Single Institution trial of anthracycline- and taxane-based chemotherapy for operable breast cancer: the ASTER study

Running title: ASTER trial chemotherapy for breast cancer

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Abstract

Purpose - The efficacy of anthracycline- and taxane-based chemotherapy for perioperative treatment of breast cancer (BC) has been established. No superiority of a cytotoxic regimen has been demonstrated, provided that administration of an anthracycline and a taxane is warranted. ASTER study was designed to investigate the safety of a 6 months peri-operative chemotherapy with doxorubicin and paclitaxel.

Methods - ASTER enrolled patients with cT2-3 N0-1 or pT1-2 N1-3 BC, from 11/2008 to 08/2011. Treatment consisted in Doxorubicin 60 mg/sm, Paclitaxel 200 mg/sm q21 (AT) for 3 cycles followed by Cyclophosphamide 600 mg/sm, Methotrexate 40 mg/sm, 5-Fluorouracil 600 mg/sm d1,8 q28 (CMF) for 3 cycles, in either neoadjuvant or adjuvant setting. Disease free and overall survival (DFS and OS, respectively) were estimated according to Kaplan-Meier method.

Results - 330 patients were enrolled. 77.9% of cases were treated in adjuvant setting; 65.5% received breast conservative surgery, 72.4% axillary dissection. 75.5% of cases presented estrogen receptor positivity, 66.7% progesterone receptor positivity; 18.5% of patients presented HER2-positive BC, 16.1% triple negative disease. 28 (8.5%) developed grade 3-4 hematologic toxicity; 9 patients (2.7%) developed grade 3 neurological toxicity. Loco-regional DFS was 99.6% at 1 year, 97.1% at 5 years, 95.9% at 7 years. Corresponding distant DFS was 98.4%, 90.2% and 88.8%. 1, 5 and 7-year OS were 99.6%, 94.9% and 91.2%, respectively.

Conclusion - Chemotherapy with ATx3→CMFx3 is confirmed safe and effective at 7 years follow-up. These results appear comparable to those reported in regulatory trials of most commonly prescribed anthracycline and taxane-based regimens.

Introduction

The role of adjuvant chemotherapy for early stage breast cancer (BC) has been established[1]. At first, 12 cycles of CMF[a] proved efficacy in prolonging survival after surgery in patients with axillary metastases[2]. Then, the introduction of anthracyclines led to the development of new therapeutic regimens such as FAC[b], FEC[c], EC[d] and AC[e][3]. In '90s taxanes were introduced in clinical practice, leading to more complex drug sequences and combinations. Indeed, the therapeutic arsenal could be enriched by regimens as AC→T[f], TAC[g], FEC→D[h] and TC[i], which improved survival against previous standards[4-7]. A parallel branch of research focused on neoadjuvant setting. At first this strategy was considered suitable for locally advanced inoperable cases, but was then extended to early cases in order to allow conservative surgery[8]. The same regimens used in adjuvant setting were proved effective with this intent and no differences in long term outcome were seen between neoadjuvant and adjuvant treatments[9,10]. With the progressive survival prolongation, more and more attention was paid to toxicity concerns[11]. Given the known relation between paclitaxel cumulative dose and incidence of

toxicity, in particular the neurologic one, we hypothesized that a reduced treatment duration may diminished moderate and severe neurologic adverse events (AEs)[12]. Previous studies tested different durations of chemotherapy, without finding a benefit when the treatment was extended beyond 4 months[13]. Moreover, no differences in efficacy could be evidenced between cytotoxic regimens, provided that a sequence or a combination of anthracyclines and taxanes was granted[3]. On these bases we designed a study comparing the incidence of toxicity between patients treated with AT^{j}x3→CMFx3 and with ATx4→CMFx4. This study was named ASTER (Adjuvant Safety Taxol Event Related) and was a phase II single arm safety trial. The regimen chosen was developed based on the previous results of European Cooperative Trial in Operable Breast Cancer (ECTO). This phase III study evaluated the addition of Paclitaxel to Doxorubicin followed by CMF, as adjuvant or primary systemic therapy. The study had shown a better performance of AT→CMF *versus* Doxorubicin alone followed by CMF, with acceptable toxicity, and without differences between neoadjuvant and adjuvant setting[14,15]. Herein we present the results of ASTER study after a median follow-up of 6.7 years.

