

# Adjuvant chemotherapy in early breast cancer

The experience of the Danish Breast Cancer Cooperative Group

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1. Ejlersen B, Jensen MB, Mouridsen HT. Excess mortality in postmenopausal women who only receive adjuvant endocrine therapy for estrogen receptor positive breast cancer. *Acta Oncol* 2014;53:174-85.
2. Ejlersen B, Jensen MB, Elversang J, Rasmussen BB, Anderson M, Andersen J, Nielsen DL, Cold S, Mouridsen HT. One year of adjuvant tamoxifen compared with chemotherapy and tamoxifen in postmenopausal patients with stage II breast cancer. *Eur J Cancer* 2013;49:2986-2994.
3. Ejlersen B, Mouridsen HT, Jensen MB, Andersen J, Anderson M, Kamby C, Knoop AS; Danish Breast Cancer Cooperative Group. Cyclophosphamide, methotrexate, and fluorouracil; oral cyclophosphamide; levamisole; or no adjuvant therapy for patients with high-risk, premenopausal breast cancer. *Cancer* 2010;116:2081-9.
4. Ejlersen B, Jensen MB, Nielsen KV, Balslev E, Rasmussen BB, Willemoe GL, Hertel PB, Knoop AS, Mouridsen HT, Brünner N. HER2, TOP2A, and TIMP-1 and responsiveness to adjuvant anthracycline-containing chemotherapy in high-risk breast cancer patients. *J Clin Oncol* 2010;28:984-90.
5. Ejlersen B, Jensen MB, Rank F, Rasmussen BB, Christiansen P, Kroman N, Kvistgaard ME, Overgaard M, Toftdahl DB, Mouridsen HT; Danish Breast Cancer Cooperative Group. Population-based study of peritumoral lymphovascular invasion and outcome among patients with operable breast cancer. *J Natl Cancer Inst* 2009;101:729-35.
6. Ejlersen B, Jensen MB, Mouridsen HT, Andersen J, Cold S, Jakobsen E, Kamby C, Sørensen PG, Ewertz M. DBCG trial 89B comparing adjuvant CMF and ovarian ablation: similar outcome for eligible but non-enrolled and randomized breast cancer patients. *Acta Oncol* 2008;47:709-17.
7. Ejlersen B, Mouridsen HT, Jensen MB. Adjuvant cyclophosphamide, methotrexate, and fluorouracil in premenopausal patients with node-positive breast cancer: Indirect comparison

of dose and schedule in DBCG trials 77, 82, and 89. *Acta Oncol* 2008;47:662-71.

8. Ejlersen B, Mouridsen HT, Jensen MB, Andersen J, Cold S, Edlund P, Ewertz M, Jensen BB, Kamby C, Nordenskjöld B, Bergh J. Improved outcome from substituting methotrexate with epirubicin: results from a randomised comparison of CMF versus CEF in patients with primary breast cancer. *Eur J Cancer* 2007;43:877-84.
9. Ejlersen B, Mouridsen HT, Jensen MB, Bengtsson NO, Bergh J, Cold S, Edlund P, Ewertz M, de Graaf PW, Kamby C, Nielsen DL. Similar efficacy for ovarian ablation compared with cyclophosphamide, methotrexate, and fluorouracil: from a randomized comparison of premenopausal patients with node-positive, hormone receptor-positive breast cancer. *J Clin Oncol* 2006;24:4956-62.

Part of 5. has been included in the PhD thesis *Timp-1 as a predictive marker for chemotherapy in primary breast cancer* (Hertel P, University of Copenhagen, 2010).

## 1. INTRODUCTION

The Danish Breast Cancer Cooperative Group (DBCG) has devoted more than three decades of research to the improvement of breast cancer diagnosis and treatment. Local treatments (surgery and radiotherapy) were established when the DBCG was founded in 1976, and subsequently both have been further developed and improved upon. In addition, the DBCG has made a substantial contribution to the introduction of systemic therapies (endocrine therapy, HER2-directed therapy and chemotherapy). This review focuses on the contribution of the DBCG in defining the optimal use of adjuvant chemotherapy for women with early breast cancer [1-13].

Randomised clinical trials (RCTs) are the source of the highest level of evidence regarding specific treatments and for comparing the benefit of alternative treatments. By enrolling more than 7,000 breast cancer patients into adjuvant chemotherapy trials, the DBCG investigators have made a significant contribution. A distinctive feature of the DBCG is its capacity to perform long-term follow-up on all events and a life-long follow-up on survival. Despite contributions from an increasing number of RCTs, gaps remain in the preparation of treatment guidelines. Data from observational studies may partially fill this gap, but these sources are heterogeneous in terms of the collected data elements and their completeness. The DBCG has continuously provided guidelines for diagnosis and treatment of breast cancer, and a high compliance has allowed the assembly of large cohorts of well-characterised and similarly treated patients. The clinical DBCG database was established 1977 in the context of a continuous nationwide quality assurance programme, and has the additional

advantage of capturing detailed clinical data and long-term follow-up. The clinical DBCG database receives patient characteristics as well as pre-specified data on tumour characteristics and treatment from the examining pathologist and the treating physicians. Data are entered prospectively and monitored through the Danish National Registry of Patients and the Nationwide Register for Pathology. Being population-based, survival may be estimated relatively by linkage to Statistics Denmark which holds information on emigration and date of death, when applicable, by using the Danish Civil Personal Registration number. Furthermore, direct linkage to the Nationwide Register for Pathology enables correlative studies in pathology and molecular biology within the large RCTs and cohorts. Furthermore, by linkage to the Danish National Patient Register, information on co-morbidities is provided.

The two first DBCG programmes used the randomised consent design or Zelen's randomisation method [14]. Consent to participate was sought after randomisation, and only patients allocated to the experimental treatment arm were asked for consent. There may be several justifications for using Zelen's method, but the main argument was that patients randomised to the standard arm would be recommended the same treatment outside of the clinical trial. Zelen's consent design was abandoned by the DBCG in 1988, primarily to comply with the Helsinki Patient Charter's statement on seeking informed consent before a patient is entered onto a clinical trial. This resulted in a significant decrease in the ratio of randomised to eligible nonrandomised patients [15]. From January 1990 through April 1998, the DBCG identified 1,628 patients who were eligible for the 89B trial, but only 525 patients participated in the randomisation [8]. Nearly all patients were included in randomised trials when Zelen's design was applied so when the proportion of eligible patients who were randomised in the 89B declined from 59% in 1993 to 14% in 1998, this triggered concern regarding the external validity of the trial. Among the 1,103 eligible but non-enrolled patients, 970 (88%) received treatment as per protocol and 583 self-selected cyclophosphamide, methotrexate and fluorouracil (CMF), while 387 selected ovarian ablation. Eight non-enrolled and relapse-free patients were prescribed tamoxifen and six non-enrolled and two randomised patients received hormone replacement therapy. The 10-year disease-free survival (DFS) was 47.2% (95% CI 42.6 to 51.9%) in randomised and 48.9% in non-enrolled (95% CI 45.4 to 52.4%). The unadjusted hazard ratio (HR) for DFS was 1.06 (95% CI 0.91 to 1.24), and the unadjusted HR for overall survival (OS) was 1.06 (95% CI 0.90 to 1.26%). Adjustment for age, tumour size, nodal status, histological type, malignancy grade, oestrogen receptor/progesterone receptor (ER/PR) status and treatment did not affect these estimates. There were, however, a difference in toxicity, as 36% of patients randomised to CMF reported moderate or severe nausea and vomiting compared with 21% of the non-enrolled CMF treated patients ( $p < 0.01$ ). Overall, there was no support for a differential outcome regarding treatment benefits according to enrolment or not in the DBCG 89B, and this validation is an example of the methodological advantages achieved by the structure of the DBCG. In contrast, a significant difference in toxicity was observed according to enrolment. Toxicity from treatment regimen used in PACS-01 (Table 7) was evaluated in several retrospective studies. The risk of febrile neutropenia following docetaxel was substantially higher in Danish and UK settings than in a French setting identical to the trial setting. The French study reported that the observed risk was similar to the one reported in PACS-01 [16-19]. Similarly, a high external validity has been reported for therapeutic benefit by other col-

laborative groups engaged in developing standards of cancer care as well as conducting RCTs [20-22], while comparisons between participants in RCTs and registries, i.e. the Surveillance, Epidemiology, and End Results (SEER) Program, not encountering information on systemic therapies have been difficult to interpret [23-28].

The majority of randomised controlled trials will be analysed using the intention-to-treat principle in order to preserve the control for confounding that is achieved by randomisation. In principle, this means that the decision to offer a new against a standard therapy is analysed regardless of adherence to the regimen. When the exposure-disease relationship is not taken into account, this may affect the generalisability of results from randomised trials to the general population.

## 2. NATURAL HISTORY OF BREAST CANCER

In 1962, Bloom and colleagues described the natural history of breast cancer by combining information on 250 women who were diagnosed with the disease between 1805 and 1933 [29]. Few of the patients had early breast cancer (none in Stage 1 and 2.4% in Stage 2), 23.2% in Stage 3 and the remainder in Stage 4 (74.4%). Only 3.6% were alive at 10 years, and spontaneous regression was not observed in any patient. The historical estimates may be subject to publication bias and other serious methodological errors. Other historical cohorts confirm that long-term survival is extremely rare in untreated breast cancer patients [30]. In collaboration with the Danish Cancer Registry, the DBCG aimed at making an unbiased estimate of survival for untreated breast cancer [31]. Among 49,058 women with histologically or cytologically verified breast cancer between 1978 and 1995, only 17 women initially declined treatment for no specific reason and five of these subsequently received treatment. Nine of the 12 persistent treatment objectors died before 2001, and the remaining three were alive at the end of follow-up. Overall, the joint report from the DBCG and the Danish Cancer Registry shows that in Denmark it is not possible to assess the prognosis in untreated patients. From the earliest times, physicians have been puzzled by the natural history of breast cancer, and the contemporary hypotheses has its beginning in 1858 when Vichow launched the theory of lymphatic spread with lymph nodes acting as defensive barriers. Forty years later, when Heidenhain described the localisation of recurrences in breast cancer patients following simple mastectomy, this prompted Halsted to suggest that breast cancers progress centrifugally from the breast through regional lymph nodes to distant sites [32]. The Halsted model was challenged by clinical observations including a long-term follow-up of 950 consecutive patients treated by Halsted and his successors who concluded that breast cancer patients are rarely cured by radical surgery [33]. Recognition that some patients will develop distant metastases without prior regional metastases, however, was inconsistent with the Halsted model [34]. In addition, circulating cancer cells were demonstrated after mastectomy and later even before surgery [35-37]. In animal experiments, regional lymph nodes did not seem to be capable of filtering cancer cells, and Fisher formulated the theory that the metastatic potential of cancer cells is predetermined [38]. Still, the survival benefits obtained by post-mastectomy irradiation and by mammography screening to some degree support the Halsted model [39, 40]. The notation that both dogmas are too restrictive has been attributed to Hellman [41], and it has increasingly been recognised that insufficient biological insight warrants a broad therapeutic perspective with emphasis on loco-regional therapy as well as systemic therapy.

