Triple-negative high-risk breast cancer derives particular benefit from dose intensification of adjuvant chemotherapy: results of WSG AM-01 trial

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Background: This paper evaluates the prognostic and predictive impact of protein expression of various molecular markers in high-risk breast cancer (HRBC) patients with >9 involved lymph nodes, who received different chemotherapy dose-intensification strategies within a prospective randomized WSG AM-01 trial.

Materials and methods: Paraffin-embedded tumors from 236 patients, who were randomly assigned to dose-dense conventional chemotherapy with four cycles of E90C600 followed by three cycles of C600M40F600 every 2 weeks (DD) or a rapidly cycled tandem high-dose regimen with two cycles of E90C600 every 2 weeks followed by two cycles of E90C3000Thiotepa400 every 3 weeks (HD), were available for retrospective central pathological review (116 HD/120 DD). Expression of estrogen receptor (ER), progesterone receptor (PR), MIB-1, epidermal growth factor receptor, and Her-2/neu was evaluated immunohistochemically using tissue microarrays. Results were correlated with follow-up data and treatment effects by proportional hazard Cox regression models (including interaction analysis).

Results: After a median follow-up of 61.7 months, 5-year event-free survival (EFS) as well as overall survival (OS) rates for the 236 patients were significantly better in the HD arm: EFS: 62% versus 41% [hazard ratio (HR) = 0.60, 95% CI 0.43–0.85, P = 0.004]; OS: 76% versus 61% (HR = 0.58, 95% CI 0.39–0.87, P = 0.007). In multivariate analysis, HD, tumor size <3 cm, positive PR, negative MIB-1 staining, and grade 1/2 were associated with favorable outcome. Interaction analysis showed that regarding predictive effects, triple negative (ER/PR/Her-2/neu) and G3 tumors derived most benefit from HD.

Conclusion: Tandem HD improves both EFS and OS in HRBC. This therapy effect may be partly attributable to superior efficacy in the subgroup of triple-negative tumors and/or G3 with their poor prognostic marker profile.

Key words: basal-like, breast cancer, high dose chemotherapy, triple negative, breast cancer

introduction

Breast cancer patients with multiple positive lymph nodes (LN)s have a poor prognosis. The average annual lethality rates in patients with ≥10 involved LNs are five times higher than for N0 patients [1]. The 5-year event-free survival (EFS) without application of adjuvant therapy for this subgroup is <30% [2]. Anthracycline-containing adjuvant chemotherapy improves outcome [3], but subgroup analysis shows that in this high-risk subgroup few standard regimens have achieved a 5-year EFS exceeding 40%–52% [4–6]. The benefit from taxanes in this subgroup remains unclear on the basis of published retrospective, unplanned subgroup analyses from trials evaluating third-generation taxane combinations. Up to now, findings from trials investigating the effects of E90C3000Thiotepa400 every 3 weeks high dose chemotherapy (HD) in this high-risk group remain controversial. Some studies have shown a significant superiority of HD in terms of disease-free survival, particularly in breast cancer patients with ≥10 positive LN [7–9]. Other studies showed a trend to better efficacy of HD but did not reach the level of significance [10, 11], while other results did not reveal any advantage for HD [6, 12, 13]. Up to now, our previously reported WSG AM-01 study
is the only study demonstrating that the tandem HD arm compared with dose-dense Epirubicine and cyclophosphamide/methotrexat and 5-fluorouracil therapy was significantly associated with reduced risk of both relapse and death [9]. Published trial designs are very heterogeneous in terms of regimens, HD strategy, control arms, and patient selection criteria. Considering the heterogeneity of the published data, the aim should now be to identify promising HD strategies and biological tumor subtypes deriving maximum benefit from HD. To date, the predictive value of established prognostic factors such as grade, estrogen receptor (ER), tumor size, Her-2/neu, or age is still under debate in the HD setting [14–16].

Gene expression studies on the basis of DNA microarray analysis have enabled molecular subtyping of breast tumors with distinct patterns of proliferation, apoptosis, and DNA repair as well as with distinct prognostic implications [17]. Luminal, hormone receptor expressing tumors have better prognosis than Her-2/neu and triple-negative [ER-/progesterone receptor (PR)/Her-2/neu]/basal-like subtypes. The management of triple-negative tumors poses a clinical challenge due to the limited therapeutic options in this subtype. The clinical relevance of the triple-negative subgroup was reemphasized in the recent 2007 St Gallen conference (oral communication).

