Optimal approach in early breast cancer: Adjuvant and neoadjuvant treatment

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1. Introduction

The treatment of early breast cancer (EBC) is becoming increasingly complex, but also more effective as a better understanding of cancer biology is achieved with evolving research. Longer follow-up of prospective trials is crucial to evaluate the impact of current standard treatments in long-term outcome and safety. In this review we will summarise the current evidence for optimal treatment of EBC.

2. Which EBC patients can safely avoid adjuvant chemotherapy?

In the 1980s there were substantial advances in the treatment of breast cancer (BC), and the results of several large randomised trials indicated that adjuvant systemic therapy could decrease breast-cancer mortality by about 20%. In fact, the widespread application of adjuvant systemic therapy is considered the main cause for the declining breast cancer mortality observed in the Western world.

Treatment decisions are based on clinical (biological age, comorbidities, performance status) and pathological variables – tumour size, lymph-node status, histological grade, oestrogen receptor (ER), progesterone receptor (PR), HER2 and proliferation – that can be combined in the form of algorithms (e.g. Adjuvant!Online, Nottingham prognostic index) and form the basis of treatment for guidelines such as the ones from the European Society for Medical Oncology (ESMO), the National Comprehensive Cancer Network (NCCN), and St Gallen. However, it is clear that still too many patients receive this therapy with little likelihood of benefit and substantial toxicity.

In this section, available data on biomarkers and molecular tests related to prognostication will be reviewed. In the first part we will address the evidence and utility for adjuvant treatment decisions of biomarkers of proliferation (namely Ki67) and urokinase plasminogen activator (uPA)/plasminogen activator inhibitor (PAI-1). In the second part we will assess the practical contribution of gene expression profiling in breast cancer.

2.1. *Biomarkers*

2.1.1. *Markers of proliferation – Ki67*

Uncontrolled proliferation is a driver for cancer and is one of the hallmarks of this disease. In general, markers of an elevated proliferative rate correlate with a worse prognosis in untreated patients and may add predictive information regarding benefit from chemotherapy (CT) [1]. The most commonly used method to measure proliferation involves immunohistochemical (IHC) detection of the nuclear non-histone protein ki67, which is detected only in proliferating cells. Ki67 expression is commonly assessed using the mindbomb E3 ubiquitin protein ligase 1 antibody (MIB1) and reported as a percentage of cells positive for Ki67.

2.1.2. *Prognostic marker*

Various studies have investigated the role of Ki67 as a prognostic marker. In a meta-analysis of 40 studies, involving over 11,000 patients, baseline Ki67 was found to have a modest prognostic value in multivariable analysis, which was more evident in lymph-node-negative patients [2]. In another meta-analysis of 46 studies including over 12,000 patients, Ki67 positivity (using cut-offs defined by individual authors) was associated with a higher risk of relapse and a worse survival in patients with EBC [3]. One must highlight several limitations of these data: namely the facts that these are retrospective studies, many include heterogeneous groups of patients who were treated and followed in various ways that are often incompletely documented, and ki67 methodology and cutoff varied widely.

The clinical utility of Ki67 as a prognostic marker is more apparent when it is considered within more narrowly defined tumour subgroups and/or as part of a multiparameter panel of biomarkers, as for example in the IHC4 [4]. Other investigators have reported that Ki67 is an important part of a prognostic

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algorithm for residual risk in EBC patients treated with letrozole or tamoxifen [5].

2.1.3. *Predictive marker*

Studies have focused on the predictive value of this biomarker regarding benefit from CT or even from specific CT agents. In the ER-positive BC the results are contradictory. In the recently reported PACS 001 and BCIRG 001, high levels of Ki67 were predictive of benefit from adding docetaxel to fluorouracil, epirubicin and cyclophosphamide (FEC) CT as adjuvant treatment [6]. However, these results contrast with those from the International Breast Cancer Study Group Trials (IBCSG) VIII and IX that found no predictive value of Ki67 levels for the addition of cyclophosphamide, methotrexate and fluorouracil (CMF) to endocrine therapy (ET) in endocrine-responsive node-negative disease [7]. For ER-negative BC data to suggest that Ki67 predicts adjuvant chemotherapy response are scarce. However, taking into account all the available evidence that these tumours as a group are more responsive to chemotherapy than ER-positive tumours [8,9], one can hypothesise that higher chemotherapy sensitivity observed in patients with ER-negative tumuors is at least partially due to the consistently higher rates of proliferation of these tumours. If so, Ki67 levels may be helpful in identifying those patients most likely to benefit from chemotherapy [10].

In spite of consistent data on Ki67 as a prognostic marker in early breast cancer, its role in breast cancer management remains uncertain [11], mainly because of the lack of standardisation. In 2007 the ASCO Tumour Marker Guidelines stated that evidence supporting the clinical utility of Ki67 was insufficient to recommend its routine use for prognostic purposes in patients with newly diagnosed breast cancer [12]. However, in the St Gallen Consensus guidelines from 2011 [13] and 2013 most panelists recommend the use of Ki67 for BC subtyping classification, prognostication and prediction of response to CT, although there is no consensus on the best cut-off to be used.

The limitations of this assay are largely related to the difficulty in interpreting the literature due to lack of standardisation of assay reagents, procedures and scoring. To overcome these constraints in 2011 the International Ki67 in Breast Cancer Working Group published recommendations for Ki67 assessment in breast cancer [14]. These guidelines aim to minimise pre-analytical and analytical variables in Ki67 assessment and harmonise scoring methodology and data handling, facilitating its routine use in clinical practice.

2.1.4. *Urokinase plasminogen activator/plasminogen activator inhibitor*

uPA and PAI-1 biomarkers are invasion biomarkers analysed by a protein-based enzyme-linked immunosorbent assay (ELISA). They can be used to determine the recurrence risk in patients with node-negative EBC with the aim of better refining the decision to recommend CT in this patient population.

uPA is a serine protease with an important role in cancer invasion and metastases [15]. When bound to its receptor (uPAR), uPA converts plasminogen into plasmin and mediates degradation of the ECM during tumour-cell invasion. PAI-1 levels are high in tumour tissue and plasma, and PAI-1 is inactivated when bound to uPA.

Several retrospective studies [16,17] and a large pooled analysis of individual patient data from 8377 women treated in clinical trials by the European Organisation for the Research and Treatment of Cancer (EORTC) [18], in which tumour uPA and PAI-1 levels were determined in primary tumour tissue extracts, proved that high levels of uPA, uPAR, and PAI-1 are associated with shorter survival in women with both node-negative and node-positive disease.

The Chemo N0 is a prospective, multicentre randomised trial in which researchers stratified patients with node-negative BC into two groups according to the presence of low or high uPA/PAI-1 values. Those with low values of both uPA and PAI-1 received observation only, whereas those with high uPA and/or PAI-1 values were randomised to receive either CMF or observation. The 10-year follow-up updated analysis showed that: low-risk N0 patients according to the uPA/PAI-1, thus without any systemic therapy, had an excellent prognosis, with a 10-year survival rate of almost 90% [19], while the high-risk patients according to the uPA/PAI-1 had a 1.84-fold higher disease recurrence risk ($P = 0.017$) than the low-uPA/PAI-1. Additionally, the assay predicted, in the high-risk population, the benefit from CT [20]. These results provide for the first time long-term follow-up from a prospective biomarker-driven clinical trial in cancer.

The Node-Negative Breast Cancer (NNBC)-3 study is a prospective multicentre phase III therapy trial, with the aim of comparing risk assessment and clinical outcome on the basis of tumour-biological factors uPA/PAI-1 with those based on established, clinical and pathomorphological factors in high-risk node-negative BC patients. It enrolled more than 4000 patients, stratified into low-risk and high-risk groups according to the uPA/PAI-1 value or according to the clinical pathological algorithm. Those classified as low risk did not receive CT, whereas those classified as high risk received either six cycles of FEC or three cycles of FEC and three cycles of docetaxel [21]. In the West German Study Group Plan B trial, a prospective comparison of recurrence score (RS) – OncotypeDx – and independent central pathology assessment of prognostic tools was performed. The study randomised 2361 patients; 18% had a recurrence score of 0–11 (low risk), 61% had a recurrence score of 12–25 (intermediate risk), and 21% had a recurrence score of >25 (high risk). A weak correlation was found between uPA/PAI-1 and RS. These data showed that high-risk status according to RS is well correlated with high risk by uPA/PAI-1; however, there was substantial heterogeneity in risk assessment in the low- and intermediate-risk RS groups in which some patients are still considered to be high risk according to uPA/PAI-1 [22].

2.2. *Gene-expression-based assays*

Gene expression profiling has identified several molecular signatures that mostly have prognostic value and some prediction value.