### 3. CHEMOTHERAPY

#### BACKGROUND

The early concept of using chemotherapy as an adjunct to loco-regional treatment of early and apparently localised breast cancer originated in the 1950s. Circulating cancer cells were demonstrated following mastectomy, and were initially assumed to have detached during surgery [35, 36]. This led to the initiation of trials of short-term perioperative chemotherapy as reviewed by Tormey in 1975 [42]. The term “perioperative” refers to chemotherapy administered at surgery or within the first two months after surgery. Between April 1958 and October 1961, the NSABP recruited 826 participants to their first perioperative thiothepa trial, later named B01. At five years, the recurrence rate was significantly different in the two groups, and overall survival was 63% in the thiothepa and 62% in the control group [43, 44]. From January 1965 to 1971, Nissen-Meyer and his Scandinavian colleagues conducted an RCT including 1,026 patients in which they demonstrated that short-course cyclophosphamide given immediately after surgery reduces the risk of recurrence ( $p < 0.001$ ) and mortality ( $p < 0.01$ ) [45-47]. In the Ludwig Breast Cancer Study Group trial, V 1275 node-negative patients were randomised to one cycle of perioperative CMF against control, and CMF was associated with a significant improvement in DFS (HR=0.77 95% CI 0.61 to 0.98) [48]. Incomplete reports have been published from a few other, generally small trials of perioperative cyclophosphamide, thiothepa, 5-FU and mitomycin [42].

Even before the results of perioperative chemotherapy were fully presented, attention was directed towards the systemic theory, i.e. that at a very early stage, breast cancer may be divided according to its ability to form distant metastasis [34]. Furthermore circulating tumour cells were demonstrated both before surgery and in patients who had not undergone surgery. Among 28 potentially curable breast cancer patients, 14 were alive at four years, and the fact that 10 of these were without circulating cancer cells postoperatively led the authors to propose a greater focus on systemic treatment [37]. The clinical significance or utility of circulating tumour cells has yet to be elucidated [49]. In the same time-period, pre-clinical experiments indicated an inverse relationship between the size of a tumour and its response to cytotoxic drugs [50, 51]. Subsequently, in an animal study Skipper showed that a complete remission could be achieved by early administration of chemotherapy, while cancers became incurable when treatment was delayed [52].

Preoperative chemotherapy is the standard of care for patients with inflammatory and inoperable breast cancer. Indications for preoperative therapy may be expanded to include patients with large primary tumours who are interested in breast preservation or in order to obtain a better chance of good cosmetics following breast conserving surgery (BCS) [53].

#### ADJUVANT CHEMOTHERAPY

This review will summarise the development of adjuvant chemotherapy. When systemic therapy was introduced, radical surgery was already established and a requisite for cure of localised breast cancer. Therefore, systemic therapy was referred to as adjuvant systemic therapy. Most breast cancer patients will start systemic therapy days to weeks after surgery; and unless otherwise specified, it may be assumed that adjuvant systemic therapy is initiated postoperatively and continued for months or years.

#### SINGLE-AGENT CHEMOTHERAPY

Adjuvant single-agent cyclophosphamide has only been assessed in the DBCG trial 77B, which compared DFS and OS in premenopausal breast cancer patients randomised to one of the following: mastectomy plus radiotherapy, radiotherapy plus 12 cycles of oral cyclophosphamide (C) 130 mg/m<sup>2</sup> days 1 through 14 every four weeks, radiotherapy plus 12 cycles of CMF (C 80 mg/m<sup>2</sup> orally on days 1 through 14, methotrexate 30 mg/m<sup>2</sup> and 5-fluorouracil 500 mg/m<sup>2</sup> intravenously on days 1 and 8) with four-weekly intervals, 12 cycles or levamisole 2.5 mg/kg on two consecutive days each week for 48 weeks. An immune-stimulant effect was anticipated from levamisole when 77B was designed. Participants were required to have axillary lymph node metastases, tumours > 5 cm, or invasion of deep fascia and no distant metastases [1]. Randomisation opened in November 1977 and safety concerns led to the closure of the levamisole arm in December 1979. Furthermore, patients on levamisole were discontinued in case of side effects. In January 1981, a succeeding interim analysis led to the closure of the control arm [54]. Cyclophosphamide significantly improved disease-free and overall survival at 10 years as compared with control. With prolonged 25 years follow up, there was significant difference in survival when adjusting for baseline characteristics (HR 0.66; 95% CI 0.51-0.86; P=0.002). Only marginal benefits were observed from melphalan in the NSABP B-05 and Manchester II/Guy's trials, and a period of two years of melphalan was later shown to be inferior to one year of CMF in SWOG 7436 [55-58]. DNA synthesis inhibitors, anthracyclines and taxanes largely replaced or were added to the existing adjuvant regimens, and patients were highly selected in the few trials that examined the effect of single agents. No significant benefit was observed in DFS or OS from six three-weekly cycles of oral capecitabine 2,000 mg/m<sup>2</sup> daily for two weeks against no adjuvant chemotherapy in the ICE (BIG 4-04) trial [59]. The moderate sized ICGG and the small FASG trial both demonstrated a significant improvement in DFS but not in OS when comparing tamoxifen plus i.v. epirubicin to the same tamoxifen regimen for 3-4 years [60, 61]. The CALGB 40101 Alliance trial had a 2 by 2 factorial design, and was unable to demonstrate non-inferiority (HR 1.26 for RFS with a one-sided upper 95% CI limit of 1.48) of single-agent paclitaxel [62]. In a correspondence, the authors of the 40101 trial has subsequently made HER2 status available for 97% of the patients and found no evidence in support of an interaction between HER2 status and outcome [63].

#### COMBINATION CHEMOTHERAPY

##### **CMF combinations**

In July 1973, the first patient was randomised in the first adjuvant Milan CMF trial, and the early results were published by Bonadonna and collaborators in 1976 [64]. The original Milan CMF regimen consisted of oral cyclophosphamide (100 mg/m<sup>2</sup> from day 1 to 14), combined with intravenous methotrexate (40 mg/m<sup>2</sup> days 1 and 8) and fluorouracil (600 mg/m<sup>2</sup> days 1 and 8), and repeated every 4 weeks. The 1<sup>st</sup> Milan trial included women younger than 76 years with early and node-positive breast cancer, and randomisation was stratified according to age and number of positive axillary nodes. None of the patients received radiotherapy or endocrine therapy. As compared with control CMF, significantly improved relapse-free (P=0.004) and OS (P=0.04) was observed at a median follow-up of 19.4 years (Table 1) [65]. Limitations were lack of a predefined statistical design, that participants were not offered multimodality adjuvant therapy and lack of knowledge of molecular subtypes. Following a small pilot trial

(N=90) in node-negative breast cancer, the Milan collaborators concluded that a similar outcome could be achieved with an intravenous regimen [66]. The DBCG evaluated CMF against no adjuvant chemotherapy in separate trials for pre- and postmenopausal breast cancer patients. The 77B trial included premenopausal patients with either positive axillary node(s), a tumour larger than 5 cm, or invasion of the deep fascia and randomised participants to one of four treatment options; no adjuvant systemic therapy; levamisole for 48 weeks; oral cyclophosphamide for 48 weeks; and classic CMF for 48 weeks. As explained previously, a safety analysis led to early closure of the control arm. Between November 1977 and January 1981, Trial 77B randomised 193 patients to CMF and 187 to the control arm. At three years, a significantly longer DFS was observed following CMF as compared with no adjuvant systemic therapy [73, 74]. With a median estimated potential follow-up of 10 years, DFS as well as OS were significantly improved (Table 1) [1]. An extended follow-up furthermore demonstrated that the survival benefit persisted with prolonged 25 years of follow-up (adjusted HR, 0.59; 95% CI, 0.45-0.77; P=0.0001).

**Table 1.**

**Randomised trials of CMF versus no CMF**

Study	Regimens	N	DFS; 95% CI	OS; 95% CI
1 <sup>st</sup> Milan	12-oCMF	207		
Bonadonna <sup>65</sup>	Control	179	0.71; 0.56-0.90	0.78; 0.62-0.99
DBCG 77B	12-oCMF	193		
Ejlertsen <sup>1</sup>	Control	187	0.70; 0.53-0.93	0.70; 0.52-0.94
DBCG 82C	TAM+9-CMF	709		
Ejlertsen <sup>5</sup>	TAM	736	0.82; 0.71-0.93	0.95; 0.85-1.08
NSABP B20	TAM+6-oCMF	768		
Fisher <sup>67</sup>	TAM+6-CMF	767	0.65; 0.50-0.84	0.64; 0.42-0.95
	TAM	771	0.72; 0.56-0.93	0.67; 0.45-0.99
ABC UK/Asia Bliss <sup>68</sup>	pTAM+CMF#	987		
	TAM	1004	0.89; 0.76-1.04	0.86; 0.73-1.03
NCIC MA.5	TAM+8-CMF	353		
Pritchard <sup>69</sup>	TAM	352	0.97; 0.77-1.23	1.01; 0.75-1.36
Ludwig III	TAM+12-oCMF	154		
Goldhirsch <sup>70</sup>	pTAM	153	NA*	NA
	Control	156		
IBCSG IX	3-oCMF→TAM	811		
Gertsch <sup>71</sup>	TAM	835	NA†	NS
Guy's/Man.	12-oCMF	193		
Richards <sup>72</sup>	Control	198	NA‡	NS

DFS: disease-free survival; OS: overall survival; CI: confidence interval; oC: oral cyclophosphamide; C: cyclophosphamide; F: fluorouracil; M: methotrexate; TAM: tamoxifen; p: prednisone.

NA: non-available; NS: non-significant.

\*: P < 0.05; †: P < 0.01; ‡: P = 0.05; #: 87% received some kind of CMF.

Trial 77C DBCG demonstrated a clinical benefit in node-positive postmenopausal patients from one year of tamoxifen and designed the DBCG 82C to evaluate whether a further improvement could be obtained by adding chemotherapy or radiotherapy to tamoxifen [75]. The DBCG trial 82C included post-menopausal breast cancer patients, but otherwise had inclusion criteria identical to the as 77B, e.g. positive axillary node(s), a tumour larger than 5 cm or invasion of the deep fascia. Between October 1982 and March 1990, eligible patients were randomised to tamoxifen 30 mg daily for 52 weeks, tamoxifen with concurrent CMF (600:40:600) intravenously on day 1 every four weeks for nine cycles (CMFT), or to tamoxifen with postmastectomy radiothera-

py. At four years, recurrence-free survival was 49% in the tamoxifen group as compared with 60% in the tamoxifen plus radiotherapy group, and 56% in the tamoxifen plus CMF group (P=0.03) [4]. At 10 years, the addition of CMF to tamoxifen significantly improved DFS (Table 1), but not OS [5].