Currently, there is only limited experience with regard to the response of molecular subtypes to different cytotoxic therapies [18], but ER and growth factor such as Her-2/neu and epidermal growth factor receptor (EGFR) play a key role in both molecular classification of breast cancer [17] and in prediction of chemotherapy effects [3, 19, 20].

The aim of our study was to evaluate the benefits from HD in clinically relevant subgroups, particularly triple-negative patients, utilizing tumor samples from a retrospective, central pathological blinded review with updated follow-up of the randomized prospective WSG AM-01 trial in high-risk breast cancer (HRBC).

materials and methods

patients

Patients included in our analysis took part at the randomized multicenter WSG AM-01 trial, which compared tandem HD versus C50M40F600 every 2 weeks (DD) conventional chemotherapy. Eligible patients were 18–60 years old, had histologically proven breast cancer, and ≥2 positive axillary LN. Absence of distant metastases was verified by normal findings in chest X-ray, liver ultrasonography, and bone scan.

The detailed protocol has been described elsewhere [9]. HD patients received an induction regimen with two courses of E90C600 every 2 weeks with granulocyte colony-stimulating factor (G-CSF) support from day 5 to day 12. Patients received HD on day 5 to day 12. Peripheral blood stem cells were given on day 0. DD consisted of four courses of E90C600 every 2 weeks (DD) conventional chemotherapy. Eligible patients were 18–60 years was started in all patients with hazard ratio (HR)-positive disease after the completion of chemotherapy.

The WSG AM-01 study was approved by the ethics committees of all 73 participating institutions. All patients provided written informed consent. The trial was conducted according to the Helsinki Declaration of 1975 as revised in 1983.

prognostic factors and histopathological analysis

At time of diagnosis, the following baseline characteristics were determined: age, histopathology, tumor size, grade, number of examined and positive LNs, and ER/PR status.

tumor samples

Paraffin-embedded tumor blocks were requested from all 403 patients participating in the trial. Representative sections from 252 of these (63%) were received. Sufficient primary tumor tissue was available in 236 (59%) tumor samples; these sections were reviewed and analyzed at our central laboratory by two experienced breast pathologists (RD-D and CP) for specific morphologic features, including grade (based on the criteria of Elston and Ellis) and vascular invasion.

tissue microarrays and antibodies for immunohistochemistry

Expression of ER, PR, Her-2/neu, MIB-1, and EGFR was analyzed immunohistochemically using tissue microarrays, constructed from core specimens (internal diameter 2 mm) of representative tumor areas. Immunohistochemical staining was carried out on 3 μm paraffin sections. Pretreatment for antigen retrieval was mainly carried out by pressure cooker except for EGFR, where pronase was used. After blockage of biotin and peroxidase, immunohistochemical staining was carried out on an automated immunostainer (Biogenex, 6000, San Ramon, CA) using the standard labeled streptavidin–biotin method (UltraTek Reagent Detection Kit, Scy Tek, Logan, UT) followed by 3,3′-diaminobenzidine enzymic development. Sections were counterstained blue with hematoxylin. Omission of the primary antibody served as negative control.

Consecutive sections were stained with antibodies against Her-2/neu (polyclonal rabbit antibody c-erbB-2, dilution 1 : 500, DAKO, Glostrup, Denmark), ER (rabbit monoclonal antibody, clone SP1, 1 : 800, DCS, Hamburg, Germany), PR (rabbit monoclonal antibody, clone SP2, 1 : 800, DCS), MIB-1/Ki-67 (mouse monoclonal antibody, MIB-1, 1 : 1000, DAKO), and EGFR (mouse monoclonal antibody, clone E 30, 1 : 100, Merck, Darmstadt, Germany).

immunohistochemical analysis

Expression of Her-2/neu oncoprotein was categorized in three groups according to the HercepTest score—score 0: no staining at all or membrane staining in <10% of the tumor cells; score 1+: faint, incomplete membranous staining; 2+: moderate, complete membranous staining; 3+: strong membranous staining, observed in >10% of the tumor cells, respectively. A score of 3+ was designated as positive. Cases with 2+ scores were further evaluated by FISH to evaluate Her-2/neu gene amplification.