2.2.1. *First-generation prognostic signatures – MammaPrint™*

MammaPrint™ is a microarray-based gene-expression-profiling assay that measures the levels of expression of 70 genes related to proliferation, invasion and angiogenesis. The assay accurately categorises patients in poor and good prognosis

groups on the basis of the development or not of distant metastases within 5 years. Initially requiring fresh or frozen samples, it can now be effectively performed in formalin-fixed paraffin-embedded specimens (FFPE).

The initial data were derived from 78 patients with node-negative BC, ⩽5 cm, the vast majority of whom had ER-positive tumours and did not receive adjuvant systemic treatment [23]. The validation cohort included 295 node-negative patients, of whom 61 were from the initial study, and confirmed MammaPrint™ independent prognostic value beyond standard clinicopathological variables in this patient population [24]. The TRANSBIG consortium carried out an independent retrospective validation of MammaPrint™ using samples from nine European countries, which further confirmed the prognostic value of this tool [25]. Additional validation studies were performed in node-positive EBC patients [26] and in HER2+ EBC patients [27].

MammaPrint™ is the first FDA-approved gene-expression-based assay to be used as a prognostic test in EBC patients. The clinical utility of this assay is being prospectively evaluated in the large, randomised MINDACT trial that enrolled 6690 EBC N0–N3 patients [28].

2.2.2. *Oncotype Dx™ recurrence score*

Oncotype Dx™ is a quantitative reverse transcriptase–polymerase chain reaction- (qRT–PCR-) based signature that measures the expression of 21 genes (16 cancer-related and five reference genes), performed using RNA from FFPE tumour tissues. With this multigene predictor assay a mathematical function (named recurrence score, RS) aiming at predicting the risk of distant relapse for patients with ER-positive, lymph-node-negative breast cancer treated with tamoxifen was developed based on the analysis of clinical samples from the NSABP B-20 clinical trial [29]. The RS is a continuous variable, ranging from 0 to 100, which translates into three risk-group categories: low (RS < 18), intermediate (RS from 18 to <31) and high (RS < 31).

OncotypeDx™ was then validated in a large cohort of ER-positive, node-negative, tamoxifen-treated BC patients from the NSABP-B14 trial [30]. The assay was able to stratify a generally good prognosis population into distinct subgroups (low, intermediate, or high score) with different rates of distant recurrence at 10 years (7%, 14% and 31% respectively. OncotypeDx™ RS was shown to be strongly associated with survival from breast cancer and independent from standard clinicopathological variables [30,31]. Subsequent analysis revealed that RS also seems to correlate with benefit from chemotherapy in ER-positive BC [32]. The optimal management of the intermediate-risk group is being addressed in the TAILORx trial (NCT00310180) in which 11,248 patients with ER-positive, node-negative breast cancer and intermediate risk (RS 11–25) were randomly assigned to hormone treatment either alone or in combination with chemotherapy.

Additional validation studies evaluated OncotypeDx™ in EBC patients with ER-positive disease treated with AI [33] and ER-positive node-positive BC patients [34].

The RxPONDER trial will randomise 4000 women with N1 disease and an RS of ⩽25 to endocrine therapy with or without chemotherapy [35].

While waiting for MINDACT and TAILORx results, international recommendations support the selected use of MammaPrint™ and Oncotype Dx™ in the ER + EBC patients in whom standard clinical/pathological factors are considered insufficient for adjuvant CT decisions.

2.2.3. *PAM50*

PAM50 assay provides a risk-of-relapse (ROR) score prognostic of relapse-free survival for patients with node-negative BC who did not receive adjuvant systemic therapy [36]. This assay is composed of 50 genes (derived from tumour samples of 220 patients in the training set who had ER-positive or ER-negative tumours and HER2+ or HER2-negative tumours) related to proliferation, ER-regulated genes, HER2, and basal and myoepithelial characteristics. It is compatible with FFPE-derived RNA or qRT-PCR using FF tissue.

The prognostic ability of the PAM50 has been validated in an independent test set of 786 patients with ER-positive disease treated only with adjuvant tamoxifen [37].

An ROR model containing a proliferation component (derived using 11 genes associated with cell-cycle function) was recently added to the original model.

2.2.4. *Genomic grade index*

The genomic grade index (GGI) is a gene expression signature developed to better define histological grade assessment with the ability to divide classic histological grade into low and high risk. It was developed to overcome the limitation issues, namely reproducibility, associated with the histological grade assessment and was developed using a "bottom-up" approach whereby 97 genes associated with histological grade were identified and subsequently related to clinical outcome [38].

The intrinsic prognostic information of proliferation genes seems to be better evaluated with the GGI than with classic histological grade as shown in a population of 570 patients for which complete recurrence-free survival (RFS) and histological grade was available [24,39,40]. The GGI was able to further stratify the subset of histological grade 2 patients into two subgroups: a grade 1 gene-like profile and a grade 3 gene-like profile with clearly different rates of relapse. Patients falling in the HG2-GGI3 category revealed a significantly higher rate of relapse than the HG2-GG1 (HR = 3.61; confidence interval (CI) 2.25–5.78; $P < 0.001$). In the overall population the GGI was also able to stratify patients into two risk categories with significant differences in RFS rates (high versus low risk; HR = 2.83; CI 2.13–3.77; $P < 0.001$).

In addition to prognostic prediction, the GGI ability to predict response neoadjuvant CT has also been evaluated [41]. In a study with 229 tumour samples collected before the beginning of CT with docetaxel and to fluorouracil, doxorubicin and cyclophosphamide (FAC) a high GGI was associated with greater response than low-risk GGI (40% versus 12%; $P < 0.001$).

2.2.5. *Second generation prognostic signatures – genes related to immune response*

Several studies have recently analysed the prognostic role of tumour-associated lymphocytes (TIL) in breast cancer, mainly in the triple-negative subtype [42,43]. A link between increased

lymphocytic infiltrate and reduced relapse rate and improved survival has been suggested. The expression of genes related to immune response has also been shown to provide important prognostic information in ER-negative and in highly proliferative ER-positive BC [44–47]. Jointly there is evidence to suggest that both the concentration of inflammatory infiltrate defined by IHC and expression of B-cell or plasma-cell metagene defined by microarray-based gene-expression profiling are likely to provide important prognostic information.

2.2.6. *Stroma-related prognostic gene signatures*

The development of stroma-related prognostic gene signatures is an evolving field of great scientific interest; however, independent validation of their prognostic accuracy is still needed before clinical application.

3. Which are the current major challenges regarding neoadjuvant systemic therapy?

3.1. *Advantages of neoadjuvant systemic treatment and end-points for clinical trials*

Neoadjuvant therapy (NT) is the standard of care for women with locally advanced, inflammatory or inoperable primary breast cancer (BC) [48–51]. Currently, based on the results of landmark trials NSABP B-18 and NSABP B-27, NT is mainly used in operable disease to improve the surgical options, to determine the response to chemotherapy and to obtain long-term DFS [52–56].

Pathological complete response (pCR) has been considered predictive of long-term outcome in several neoadjuvant trials [57], and this finding has been confirmed in two recent studies [58,59]. The meta-analysis from the Collaborative Trials in Neoadjuvant Breast Cancer [58] included 12 randomised neoadjuvant trials (n = 13,125) and results have shown that individual patients who achieved a pCR (ypT0ypN0 or ypT0/isypN0) had a more favourable long-term outcome. This effect was only seen in HR^+/grade 3, triple-negative and HER2+ tumours and not in low-grade hormone-receptor-positive tumours. Similarly, in the pooled analysis of seven prospective trials (n = 6000) published by the German Group [59] pCR was associated with improved DFS in tumours luminal B/HER2-negative, HER2+/nonluminal, and triple-negative. These recent data establish pCR as a surrogate marker for survival but emphasise that it is not an adequate endpoint for slow proliferative tumours (grade 1 or 2, HR^+). Additionally, it was not possible to determine the magnitude of increase in pCR rates predictive of superior long-term outcome of a specific therapy of a clinically meaningful improvement in survival [60]. These findings led the FDA to support certain drug development programmes throughout NT trials using pCR for accelerated approval [61]. Neoadjuvant trials are also recognised as important research tools, particularly in the field of biomarkers.

3.2. *Which chemotherapy and targeted therapy regimens in the neoadjuvant setting. Are there predictive markers?*

Anthracycline/taxane-based CT regimens have been the most extensively studied in the neoadjuvant setting, but so far no specific regimen has been found to be clearly superior. Incorporation of taxanes has increased the response rates [54,62,63] with large phase III trials reporting pCR rates of 15–20% [57,64,65]. The studies that have accessed tailoring treatment to response [63,65–67] have not confirmed a clear benefit from changing to a non-cross-resistant regimen.