Materials and methods

Study design and inclusion/exclusion criteria

ASTER was a phase II single arm safety trial. Its primary objective consisted in evaluating whether an inferior cumulative dose of Paclitaxel was associated to a reduced incidence of neurologic toxicity graded ≥2 according to Common Terminology Criteria for AEs (CTCAE) version 3.0[16]. Its secondary objective was to report any toxicities related to study treatment. Although efficacy was not a predefined outcome, also data about disease free and overall survival (DFS and OS, respectively) of the trial population were analyzed. Women aged 18 to 70 years-old were eligible for inclusion in the study when presenting a histological diagnosis of invasive BC, staged cT2-3 cN0-1, or pT1-2 pN1-3. Normal baseline hematologic values, no active viral hepatitis and HIV infection, as well as normal cardiac functioning, were also required. Written informed consent was obtained in all patients. The protocol was approved by the Ethics Committee of our Institution.

Protocol treatment

The patients received Doxorubicin 60 mg/sm i.v. bolus q21 and Paclitaxel 200 mg/sm i.v. 3 hours infusion q21 for 3 cycles followed by Cyclophosphamide 600 mg/sm i.v. bolus d1,8 q28, Methotrexate 40 mg/sm i.v. bolus d1,8 q28, 5-Fluorouracil 600 mg/sm i.v. bolus d1,8 q28 for 3 cycles. At the end of study treatment, the patients received endocrine therapy according to current guidelines, in case of HR positivity; targeted therapy with trastuzumab for 1 year, in case of HER2 over-expression or amplification; complimentary radiotherapy according to institutional guidelines.

Study procedures and follow-up

Screening procedures required anamnesis and physical examination, breast biopsy and histological exam with tumor grade, HR and HER2 status determination. HR positivity were defined according to local cut-off levels for positivity; HER2 over-expression was assessed by immunohistochemistry (IHC; HER2 3+) or fluorescence *in situ* hybridization (FISH) or by both techniques (if HER2 2+). Staging exams consisted of chest X-ray, abdomen ultrasound, bone scan and cardiac examination. Hematological and biochemistry evaluations with complete blood cell count, renal and liver function tests, viral hepatitis and HIV tests were performed in all cases. Caliper measurement and tattoo of the breast nodule were performed in all patients treated with neoadjuvant intent. In this group, the patients were clinically evaluated for response before each treatment cycle, whereas mammography and breast ultrasound were repeated after the first 3 cycles of chemotherapy and before definitive surgery. Laboratory monitoring was performed before the administration of each cycle of treatment. Evaluation of AEs was repeated before each cycle. Anamnesis and neurological examination was regularly performed during the therapy, then at least once a year. Assessment of the left ventricular ejection fraction (LVEF) had the same schedule for the whole period of the study. The patients were followed-up after the end of treatment with physical examination, hematological and biochemistry evaluations every 6 months for 5 years; breast X-ray and ultrasound, chest X-ray, abdomen ultrasound and bone scan once a year for 5 years.

Loco-regional treatment

After the conclusion of neoadjuvant chemotherapy or in case of progression during pre-operative treatment, the patients underwent breast surgery within 4 weeks. A conservative approach was preferred, whenever feasible and according to the patients' preference. Sentinel lymph node biopsy was performed in cases staged cN0 at the initial evaluation. The patients treated with conservative surgery were candidate to complimentary radiation therapy, which was started within 6 weeks after the surgical intervention, or the conclusion of chemotherapy in cases treated in adjuvant setting. Radiotherapy was administered with standard fractioning, up to a final dose of 60 Gy (50 Gy on whole breast + 10 Gy boost on tumor bed). Patients treated with radical surgery received radiation therapy in locally advanced cases only.