In B20, the NSABP similarly evaluated the addition of six cycles of classic CMF or MF (methotrexate and fluorouracil) chemotherapy to tamoxifen in patients with operable node-negative and ER-positive breast cancer. Both MF and CMF were associated with a significant reduction in RFS events and deaths, and the benefit was seen regardless of age, tumour size and ER expression level (Table 1) [67]. The ABC trial also explored adding chemotherapy, in 89% some kind of CMF, to five years of tamoxifen (with or without ovarian suppression) and was able to show a significant improvement in overall survival only after adjustment for nodal status, ER and age (P=0.03; Table 1) [68]. Trastuzumab was not available in the ABC trial for participants with HER2-positive tumours. In MA.4, the NCIC found no significant benefit from adding eight cycles of intravenous CMF to tamoxifen (Table 1) [69], while the Ludwig III and IBCSG X demonstrated a significant improvement in DFS, but not in OS (in both studies, detailed analyses were only presented according to ER status) [70, 71]. A small trial from Guy's and Manchester reported a significant benefit in DFS, but not in OS from CMF compared with control (Table 1) [72].

**Anthracycline combinations**

Three trials have evaluated addition of anthracycline-based chemotherapy to tamoxifen compared with tamoxifen alone in patients with ER-positive breast cancer. In NASBP B-16, four cycles of AC (60:600) given concomitantly with five years of tamoxifen significantly improved DFS (p=0.0004) and OS (p=0.04) [76]. Six cycles of CAF significantly improved DFS when given before (HR=0.70; 95% CI 0.57 to 0.85) as well as concomitantly (HR=0.83; 95% CI 0.64 to 0.91) with five years of tamoxifen in SWOG-8814/INT-0100, but only gave an improvement in OS when given before (HR=0.79; 95% CI 0.63 to 0.98) and not when given concomitantly (HR=0.87; 95% CI 0.70 to 1.08) with tamoxifen [77]. However, no significant difference was observed in DFS or OS when sequential CAF plus tamoxifen was compared to concurrent CAF plus tamoxifen [77]. FASG 02 compared tamoxifen plus FEC50 to control, and FASG 07 compared tamoxifen plus FEC50 to tamoxifen and showed a benefit in DFS (p=0.0008), but not in OS (p=0.11) when analysed jointly [78]. The Genoa, Geicam 9401, and GONO-MIG trials also evaluated concurrent versus sequential chemotherapy and tamoxifen, but were underpowered [79-81]. In comparisons of anthracyclines with other chemotherapy, CMF was widely used in the control group, but involved several variants of CMF, e.g. oral as well as intravenous cyclophosphamide and different schedules. An even greater variability is seen in anthracycline regimens, and anthracyclins were co-administered with other drugs in some trials while a sequential approach was adopted by others. Six trials compared CEF or CAF with CMF using the same number of drugs, schedule and treatment duration in both regimens, and ten-year results from three of these trials have been published (Table 2).

The Canadian MA.5 trial compared six cycles of classic CMF to CEF with epirubicin 60 mg/m<sup>2</sup> i.v. on days 1 and 8 in premenopausal patients without use of tamoxifen (Table 2) [87]. A significant improvement in RFS was achieved with CEF at five years, and this effect was maintained at 10 years (52% versus 45%, P=0.007),

whereas a significant improvement in OS observed at five years not was sustained at ten years [82].

**Table 2.**

**Randomised symmetrical trials of CEF or CAF versus CMF**

Study	Regimens	N	DFS; 95% CI	OS; 95% CI
NCIC MA.5	6-oCMF	359	1.31; 1.06-1.61	1.18; 0.94-1.49
Levine <sup>82</sup>	6-oCE <sub>60</sub> F days 1+8	351		
DBCG 89D	9-CE <sub>60</sub> F	615	0.84; 0.71-0.99	0.79; 0.66-0.94
Ejlertsen <sup>2</sup>	9-CMF	584		
ICCG	6-oCMF	179	NS	NS
Coombes <sup>83</sup>	6-CMF	185		
	8-CE <sub>50</sub> F q 21	173		
	6-CE <sub>50</sub> F q 28	191		
INT-0102	6-oCMF		1.09; 0.94-1.27	1.19; 0.99-1.43
Hutchins <sup>84</sup>	6-oCMF+TAM	1350		
	6-oCA <sub>30</sub> F days 1+8	1340		
	6-oCA <sub>60</sub> F+TAM			
GEICAM	6-CMF	405	1.2*	1.3
Martin <sup>85</sup>	6-CA <sub>50</sub> F	480		
SECSG	6-CMF	268	NA	NS
Carpenter <sup>86</sup>	6-CA <sub>50</sub> F	260		

DFS: disease-free survival; OS: overall survival; CI: confidence interval; A: doxorubicin; oC: oral cyclophosphamide; C: cyclophosphamide; E: epirubicin; F: fluorouracil; M: methotrexate; TAM: tamoxifen. NA: non-available; NS: non-significant; \*: P<0.05.

The DBCG 89D trial (Table 2) compared nine cycles of intravenous CEF (600; 60; 600 mg/m<sup>2</sup>) with CMF (600; 40; 600 mg/m<sup>2</sup>). After a potential ten-year median follow-up, the trial showed a statistically significant reduction in DFS events (P<0.04) and mortality (p<0,001) from substitution of methotrexate with epirubicin [2]. No significant benefit was observed by the International Collaborative Cancer Group (ICCG) from substituting methotrexate with epirubicin, but this trial had only limited power which was aggravated by use of two different schedules according to centre [83]. Six cycles of CAF with intravenous doxorubicin 30 mg/m<sup>2</sup> on days 1 and 8 was not superior to classic CMF in the Intergroup 0102 trial (Table 3) [88], while the GEICAM trial demonstrated a significant decrease in the risk of recurrence (P < 0.05), but not in mortality from three-weekly intravenous CAF compared with CMF [85]. The South-eastern Oncology Group compared CAF to CMF; unfortunately the trial was never fully published, but five-year survival was reported in abstract form and was not significantly different despite a 22% relative reduction in mortality [86].

A significant difference was not achieved in the four asymmetrically designed trials that compared EC or AC (Table 3) to classic CMF. A small Belgian trial used eight cycles of EC with 60 or 100 mg/m<sup>2</sup> of epirubicin in a three-arm trial [89, 90], while NSABP B-15 in a three-arm trial compared intravenous anthracycline-based therapy for 12 weeks or the same AC with addition of intravenous CMF for nine weeks to 24 weeks of CMF [91]. In a two-by-two randomisation NSABP B-23 compared AC for 12 weeks to 24 weeks of CMF with or without 20 mg tamoxifen daily for 5 years [92].

**Table 3.**

**Randomised trials of EC or AC versus CMF**

Study	Regimens	N	DFS; 95% CI	OS; 95% CI
Brussels	6-oCMF	255	0.84; 0.71-0.99	0.84; 0.71-0.99
Piccart <sup>89</sup>	8-EC60	267		
de Azambuja <sup>90</sup>	8-EC100	255		
NSABP B-15	6-oCMF	762	NS	NS
Fisher <sup>91</sup>	4-AC60	734		
	4-AC60+3-CMF	728		
NSABP B-23	6-oCMF	503	NS	NS
Fisher <sup>92</sup>	4-AC60	501		
	6-oCMF+TAM	502	NS	NS
	4-AC60+TAM	502		
GOCNE	6-oCMF	103	NA	NA
Galligione <sup>93</sup>	4-EC120	104		

DFS: disease-free survival; OS: overall survival; CI: confidence interval; A: doxorubicin; oC: oral cyclophosphamide; C: cyclophosphamide; E: epirubicin; F: fluorouracil; M: methotrexate; TAM: tamoxifen. NA: non-available; NS: non-significant.

Six trials compared doxorubicin or epirubicin in sequence with CMF to some duration of CMF, and results have been presented from three of these trials (Table 4). The two UK trials, NEAT and BR9601, were planned and analysed jointly, while the GUN-3 and Bergonie trials were too small to show an effect individually. In NEAT, four cycles of three-weekly epirubicin 100 mg/m<sup>2</sup> were followed by four cycles of classic CMF and compared with CMF for a similar duration, while in the Scottish BR9601 the same epirubicin was followed by four cycles of three-weekly intravenous CMF and compared with CMF alone for a similar duration. The pre-planned joint analysis (Table 4) demonstrated a significant clinical benefit without pronunciation of toxicity [94-97]. No significant benefit was observed in the small Italian GOIRC trial from weekly epirubicin as compared to intravenous CMF [100]. Additional drugs were included in SWOG 8313, ECOG 5181 and a small OncoFrance trial, and none of them were able to demonstrate a difference in effect by regimen [101-104].

**Table 4.**

**Randomised trials of sequential epirubicin versus no epirubicin**

Study	Regimens	N	DFS; 95% CI	OS; 95% CI
NEAT/BR9601	6-oCMF	1,012	0.75; 0.65-0.86	0.76; 0.65-0.89
Poole <sup>94</sup>	4-E100+4-CMF	1,009		
Earl <sup>95</sup>	8-CMF	190		
	4-E100+4CMF	180		
GUN-3 Naples	6-oCMF	115	0.75; 0.65-0.86	0.75; 0.65-0.86
De Placido <sup>98</sup>	3-CMF→3-EV	105		
Bergonie	9-CMF	115	NS	NS
Mauriac <sup>99</sup>	3-MiTVd+3-EVM	113		

DFS: disease-free survival; OS: overall survival; CI: confidence interval; oC: oral cyclophosphamide; C: cyclophosphamide; E: epirubicin; F: fluorouracil; M: methotrexate; Mi: mitomycin C; T: thiotepa; V: vincristine; Vd: vindesine; NS: non-significant.

There is no consensus on whether comparisons between doxorubicin and epirubicin should be based on equimolar, equitoxic or maximally effective doses. The MA.21 trial randomised 2,104 high-risk patients to eight cycles of CEF, dose-dense EC (epirubicin 120 mg/m<sup>2</sup> and cyclophosphamide 830 mg/m<sup>2</sup> every two weeks for 6 cycles) followed by T (paclitaxel 175 mg/m<sup>2</sup> every three



weeks for 4 cycles) or AC (doxorubicin 60 mg/m<sup>2</sup> and cyclophosphamide 600 mg/m<sup>2</sup> every three weeks for four cycles) followed by T. The three-year adjusted RFS rates were 90.1%, 89.5%, and 85% (P=0.001). The pairwise comparison of AC/T versus EC/T demonstrated a significantly higher risk of RFS events from AC/T than from EC/T (HR 1.68; 95% CI 1.25 to 2.25; P=0.0006) [105]. With only 47 deaths among patients in the EC/T arm and 65 deaths in the AC/T, there were too few events to allow for an analysis of survival. No significant differences in DFS or OS was shown in the NSABP B-36 comparing four cycles of AC with six cycles of CEF (Table 5) [106].