Ki-67/MIB-1 expression was defined as a strong nuclear staining in at least 10% of the tumor cells. ER and PR were scored by an intensity and proportion score (1–3 negative and >4 positive).

statistical analysis

Bivariate correlations of individual markers were measured by Pearson’s correlation. Association of markers with triple negativity was assessed by Fisher’s exact test. Survival analysis was carried out using the Kaplan–Meier method including the log-rank test group comparisons. Uni- and multivariate analyses of EFS and overall survival (OS) used proportional hazard Cox regression models. Multivariate analyses (including treatment interaction analyses) were carried out by backward elimination using the Wald test; HRs for borderline significant factors and interactions are included; 95% confidence intervals are presented elsewhere [9].
From May 1995 to June 2002, 403 patients were randomly assigned to receive to tandem HD (201 patients) or DD conventional chemotherapy (202 patients). From 236 patients (59%), paraffin-embedded tumor blocks with primary tumor tissue were retrieved for central pathological review and further immunohistochemical investigations (116 from HD and 120 from DD). Patient and tumor characteristics are detailed in Table 1. The collective data available for the immunohistochemistry analyses reported here were representative of the original study population. There were no significant differences in the distributions of clinicopathological factors.

With regard to clinically interesting subgroups, 66 patients had tumors that were triple negative, i.e. that stained negative for ER, PR, and HER2. Triple-negative tumors were significantly associated with younger age (median: 45.2 years ± 9.0 versus 51.2 ± 8.1; P < 0.001, Fisher’s exact test), G3 (57% versus 31%; P = 0.001), and EGFR overexpression (34% versus 7%; P < 0.001). Moreover, HR-negative tumors as a whole were significantly associated with G3 (60% versus 23.2, P = 0.001), as well as overexpression of MIB-1 (67% versus 52%, P = 0.028), Her-2/neu (31% versus 9.6, P < 0.001), and EGFR (28% versus 4%, P < 0.001).

patient outcome according to study arm
In all, 236 patients (116 HD and 120 DD) were followed up until November 2005, leading to a median follow-up time of 61.7 months in patients still alive at time of analysis with a range of 4.2–121.9 months (HD: 67.9 months, DD: 56.3 months). In all, 56 relapses were reported in the HD and 76 in the DD group. Estimated 5-year EFS rates were 62% for HD group and 41% for DD (HR = 0.60, 95% CI 0.43–0.85, P = 0.004). A total of 99 deaths were reported (39 in HD; 60 in DD); 5-year OS was 76% in HD arm and 61% in DD arm (HR = 0.58, 95% CI 0.39–0.87, P = 0.007) (Figure 1).

patient outcome according to prognostic factors
Tumors from 211 patients with available staining results for all protein markers were included in univariate and multivariate analyses.

In univariate analysis, the following factors were significantly associated with better EFS: HD, positive PR status, tumor size <3 cm, G1/2, and negative status of Her-2/neu and of MIB-1. The same factors as well as positive ER status and negative EGFR were significantly associated with longer OS.

Multivariate analysis was carried out including the factors therapy, tumor size, ER, PK, grade, HER-2/neu, MIB-1, and EGFR. HD therapy (HR = 0.48, P = 0.001) was significantly associated with reduced relapse risk. There was a similar but nonsignificant trend in positive PR status (HR = 0.65, P = 0.03). Positive MIB-1 status (HR = 1.90, P = 0.003) and larger tumor size ≥3 cm (HR = 1.66, P = 0.008) were significant factors for poor EFS.

For OS, again HD therapy (HR = 0.43, P < 0.001) and positive PR status (HR = 0.43, P = 0.001) were favorable, whereas in addition to positive MIB-1 status (HR = 1.73, P = 0.03), G3 (HR = 1.64, P = 0.05) and larger tumor size ≥3 cm (HR = 1.76, P = 0.01) were associated with increased risk of death. Detailed results for univariate and multivariate analysis are shown in Table 2.