In this regard efforts have been made to study biological markers predictive of pCR. The integrated meta-analysis [68] on individual data from the German Breast Group and the AGO Breast Group, on 6402 patients enrolled in neoadjuvant trials has shown that a greater chance of pCR was seen in ER-negative patients (OR 3.2; $P < 0.0001$), HER2+ disease (OR 2.2; $P < 0.0001$), higher grade (OR 1.8; $P < 0.0001$), younger age (OR 1.3; $P = 0.0001$), non-lobular type tumours (OR 1.7; $P = 0.001$) and smaller tumour size (OR 1.5; $P = 0.0006$). Furthermore, this group recently published a pooled analysis assessing the prognostic impact of different definitions of pCR and the outcome regarding the biological intrinsic breast cancer subtypes [59]. It was found that pCR was associated with improved DFS in tumours luminal B/HER2-negative ($P < 0.005$), HER2+/nonluminal ($P < 0.001$) and triple-negative tumours ($P < 0.001$) but not in luminal A ($P = 0.39$) or in luminal B/HER2+ ($P = 0.45$) tumours. Despite the fact that tumours lacking expression of ER have higher pCR, exceeding 40% in some studies, overall patient survival with this phenotype is still shorter than in patients with hormone-receptor-positive disease [9]. However, recent data show that patients with HER2+/nonluminal and triple-negative disease who achieved pCR have an excellent prognosis [58,59].

Mutations in p53 were shown not to be predictive of response to taxanes in the large randomised multicentric neoadjuvant trial EORTC 10994/BIG 1-00 [69]. Some studies have generated preliminary gene signatures with potential predictive value for docetaxel and paclitaxel plus FAC [70,71] but these signatures have not yet been validated in subsequent studies and are not ready for use in clinical practice. More recent studies suggest that prediction of response to a specific CT agent is different among the different BC subtypes and is more likely to be achieved by using multifactorial tools [59,72–75]. HER2 over-expression/amplification predicts response to treatment with the monoclonal antibody trastuzumab [76], and has also been associated with a better response to anthracyclines [77,78]. It is uncertain whether the latter effect is linked to the co-amplification of topoisomerase IIα as mixed results have been obtained [78,79]. The association between HER2+ and response to taxanes suggested in some studies [80] needs to be confirmed with further research. In HER2+ disease the incorporation of *trastuzumab* (H) into NT chemotherapy regimens is considered standard of care [57]. The first reported randomised trial from the MDACC showed a very high pCR rate of 65.2% in patients treated with trastuzumab (versus 26%) [81,82] which led to a premature closure of the study. Data from two randomised phase III studies were subsequently available, the NOAH trial [83] and the GeparQuattro trial [65,84]. The addition of trastuzumab to an anthracycline/taxane-based regimen led to an improvement in event-free survival at 3 years (HR 0.59; 95%CI: 0.38–0.90) in the NOAH trial and a significant increase in pCR rate in the GeparQuattro trial (31.7% in HER-2-positive disease versus 15.7% in HER-2-negative disease).

Lapatinib (L) has been tested in the NT setting, both as single agent and in combination with trastuzumab in two phase III studies. In the NeoALTTO study [85], 455 patients were randomly assigned to L, H, or L plus H, given alone initially and then combined with weekly paclitaxel before surgery. Combination of L and H yielded a significantly higher pCR rate than the monotherapy arms. The dual combination was associated with higher toxicity, mainly diarrhoea and a transient reversible rise in transaminases. In the Geparquinto trial [84] 620 HER2+ patients with operable or locally advanced BC were randomised to four cycles of epirubicin plus cyclophosphamide and four cycles of docetaxel 3 weeks, with either concurrent H or L. The H arm had a significantly higher pCR (30.3%) compared with L (22.7%). Taken together, the results of these two studies have led to the recommendation that lapatinib should not be used as a single (neo)adjuvant anti-HER2 target outside clinical trials. Furthermore, the lapatinib monotherapy arm in the large adjuvant ALTTO trial has been STOPPED and patients in that arm were informed and proposed to receive adjuvant trastuzumab.

Dual-HER2 blockade has also been tested in the NeoSphere trial [86], a phase II randomised trial designed to test the antitumour activity and tolerability of the combination of docetaxel, trastuzumab and pertuzumab (THP), compared with trastuzumab plus pertuzumab (HP), docetaxel and pertuzumab (TP) and docetaxel and trastuzumab (TH). The pCR was significantly higher ($P = 0.014$) for the combination of docetaxel with both anti-HER2 target agents (THP), with good tolerability, namely cardiac safety. These studies plus two trials in the metastatic setting [87,88] represent growing evidence that the dual blockade of the HER2 receptor has superior efficacy and may soon become standard of care. Still, it is not known which is the optimal combination of anti-HER2 agents; the best chemotherapy regimen to use with these agents, the role of dual HER2 blockade in combination with endocrine therapy for HER2+ and HR^+ BC are among other questions.

Triple-negative phenotype (TNBC) has higher response rates to NT compared to non-triple-negative tumours in several studies [59,72,89–91], but only if pCR is obtained can it be translated into a better prognosis. At the present time, CT is the only proven therapy for TNBC and international guidelines recommend the use of the same regimens as for non-TNBC, i.e. an anthracycline/taxane-based regimen. Small studies have suggested that platinums may be particularly effective in this subset, with pCR rates of 54.6% for docetaxel and carboplatin [92], 40% for epirubicin, cisplatin, and fluorouracil followed by weekly paclitaxel [93], and 80% with cisplatin in a BRCA1 mutation patient population [94]. However, pCR rates of 20% have also been reported with neoadjuvant cisplatin monotherapy [95]. These results need further validation in large randomised studies, especially in the non-BRCA population.

4. What is the optimal adjuvant chemotherapy regimen?

4.1. *State-of-art regimens according to breast cancer subtype*

So far available data do not allow for different regimen recommendations according to BC subtype. Therefore, the considerations below apply to all subtypes of BC when CT is deemed necessary, with some specific points for HER2+ EBC.

The rationale and support for adjuvant CT for patients with BC are derived from many large, randomised trials and from the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis. In the last update analysis [96] the use of adjuvant CT, with either an anthracycline-based or a CMF regimen, was shown to be superior to no treatment in terms of risk of recurrence, breast cancer, or overall mortality (Table 1). The application of adjuvant CT translated to an absolute benefit of 5.0%.

There is no single standard adjuvant chemotherapy regimen in the treatment of EBC.

When choosing a particular regimen various factors must be taken in account: namely the recurrence risk, co-morbid illness and patient preference. The following discussion is organised along the lines of debate concerning CT regimens: anthracyclines versus CMF and anthracyclines versus taxanes.

4.2. *Anthracyclines versus CMF*

Several randomised trials and the EBCTCG overview (Table 2) support the superior efficacy of anthracycline-based regimens over CMF with level I based evidence. However, some caveats must be highlighted. In the 2011 Oxford Overview anthracycline-based regimens were divided into standard doses (e.g. cumulative doses of 240 mg/m^2 of doxorubicin) or higher doses (i.e. cumulative doses > 240 mg/m^2 of doxorubicin or 360 mg/m^2 of epirubicin) [96]. The improvement in the risk of recurrence, breast cancer or overall mortality was present only with the use of higher cumulative doses of anthracyclines (Table 2). This suggests that a real difference between these regimens exists but is limited to anthracycline regi-

Table 1 – Early Breast Cancer Trialists' Collaborative Group (EBCTCG) overview results comparing adjuvant chemotherapy (CT) with no CT in early breast cancer (EBC).

Risk of recurrence	Breast cancer mortality	Overall mortality
Anthracycline-based regimen versus no CT		
RR: 0.73, 95% confidence interval (CI) Absolute gain: 8%	RR: 0.79, 95%CI Absolute gain: 6.5%	RR: 0.84, 95%CI Absolute gain: 5%
Cyclophosphamide, methotrexate and fluorouracil (CMF) regimen versus no CT		
RR: 0.70, 95%CI Absolute gain: 10.2%	RR: 0.76, 95%CI Absolute gain: 6.2%	RR: 0.84, 95%CI Absolute gain: 4.7%

Table 2 – Adjuvant anthracycline versus cyclophosphamide, methotrexate and fluorouracil (CMF) trials in early breast cancer (EBC).