### Contribution from DNA synthesis inhibitors

Decades ahead of others, the DBCG realised the need to evaluate whether DNA synthesis inhibitors adds benefit to cyclophosphamide and continued randomisation in the DBCG 77B to the cyclophosphamide and CMF following closure of the control and levamisole arms (Table 5).

The ten-year survival rates were 60% and 62% for the cyclophosphamide and CMF arms, respectively. No significant difference was observed in outcome between the cyclophosphamide and CMF arms at ten years (Table 5) or in survival between the two chemotherapy arms at 25 years (HR 1.09; 95% CI 0.92 to 1.29). The DBCG 77B was, however, not designed to demonstrate non-inferiority of single agent cyclophosphamide as compared with CMF [1].

**Table 5.**

#### Randomised trials of DNA synthesis inhibitor(s) versus no such inhibitors

Study	Regimens	N	DFS; 95% CI	OS; 95% CI
DBCG 77B	Single oC	424	0.95; 0.77-1.16	1.09; 0.92-1.29
	oCMF, q28	423		
NSABP B-36	4-AC, q21	1361	1.03; 0.85-1.26	0.94; 0.71-1.25
	6-CEF, q21	1361		
GIM2	4EC→4P q21	545	0.98; 0.83-1.17	0.93; 0.73-1.19
	4CEF→4P q21	544		
	4EC→4P q14	502		
	4CEF→4P q14	500		
USON 01062	3EC→3D	1304	0.84; 0.67-1.05	0.68; 0.51-0.92
	3EC→3DX	1307		
FinXX	3D→3CEF	118	0.81; 0.63-1.04	0.74; 0.53-1.03
	3DX→3CEFX	96		

DFS: disease-free survival; OS: overall survival; CI: confidence interval; A: doxorubicin; oC: oral cyclophosphamide; C: cyclophosphamide; D: docetaxel; E: epirubicin; F: fluorouracil; M: methotrexate; P: paclitaxel; X: capecitabine.

NSABP B-36 was originally designed as a factorial 2-by-2 trial with randomisation to four cycles of AC (60:600) against six cycles of CEF (500:100:500), and to celecoxib against placebo. Randomisation to celecoxib was suspended in B-36 due to safety issues, and the protocol was furthermore amended to allow HER2-positive patients access to trastuzumab. With a median eight-year follow-up, there was no significant difference in DFS or OS [106]. The GIM-2 trial (Table 5) found no evidence of an improvement in DFS or OS from the addition of fluorouracil (600 mg/m<sup>2</sup>) to cyclophosphamide and epirubicin (CEF) against the same EC (90:900). Integration of capecitabine with docetaxel has been evaluated in two trials; USON 01062 and FinXX (Table 5). The addition of capecitabine 825mg/m<sup>2</sup> orally twice daily for four cycles to docetaxel was not associated with significant improvement in DFS (HR=0.84,

P=0.12) in a preliminary analysis of USON 01062. With just about 200 participants, the FinXX trial was underpowered, but showed a trend towards additional benefit from including capecitabine in a combination regimen [108, 109].

### Taxane combinations

In the metastatic setting, the activity of taxanes and anthracyclins is comparable and partially non-cross resistant. This has provided a rationale for largely studying combinations including a taxane and an anthracycline compared with an anthracycline combination. Having major side effects, especially in early trials, taxanes were given in sequence with other types of chemotherapy. As side effects became manageable, taxanes in general and docetaxel in particular were more widely given concurrently with an anthracycline. The results from taxane trials may, however, be confounded by choice of taxane and drug schedule as demonstrated by Sparano and colleagues in ECOG 1199 by inclusion of almost 5,000 node-positive patients in a factorial two-by-two design [110]. No significant difference in DFS was observed between docetaxel and paclitaxel, but a significant improvement was demonstrated by weekly paclitaxel compared with weekly docetaxel, and from three-weekly docetaxel compared with three-weekly paclitaxel [110].

The CALGB 9344 and NSABP B28 trials both evaluated addition of four cycles of three-weekly paclitaxel 175 mg/m<sup>2</sup> to four cycles of AC (Table 6).

**Table 6.**

#### Randomised trials of sequential paclitaxel versus no paclitaxel

Study	Regimens	N	DFS; 95% CI	OS; 95% CI
CALGB 9344	4AC	1,580	0.83; 0.73-0.94	0.82; 0.71-0.95
	4AC→4P	1,590		
NSABP B-28	4AC	1,529	0.83; 0.72-0.95	0.93; 0.78-1.12
	4AC→4P	1,531		
NCIC MA.21	8CEF	701	0.89; 0.64-1.22	NA
	4EC→4P	701		
	4AC→4P	702		
MDACC	8FAC	252	NA§	NA
	4P→4FAC	259		
HE10-97	4E→4CMF	604	0.65; 0.48-0.90	2.42; 1.17-4.99
Fountzilas <sup>114</sup>	3E→3P→3CMF	604	0.65; 0.48-0.90	2.42; 1.17-4.99
ECTO	4A→4CMF	453	0.73; 0.57-0.97	0.80; 0.56-1.14
Gianni <sup>115</sup>	4AP→4CMF	451	0.73; 0.57-0.97	0.80; 0.56-1.14
AERO B2000	6CEF	471	0.99; 0.77-1.26	0.85; 0.62-1.15
Delbaldo <sup>116</sup>	4CEF→4P	420		
GIECAM 9906	6CEF	634	0.77; 0.62-0.95	NS
Martin <sup>117</sup>	4CEF→weekly P	614		

DFS: disease-free survival; OS: overall survival; CI: confidence interval; A: doxorubicin; C: cyclophosphamide; E: epirubicin; F: fluorouracil; M: methotrexate; P: paclitaxel.

NA: non-available; NA§ not reported but for RFS HR= 0.70; 95% CI 0.47-1.07 in a multivariate Cox model; NS: non-significant.

Addition of paclitaxel translated into a significant improvement in DFS in both trials, but only CALGB 9344 demonstrated a significant decrease in mortality (Table 6) [111, 112]. In these two trials, the reference anthracycline arm could be of reduced strength as superiority of four cycles of AC was not shown in NSABP B-15 when compared with classic CMF (Table 3), and increasing the doxorubicin dose did not provide additional benefit in CALGB 9344. None of the other moderately sized trials comparing CAF or CEF to a sequence in which part of the cycles were substituted by

three-weekly paclitaxel did individually show a statistically significant improvement in OS (Table 6) [105,113-116]. GEICAM 9906 is the only trial assessing weekly taxane and compared four cycles of CE<sub>90</sub>F with the same CEF plus weekly paclitaxel for eight cycles in 1,246 patients. At a median follow-up of 66 months, no significant reduction in mortality was obtained [117].

Identical results have not been obtained from replacing CEF or CMF with three-weekly docetaxel 100 mg/m<sup>2</sup> (Table 7). Differences are evident among the large and adequately sized trials and are not easily explained as the PACS 01 and WSG/AGO trials reported a significant DFS and OS benefit, while TACT and NSABP B-27 were negative trials despite adopting a full-dose (100 mg/m<sup>2</sup>) sequential docetaxel schedule [118-122]. The moderately sized trials do not provide greater clarity, as the Mansoura trial reported a survival benefit from sequential docetaxel, whereas the BIG 2-98 and HORG trials reported a benefit in DFS but not in OS, and TAXIT 216 and GOIM 9902 reported no benefit [123-128].

**Table 7.**

**Randomised trials of sequential docetaxel versus no docetaxel**

Study	Regimens	N	DFS; 95% CI	OS; 95% CI
PACS 01	6CEF	996		
Codert <sup>119</sup>	3CEF→3D	1,003	0.85; 0.73-0.99	0.75; 0.62-0.92
WSG-AGO	6CMF or CEF	972		
Nitz <sup>120</sup>	4EC→4D	978	0.74; 0.57-0.97	0.70; 0.49-0.99
UK TACT	4CEF/4E→4CMF	2,089		
Ellis <sup>121</sup>	Same→4D	2,073	0.95; 0.85-1.08	0.99; 0.86-1.44
NSABP B-27	4AC→Surgery	802		
Bear <sup>122</sup>	4AC→4D→Surg.	803	0.90; 0.76-1.06	NA
	4AC→Surg.→4D	799	Group 2+3 vs 1	
Mansoura	6CEF	327		
Sakr <sup>123</sup>	3CEF→3D	330	0.83; §NA	0.73; 0.56-0.94
BIG 2-98	4A→3oCMF	481		
Oakman <sup>125</sup>	3A→3D→3CMF	960	0.81; 0.67-0.99	0.85; 0.67-1.11
HORG	4D→4EC	378		
Polyzos <sup>126</sup>	6CEF	378	1.31; 1.01-1.69	1.09; 0.79-1.51
TAXIT	4E→4CMF	486		
Bianco <sup>127</sup>	4E→4D→4CMF	486	0.80; 0.62-1.03	0.74; 0.51-1.07
GOIM 9902	4EC	374		
Vici <sup>128</sup>	4D→4EC	376	0.99; 0.75-1.31	0.84; 0.54-1.31

DFS: disease-free survival; OS: overall survival; CI: confidence interval; A: doxorubicin; oC: oral cyclophosphamide; C: cyclophosphamide; D: docetaxel; E: epirubicin; F: fluorouracil; M: methotrexate. NA: non-available; NS: non-significant.

The BCIRG 001 and GEICAM 9805 trials consistently showed a significant reduction in DFS events from substituting 5FU with docetaxel 75 mg/m<sup>2</sup> in the CAF (500; 50, 500) combination (Table 8), but BCIRG001 – in contrast to GEICAM 9805 – also showed a significant reduction in mortality [117, 129, 130]. The concurrent anthracycline-taxane arm in BIG 2-98 showed no benefit in DFS or OS compared with standard AC followed by CMF (Table 8) [125]. Substitution in standard AC (60:600) of cyclophosphamide with docetaxel 60 mg/m<sup>2</sup> did not improve DFS or OS in the Intergroup trial E 2197; no efficacy data were reported from the RAPP 01 trial following early discontinuation for safety reasons and only data from the HER2-positive subset in PACS 04 have been fully published (Table 8) [131-133].