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HR, Her-2/neu, and EGFR status; and triple-negative tumors (ER/PR/Her-2/neu) as well as positive MIB-1 staining.

As noted regarding Figure 2, significant benefits from HD versus DD was seen in the HR negative but not in the HR-positive subgroup (see Figure 3). Within the HD arm, HR status did not have a significant impact on EFS ($P = 0.592$), whereas within the DD arm, HR-positive tumors had significantly better EFS ($P = 0.017$). Her/2-neu-negative tumors were significantly associated with better EFS in both therapy subgroups (Figure 4).

In triple-negative tumors, the most pronounced effect of HD was observed. In this subgroup, median EFS was not reached in the HD arm, whereas it was only 32.3 months in the DD arm. This translates into an estimated 5-year EFS of 71% in the triple-negative cohort treated by HD compared with only 26% in the DD arm. There was no significant outcome difference by therapy arm associated with the remaining tumors (see Figure 5).

adjuvant therapy interactions in multivariate analysis

An additional perspective on predictive significance of factors for optimal individualized adjuvant therapy is provided by multivariate interaction analysis: Each marker that was significant in univariate Cox analysis was considered both as a potential ‘main effect’ and as a potential interaction with adjuvant therapy (i.e. HD versus DD) in a proportional hazards model for EFS, the primary end point of this randomized prospective trial. (Interaction analysis for EFS provides predictive information about adjuvant therapy efficacy that is not confounded by possibly differing palliative therapy strategies after relapse.)

The resulting model is given in Table 3, which includes the $\beta$ coefficients of the underlying Cox model to aid in the interpretation. We first note that according to the first prognostic model, tumor size, PR, and MIB-1 are purely

![Figure 1](https://example.com/figure1.png)

**Figure 1.** (A) Event-free survival (EFS) and (B) overall survival (OS).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Event-free survival</th>
<th>Overall survival</th>
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<tbody>
<tr>
<td>Therapy</td>
<td>Univariate $P$</td>
<td>Multivariate $P$</td>
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<tr>
<td>HD versus DD</td>
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<td>0.001</td>
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<td>Tumor size</td>
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<td>Her-2/neu</td>
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<tr>
<td>MIB-1</td>
<td>Positive versus negative</td>
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<td>EGFR</td>
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<tr>
<td>Age</td>
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</table>

$^a$Log-rank test
$^b$Cox proportional hazards model adjusted for age.

CI, confidence interval; HD, $E_{90}C_{600}$Thiotepa$_{400}$ every 3 weeks; DD, $C_{600}M_{400}F_{600}$ every 2 weeks; ER, estrogen receptor; PR, progesterone receptor; EGFR, epidermal growth factor receptor.
prognostic, whereas EGFR and grade are both prognostic and predictive, and triple-negative status is purely predictive. HD benefit (compared with DD) is entirely attributable to interactions in this model.

Controlling for status of tumor size, PR and MIB-1, HD is associated with relative benefit in triple-negative tumors (HR = 0.38, 95% CI 0.16–0.91, P = 0.03) and in G3 tumors (HR = 0.20, 95% CI 0.10–0.40, P < 0.001), whereas it is associated with relative risk in EGFR-positive tumors (HR = 6.76, 95% CI 1.78–25.62, P = 0.005). More precisely, for an individual patient, the composite HD HR (a number less than unity if there is relative benefit) can be calculated from the model according to the formula

$$HR_{HD \text{ versus } DD} = \exp\left(\sum_{j=1}^{3} \beta_j x_j\right),$$

where \(j\) refers to the binary factor (triple negativity, G3, or EGFR positivity), \(x_j\) is 1 if the factor \(j\) is positive (e.g. a triple-negative tumor) and 0 otherwise, and \(\beta_j\) is the appropriate entry from the last three rows of Table 3.

For example, according to the model, a G3 patient who is EGFR negative and not triple negative would have an HR of 0.16 compared with a G1 or G2 patient with the same tumor size class, PR status, and MIB-1 status if both receive DD. If both receive HD, then the G1 or G2 patient would not benefit, whereas the G3 patient would have a benefit corresponding to an HR of 0.20 compared with the same patient with DD and would even have a favorable HR of close to 0.5 compared with the G1/G2 patient (with either therapy).