Toxicity and efficacy evaluations

Safety data were descriptively analyzed in all patients who received at least one full dose of study medication. Data about neurologic toxicity were mainly compared with those reported in National Surgical Adjuvant Breast and Bowel Project (NSABP) B-28[6]. A comparative assessment was also done with ECTO trial[14,15]. The B-28 study treatment regimen was different from ASTER one, but was chosen because it was the trial leading to Paclitaxel approval in the adjuvant setting. Evaluations on all other AEs were performed exclusively in comparison to ECTO, based on a longer version of the same regimen[14]. Efficacy assessment in patients receiving neoadjuvant therapy was based on the clinical measurement of breast lesion and was performed before each cycle of treatment,. Radiological exams were repeated

after 3 cycles of chemotherapy and before surgery. Disease response was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1[17]. We defined pathologic complete response (pCR) as the absence of infiltrating tumor in both breast and lymph node. Presence of *in situ* lesions was therefore considered as a pCR[18]. Progressive disease, inacceptable toxicity and insurgence of other serious conditions constituting a risk for treatment continuation and/or surgery were considered criteria for patients' early discontinuation of the study.

Statistical evaluation

Progression free survival (PFS) was defined as the time interval between surgery, and relapse or follow-up. OS was defined as the time interval between surgery, and death or follow-up. PFS and OS were estimated by Kaplan-Meier method. Comparisons of survival curves between different groups were analyzed with log-rank test. The significance level was set at 0.05. Statistical analyses were performed with GraphPad Prism® version 5.02.

Results

Patients' characteristics

Three hundred thirty patients were enrolled, with a median age of 51 years (range: 23-74 years). Tumor stage was mostly pT1 and pT2 (216 and 100 cases, respectively). Seven patients (2.1%) obtained a pCR after pre-operative treatment. Nodal stage was pN0 in 27.3% of cases, pN1 in 59.4% of cases, pN2 in 6.7% of cases and pN3 6.3% of cases. A higher proportion of the neoadjuvant subpopulation presented pathologic node negativity (57.5% *versus* 18.7%) in comparison with the adjuvant one, as predictable consequence of pre-operative treatment. Tumor grade was most frequently intermediate (52.4% of cases) and high (44.3% of cases), without significant imbalance between neoadjuvant and adjuvant setting. Estrogen receptors (ERs) were expressed in 249 patients (75.5%), progesterone receptors (PRs) in 220 patients (66.7%). Pre- and post-operative subgroups were homogeneous in the expression of HRs. Most cases were HER2 negative (81.5% of cases), with a slight prevalence in the neoadjuvant group (90.4% *versus* 79.0%). Fifty-three patients (16.1%) had triple negative BC, 16 of them in the pre-operative setting. Ki-67 was equal or above 14% in 139 cases (42.1%). A larger proportion of neoadjuvant patients presented high Ki-67 at surgery (43.8% *versus* 41.6%), in comparison with the adjuvant counterpart.

Treatment characteristics Two hundred fifty-seven cases (77.9%) underwent adjuvant chemotherapy, 73 (22.1%) were treated in the neoadjuvant setting. Two hundred sixteen patients (65.5%) received conservative surgery, the remaining ones radical mastectomy. Axillary dissection was performed in 239 cases (72.4%), while 91 patients (27.6%) underwent sentinel node biopsy. Comparing the two study groups, a larger proportion of neoadjuvant patients were treated with radical breast surgery than the adjuvant counterparts (mastectomy in 43.8% *versus* 31.9% of cases); the opposite observation can be made for nodal surgery (axillary dissection in 50.7% *versus* 78.6% of cases). Chemotherapy was administered for a median of 6 cycles (range: 2-6 in the neoadjuvant setting, 1-6 cycles in the adjuvant one). Two

hundred fifty patients (75.8%) received subsequent endocrine therapy. Neoadjuvant or adjuvant trastuzumab was administered in 61 cases (18.9%). No significant differences in endocrine and targeted treatment were observed between the two study subgroups. All the patients treated with conservative surgery received subsequent radiotherapy on residual breast tissue.