**Table 8.**

**Randomised trials of concurrent docetaxel versus no docetaxel**

Study	Regimens	N	DFS; 95% CI	OS; 95% CI
BCIRG 001	6FAC	746		
Makey <sup>129</sup>	6DAC	745	0.80; 0.68-0.93	0.74; 0.61-0.90
GEICAM 9805	6FAC			
Martin <sup>130</sup>	6DAC	1,059	0.68; 0.49-0.93	0.76; 0.45-1.26
BIG 2-98	4AC→4CMF	487		
Oakman <sup>125</sup>	4AD→4CMF	959	1.02; 0.84-1.23	0.96; 0.76-1.21
E 2197	4AC	1,476		
Goldstein <sup>131</sup>	4AD	1,476	1.02; 0.86-1.22	1.06; 0.85-1.31
RAPP 01	4AC	316		
Brain <sup>132</sup>	4AD	311	NA	NA
PACS 04	6CEF	1,518		
Spielmann <sup>133</sup>	6ED	1,492	NA	NA

DFS: disease-free survival; OS: overall survival; CI: confidence interval; A: doxorubicin; C: cyclophosphamide; D: docetaxel; E: epirubicin; F: fluorouracil; M: methotrexate. NA: non-available.

The Italian MIG-5 and ECTO trials evaluated paclitaxel given concurrently with an anthracycline against non-taxane regimens, but have not yet been fully published [115, 134]. In US Oncology trial 9735, doxorubicin in standard dose AC (60:600) was substituted by docetaxel 75 mg/m<sup>2</sup>, and at eight-year follow-up DC was associated with a significantly superior DFS (HR=0.67; 95% CI 0.56 to 0.98) and OS (HR=0.69; 95% CI 0.50 to 0.97) [135]. Among HER2-negative patients, the replacement of docetaxel with vinorelbine resulted in a significantly shorter distant disease-free survival (DDFS) (HR=0.66; 95% CI 0.49 to 0.91) in the FinHER trial [136, 137].

In addition, some trials have compared sequential and concurrent taxane regimens. In the NSABP B-30, AC followed by T was superior to four cycles of TAC (HR for DFS 0.83; P=0.01). The NSABP B-38 randomised 4,894 patients to six cycles of TAC against two dose-dense sequential paclitaxel regimens; and no significant differences were observed in efficacy between TAC and dose-dense AC followed by paclitaxel, and addition of gemcitabine to paclitaxel (AC→PG) did not improve the outcome [138]. In the BCIRG 005, eight cycles of sequential AC followed by docetaxel was as effective (HR for DFS 1.0; 95% CI 0.86 to 1.16, and OS 0.91; 95% CI 0.75 to 1.11) as six cycles of TAC [139]. In a secondary comparison, sequential doxorubicin followed by docetaxel was superior to concurrent doxorubicin and docetaxel in BIG 2-98, for both DFS (HR= 0.84; 95% CI 0.72-0.99; P=0.035) and OS (HR= 0.79; 95% CI 0.65-0.98; P=0.028) [124, 125].

**CHEMOTHERAPY DOSE**

The dose intensity of chemotherapy can be intensified by increasing the dose per administration (escalation), by decreasing the interval between administrations (dose density) and by combining the two approaches. Dose escalation and higher total doses of intravenous cyclophosphamide did not improve recurrence-free survival (RFS) or OS in the NSABP B-22 or B-25 [140, 141]. However, the CMF regimen initially introduced by Bonadonna was later modified in subsequent trials from the Milan groups as well as by others, and oral cyclophosphamide given daily for two weeks in the classic CMF regimen has never been compared with intravenous administration in a randomised trial [142]. The Milan group made an indirect comparison and found no detrimental effects on DFS or OS when switching from classic CMF to 12 cycles of three weekly intravenous CMF (600, 40, 600 mg/m<sup>2</sup>) [143]. However,

the comparison made by the Milan group had major limitations, in particular due to lack of documentation of received drug doses and selection of different patient populations without sufficient assurance of adjustments for differences in patients' characteristics within trials.

To minimise the risk of selection bias, to avoid any interaction from other systemic therapies and to allow adjustment for administered drug doses, patient- and tumour characteristics, we identified three cohorts of premenopausal node-positive patients within the population-based DBCG database. None of the patients received adjuvant endocrine therapy, the 77B cohort received classic CMF (12 cycles of cyclophosphamide 80 mg/m<sup>2</sup> orally on days 1-14, and methotrexate 30 mg/m<sup>2</sup> plus 5-fluorouracil 500 mg/m<sup>2</sup> both intravenously on day 1 and 8 every four weeks), the 82B cohort received a low dose-intensity intravenous CMF (nine four-weekly cycles of intravenous cyclophosphamide 600 mg/m<sup>2</sup>, methotrexate 40 mg/m<sup>2</sup> and fluorouracil 600 mg/m<sup>2</sup>), and the 89B cohort received a intermediate moderate dose-intensity intravenous CMF (nine three-weekly cycles of intravenous cyclophosphamide 600 mg/m<sup>2</sup>, methotrexate 40 mg/m<sup>2</sup> and fluorouracil 600 mg/m<sup>2</sup>). In the DBCGs 89 programme, one or two cycles of single-agent cyclophosphamide (850 mg/m<sup>2</sup>) were administered concomitantly with radiotherapy followed by CMF to a total of nine cycles of three-weekly chemotherapy [2, 5, 7]. Major differences were observed in patients' characteristics across the 77, 82 and 89 cohorts, and we found no statistically significant difference in the unadjusted pairwise comparisons of DFS or OS. When adjusting for treatment cohort, age, nodal status, tumour size, hormone receptor status, and histological type and grade in a Cox model, DFS was significantly longer in the 77B cohort than in the 82B (HR 1.31; 95% CI, 1.08 to 1.58, P<0.01) and the 89B (HR 1.30; 95% CI, 1.05 to 1.62, P=0.02) cohort. Likewise, in the adjusted analysis, we found a significant difference in OS between the 82 and 77 cohorts (HR 1.40; 95% CI, 1.14 to 1.72, P<0.01), but not between the 89 and 77 cohorts (HR 1.13; 95% CI, 0.89 to 1.43, P=0.32) [6].

Standard anthracycline dose-intensities have been compared with an experimental dose per cycle only in a few major randomised trials. CALGB 8541 was compared the standard dose of CAF for six cycles to a low and very low dose by reducing doxorubicin from 60 to 40 or 30 mg/m<sup>2</sup> and simultaneously reducing cyclophosphamide and fluorouracil from 600 to 400 or 300 mg/m<sup>2</sup> [144]. The final results demonstrated an increase in mortality (P=0.004) and DFS events (P<0.0001) in the very low dose arm, while no significant differences were observed in the low dose arm. However, doubt exists as to whether this effect should be attributed to all three drugs. A subsequent study, CALGB 9344 (NAI 0148), demonstrated no evidence of benefit from escalating doxorubicin from a 60 mg/m<sup>2</sup> standard dose to 75 or 90 mg/m<sup>2</sup> when combined with a fixed dose of cyclophosphamide (AC) [111]. PACS 05 and a small Belgian trial demonstrated the standard dose of epirubicin (100 mg/m<sup>2</sup>) to be significantly more efficacious when compared with about half the standard dose (50 or 60 mg/m<sup>2</sup>) [90, 145].

In the mid-eighties, results from preclinical studies once again gained a decisive influence, and high-dose chemotherapy was included in the design of clinical trials and was even introduced in clinical practice in some countries. Particular emphasis was placed on the preclinical observation of steep dose-response relationships for alkylating agents, without waiting for the results of the NSABP trials which would later demonstrate a lack of benefit from dose escalation and higher total dose of cyclophosphamide

[141, 146]. In the early nineties, considerable support for the use of high-dose chemotherapy came from an un-controlled phase 2 trial and early results from a publication on metastatic breast cancer authored by dr. Bezwoda. An audit later documented that the trials by dr. Bezwoda and collaborators were fraudulent [147, 148]. The published randomised phase 3 trials do not have a sufficient sample size (48 to 885 patients), have heterogeneous patient selection criteria and vary considerably in the choice of standard and high-dose chemotherapy. Therefore, no attempt has been made to compare the results of the individual trials of high-dose chemotherapy [149].

The development of granulocyte colony-stimulating factor allowed shortening of the interval between chemotherapy without reducing the dose per cycle. In what is considered the pivotal dose-dense trial, CALGB 9741 showed a significant reduction in mortality and DFS events by the condensed approach compared with three-weekly administration of the same chemotherapy in patients with node positive breast cancer [150] (Table 9).

**Table 9.**

**Major randomised trials on dose-dense chemotherapy versus no dose-dense chemotherapy**

Study	Regimens	N	DFS; 95% CI	OS; 95% CI
CALGB 9741	AC→P/A+DCP	493	0.80; 0.73-0.95	0.81; 0.66-1.00
Citron <sup>150</sup>	AC→P/A+P+C	484		
GIM 1	CEF q 21	610	0.88; 0.71-1.08	0.87; 0.67-1.13
Venturini <sup>151</sup>	CEF q 14	604		
AGO	4EC→4D	590	0.64; 0.49-0.83	NA
Moebus <sup>152</sup>	3E→3D→3C	584		
INT 0137	6AC q 21	1590	1.09; 0.95-1.26	1.11; 0.93-1.32
Linden <sup>153</sup>	A →C q 14	1524		
GeparDuo	4AD, q 14	451	0.94; 0.73-1.22	0.79; 0.54-1.17
Minckwitz <sup>154</sup>	4AC→4D q 21	453		
EORTC- SAKK	6EC q 21	224	NA	0.99; 0.76-1.29
Therasse <sup>155</sup>	6oCMF	224		
NCIC MA.21	CEF q 28	701	0.89; 0.64-1.22	NA
Burnell <sup>105</sup>	EC q14→D q21	701		
GIM 2	4EC→4P q 21	545	0.78; 0.66-0.94	0.68; 0.52-0.87
	4CEF→4P q 21	544		
	Cognetti <sup>107</sup>	4EC→4P q 14		
PREPARE	4EC→4P q 14	500	0.70; 0.54-0.92	0.66; 0.38-1.15
	4EC→4D	333		
Uncht <sup>156</sup>	3E→3D→CMF	335		

DFS: disease-free survival; OS: overall survival; CI: confidence interval; A: doxorubicin; C: cyclophosphamide; D: docetaxel; E: epirubicin; F: fluorouracil; M: methotrexate; P: paclitaxel. NA: non-available

In contrast, Venturini and colleagues found no significant benefit in DFS or OS from two-weekly compared to three-weekly CEF (600; 60; 600 mmg/m<sup>2</sup>) in the Gruppo Italiano Mammella (GIM) trial 1 [151].