If the patient is triple negative, then the model implies a benefit from HD for any grade if the patient is EGFR negative (the usual). If the patient is EGFR positive, the model formally implies that the benefit of HD (to triple-negative patients) would occur only for grade G3, i.e., not for G1 or G2; however, this is a rare occurrence: all but one of the triple-negative and EGFR-positive patients were G3.

### Discussion

WSG AM-01 was the first trial to report significant OS benefit for patients with ≥9 involved LN after HD compared with a DD anthracycline-based sequential regimen [9]. For a representative subgroup of 236 patients from this trial, paraffin-embedded tumor tissue was available for central pathological review and immunohistochemical analysis of a panel of molecular markers. At a median follow-up of 61.7 months, the EFS and OS rates favor HD.

Results from HD trials in HRBC are controversial, some reporting significant improvement in terms of EFS [7, 8, 20] or nonsignificant trends to improved relapse-free survival [10, 11, 13, 21] or OS [10, 11] and others reporting no survival differences [6, 12, 22]. Designs and patient inclusion criteria differ substantially among these trials. Definitive evaluation of
the role of HD from this large body of heterogeneous data is impossible, but further analysis of these studies could indicate successful strategies and/or subsets of patients with maximum benefit.

In the present study collective, we have analyzed established parameters such as age, tumor size, number of involved LN, grade as well as HR, Her-2/neu MIB-1, and EGFR, which are important switching points of proliferation, apoptosis, DNA repair, and related pathways. In multivariate analysis, the only established parameters correlating significantly with poor outcome were tumor size ≥3 cm (EFS and OS) and negative PR (OS). Of the evaluated molecular markers, only positive MIB-1 (EFS and OS) and G3 (OS) were independent prognostic factors for increased risk of relapse or death. The triple-negative phenotype, which was the spotlight of our interest, had no independent prognostic impact with our study population. Other phase II and III trials in HRBC also demonstrated an association of tumor size [10, 14, 15, 23], negative HR [6, 8, 10, 14, 15, 23], poor differentiation (G3) [10, 14], and higher proliferation [23] with poor outcome. In contrast, the recently published study of Kroger et al. [16] did not show a prognostic effect of MIB-1. In view of the limited data on molecular markers in HRBC, further analyses of prognostic markers are, however, still needed.

Within most of the above-mentioned trials as well in the WSG AM-01 trial, young patient age was demonstrated as a predictive factor for benefit from dose intensification and/or dose-dense chemotherapy [3, 5, 10, 13, 21, 24]. Ovarian ablation due to HD has been extensively discussed as one potential mechanism of action in hormone-sensitive disease. Three trials reported a trend to better survival for HD within the HR-positive subgroup [10, 11, 25]. Nevertheless, as reported for other ‘dose-dense’ trials, the strongest benefits from HD in our own trial were found in HR-negative disease [9, 20, 21]. These effects are very similar to overall chemotherapy efficacy reported for conventional chemotherapy trials [3, 20]. Rates of chemotherapy-induced amenorrhea were comparably high in both therapy arms of
our study. It, however, appears that biological characteristics such as proliferation and drug resistance within the luminal molecular classification subtype may be the decisive mechanisms for this correlation. This hypothesis is supported by our previous molecular classification study, where luminal A and B tumors had better prognosis than Her-2/neu and basal-like subtypes and were associated with several factors of drug resistance as e.g. bcl-2 [26].

In a clinical decision framework (HD versus DD), the presence of three therapy interactions as found here by multivariate interaction analysis would imply that the benefit or risk associated with HD depends on which of the eight possible combinations (some of which are rare due to correlations) of the binary variables for grade, EGFR status, and triple-negative status is present.

Poor nuclear grade, which is frequently associated with high proliferation and negative HR, was the strongest predictor for benefit from HD in our study and in other trials [10]. The predictive impact of G3 has been shown here by multivariate interaction analysis for the first time. More precisely, G3 patients in HRBC appear to benefit from HD rather than DD, with the exception of those few EGFR-positive patients who are not triple negative, who would appear to fare better with DD, all other things being equal. In only one study have G1 and low mitotic activity been reported to correlate with benefit from HD [5].