Study	Population (*n*)		Median follow-up	Treatment	Disease-free survival(DFS) (*P*-value)	Overall survival (OS) (*P*-value)
INT 0102	Node-negative high risk EBC (*n* = 2691)		60	CAF × 6 CMF × 6	85% versus 82% *P* = 0.03	92% versus 90% *P* = 0.03
NSABP B-23	Node-negative,ER-negative EBC (*n* = 2008)		60	AC × 4 CMF × 6	87% versus 87% *P* = 0.9	90% versus 89% *P* = 0.4
Belgian study	Node-positive EBC (*n* = 777)		50	CMF × 6 EC × 8 full dose		85% versus 86%
Mam-1 GOCSI	Node-positive premenopausal EBC (*n* = 466)		60	A → CMF CMF	82% versus 75% *P* < 0.001	HR: 0.79 *P* = 0.31
NEAT/BR969	Node-negative and node-positive EBC (*n* = 2391)		48	E × 4 → CMF × 4 versus CMF × 6/8	76% versus 69% *P* < 0.001	82% versus 75% *P* < 0.001
EBCTCG Overview: anthracyclines versus CMF						
Standard-strength anthracycline-based regimen versus standard or near-standard CMF		Recurrence RR: 0.89 (*P* = 0.003)		BC mortalitiy RR: 0.80 (*P* = 0.00001)		Overall mortality RR: 0.84 (*P* = 0.0002)
Low-strength anthracycline-based regimen versus standard or near-standard CMF		Recurrence RR: 0.99 (*P* = 0.76)		BC mortalitiy RR: 0.98 (*P* = 0.67)		Overall mortality RR: 0.97 (*P* = 0.55)

AC, doxorubicin and cyclophosphamide; CAF, cyclophosphamide, doxorubicin, and fluorouracil; EC, epirubicin and cyclophosphamide; A, doxorubicin; E, epirubicin.

mens containing three agents (e.g. CEF, CAF) and given for at least six cycles. Standard dosing of anthracycline-based therapy (four cycles of a two-drug regimen, e.g. 4AC) seems to be equivalent to CMF.

4.2.1. *Anthracyclines versus Anthracylines + taxane based therapy*

The role of taxane-base CT as adjuvant treatment of EBC is an extensively studied but still controversial issue. We currently have 21 clinical trials of first-generation taxanes, several pooled analyses, meta-analyses, and since 2012 the role of these agents is also evaluated in the analysis of the EBCTCG overview.

Some of the key first-generation taxane trials are presented in Table 3. When analysing the 12 first-generation trials using low-strength anthracycline reference regimens, eight suggest a benefit in terms of DFS for the taxane regimen (CALGB 9344; NSABP B-28; the MD Anderson Neoadjuvant Trial; FinHER; BCIRG 001; HORG; GEICAM 9805; US Oncology Group 9735) and only three of the 10 trials that reported survival showed a benefit in OS (CALGB 9344, BCIRG 001, and US Oncology 9735).

Several pooled analyses and meta-analyses have been undertaken aiming to clarify the benefit of taxane-based therapy (Table 3). Overall they support a modest improvement in DFS and overall survival (~5% and ~3% absolute benefit, respectively) when taxane-based regimens are compared with standard anthracycline polychemotherapy, irrespective of the type of taxane, schedule of administration, extent of nodal involvement and hormone-receptor expression status [97].

In the EBCTCG 2012 meta-analysis the incorporation of a taxane into an anthracycline CT regimen resulted in reduction in the recurrence risk, risk of breast cancer and overall mortality (Table 3) independently of age, nodal status, tumour size, tumour grade or ER status.

However, we must underscore that treatment comparisons varied greatly, which complicates the analysis. In this regard, the effect of taxanes was analysed taking into account how the CT regimen in the control group compared with the non-taxane CT in the taxane group (same, doubled or intermediate). The major effect of these agents was seen in the trials where the same control regimen was used in both arms (*n* = 11,167 women) with a reduction in the risk of recurrence, breast cancer and overall mortality that translated into an absolute gain of 4.6%, 2.8% and 3.2%, respectively [96]. When considering this benefit we must acknowledge that in these trials a 'week' anthracycline-based regimen was used and greater treatment duration was obtained with the additional four cycles of a taxane to the anthracycline regimen. As a matter of fact, when the number of cycles in the control anthracycline regimen was doubled (to mirror the addition of four cycles of taxanes to anthracyclines in the experimental arm) there was little difference in recurrence, breast cancer or overall mortality (Table 3).

4.2.2. *HER2 positive breast cancer*

The optimal anti-HER2 adjuvant treatment will be addressed below

Table 3 – Adjuvant taxane trials in early breast cancer (EBC).

Study	Population	Median follow-up (months)	Treatment	DFS (*P*-value)	OS (*P*-value)
"Low-strength" sequential anthracycline					
CALGB 9344	Node-positive EBC ($n = 3170$)	69	AC × 4 versus AC × 4 – Pac × 4	7 years: 64% versus 58% (HR: 0.83; $P = 0.001$)	7 years: 74% versus 68% (HR: 0.82, $P = 0.01$)
NSABP B-28	Node-positive EBC ($n = 3060$)	34	AC × 4 versus AC × 4 – Pac × 4	5 years: 76% versus 72%; (HR: 0.83; $P = 0.002$)	5 years: 85% versus 85% (HR: 0.93; $P = 0.46$)
MDACC	EBC ($n = 524$)	60	FAC × 8 Pac × 4 – AC × 4	86% versus 83% (HR: 0.70; $P = 0.009$)	NR
NSABP B-27	T1–T3 operable BC ($n = 2411$)	102	S → AC → Doc versus S → AC AC → S → Doc versus S → AC	71% versus 68% (HR: 0.92; $P = 0.29$) 70% versus 68% (HR: 0.92; $P = 0.29$)	83% versus 82% (HR: 0.93; $P = 0.46$) 82% versus 83% (HR: 0.97; $P = 0.7$)
"Low-strength" concurrent anthracycline[a]					
BCIRG-001	Node-positive EBC ($n = 1491$)	124	DAC × 6 FAC × 6	62% versus 55%, $P = 0.0043$	76% versus 69%, $P = 0.002$
GEICAM 9805	Node-negative EBC ($n = 1060$)	77	DAC × 6 FAC × 6	87.8% versus 81.8% (HR: 0.68; 95%CI; $P = 0.01$)	95.2% versus 93.5% (HR: 0.76; 95%; P = NS)
"Standard strength" sequential anthracycline[*]					
GEICAM 9906	Node-positive EBC ($n = 1246$)	66	FECq3w × 6 FEC × 3 – Pac × 8w	78% versus 72% (HR: 0.74; $P = 0.006$)	90% versus 87% (NR; $P = 0.11$)
PACS 01	Node-positive EBC ($n = 1999$)	60	FECq3w × 6 FEC × 3 – Doc × 3w	78% versus 73% (HR: 0.82; $P = 0.034$)	91% versus 87% (HR: 0.73; $P = 0.014$)
WGSG/AGO EC-Doc Trial	1–3 Positive lymph node ($n = 2011$)	41	4 × EC – 4 × Doc 6 × FEC100 6 × CMF[b]	Estimated 5 years EFS 91% versus 85% (HR: 0.58, $P = 0.004$)	Estimated 5 years OS 95% versus 91% ($P = 0.03$)
Meta-analysis					
Meta-analysis	13 Studies EBC ($n = 22,903$)	–	–	HR: 0.83 (95%CI, 0.79–0.87; $P < 0.00001$)	HR: 0.85 (95%CI, 0.79–0.91; $P < 0.00001$)

EBCTCG overview – taxane-plus-anthracycline versus anthracycline-based regimen			
Results for all trials that test taxane effect ($n = 44,000$)	Distant recurrence RR: 0.87 Any recurrence RR: 0.86 ($P = 0.00001$)	BC mortality RR: 0.87 ($P = 0.00001$) Other mortality RR: 0.99	Overall mortality RR: 0.89 ($P = 0.0001$)
Unconfounded trials[a] (taxane versus control group)	8-year recurrence: 30.2% versus 34.8% (absolute gain 4.6%)	8-year BC mortality: 21.1% versus 23.9% (absolute gain 2.8%)	8-year overall mortality 23.5% versus 26.7% (absolute gain 3.2%)
Counfounded trials[a] (taxane versus control group)	8-year recurrence: 19.2% versus 22% (absolute gain 2.9%)	8-year BC mortality: 10.1% versus 11.5% (absolute gain 1.4%)	8-year overall mortality 11.2% versus 12.4% (absolute gain 1.2%)

FEC, cyclophosphamide, epirubicin, and fluorouracil; AC, doxorubicin and cyclophosphamide; Pac, paclitaxel; FAC, fluorouracil, doxorubicin and cyclophosphamide; Doc, docetaxel; S, surgery; DAC, docetaxel, doxorubicin, cyclophosphamide; EC, epirubicin and cyclophosphamide; CMF, cyclophosphamide, methotrexate, and fluorouracil.

[a] Anthracycline-based adjuvant breast cancer regimens are categorized into 'standard-strength' and 'low-strength' regimens based on cumulative doses of doxorubicin >240 mg/m^2 and epirubicin >360 mg/m^2. Example: standard strength: FEC100; FEC90; CEF; CAF:A75 or E100 followed by CMF; reduced strength: FEC75; FEC60; FEC50; FAC; AC; EC.

4.3. *Should anthracyclines be avoided in the adjuvant setting?*

Anthracyclines are amongst the most active chemotherapeutic agents for the treatment of breast cancer. Multiple trials in the past two decades demonstrated that anthracycline-based chemotherapy was associated with lower rates of breast cancer recurrence and improved survival when compared with non-anthracycline chemotherapy regimens, such as CMF [96]. However, these agents are associated with increased risk of cardiovascular complications, dependent on cumulative dose and schedule, and are often irreversible.