Seven other trials (Table 9) have examined an experimental dose-dense regimen without a conserved standard comparison or, additionally, have varied the number of cycles, dose-intensity, or have used different drugs or sequences. At this point, it is not clear whether CALGB 9741 supports dose-density as a concept or merely confirms that paclitaxel, but not docetaxel should preferably be administered at shorter than three-weekly intervals.

**Duration of chemotherapy**

One cycle of CMF compared with six cycles was associated with a significantly higher risk of DFS events (p<0.0001) and mortality



( $p=0.011$ ) in the Ludwig Breast Cancer Study Group trial including 1,229 node-positive breast cancer patients [157]. The second Milan trial found no significant differences in outcome from 12 compared to six cycles of CMF, but only recruited 324 participants and was underpowered (Table 10) [158].

Several other small trials (Table 10) addressed the same question, but were individually unsuited for detection of a modest benefit. In succeeding trials, the German Breast Group and the IBCSG further reduced duration of chemotherapy to 12 weeks of classic CMF as compared with 48 weeks [20, 159]. Both trials were underpowered, and a clear conclusion could not be reached (Table 10). No significant difference was observed in DFS or OS in NSABP B-15 from four cycles of AC as compared with six cycles of CMF, but this comparison might be confounded by exchanging methotrexate and fluorouracil with doxorubicin [91].

**Table 10.**

**Duration of adjuvant chemotherapy**

Study	Regimens	N	DFS; 95% CI	OS; 95% CI
2 <sup>nd</sup> Milan trial	12-oCMF	243		
Tancini	6-oCMF	216	NS	NS
GBSG	6-CMF	235	0.96; 0.76-1.22	0.90; 0.69-1.18
Sauerbrei	3-CMF	238		
IBCSG VI	6-oCMF	375	1.04; 0.85-1.27	NA
Pagani	3-oCMF	360		
NSABP B-15	4AC	734	NS	NS
Fisher	6-oCMF	732		
CALGB 40101	6AC or P	1578	1.03; 0.84-1.28	1.12; 0.84-1.49
Shulman	4AC or P	1593		

DFS: disease-free survival; OS: overall survival; CI: confidence interval; A: doxorubicin; C: cyclophosphamide; E: epirubicin; F: fluorouracil; M: methotrexate; P: paclitaxel. NA: non-available; NS: non-significant.

The CALGB 40101 Alliance trial had a 2-by-2 factorial design, and demonstrated that six cycles was not superior to four cycles of three-weekly adjuvant chemotherapy (Table 10); and in the other comparison this trial aimed to investigate whether single agent paclitaxel was non-inferior to standard AC [160]. As reflected by the wide confidence interval, the survival analysis was based on only 191 events despite the large sample size.

**TOXIC EFFECTS**

Trials on adjuvant chemotherapy have predominantly focused on breast cancer outcome and short-term patient safety. Less attention has been given to non-fatal acute adverse effects and to long-term effects that may potentially compromise rehabilitation.

**Neutropenia**

Neutrophils are the first and main defence against bacteria and fungi, and with a half-life of about seven hours in the blood circulation, they are very susceptible to chemotherapy. Patients with neutropenia are at risk of developing life-threatening infections and, even with adequate treatment, febrile neutropenia carries an overall mortality approaching 5% [161]. The white blood cell count (WBC) only partly reflects neutrophils and is a rather blunt instrument used in early trials to adjust chemotherapy doses according to myelotoxicity. Patients experiencing febrile neutropenia during chemotherapy will often be subject to dose reductions and/or delays, interruptions or early discontinuation of their chemotherapy and thus receive a potentially less effective treatment [162-164]. A working group established by European Society

for Medical Oncology (ESMO) has proposed febrile neutropenia to be defined as: An absolute neutrophil count (ANC) of  $< 0.5 \times 10^9/L$  or  $< 1.0 \times 10^9/L$  predicted to fall below  $0.5 \times 10^9/L$  within 48 hours, with fever or clinical signs of sepsis [165]. In this setting, fever has been defined as a rise of axillary temperature to  $> 38.5^\circ C$  sustained for a minimum of one hour. The risk of febrile neutropenia varies widely by chemotherapy regimen and established risk factors [166, 167]. Several societies have provided guidelines for prophylactic use of antibiotics and granulocyte colony-stimulating factor (G-CSF) [167], and a recent meta-analysis concluded that prophylactic G-CSF reduces overall mortality, while prior systematic reviews and a meta-analysis found there was not enough evidence to allow for the development of guidelines [168-171]. Fewer episodes of neutropenia were reported in the PACS 01 trial (Table 7) from sequential CEF followed by docetaxel (compared with the same duration of CEF) in node-positive breast cancer, and the sequential regimen was introduced in clinical praxis without prophylactic G-CSF [119]. The DBCG later established that 27.5% of patients who, according to nationwide guidelines, received three cycles of epirubicin  $90 \text{ mg/m}^2$  and cyclophosphamide  $600 \text{ mg/m}^2$  followed by three cycles of docetaxel  $100 \text{ mg/m}^2$  in 2007 developed febrile neutropenia and that the frequency was reduced to 10% when the following year G-CSF was given after administration of docetaxel in a cohort treated with the same regimen according to precisely the same guidelines [17]. Similarly, a discrepancy has been observed between the published 5% risk of febrile neutropenia in the US Oncology 9735 trial and a 25-33% risk observed in community practice [172, 173]. Participants in clinical trials are generally younger and have less comorbidity, and side effects may be under-reported if sufficient focus not has been directed to recording adverse events [174].

**Secondary non-breast cancer**

Population-based studies have consistently shown that breast cancer survivors remain at an increased risk, albeit small, of developing a secondary non-breast cancer (SNBC) [175]. The increased risk of SNBC may in part be explained by the same cause as the first cancer. A common hereditary predisposition and inherited disease-causing mutations are associated with an increased risk of SNBC, including ovarian cancer (BRCA1, BRCA2, and RAD51C), gastric cancer (CDH1), thyroid cancer (PTEN) and sarcoma (TP53). Environmental factors contributing to breast cancer may also increase the risk of SNBC as may hormonal and reproductive factors. Part of the SNBC risk will derive from the treatment of the first breast cancer, and the clinical trials programme of the DBCG has facilitated the description of the part of SNBC inflicted separately by chemotherapy, endocrine therapy and radiotherapy. With a median estimated potential follow-up of 25 years, 100 patients (9%) among the 1,146 participants in the DBCG 77B (Table 1) had experienced SNBC. Thirty-one patients were diagnosed with lung cancer (SIR=2.09; 95% CI; 1.36 to 2.83) and the risk of second primary lung cancer was equally elevated in patients randomised to radiotherapy alone (SIR=2.23) and to radiotherapy plus levamisole (SIR=1.57), cyclophosphamide (SIR=1.93) or CMF (SIR=2.32) [1]. Bladder cancer was reported in five patients treated with cyclophosphamide (SIR=3.17, 95% CI; 0.39 to 5.94), three patients treated with CMF (SIR=1.93, 95% CI 0.00 to 4.12) and none of the patients in the levamisole or radiotherapy alone arms. Acute leukaemia was reported in two patients treated with cyclophosphamide (SIR=6.21, 95% CI; 0.00 to 14.8), two patients treated with CMF (SIR=6.14, 95% CI 0.00 to

14.6), one patient treated with levamisole (SIR=13.3, 95% CI 0.00 to 39.4) and none of the patients in the radiotherapy alone arm. In addition, 56 other second primaries were reported in 17 different sites without any distinctive pattern [1]. In the second DBCG programme (DBCG 82C; Table 1), SNBC was not increased among postmenopausal patients randomised to tamoxifen plus nine cycles of CMF (N=85) at data cut-off compared with patients randomised to tamoxifen alone (N=96; P=0.63; F test) [5]. In the third DBCG programme, methotrexate was substituted by epirubicin, and the 89D trial randomly assigned 1,224 patients to CEF against CMF. At a potential ten-year follow-up, 28 patients (2.3%) had developed SNBC [2]. No significant differences were observed in the occurrence of SNBC according to treatment arm. One patient in each group developed acute myeloid leukaemia (AML) and one patient in the CMF group developed myelodysplastic syndrome (MDS) [2].

In contrast, Fisher and colleagues reported the incidence of leukaemia to be increased in 8,483 women participating in seven NSABP trials [176]. Among the 2,068 patients who were treated with surgery alone, three developed leukaemia within ten years, while five of 646 developed leukaemia following adjuvant radiotherapy without systemic therapy. The incidence increased to 27 of 5,299 (0.5%) following L-phenylalanine as well as seven cases of myeloproloferative syndrome (MDS) [176]. In a subsequent work, Smith et al. demonstrated that the cumulative incidence of AML/MDS increased from 0.21 (95% CI 0.11-0.43) with standard AC to 1.01 (95% CI 0.63-1.62) in patients who received two or four cycles of 2,400 mg/m<sup>2</sup> of cyclophosphamide with granulocyte colony-stimulating factor (G-CSF) [177]. Furthermore, a higher risk of secondary AML/MDS was observed with the addition of radiotherapy (RR=2.38; P=0.006).

In a joint analysis from four Scandinavian cancer registries, Brown and colleagues identified 23,158 second non-haematological non-breast malignancies in 376,825 one-year survivors of breast cancer diagnosed between 1943 and 2002 and calculated standard incidence ratios (SIR) [175]. The overall SIR for second cancers was 1.15 (95% CI 1.14-1.17) and small compared with the risk of dying from breast cancer. The largest SIRs were found for women diagnosed with localised breast cancer before the age of 40 years, and at 20 years of follow-up the absolute risk ranged between 0.6% and 10.3% depending on age and stage. While a major proportion of non-haematological SNBC were attributable to radiation therapy or endocrine therapy, a proportion attributable to chemotherapy could not be specified. A recent DBCG study and a meta-analysis confirmed that the risk of non-haematological SNBC is not increased in non-radiation-associated sites [178, 179]. About five out of a thousand new cancers registered in the UK are radiotherapy-related second cancers, and just over half were seen in individuals aged 75 or over [180].