Our results regarding HD are fully consistent with chemosensitivity profiles on the basis of negative HR, G3, and MIB-1 positivity, as reported in patients intensively monitored under neo-adjuvant chemotherapy [19].

Her-2/neu status has been identified in several randomized studies as well in our study as a reliable prognostic factor for poorer outcome in HRBC [16, 27] as well as in unselected cohorts of patients.

In our study, HRs for therapy are comparable in both groups of Her-2/neu status with significant benefit of HD in Her-2/neu negative and nonsignificant in Her-2/neu-positive disease. These results are in line with other trials which used similar anthracycline doses in both treatment arms [16].

**Figure 5.** (A and B) Kaplan–Meier plot of event-free survival (EFS) in the $E_{90C30}$Thiotepa$_{400}$ every 3 weeks (HD) versus $C_{600M40F600}$ every 2 weeks (DD) arms in patients with triple-negative (estrogen receptor/progesterone receptor/Her-2/neu) and non-triple-negative tumors.

**Table 3.** Multivariate adjuvant therapy interaction analysis for event-free survival

<table>
<thead>
<tr>
<th>Comparison</th>
<th>$P$</th>
<th>$\beta$ (log HR)</th>
<th>HR* (95% CI)</th>
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<tr>
<td>Therapy</td>
<td>HD vs DD</td>
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<td>Tumor size</td>
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<tr>
<td>Grade</td>
<td>G3 vs G1/2</td>
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<tr>
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<td>MIB-1</td>
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<td>Triple-negative status</td>
<td>Triple negative vs others</td>
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<td>Interaction: EGFR * therapy</td>
<td>Positive and HD vs all others</td>
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<td>1.91</td>
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<tr>
<td>Interaction: triple-negative status * therapy</td>
<td>Triple and HD vs all others</td>
<td>0.030</td>
<td>−0.98</td>
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</table>

HR, hazard ratio; CI, confidence interval; HD, $E_{90C30}$Thiotepa$_{400}$ every 3 weeks; DD, $C_{600M40F600}$ every 2 weeks; NS, non-significant; ER, estrogen receptor; PR, progesterone receptor; EGFR, epidermal growth factor receptor; NS, not significant.
Negative Her-2/neu status, however, correlates significantly with benefit from HD, as reported by Rodenhuis et al. [7], who used a 25% lower cumulative anthracycline dose in the HD arm compared with the standard arm. The proposed explanation of this observation (as being due to associated Topo IIα amplification in Her-2/neu-positive disease as a target for anthracycline efficacy) was not confirmed in the recently published study of Hannemann et al. [25]. Only 22% of the tumors in the Dutch study had coamplification of Topo IIα and Her-2/neu, and there were no survival differences between both therapy groups in relation to Topo IIα status [25].

In contrast to these results, patients with positive Her-2/neu status and associated Topo IIα amplification (37%) had the most prolonged benefit from tailored dose-escalated 5-fluorouracil, epirubicin and cyclophosphamide compared with HD (cyclophosphamide, thiopeta and carboplatin) in the substudy of Tanner et al. [27] in 396 tumors (73%) from the Scandinavian high-dose trial, where in the control arm the median total anthracycline dose was 4.3 times higher than in the HD arm. Several studies have identified positive Her-2/neu status as a global marker, particularly for dose/schedule–response anthracycline sensitivity [21, 28] and for resistance to alkylating compounds [29], particularly in HD regimens in phase II trials [23, 30, 31]. A recently presented meta-analysis of 5099 patients supports the conclusion of significant benefit of anthracycline-based versus non-anthracycline-based chemotherapy only in Her-2/neu-positive BC [32].

Beyond trastuzumab, optimal chemotherapy regimens in Her-2/neu-positive breast cancer and its combination with HD in HRBC [33] remain to be defined. In particular, further investigations of the impact of several cytotoxic agents and their interaction with Topo IIα are needed in Her-2/neu-positive HRBC, and this issue should preferentially be addressed by randomized trials.