Efficacy results Median follow-up was 6.7 years (range: 0.2-8.9). In the overall population, neither loco-regional nor distant median DFS was reached. Loco-regional DFS was 99.6%, 99.0%, 97.1% at 1, 2 and 5 years, respectively. Correspondent distant DFS was 98.4%, 95.9% and 90.2%. Median global progression free survival (PFS) was not reached. PFS was 98.4% at 1 year, 95.3% at 2 years and 89.3% at 5 years. In the overall population, median OS was not reached. 1-, 2- and 5-year OS was 99.6%, 98.7% and 94.9%, respectively. Data about PFS and OS for the two study subgroups are detailed in Table 1. Analyzing outcome data in the specific population of triple negative patients, results appeared similar to those of the overall population, with median PFS and OS not reached. In particular, PFS was 92.2%, 76.5% and 68.% at 1, 5 and 7 years. Corresponding OS was 98.0%, 82.3% and 80.1%. Patients obtaining a pCR after neoadjuvant therapy had a median PFS of 82 months and a particularly favorable OS, with 100% of cases alive at the date of data lock.

Toxicity results

The treatment was well-tolerated. In particular, 299 patients (90.6%) did not have hematologic toxicities, 203 (61.5%) did not experience neurologic AEs. Grade 3 or 4 hematologic toxicity occurred only in 28 patients (8.5%). As regards neurologic toxicity, 9 (2.7%) grade 3 neurologic AEs were registered. Other relevant (i.e. graded ≥2) AEs included 4 cases of severe mucositis, 1 grade 3 aminotransferases elevation and a single case of allergic reaction to paclitaxel. No cases of moderate to severe cardiac toxicity were reported, as well as long term or delayed AEs were observed.

Conclusion

The retrospective parallel between ASTER and NSABP B-28 results shows an apparent reduction in the incidence of neurologic AEs. In fact, NSABP B-28 registered severe neurotoxicity in 18.0% of patients, while only 9 patients (2.7%) developed this AE in ASTER[6]. The incidence of neurologic toxicity with ASTER regimen appears also lower of that reported in ECTO. In fact, grade 2 neuropathy was recorded in 20.5% of ECTO patients, but only in 6.1% of ASTER patients[15]. With the limitations of a retrospective comparison, these observations seem to confirm that a short course chemotherapy is able to limit the incidence of a potentially invalidating toxicity.

AEs different from the neurologic ones showed a modest reduction, in comparison with the historical cohort of NSABP B-28[6]. In particular, grade ≥3 hematologic toxicity decreased from 9.4% to 8.7% and mucositis from 6.7% to 2.9%. This observation is consistent with a reduced exposure to anthracyclines and taxanes. Though such AEs have an acute onset and generally do not entail long term *sequelae*, they can limit patients' quality of life during chemotherapy and be

life threatening in severe cases. Indeed, the reduced incidence of early onset AEs is a comparably desirable result of short course treatment, potentially implementing patients' compliance.

Another important observation can be done as regards cardiac toxicity. ECTO trial reported grade ≥2 decrease in LVEF in 16.2% of patients, while no cases of grade ≥3 cardiotoxicity were registered in ASTER[14,15]. It has to be underlined that 18.6% of the study population was also treated with neoadjuvant or adjuvant trastuzumab, without apparent worsening of safety profile.

Efficacy results can also be evaluated through retrospective comparison with data from ECTO. 5-years DFS was 76% and 72% in adjuvant and neoadjuvant treatment arms of ECTO, respectively. Corresponding OS was 85% and 84%[14,15]. ASTER documented a 5-year DFS of 92.2% and 78.9% in post- and pre-operative setting, respectively. Corresponding OS was 94.9% and 95.8%. Again, it has to be underlined that the retrospective nature of this parallel does not allow to draw conclusions about the relative efficacy of the two experimental regimens. However, outcome data seem to suggest that a short course chemotherapy is at least as effective as standard regimen.