### **Cardiac toxicity**

There has been considerable concern about anthracycline-induced cardiac toxicity. The risk increases exponentially with the cumulative anthracycline dose administered, and important risk factors in addition to dose include previous mediastinal radiotherapy, old age, hypertension and pre-existing coronary artery disease [181-185]. The incidence of congestive heart failure (CHF) is about 5% when giving a cumulative dose of either 400 mg/m<sup>2</sup> of doxorubicin or 920 mg/m<sup>2</sup> of epirubicin [183, 185]. Lower cumulative doses were, however, administered to women with early breast cancer in the early anthracycline trials and have been further reduced following the introduction of taxanes. The inci-

dence of heart failure was similar following CMF and CEF in a registry-based long-term follow-up of women participating in the DBCG trial 89D, but marginally higher plasma-NTproBNP following CEF could indicate a moderate increase in later risk of cardiac events [186]. Instead of reporting symptomatic CHF, the majority of individual adjuvant trials have reported asymptomatic changes by different definitions and methods. With a median follow-up of 11 years after completion of adjuvant chemotherapy, Zambetti and colleagues performed an echocardiogram in 355 patients who were free of relapse [187]. A systolic dysfunction was observed in 8% of patients who received doxorubicin (median cumulative dose 295mg/m<sup>2</sup>) compared with 2% following CMF [187]. Similarly, Ganz and colleagues assessed left ventricular ejection fraction (LVEF) by MUGA scan in participants from the SWOG S8897 at five to eight years after completion of CAF or CMF, and found no significant differences in the proportion who had an LVEF below 50% (5% vs. 7%) [188]. The French Adjuvant Study Group compared assessed cardiac function by echocardiogram at eight years after completion of chemotherapy and found an asymptomatic drop in LVEF in 18 of 85 patients allocated to FEC100 compared with two of 65 patients allocated to FEC50 [189]. A more meaningful insight into the importance of cardiotoxicity may be obtained from the DBCG trial 89D where we analysed death without recurrence among 1,224 participants and found no significant difference in the proportion who died of cardio-vascular causes among patients randomised to CEF (4 of 36) compared with CMF (3 of 31) [2]. In order to reduce the risk of cardiotoxicity, most guidelines in the metastatic setting suggest an upper limit of the cumulative dose of anthracyclines and cardiotoxicity, and cardiotoxicity may further be reduced by using analogues, e.g. epirubicin, liposomal encapsulation, weekly schedules, longer infusion time and a cardioprotector [190, 191]. The cumulative doses used in the adjuvant setting is substantially below the recommended upper limit; and apart from using epirubicin as opposed to doxorubicin the relatively low risk of cardiotoxicity has not been considered sufficient reason to risk testing potential, less cardiotoxic regimens as these regimens may simultaneously be less effective.

Takotsubo syndrome (TTS) or stress-induced cardiomyopathy mimics acute myocardial infarction in the absence of coronary artery disease, and should be considered in patients developing chest pain and in case of electrocardiographic changes or abnormal cardiac biomarkers during chemotherapy in general and fluorouracil in particular [192, 193]. Cardiotoxicity has only been observed after cyclophosphamide, but only when given in high doses and even then with varying degree [194, 195].

### **Ovarian suppression and loss of fertility**

Premature ovarian failure (POF) results from the loss of primordial follicles and an immediate toxic effect of chemotherapy on the granulosa cells of growing follicles has demonstrated that a range of mechanisms and different target cells are probably involved [196, 197]. Depending on age at diagnose, the residual pool of primordial follicles, and in the very young the degree of leukocyte depletion and cumulative doses of chemotherapy, a proportion of pre- and peri-menopausal women will develop transient amenorrhoea or menopause [198, 199]. POF may not only reduce or discontinue fertility, but also leads to menopausal symptoms, sexual dysfunction and bone loss.

#### 4. PROGNOSTIC FACTORS

Prognostic factors may be defined as a single marker or a combination of markers that separates patients following a standard treatment according to clinical outcome [200]. Together with distant metastasis, tumour size and axillary lymph node status are fundamental elements when describing the extent of the disease. These factors are also decisive for treatment selection in patients with newly diagnosed breast cancer and they are simultaneously the most significant prognostic factors [201-203]. Prognostic significance may be related to the method of cancer detection, as may use of sentinel node techniques [204, 205]. Furthermore, the prognostic impact of tumour size may be modified by molecular subtype [206]. Approximately 80% of breast cancers are uniformly classified as invasive ductal carcinomas [207]. The distribution of other invasive histologic types, however, varies considerably over time and between institutions [208, 209]. Rare types including tubular, cribriform, mucinous, and medullary carcinomas are associated with a favourable outcome, while infiltrating lobular carcinomas carry a different biology, but not unambiguously a more favourable prognosis [210, 211]. Histological grade combines scores of tubule formation (glandular differentiation), nuclear pleomorphism and mitotic counting and was standardised for use in the Nottingham Prognostic Index by Elston and Ellis [212, 213].

In the absence of endocrine therapy and after about 5 years, the positive prognostic impact of a positive ER status is altered to a slightly inferior prognosis as compared with patients with ER-negative tumours [214-216]. This suggests that ER is a proliferation marker rather than a marker of inherent metastatic potential. Human epidermal growth factor receptor 2 (HER2, also referred to as ERBB2) was identified in 1985 and is positive (amplified or overexpressed) in about 12% of breast cancers [217-220]. This population-based rate is lower than the rate (15-25%) reported in historical and highly selected studies. HER2 is a strong prognostic factor in the absence of HER2-directed therapies and is strongly predictive of benefit from anti-HER2 therapies [5, 9, 75, 221-223]. Topoisomerases (topo) are essential for DNA topology, and only topo-II is significantly involved in double-stranded passages [224]. DBCG investigators as well as some others have evaluated TOP2A aberration as a predictive marker with the purpose of evaluating a possible incremental benefit from adding an anthracycline to a standard chemotherapy regimen. Conflicting results have emerged regarding the prognostic implications of TOP2A aberrations irrespective of whether TOP2A was evaluated in patients receiving anthracyclines or not [225-230]. Chromosomal instability (CIN) is a hallmark of solid tumours, although the biology behind this mechanism is poorly understood [231]. Aneuploidy is a consequence of CIN, and attempts have been made to create a measure of overall chromosomal imbalance [232]. However, the prognostic impact of CIN is complex as excessive CIN may negatively impact cancer cells [231, 233]. Copy number alterations of centromere 17 (CEP17) have been proposed as one way to quantify CIN in breast cancer as it houses multiple genes involved [234, 235].

The prognostic impact of lymphatic and blood vessel invasion (LBVI), also referred to as lymphovascular invasion, has been evaluated in numerous studies, but only five of these have included more than 1,500 patients, and the study from the DBCG comprises a larger sample size than the other four combined [13, 236-239]. LBVI was associated with a significantly increased risk of a DFS event (HR=2.48; 95% CI 2.32 to 2.66) and mortality (HR=2.74; 95% CI 2.55 to 2.94). When assessed separately in the low and high risk subsets, LBVI remained a highly significant prog-

nostic factor in the high-risk group, but did not have sufficient prognostic influence as a single criterion to move patients from the low to the high-risk group [13]. Invasion of blood vessels may, in theory, precede circulating tumour cells, while invasion of lymphatic vessels may precede lymph node metastasis. The two kinds of vessels are inseparable by H&E staining, and immunostaining may aid a more specific evaluation in future. Lymphocyte infiltration is a hallmark of both medullary breast cancer and BRCA1-associated breast cancer, and these two subtypes have very different prognoses [210, 240]. Lymphocytic infiltration has traditionally been evaluated by H&E staining, but may be subdivided into cytotoxic (CD8+) T cells and T-regulatory (FOXP3+) cells by IHC. Presence of CD8+ T cells in the breast has consistently been associated with a reduction in DFS events and mortality and might be considered as a standard [241, 242]. The occurrence of micrometastatic tumour cells has been known for more than half a century and these cells are referred to as disseminated tumour cells if derived from bone marrow, and as circulating tumour cells (CTC) if derived from peripheral blood [243, 244]. Detection of CTC is associated with minimal residual disease after breast surgery and has recently been associated with DFS and OS in large randomised studies, both when evaluated before and after adjuvant chemotherapy [245, 246]. Discordant ER and HER2 status has been reported when comparing CTC and breast cancer metastasis, which could affect adjuvant therapy if demonstrated in early breast cancer [247]. Small amounts of circulating cell-free DNA likely to originate from cancer cells have been identified in plasma from breast cancer patients and may be associated with RFS and OS [248]. Recent technological advances may allow the use of "liquid biopsies" as a complementary tool for identifying molecular targets and estimating prognosis [249]. Numerous additional potential prognostic markers have been investigated in early breast cancer, but only the combination of uPA and PAI1 has reached the highest possible level of evidence [250-252]. Nevertheless, in a prospective validation study, node-negative patients with a low uPA/PAI1 had a ten-year disease recurrence rate of 12.9% (95% CI 9.1 to 18.1) in the absence of systemic therapy [253]. This may translate into an excess mortality, but it is still uncertain how uPA/PAI1 should be combined with other prognosticators. Furthermore, the requirement of fresh-frozen tumour tissue and use of enzyme-linked immunosorbent assay have further limited its clinical utility. Matrix remodelling is crucial for cancer progression, and expression of matrix metalloproteinase (MMP) 2 and 9 in tumour tissue has been associated with a poor prognosis in breast cancer patients [254, 255]. High TIMP1 protein expression in tumour tissue has been associated with a poor prognosis, but the analytic validity is low, which may be due to lack of standardisation [256-259]. Partly conflicting results have been obtained using tissue mRNA levels and plasma levels [260-264]. Expression of the nuclear antigen Ki67 has been linked to tumour cell proliferation rates and expression of proliferation-associated genes [265]. The vast majority of proliferation-associated genes are cell-cycle regulated, and an expanding number of small non-coding RNAs seems to impact the post-transcriptional regulation of the many identified protein-coding proliferation-associated genes [266-268]. Ki67 has consistently possessed independent prognostic information when evaluated retrospectively in randomised trials [120, 227, 269, 270]. Nevertheless, the clinical utility of Ki67 is low due to its limited reproducibility [271].

