegylated liposomal doxorubicin versus vinorelbine or mitomycin C plus vinblastine in women with taxane-refractory advanced breast cancer. J Clin Oncol 2004; 22: 3893-901.

33. Lefrak EA, Pitha J, Rosenheim S, Gottlieb JA. A clinicopathologic analysis of adriamycin cardiotoxicity. Cancer 1973; 32: 302-14.

34. Von Hoff DD, Layard MW, Basa P, Davis HL Jr, Von Hoff DL, Rozencweig M, Muggia FM. Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 1979; 91: 710-7.

35. Greene J, Hennessy B. The role of anthracyclines in the treatment of early breast cancer. J Oncol Pharm Pract 2015; 21: 201-12.

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