The benefit of taxanes when incorporated into the adjuvant setting for women with newly diagnosed breast cancer was analysed in several trials and has been discussed above. It is, however, unknown whether the benefit seen from add-

ing a taxane in the adjuvant setting will obviate the need for anthracyclines in a subset of patients, since the great majority of studies evaluated the addition of a taxane to an anthracycline regimen and not its replacement. A phase III randomised trial, the US Oncology Research Trial 9735 [98], enrolled 1016 women with stages I–III HER2-negative breast cancer and randomly assigned therapy with four cycles of AC or four cycles of docetaxel plus cyclophosphamide (TC). With a median follow-up of 7 years, TC resulted in a significantly higher DFS (81% versus 75%) and OS (87% versus 82%). However, how the TC regimen compares with stronger anthracycline-based regimens such as FEC/FAC and with third-generation regimens, which incorporate both an anthracycline and taxane, is still unknown. Therefore, most international guidelines continue to recommend an anthracycline- and taxane-containing regimen for most women, particularly those with higher-stage tumours, and for those with triple-negative or HER2+ BC, unless there are clear contraindications for the use of anthracyclines [13].

The role of anthracycline regimens in the HER2+BC is also a matter of intense research. Several CT regimens used with trastuzumab have been evaluated in large prospective studies, and historically anthracyclines have been considered critical for the management of HER2+ BC. A number of studies from the pre-trastuzumab era support this concept. Retrospective subset analyses of anthracycline-based adjuvant CT studies have suggested that the major benefit for these regimens is seen in HER2-over-expressing tumours [99]. The value of HER2 and TOP2A as predictive markers of response to anthracycline-based therapy has been extensively studied. In the meta-analysis by Di Leo et al., although HER2 amplification and combined TOP2A amplification and deletion had some value in prediction of responsiveness to anthracycline-based therapy, the overall findings did not support the routine use of TOP2A to select the adjuvant CT regimen in this patient population [78].

With the advent of trastuzumab, concerns have been raised regarding the use of anthracycline-based regimens in HER2+ early BC due to potential cardiotoxicity. Previous or concurrent anthracyclines are a risk factor for trastuzumab-related cardiotoxicity. Notwithstanding the increased incidence of cardiac events, these still remain in very acceptable ranges for all types of CT regimens used in the adjuvant setting. Rates of severe congestive heart failure in adjuvant trials ranged between 0.4% and 3.5%, depending on the regimen and schedule used.

Combining trastuzumab with a non-anthracycline-containing CT regimen was evaluated in the BCIRG 006 trial with the aim of investigating whether the association of trastuzumab, carboplatin and docetaxel could be better tolerated and superior in terms of efficacy compared with an anthracycline-based schedule [79]. At a median follow-up of 65 months, the differences in DFS and OS between ACTH and TCH, although not statistically significant, were numerically different, with a trend favouring the anthracycline-containing regimen. The trial hypothesis that TCH was superior to ACTH was not proven and, since the study was not powered to detect equivalence between ACTH and TCH, this conclusion cannot be drawn. With respect to adverse events, the differences were significantly lower rates of severe (grade) neutropenia (66% versus 63%) and leucopaenia (48% versus 60%) but significantly higher rates of anaemia (6% versus 3%) and thrombocytopenia (6% versus 2%) for TCH and a higher incidence of congestive heart failure (2% versus 0.4%), subclinical and sustained loss of mean left ventricular ejection fraction (18.6% versus 9.4%) for ACTH. Based on this trial alone, TCH can only be considered an alternative treatment for patients with contraindications to anthracyclines (pre-existing cardiac conditions, borderline ejection fraction at baseline, or prior anthracycline exposure) while anthracycline-based regimens remain the standard of care.

Findings suggest that the more dramatic risk reduction when adding trastuzumab to CT is observed when using some concurrent CT and trastuzumab, and employing both anthracycline and a taxane.

4.4. *Dose-dense adjuvant chemotherapy*

The introduction of granulocyte-colony-stimulating factors has allowed the administration of CT in the dose-dense approach, thought to have higher efficacy based on mathematical models of human breast cancer growth [100]. The pivotal trial CALGB9741 [101] has shown significant improvement in DFS and OS with dose-dense concurrent AC followed by paclitaxel in women with node-positive EBC. Several trials with dose-dense regimens have shown similar results, as shown in a systematic review and meta-analysis of these studies [102] with HR of death 0.85 (95%CI = 0.77–0.93) and HR of relapse or death 0.81 (95%CI = 0.75–0.88). Another important finding was that the benefit was seen only in hormonal-receptor-negative disease. There was no statistically significant increase in adverse events. The concern about these results is related to the design of these trials that did not evaluate the same agents and doses in the conventional arm as in the investigational arm. Further prospective data will help to clarify which patients should be selected for this approach. At the moment these regimens have been mainly used in high-risk disease with features of aggressive biology.

4.5. *Sequential versus combination regimens*

Sequential single-agent doxorubicin and cyclophosphamide did not improve outcome compared with combination AC [103]. Sequential versus concurrent use of anthracyclines and taxanes in EBC has been evaluated in three studies: CALGB 9741, BIG 2-98 and NSABP B-38.

The first study, CALGB 9741 [101], randomised 2005 female patients, with node-positive disease, to sequential $A \times 4 \rightarrow T \times 4 \rightarrow C \times 4$, every 3 weeks; $A \times 4 \rightarrow 3\,T \times 4 \rightarrow C \times 4$, every 2 weeks with filgrastim; concurrent $AC \times 4 \rightarrow T \times 4$, every 3 weeks or $AC \times 4 \rightarrow T \times 4$, every 2 weeks with filgrastim. Dose-dense treatment was associated with improved DFS and OS, with no increase in toxicity.

In the BIG-2-98 [104] study 2887 patients, also with node-positive disease, were randomised to sequential $A \times 4 \rightarrow CMF \times 3$ (sequential control); concurrent $AC \times 4 \rightarrow CMF \times 3$ (concurrent control); sequential $A \rightarrow T \times 4 \rightarrow CMF \times 3$ (sequential experimental); concurrent $AT \times 4 \rightarrow CMF \times 3$ (concurrent experimental). The updated analysis [105] revealed that sequential docetaxel was associated with significant

improvement of DFS compared with control arms and with concurrent AT.

Preliminary results of NSABP B-38 were recently presented [106]. The trial randomised 4894 women (65% node-positive) to dose-dense ACT, dose-dense AC followed by the combination of paclitaxel and gemcitabine (ACTG), or TAC. Five-year DFS and OS were similar between groups, but the TAC regimen was associated with more grade 3/4 toxicity, namely febrile neutropenia and diarrhoea. Based on the tolerability profile, and on the possible higher efficacy, sequential anthracycline–taxane-based regimens are preferred to combination regimens.

4.6. *Are there predictive biomarkers to help select the optimal regimen?*

Identification of markers that predict chemosensitivity in BC is a research priority. Several approaches and technologies have been used to identify these predictive markers. The aim is to answer two questions: (a) can we use gene signatures to identify tumours, which are more likely to respond to chemotherapy? and (b) when chemotherapy is indicated, what is the optimal chemotherapy regimen for a specific tumour or group of tumours?

4.6.1. *Markers predicting general chemosensitivity*

Since patients with poor prognosis disease defined by first-generation signatures have tumours with high expression of proliferation-related genes, and cytotoxic agents target the proliferating fraction of tumours, the finding that first-generation prognostic signatures also predict benefit from conventional multidrug CT regimens is not surprising [45,107–110].

With respect to OncotypeDx, two retrospective studies have reported its predictive value for chemosensitivity [32,111]. In the NSABP trial B-20, 651 patients with node-negative, hormone-receptor-positive tumours were randomised to tamoxifen alone ($n = 227$) or tamoxifen plus CT (methotrexate–fluorouracil or CMF) ($n = 424$) [32]. A high recurrence score predicted benefit from CT [hazard ratio (HR) = 0.26; 95% CI = 0.13–0.53], with little or no benefit from CT in the low and intermediate recurrence score groups. The predictive value of the OncotypeDx was also assessed in a subset of patients more than 50 years old with node-positive hormone-receptor-positive tumours included in the SWOG 8814 trial [111]. In this trial, patients were randomised to receive either tamoxifen alone ($n = 361$); CAF followed by tamoxifen for 5 years ($n = 566$); or concurrent CAF and tamoxifen ($n = 550$). The 21-gene recurrence score was assessed in 367 of these patients. The addition of CT to tamoxifen resulted in no difference in DFS or OS in the low recurrence score group, but a clear benefit in DFS and OS in the high recurrence score group. There appeared also to be a benefit for patients in the intermediate recurrence score group, but the confidence intervals were wide due to the small sample size.