Notably, efficacy results were confirmed in ASTER triple negative subpopulation, whose median PFS and OS were not reached. Indeed, short course chemotherapy does not seem to increase the risk of relapse or death even in the group of patients with the worst prognosis.

ASTER study confirms pCR to be a strong positive prognostic factor in the neoadjuvant setting. In fact, all patients obtaining complete remission of disease were alive at the time of data lock.

This study presents some weak points, in particular the absence of a control group which allows only retrospective observations. However, it also has the value of focusing on a large number of cases (considering the phase II nature of the study), treated within the same Institution within a limited range of time. Given these considerations, ASTER showed that a peri-operative treatment with ATx3→CMFx3 has satisfactory long term efficacy and toxicity profile in BC patients.

We are well aware that no definitive conclusions are possible, due to the non-randomized phase II nature of the trial. However, these evidences seem to suggest an advantage in prescribing a short term chemotherapy to patients with operable BC. The particular regimen studied in ASTER seems to be sufficiently safe and effective to be proposed for use in clinical practice. Large randomized trials are needed to confirm this point and to establish the potential superiority of a specific drug combination over the others.

Legend

[a](Cyclophosphamide 600 mg/sm i.v. + Methotrexate 40 mg/sm i.v. + Fluorouracil 600 mg/sm i.v. d1,8 q28)x6

[b](Fluorouracil 600 mg/sm i.v. + Doxorubicin 60 mg/sm + Cyclophosphamide 600 mg/sm i.v. d1 q21)x6

[c](Fluorouracil 600 mg/sm i.v. + Epirubicin 75 mg/sm + Cyclophosphamide 600 mg/sm i.v. d1 q21)x6

[d](Epirubicin 90 mg/sm i.v. + Cyclophosphamide 600 mg/sm i.v. d1 q21)x4

[e](Doxorubicin 75 mg/sm + Cyclophosphamide 600 mg/sm i.v. d1 q21)x4

[f](Doxorubicin 75 mg/sm + Cyclophosphamide 600 mg/sm i.v. d1 q21 x 4 cycles → Docetaxel 100 mg/sm i.v. d1 q21)x4

[g](Docetaxel 75 mg/sm i.v. + Doxorubicin 50 mg/sm i.v. + Cyclophosphamide 500 mg/sm i.v. d1 q21)x6

[h](Fluorouracil 500 mg/sm i.v. + Epirubicin 100 mg/sm + Cyclophosphamide 500 mg/sm i.v. d1 q21)x3→(Docetaxel 100 mg/sm i.v. d1 q21)x3

[i](Docetaxel 75 mg/sm i.v. + Cyclophosphamide 600 mg/sm i.v.d1 q21)x4

[j] (Doxorubicin 60 mg/sm i.v. + Paclitaxel 200 mg/sm i.v. d1 q21)x 3-4

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Table 1 Efficacy data

	Global population	**Neoadjuvant subgroup**	**Adjuvant subgroup**
Loco-regional DFS, %			

Median	Not reached	Not reached	Not reached
1-year-DFS	99.6	98.6	100.0
2-year-DFS	99.0	97.2	99.5
5-year-DFS	97.1	92.6	98.3
Distant DFS, %			
Median	Not reached	Not reached	Not reached
1-year-DFS	98.4	94.5	99.6
2-year-DFS	95.9	90.3	97.5
5-year-DFS	90.2	81.5	93.0
OS, %			
Median	Not reached	Not reached	Not reached
1-year-OS	99.6	98.6	100.0
2-year- OS	98.7	95.8	99.5
5-year- OS	94.9	90.2	96.3

Figure 1 Loco-regional and distant DFS (LR DFS and d DFS, respectively), and OS curves for global population.