In our study, young triple-negative (defined as ER, PR, and Her-2/neu negative) patients benefited most from rapidly cycled tandem HD. This observation was confirmed at borderline significance by other research groups [25]. Microarray and immunohistochemical studies as well our recent results of k-cluster analysis of protein profiling have identified triple-negative tumors as a biologically distinct breast cancer entity, often associated with basal-like subtype [17, 26]. This subtype correlates with G3, p53 mutations, and poor prognosis [17, 26, 34], as well as with higher sensitivity to neo-adjuvant chemotherapy [18, 35]. HD provided an independent relative benefit in the triple-negative phenotype (as long as EGFR is negative) not attributable to the association of grade with triple negativity. The finding of a predictive impact of triple negativity is also remarkable considering the absence of a prognostic impact. Several studies have also identified it as a typical phenotype of BRCA1-associated tumors [36], especially in younger patients or in patients with familiar history of breast cancer [37], which are frequently associated with p53 mutations. Loss or inactivation of BRCA1 function is thought to be associated with sensitivity to DNA-damaging (e.g. alkylating) chemotherapy, which was used for dose intensification within our study [38]. This association could be one hypothetical explanation for our results.

In our previous study, molecular classification has been identified as a very strong prognostic parameter, particularly in conventionally treated HRBC. Basal-like and Her-2/neu subtypes determined by k-clustering were strong predictors for poorer outcome [26], in line with other studies in HRBC [25]. HD efficacy was also more pronounced in these subtypes. No significant interaction between molecular subtypes and dose intensification in HRBC has yet been reported, possibly due to small subgroups. In contrast, both triple negativity and G3 status as assessed by standard clinical methodology were highly predictive for HD efficacy in HRBC, as shown in our study by interaction analysis. This effect exceeds the predictive value of molecular subtypes for selection of patients for whom dose intensification could be warranted. One possible explanation could be the heterogeneous biology within triple-negative breast cancer; these include a subset of nonbasal-like tumors, which are also associated with proliferative patterns [39], but are negative for basal markers (cytokeratin 5/6, vimentin, c-kit). Before robust and standardized subtyping using gene arrays is available, the immunohistochemical determination of triple negativity in addition to routine tumor grade seems to be feasible for patient selection for dose-intense/dense regimens in clinical routine.

In view of the clinical therapeutic challenges in management of tumors with a triple-negative phenotype up to now, the finding of a predictive impact of triple negativity by multivariate interaction analysis with adjuvant HD versus DD therapy could have very important clinical consequences. The HD approach is intended to overcome some chemoresistance mechanisms and to target rapidly proliferating tumor cells within a distinct molecular chemosensitive subgroup and would provide further information for defining of optimal chemotherapy regimen within triple-negative tumors.

In EGFR-negative patients, either G3 or triple negativity implies a benefit from HD. In EGFR-positive patients, who had poor outcome by multivariate analysis, a benefit from HD is apparent only for tumors (the large majority) that are both triple negative and G3. In view of the small numbers in this subgroup and the methodological difficulties regarding EGFR testing (e.g. determination activated/total expression) [40], our results, however, need to be substantiated before definite conclusions are possible. Yet, our preliminary results indicate the need for additional targeted therapies, e.g. the use of EGFR or multi-tyrosine kinase inhibitors in this subgroup [41] particularly in combination with adequate chemotherapeutical options.

In conclusion, tumor size, PR, and MIB-1 were identified as independent prognostic factors in multivariate analysis within our HRBC population for both EFS and OS. Age, tumor size, grade, HR, Her-2/neu, and EGFR status were predictive for effects of HD with stem-cell support. All of the observed benefit from HD can be attributed to patients with triple-negative and/or G3 tumors, as demonstrated by interaction analysis. Our results support design of randomized trials within a defined biological chemosensitive subtype, such as triple-negative breast cancer, and prospective implementation of molecular biological markers for HRBC.
authors contributions

Conception and design: OG, UAN, NH, SM, RD-D, RK, and CP. Financial support: UAN and NH. Administrative support: UAN, NH, and FW. Provision of study material or patients/collection of data: UAN, SM, GS, OG, RD-D, CJ, MF, WER, BM, WL, and HK. Data analysis and interpretation: RK, OG, UAN, NH, AH, RD-D, CP, ET, SM, and MF. Manuscript writing: OG, UAN, NH, and RK. Final approval of manuscript: all authors.

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