This signature was assessed in a series of 167 patients with tumours >5 cm or clinically positive nodes and has also been suggested to predict the response to neoadjuvant CT [112]. Pathological complete response (pCR) after neoadjuvant CT was used as a surrogate for chemosensitivity and in this trial only patients with a bad signature achieved a pCR of 20% (29/144). None of the patients with a good signature ($n = 144$) achieved a pCR (0/23). The authors concluded that patients with a good signature would be unlikely to respond to CT.

4.6.2. *Markers predicting drug-specific chemosensitivity*

There are currently no biomarker predictors of response to specific cytotoxic agents. There are several reasons for the apparent inability to develop these predictive factors, namely: (a) resistance or response to therapies may be caused by a functional alteration in only a few genes and this may not manifest itself as a detectable signal in the complex transcriptomic landscape of a tumour; (b) tumours are often composed of a mosaic of genetically heterogeneous clonal subpopulations harbouring numerous private genetic aberrations (that is, aberrations found in a single clone of a tumour [31,113]. These private genetic aberrations may be the drivers of resistance to therapy in a subpopulation and would not be detected by microarrays that survey the average expression profile of the entire tumour.

The Topoisomerase II Alpha Gene Amplification and Protein Overexpression Predicting Efficacy of Epirubicin (TOP) trial (NCT00162812) led to the development of the anthracycline-based score (A-score), which combines three predictive signatures: a TOP2A gene signature and signatures related to tumour invasion and immune response [74]. Analysis of the predictive power of the A-score was performed in the EORTC 10994/BIG (Breast International Group) 00-01 (NCT00017095) trial and from ER-negative patients from the Randomised Clinical Trial to Evaluate the Predictive Accuracy of a Gene Expression for Stage I–II Breast Cancer (NCT00336791). Both studies revealed its high negative predictive value (0.98, 95%CI 0.90–1.00) [74] suggesting, if validated, its potential clinical use for identification of patients who are unlikely to benefit from anthracyclines.

5. What is the optimal adjuvant endocrine treatment?

5.1. *Tamoxifen 5 years*

Endocrine therapy (ET) is one of the most effective treatments in women with endocrine responsive breast cancer. Tamoxifen has been the mainstay endocrine agent for both pre- and postmenopausal women. Updated analyses [114] of the EBCCTG overview assessed long-term outcomes among 21,475 women with EBC in trials of 5 years of tamoxifen compared with observation or placebo. In oestrogen-receptor- (ER-)positive disease, 5 years of tamoxifen significantly reduced recurrence rates throughout the first 10 years, independently of progesterone receptor status, nodal status, or use of CT: relative risk (RR) 0.53 during years 0–4 and RR 0.68 during years 5–9 [both $2P < 0.00001$]. For marginally ER-positive disease there was also an important risk reduction (RR 0.67). More importantly there was a reduction in breast cancer mortality by about a third throughout the first 15 years (RR 0.71 during years 0–4, 0.66 during years 5–9, and 0.68 during years 10–14; $P < 0.0001$).

5.2. *Ovarian suppression and aromatase inhibitors for premenopausal patients*

The standard adjuvant hormonal therapy in premenopausal women with ER-positive disease remains tamoxifen alone for 5 years, but benefit has also been shown with the use of luteinising-hormone-releasing (LHRH) agonists specifically in the absence of CT. Several studies have been conducted testing LHRH agonists alone, combined with tamoxifen, chemotherapy or both. In the EBCTCG overview [115] 8000 patients randomised to ovarian function suppression (OFS) or ablation by surgery/radiation had reduced recurrence and breast cancer mortality, but the benefit was seen mainly in the absence of other systemic treatments. An individual patient data meta-analysis [116] of 16 trials using LHRH identified 9022 women with ER^+ disease and assessed recurrence rate, breast cancer mortality and overall mortality. While LHRH agonists alone did not have a significant effect, adding these agents to CT, to tamoxifen or both, significantly reduced recurrence by 12.7% ($P = 0.02$) and death after recurrence by 15.1% ($P = 0.03$). Furthermore, the benefit of LHRH agonists after CT was seen in women younger than 40 years, but not in older premenopausal women. However, the data do not answer the question of whether LHRH agonist is useful only when amenorrhoea is not achieved with CT, an event that has been associated with worse outcome in some trials [117,118].

Recently a guideline from Cancer Care Ontario was published and endorsed by ASCO [119] conducting a systematic review of available literature. The guideline does not recommend the routine use of OFS added to chemotherapy, tamoxifen or a combination of both. It does acknowledge as a major difficulty in assessing its efficacy the fact that ovarian suppression has not been compared with current CT regimens (e.g. anthracyclines or anthracyclines/taxanes), which deems the benefit of these agents unclear. For chemical suppression the guideline does suggest the use of monthly injections.

The role of aromatase inhibitors (AIs) in premenopausal women was assessed in the ABCSG-12 trial [120] which randomised 1803 patients to receive goserelin monthly plus tamoxifen or anastrozole, with or without zoledronic acid for 3 years. There was no significant difference in DFS between the anastrozole and tamoxifen groups (HR = 1.10; CI 0.78–1.53), but the trial was relatively small to answer this secondary objective. Till now AIs combined with OFS are only recommended in premenopausal patients if tamoxifen is contraindicated. To better understand the role of aromatase inhibitors, as well as OFS in this setting, results from the studies TEXT, SOFT and PROMISE are eagerly awaited.

5.2.1. *Aromatase inhibitors*

For postmenopausal patients the aromatase inhibitors anastrozole, letrozole and exemestane have been extensively studied in adjuvant setting as upfront therapy for 5 years, "switch" strategy of initial tamoxifen for 2–3 years followed by an AI 2–3 years, the reverse sequence, or as an extended treatment after 5 years of tamoxifen (see Table 4) [121–129]. The meta-analysis of the adjuvant trials [130] analysed a cohort of 9856 patients where AI upfront therapy was compared with standard tamoxifen treatment, showing a significant 2.9% absolute decrease in recurrence and a non-significant absolute 1.1% decrease in breast cancer mortality. A second cohort comprising 9015 patients compared the switch strategy with standard tamoxifen treatment and showed a significant absolute decrease in recurrence and in breast cancer mortality of 3.1% and 0.7%, respectively. Current ASCO [131] and European Guidelines [132] recommend the incorporation of AIs in the endocrine treatment plan as switch (2–3 years) or upfront therapy strategy (5 years). For patients who have completed 5 years of tamoxifen the addition of an AI for a further period of 2–5 years is recommended, especially for patients with node-positive disease. On the other hand, 5 years of tamoxifen alone is still a viable option for certain patients at very low risk of recurrence.

The choice of endocrine treatment and the timing for AI treatment is nowadays based on the toxicity profile of these drugs compared with tamoxifen, general health issues of each individual and the risk of relapse. A recent meta-analysis [133] on safety reports from major adjuvant trials found that AI therapy was associated with a higher risk for cardiovascular disease (HR, 1.2) and bone fracture (HR, 1.48) than tamoxifen, but a lower risk for venous thromboembolism (HR, 0.53) and uterine cancer (HR, 0.32). Overall these risks were low, around 2% of patients, and fractures only occurred in fewer than 10% of all patients. Additional data from a population-based study [134] evaluating 44,000 women with breast cancer and age-matched women without breast cancer, have shown that breast cancer patients on ET had a lower risk for both myocardial infarction and ischaemic stroke than those who did not have breast cancer. No differences were seen between AI therapy and tamoxifen therapy in the risk for myocardial infarction or stroke, but AI therapy was associated with a higher risk for any fracture (mainly hip fractures). Guidelines [131,132] recommend surveillance of bone mineral density during AI treatment, and calcium and vitamin D supplementation or a bisphosphonate depending on the result.

5.3. *Extended ET treatment*

Because the risk of recurrence in hormone-receptor-positive disease still remains after the first decade [135], clinicians and researchers have been questioning the benefit of extended tamoxifen treatment beyond 5 years. Three prospective trials addressed this question, randomising patients after 5 years of tamoxifen treatment to additional 5 years of treatment or placebo (NSABP-B14 [136], aTTom trial [137] and ATLAS trial [138]). Except for the NSABP B14 trial, these studies together with EBCCTG [114] have shown benefit for extended tamoxifen. However, balance with side effects has to be considered as extended treatment is associated with increased incidence of endometrial cancer, which is 2.3-fold with 5 years of tamoxifen and 4-fold with 10 years [114]. On the other hand, there is some evidence that tamoxifen has a favourable effect in lipid profile [139–141]. ATLAS results suggest some protection against ischaemic heart disease and certainly no increase in stroke deaths. In the EBCCTG overview the non-significant excess of stroke deaths was balanced by a non-significant shortfall in cardiac deaths with lit-

Table 4 – Trials of adjuvant endocrine therapy.

Study	Treatment arms/ population (*n*)	Median follow-up	Recurrence	Mortality
Tamoxifen 5 years				
Overview 2011 (W164)	TAM 5 years versus no TAM 10,645 ER^+	15 years	RR = 0.53 [SE 0.03] years 0–4 RR = 0.68 [SE 0.06] years 5–9 2*P* < 0.00001 RR = 0.97 [SE 0.10] years 10–14	RR = 0.71 [SE 0.05] years 0–4 RR = 0.66 [SE 0.05] years 5–9 RR = 0.68 [SE 0.08] years 10–14 *P* < 0.0001
OFS				
Overview 2005 [115]	8000 ER^+/ER unknown, <50 years, OFS $LHRH^-$ 3408	5 years	15 years gain 4.3% (SE 1.9) 2*P* < 0.00001	15 years gain 3.2% (SE 2.0) 2*P* = 0.04
Meta-analysis [164]	11,906 Premenopausal	6.8 years	*No systemic treatment versus LHRH:* HR 0.72 (95%CI 0.49–1.04), *P* = 0.08 *CT versus LHRH:* HR 1.04 (95%CI 0.92–1.17), *P* = 0.52 *CT versus LHRH + TAM:* HR 0.90 (95%CI 0.75–1.08), *P* = 0.25 *Addition to TAM:* HR 0.85 (95%CI 0.67–1.09), *P* = 0.20 *Addition to CT ± TAM:* HR 0.88 (95%CI 0.77–0.99), *P* = 0.04	*No systemic treatment versus LHRH:* HR 0.82 (95%CI 0.47–1.43), *P* = 0.49* *CT versus LHRH:* HR 0.93 (95%CI 0.79–1.10), *P* = 0.40[a] *CT versus LHRH + TAM:* HR 0.89 (95%CI 0.69–1.15), *P* = 0.37[a] *Addition to TAM:* HR 0.84 (95%CI 0.59–1.19), *P* = 0.33[a] *Addition to CT ± TAM:* HR 0.85 (95%CI 0.73–0.99), *P* = 0.04*
AIs 5 years				
ATAC [164]	TAM 5 years versus ANA 5 years 3116/3125	120 months	HR = 0.91 (95%CI 0.83–0.99) *P* = 0.04	0.97 (95%CI 0.88–1.08) *P* = 0.6
BIG 1.98 [164]	TAM 5 years versus LET 5 years 2459/2463	76 months	HR = 0.88 (95%CI 0.78–0.99) *P* = 0.03	HR = 0.87 (95%CI 0.75–1.02) *P* = 0.08
TEAM [164]	EXE 5 years versus TAM 2–3 years followed EXE 2–3 years 4868/4898	5.1 years	HR = 0.97 (0.88–1.08) *P* = 0.60	HR = 1.00 (0.89–1.14) *P* > 0.9
Meta-analysis [164]	Cohort 1 AIs initial monotherapy versus TAM 9856	5.8 years	9.6% AI versus 12.6% TAM 2.9% absolute decrease (SE 0.7%) 2*P* < .00001	4.8% AI versus 5.9% TAM 1.1% (SE = 0.5%) absolute decrease 2*P* = 0.1
MA.27 [6]	EXE 5 years versus ANA 5 years 7576	4.1 years	HR = 1.02 (95%CI 0.87–1.18) *P* = 0.85	HR = 0.93 (95%CI 0.77–1.13) *P* = 0.46
AIs and tamoxifen in switching strategies				
BIG 1.98 [129]	LET 5 years TAM 2 years followed by LET 3 years LET 2 years followed by TAM 3 years 1546/1548/1540	71 months	HR = 1.05 (95%CI 0.84–1.32) HR = 0.96 (95%CI 0.76–1.21)	HR = 1.13 (95%CI 0.83–1.53) HR = 0.90 (95%CI 0.65–1.24)
ABCSG-8/ARNO 95 [125]	TAM 5 years versus Tam f 2 years followed by ANA for 3 years	28 months	HR = 0.60 (0.44–0.81) *P* = 0.0009	*P* = 0.16

(*continued on next page*)

Table 4 – (*Continued*)

Study	Treatment arms/ population (*n*)	Median follow-up	Recurrence	Mortality
ITA [122]	TAM 5 years versus Tam f 2 years followed by ANA	128 months	HR = 0.64 (0.44–0.94) *P* = 0.023	HR = 0.72 (0.44–1.17) *P* = 0.3
IES [124](164)(164) (164)	TAM 5 years versus Tam f 2–3 years followed by EXE 2–3 years	55.7 months	HR = 0.76 (95%CI 0.66–0.88) *P* = 0.0001	HR 0.85 (95%CI 0.71–1.02) *P* = 0.08
Meta-analysis [130]	Cohort 2 AIs T after 2–3 years of TAM versus TAM 9015	3.9 years	5.0% AI versus 8.1% TAM 3.1% absolute decrease (SE 0.6%) 2P < .00001	1.7% AI versus 2.4% TAM 0.7% (SE = 0.3%) absolute decrease 2P = 0.2
Extended treatment beyond 5 years				
ATLAS [138]	TAM 5 years versus TAM 10 years 3428/3418	NR	RR = 0.90 (95%CI 0.79–1.02) 5–9 years RR = 0.75 (95%CI 0.62–0.90) later years RR: 0.84, 95%CI 0.76–0.94; *P* = 0.002 in ER+	RR = 0.97 (95%CI 0.79–1.18) 5–9 years RR = 0.71 (95%CI 0.58–0.88) later years 639 deaths versus 722 deaths, *P* = 0.01 in ER+
NSABP-B14 [136]	TAM 5 years versus TAM >5 years 579/593	7 years	DFS = 82% TAM 5 years versus 78% TAM >5 years *P* = .03	OS7Y = 94% TAM 5 years versus 91% TAM >5 years *P* = .07
aTTOM [137]	TAM 5 years versus TAM 10 years 6934	4.2 years	415 TAM 5 years versus 442 recurrences TAM 10 years RR = 0.94 (95%CI 0.81–1.09) *P* = 0.4	NA
MA.17 [143]	TAM 5 years followed LET 5 years versusTAM 5 years 2594/2593	30 months	HR = 0.58 (95%CI 0.45–0.76) *P* < .001	HR = 0.82(95%CI 0.57–1.19) *P* = 0.03
NSABP-B33 [164]	TAM 5 years followed EXE 5 years versus TAM 5 years 779/786	30 months	DFS 4 years 91% versus 89% RR = 0.68 (*P* = 0.07)	16 deaths versus 13 *P* = 0.1
ABCSG-6a [165]	TAM 5 years followed ANA 3 years versus TAM 5 years 469/387	62 months	HR = 0.62 (95%CI 0.40–0.96) *P* = 0.031	HR = 0.89 (95%CI 0.59–1.34) *P* = 0.57

AI, aromatase inhibitor; DFS, disease-free survival; ER+, estrogen-receptor-positive patients; HR, hazard ratio; RR, event rate ratio; OS, overall survival; TAM, tamoxifen; LET, letrozole; EXE, exemestane; ANA, anastrozole; LHRH, luteinizing-hormone-releasing agonists; OFS, ovarian function suppression.

tle net effect on overall vascular mortality. Interestingly, a recent study [142], with a median follow up of 10.1 years, assessed the long-term benefits of 5 years versus 2 years of tamoxifen use in a large randomised trial of EBC women more than 50 years of age. Follow-up strategies included matching trial subjects with death data from the British National Health Service Information Center. Besides the well-known positive efficacy of tamoxifen, this study revealed a nearly statistically significant reduction in cardiovascular deaths (HR, 0.79; *P* = 0.08) with longer tamoxifen, and in women of 50–59 years there was an even greater reduction in cardiovascular events (HR, 0.65; *P* = 0.005; *P* = 0.046 for interaction between age and treatment groups).

In postmenopausal women extended use of AIs after 5 years of tamoxifen has shown improvement in DFS (see Table 4), and in one study, the MA-17 trial [143], an improvement in OS was also seen in node-positive patients. It is not known if longer use of AIs (more than 5 years) will increase outcomes without compromising safety, and it is not recommended until mature data from MA.17R and NSABP B-42 trials are available. The best regimen of ET for postmenopausal patients and the duration of ET treatment are still unanswered questions.

Table 5 – Phase III trials of adjuvant trastuzumab in patients with HER2-positive early breast cancer (EBC)

Study	Population	Median follow-up (months)	Treatment	DFS (*P*-value)	OS (*P*-value)	Cardiac dysfunction (%)
HERA [156]	Node-positive or node-negative high-risk EBC after completion of standard CT (n = 5.090)	96	No additional therapy H 1 year H 2 year	HR = 0.76, $P < 0.0001$	HR = 0.76, $P = 0.0005$	0.8 3.7
NSABP B-31/ NCCTG N9831 [157]	Node-positive Node-negative high-risk EBC (n = 4046)	100.8	AC→Pac AC→Pac→H	62.2% 73.7% ($P < 0.001$) HR = 0.6	75.2% 84.0% ($P < 0.0001$) HR = 0.63	
NCCTG N9831 [161]	Node-positive Node-negative high-risk EBC (n = 1.944)	63.6	AC→PacH AC→Pac→H	84% (5 years) 80% ($P = 0.0216$) HR = 0.77	NR NR	17 14
BCIRG 006 [79]	Node-positive Node-negative high-risk EBC (n = 3,222)	65	AC→Doc AC→Doc-H Doc–Carb–H	75% 84% HR = 0.64 ($P < 0.001$ versus CT) 81% HR = 0.75 ($P < 0.04$ versus CT)	87% 92% HR = 0.63 ($P < 0.001$ versus CT) 87% HR = 0.77 ($P < 0.038$ versus CT)	9.0 18.1 8.6
PACS-04 [159]	Node-positive EBC	47	FEC or Epi–Doc FEC or Epi–Doc→H 1 year	78% (3 years) 81% ($P = 0.41$)	96% (3 years)v 95% ($P = 2.38$)	2.2 4.2
FinHER [158]	Node-positive Node-negative high-risk EBC (n = 232)	62	Doc or Vin →FEC Doc or Vin →FEC–H	73.3% 83% HR = 0.65 ($P = 0.12$)	82.3% 91.3% (5 years) HR = 0.55 ($P = 0.094$)	
Meta-analysis 2012 [160]	All trials included			HR: 0.60; 95% $P < 0.00001$	HR: 0.66; 95% $P < 0.00001$	

FEC, cyclophosphamide, epirubicin, and fluorouracil; AC, doxorubicin and cyclophosphamide; Pac, paclitaxel; Doc, docetaxel; S, surgery; H, herceptin; Carb, Carboplatin; Vin, vinorelbin; Epi, epirubicin.

5.4. *Compliance to hormonal therapy and predictors of response to treatment*

Adherence to ET is a concern in patients with EBC as it is believed to impact on the outcome; however, the association between non-adherence and breast cancer mortality is not proven. In ET studies patients are considered to be adherent to treatment if ⩾80% of prescribed doses are taken, but the best tool for measurement of adherence is not yet defined, and has varied among studies. It has been reported that adherence to tamoxifen falls to 50% during the course of therapy [144]. Non-adherence to anastrozole has been reported to occur in 1/3 of patients [145]. In a recent population-based study of 8769 patients with BC [146], 32% discontinued treatment with tamoxifen or an AI over the 4.5-year follow-up period, and among those who continued 28% were non-adherent. Younger women were at high risk of non-adherence being 50% more likely to discontinue therapy and 40% more likely to be non-adherent ($P < 001$).

Among patients taking AIs the musculoskeletal toxicities are the main reason for treatment discontinuation/non-adherence [147–149]. Predictive factors of these adverse effects have been studied, but have not been consistent among studies. A retrospective exploratory analysis from the ATAC trial has shown that previous hormone replacement therapy, previous CT and obesity were risk factors for the development of joint symptoms. A recent exploratory analysis from a prospective study, the Exemestane and Letrozole Pharmacogenetics (ELPh) clinical trial [150], found that younger age and prior taxane-based CT were associated with a greater likelihood of treatment discontinuation, but prior tamoxifen therapy, prior hormone replacement therapy and body mass index were not predictors. One third of patients prematurely discontinued adjuvant AI therapy in this study, but it was also seen than more than one third of patients who switched drugs tolerated the second AI, confirming previous results [151].

There is no evidence to demonstrate differences in efficacy and toxicity among AIs. Anastrazol has shown efficacy similar to that of letrozol in the MA.27 trial [152]. The results from the FACE trial comparing two non-steroidal AIs, letrozole and anastrozole, are awaited.

The main predictors of response to hormonal treatment are oestrogen and progesterone receptors [114]. There is no

evidence to support HER2 status as predictive of different responses to tamoxifen or AIs [129,153]. New genomic tools such as Oncotype DX and PAM50 [30,154] have been predictive of tamoxifen treatment, but their use in clinic has been mainly as a prognostic tool.

Recently an exploratory analysis from the BIG 1-98 trial [155] of 2599 patients treated with tamoxifen monotherapy or letrozol monotherapy, with a 12-year follow-up, showed a significant interaction effect between histology subtype and degree of benefit to letrozole over tamoxifen, with greater benefit being seen with letrozol in women with lobular carcinomas compared with invasive ductal carcinomas. Although these data need further validation, it restores confidence in the use of AI in high-risk lobular tumours.

6. What is the optimal adjuvant anti-HER2 treatment?

For patients with HER2+ early BC the use of trastuzumab and CT is well established and evaluated in six adjuvant trastuzumab randomised clinical trials (Table 5) involving more than 13,000 women: the Herceptin Adjuvant trial (HERA) [156], the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 trial and the North Central Cancer Treatment Group (NCCTG) N9831 trial [157], the Breast Cancer International Research Group (BCIRG) 006 trial [79], the Finland Herceptin trial (FinHER) [158] and the Protocol Adjuvant dans le Cancer du Sein (PACS-04) trial [159], and in a 2012 meta-analysis [160].

All trials except PACS-04 yielded an improved DFS (HR between 0.6 and 0.77) and OS (HR between 0.55 and 0.77) with the administration of trastuzumab (Table 5).

Cardiac toxicity data from these trials indicate that the rate is higher when anthracyclines are used and with concurrent regimens. Nevertheless, the rates are always low and clinically acceptable.

The 2012 meta-analysis of eight studies, involving 11,991 patients, assessed the benefits of adding trastuzumab to adjuvant CT in patients with HER2+ BC [160]. The inclusion of trastuzumab resulted in an improvement in DFS with an HR = 0.60 (95%CI 0.50–0.71), regardless of trastuzumab treatment duration or administration schedule (i.e. concurrently or sequentially with CT) and an improvement in OS with an HR = 0.66 (95%CI 0.57–0.77).

6.1. *Timing of trastuzumab initiation*

The decision about whether trastuzumab should be administered concurrently or sequentially after the completion of adjuvant CT as been addressed directly in the N9831 trial. The second planned interim analysis, with a median follow-up of 6 years, indicates that although trastuzumab added sequentially to CT improves DFS, there is a strong trend towards a better outcome with concurrent trastuzumab relative to sequential administration [161].

In the 2012 meta-analysis the benefit in OS was associated with concurrent administration [HR 0.64 (95%CI 0.53–0.76)] but not with sequential treatment of CT followed by single-agent trastuzumab [HR 0.85 (95%CI 0.43–1.67)] [160]. BCIRG-006 also support the use of trastuzumab administered concurrently with CT in the adjuvant setting [79].

6.2. *Duration of trastuzumab treatment*

One year of trastuzumab is the standard of care in adjuvant therapy. In the HERA trial a comparison between 1 and 2 years of adjuvant trastuzumab after CT concluded that 2 years of treatment was not better than 1 year [162]. The PHARE trial recruited over 3380 HER2+ patients and randomly assigned them to receive either 6 months or 1 year of adjuvant trastuzumab. The trial results were reported as unable to prove the non-inferiority hypothesis of 6 months versus 1 year of adjuvant trastuzumab [163]. In the 2012 meta-analysis trastuzumab administered for 12 months was associated with an improvement in OS [HR 0.67 (95%CI 0.57–0.80)]; although trastuzumab treatment for $\leqslant$6 months also showed a trend towards an improvement in OS, it did not reach statistical significance [HR 0.55 (95%CI 0.27–1.11)] [160].

Several trials are still ongoing evaluating the optimal duration and regimen of adjuvant trastuzumab; these might lead to a different conclusion in the future. The relative benefit of 6 months versus 1 year of trastuzumab is being evaluated in the PERSEPHONE trial (which also evaluates sequential versus concurrent trastuzumab) and the HELLENIC trial (using only concurrent therapy). The SHORT-HER and SOLD trials are evaluating 9 weeks versus 12 months of trastuzumab given concomitantly with a taxane, similar to the FinHER trial.

7. Conclusions and future perspectives

(Neo)adjuvant systemic therapy has dramatically changed the natural history of EBC. Together with screening and early detection, it is responsible for the 30% decrease in mortality observed since the 1990s.

The stronger effects are seen with biologically targeted agents such as endocrine and anti-HER2 therapies. Similar advances are still lacking for the heterogeneous groups of triple-negative EBC.

Prognostication has been greatly improved in the last decade, but advances in prediction have been only minimal and remain a research priority.

New technologies and a better knowledge of the biology of the different subtypes of BC, as well as an in-depth understanding of the mechanism of cancer resistance, will hopefully enable us to achieve a true individualised/personalised medicine in the near future

Conflict of interest statement

The author has a potential conflict of interest with the following companies: Eisai, Roche, GSK, Celgene, AstraZeneca, Novartis, GE Oncology, Merck-Sharp, Merus BV, Genentech and Pfizer.

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