### **Combining prognostic factors**

The TNM system is an early example on how prognostic factors can be combined [202]. More sophisticated flowcharts have been developed nationwide, e.g. in Denmark and the Netherlands and internationally. The best known are the St. Gallen criteria [15, 272, 273]. The Nottingham Prognostic Index (NPI) was the first attempt at establishing a prognostic model by integrating tumour size, lymph node status and histological grade into an index, and the NPI has been validated in independent cohorts [213, 274, 275]. Adjuvant Online (AOL) combines a prognostic index based on US SEER registry data with estimates of treatment effect derived from the EBCTCG reports [276]. AOL has similarly been validated in independent cohorts [277-279]. Predict is also both a prognostication and treatment benefit tool that has been validated in independent cohorts and compared with Adjuvant! [280, 281]. The updated version, Predict Plus (P+), includes HER2 status [282]. Prognostic impact of even major prognosticators may be modified by the method of detection, and screen detection appears to confer a survival advantage beyond what can be explained by stage shift [204, 283, 284]. The method of detection has, however, not been taken into account when constructing the existing prognostic indexes, though this appears appropriate. The Registry of the DBCG offers a unique opportunity to examine the impact of patient and tumour characteristics on prognosis in similarly treated and well-characterised patients. Firstly, diagnostic procedures were uniformly performed according to detailed guidelines that were implemented nationwide; secondly, surgical procedures, radiotherapy, and systemic treatments were given according to a standardised algorithm. Furthermore, detailed information on patient and tumour characteristics is reported prospectively by the use of standardised forms and monitored uniformly by the DBCG data centre. Finally, the Danish population is well defined, and standardised mortality rates are available from the Central Population Registry. The clinical utility of favourable patient and tumour characteristics, i.e. the degree to which a flowchart combining favourable characteristics may identify patients who do not need adjuvant systemic therapy, was evaluated in a large cohort study from the DBCG. Within the nationwide and population-based clinical DBCG database, 3,197 systemically untreated patients were identified and contributed 41,167 person-years of follow-up. These patients were node-negative and had a low grade ER- or PR-positive invasive cancer of 20 mm or less [285]. Time at risk was defined as time from surgery until date of death from any cause, emigration or end of follow-up and was obtained through linkage to Statistics Denmark. The standardised mortality ratio (SMR) was calculated as the ratio of observed to the number of expected deaths by applying age- and calendar-year-specific female mortality figures of the general Danish population. In the absence of any systemic therapy, excess mortality was pronounced among young patients aged 35 to 39 years (SMR=5.53; 95% CI 3.11 to 8.95), gradually decreased with increasing age and failed to achieve statistical significance in patients aged 60-64 years (SMR=1.14 95% CI 0.98 to 1.32) and above [285]. Since 1985, young-onset breast cancer has consistently been associated with a poorer prognosis, although young age has variously been defined as 33, 35, 40 or 45 years [286-288]. However, in the recent DBCG cohort study, only patients who were 60 years or older with grade 1 tumours up to 10 mm had no excess mortality compared with the general female population. Although these patients represent only a small subgroup, findings from this large cohort study provided evidence that not all patients with early breast cancer need systemic treatment [285].

In the absence of factors capable of predicting who will benefit from chemotherapy, the decision to recommend chemotherapy can only be supported by prognosis. Young age has a negative prognostic impact in the absence of systemic therapy [288]. Following chemotherapy, patients younger than 40 years at diagnosis with ER-positive and HER2-negative breast cancer still may have a poorer RFS and OS than older patients, even following adjustment for other prognostic factors [289, 290]. Among postmenopausal women with endocrine-responsive breast cancers, a large subgroup may, however, not require chemotherapy on the condition that they receive adequate endocrine therapy and are at a low risk of developing distant metastases. It has clearly been demonstrated that the majority of patients with node-negative and more than a third of those with node-positive breast cancers are cured by local treatment alone [1, 75, 291]. The treatment algorithm enforced by the DBCG from January 1996 through 2004 recommended that high-risk postmenopausal patients with ER-positive breast cancer should receive five years of endocrine therapy without chemotherapy. Node-positivity was a high-risk criterion throughout the period, and a tumour size > 2 cm (from > 5 cm) and malignancy grade II-III were added subsequently. A total of 7,163 patients were recommended endocrine therapy alone according to the DBCG algorithm. Data were available for analyses from 6,259 (87%) patients, and 634 were lost to follow-up. Multivariate Cox regression models were used to identify prognostic factors and broadly identical results were obtained for RFS by categorical and fractional polynomials (MFP) models. Age, tumour size, positive lymph nodes and ER status were included in the latter as continuous factors. Tumour size, nodal status, histological type, grade ER expression level, lymphovascular invasion and loco-regional therapy were associated with prognosis ( $p < 0.0001$ ), while age was not ( $p = 0.36$ ) [13]. Since the cohort was population-based, it was possible to compare the observed number of deaths to the expected number derived from the background population. In a multivariate analysis of SMR, a highly significant association ( $p < 0.0001$ ) was observed with age, nodal status, tumour size, grade, lymphovascular invasion, ER and locoregional therapy, but not with histological type ( $p = 0.56$ ). Broadly similar results were obtained in categorical and MFP regression Cox models, and both models suggested that 75% of postmenopausal patients with an ER-positive breast are affected by excess mortality if chemotherapy is omitted [12]. ER expression levels were significantly associated with prognosis in the MFP model, but not in the categorical model. This was not unexpected as information will be lost when continuous data are transformed into binary variables, and cut-points will falsely imply a different implication of two observations located closely to the either side of the cut-point. LVI similarly had a significant impact in the MFP, but not in the categorical model, and the explanation may be a highly skewed pattern. To allow for identification of the 25% who could safely be spared chemotherapy, a flowchart algorithm was constructed from the identified risk factors, and a prognostic SMR index (PSI) was built using the regression coefficients obtained in the MFP model. Among the 1,665 patients who were classified to be free of excess mortality according to the flowchart, 462 (27%) were classified discordantly by PSI and had a significantly increased SMR [1,38; 95% CI 1.16 to 1.65]. SMR was not increased in patients classified as being free of excess mortality by PSI and discordantly classified by the flowchart. By PSI, less than 1% of patients younger than 54 years of age were allocated to the quartile without excess mortality compared with 74% of patients who were 75 years of age or older. Discontinuation of therapy was associated with a significantly higher risk of recurrence (adjusted

HR 1.45; 95% CI 1.14 to 1.85) [12]. The DBCG concluded that PSI has a good clinical utility exceeding what may be obtained with a flowchart algorithm. Since January 2014, the PSI has prospectively been estimated for all postmenopausal patients with early ER-positive breast cancer; and according to DBCG guidelines, chemotherapy is recommended to patients with a PSI in the 2<sup>nd</sup> to 4<sup>th</sup> quartiles.

#### Molecular profiles

Multigene assays have provided a new approach to breast cancer sub-typing and prognostic assessment. Already in 2000, Perou et al. described the molecular heterogeneity of breast cancer in a way that is still valid, and this classification has recently been confirmed by the Cancer Genome Atlas Network project [292, 293]. Several multigene assays have demonstrated the capacity to stratify patients with early breast cancer according to risk of recurrence, but the EGAPP and also the more recently the IM-PACT Working Group both concluded that the evidence is insufficient regarding the clinical utility of the available genomic tests [294, 295]. Attempts have been made to compare the prognostic ability of multigene assays to flowcharts such as the St. Gallen criteria, but it has only rarely been evaluated what a multigene assay might add to a prognostic index. In the RASTER study, 83% of the patients were classified by AOL as high-risk patients compared with 49% by MammaPrint, and 43% by the rather restrictive Dutch flowchart [296]. As expected, the addition of MammaPrint to P+ (AUC:0.662) resulted in an improved risk prediction as the two tests individually and P+ performed better than AOL and NPI [297]. The combined EPclin score resulting from combining EndoPredict with nodal status and tumour size assigned more than half of the patients to a low-risk group that could potentially be spared chemotherapy [298].

### 5. MODIFYING FACTORS

As knowledge of chemotherapeutic action increases, it has become likely that the effect of individual drugs is largely determined by the cancer (somatic) genome, while the patient's (germline) genome is essential for drug exposure and therefore toxicity. None of the germline variants reported so far have obtained clinical utility, but they could potentially influence decisions regarding use of prophylactic G-CSF [299-301]. The development of chemotherapeutic drugs has largely been performed without consideration of possible modifiers, and attempts to identify modifiers have generally been done after trials were completed.

#### PATIENT CHARACTERISTICS

For a long period of time, chemotherapy appeared to be more effective in younger and premenopausal women than in older women, and the prevailing view in the late 1980s was that the differential benefit apparently obtained by CMF predominantly occurred through ovarian suppression [302]. The DBCG 89B trial was designed to analyse this hypothesis and randomised 762 premenopausal node-positive patients with ER and/or PR-positive tumours to ovarian ablation (OA) by irradiation or nine courses of three-weekly intravenous CMF. After a median follow-up of 8.5 years, no significant difference was observed in DFS (HR 0.99; 95% CI 0.81 to 1.21) or with 10.5 years of follow-up in OS (HR 1.11; 95% CI 0.88 to 1.42) [7]. In an exploratory subset analysis, the treatment effect largely seemed independent of age, nodal status, tumour size, histological type, malignancy grade and PR status. However, in the subset with discordant hormone receptor

status (either ER or PR negative tumours), CMF resulted in a significant reduction of DFS events and mortality (DFS; HR=2.04 95% CI 1.04 to 4.00; and OS; HR=2.33 95% CI 1.12 to 4.85). Several trials support that ovarian ablation or suppression either alone or in combination with tamoxifen improve outcome similarly to what is achieved with CMF- and anthracycline-based chemotherapy [303]. Ten years of tamoxifen is now a standard component of adjuvant treatment in premenopausal patients with ER-positive breast cancer, but ovarian suppression versus chemotherapy has not been assessed with tamoxifen in both arms in any trial. Attempts to determine the potential benefits of adding chemotherapy to the combination of tamoxifen and ovarian suppression has failed due to poor accrual [304, 305]. Thus, the benefits obtained by chemotherapy in premenopausal breast cancer patients appear to some extent to be mediated by ovarian suppression, although it is uncertain to what extent this applies in the presence of tamoxifen.

Only few participants aged 70 years or older have been enrolled in randomised trials assessing the benefits of adjuvant chemotherapy, and results from population-based observational studies may complement our knowledge in this area. Three studies have used data drawn from the Surveillance, Epidemiology, and End Results (SEER) Medicare data set. These studies revealed a somewhat different result. Two of these analyses demonstrated that adjuvant chemotherapy was associated with a decreased all-cause mortality with the greatest benefit in older women with lymph node-positive and ER-negative breast cancers [306, 307]. In contrast, the third study reported that the benefit from adjuvant chemotherapy was restricted to those younger than 70 years of age [308]. Considerable uncertainty remains as to whether age at diagnosis modifies chemosensitivity, and whether age interacts with other possible modifying factors. While a modifying effect of age and menopausal status is unresolved for CMF-like regimens, the additional benefits have been independent of age for anthracyclins [2, 82, 95]. In trials demonstrating an additional benefit of adding a taxane or in part replacing anthracycline-containing adjuvant chemotherapy with a taxane, these effects have similarly been independent of age [111, 112, 119, 120, 129, 130].

#### TUMOUR CHARACTERISTICS

In the DBCG trial 82C, histological type other than invasive ductal and lobular carcinoma was associated with a differential benefit of adding CMF to one year of tamoxifen (HR for DFS 0.35, 95% CI 0.18 to 0.67;  $P_{interaction}=0.01$ ) [5]. Infiltrating lobular carcinoma is associated with older age at diagnosis, low proliferation and ER expression. Patients with a tumour of lobular histological type are unlikely to achieve a pCR by preoperative chemotherapy, which leads to questioning of the added benefit of chemotherapy in the adjuvant setting [309]. The Netherlands Cancer Registry evaluated the effectiveness of chemotherapy according to histological type in patients aged 50 to 70 years and diagnosed with early breast cancer between 1995 and 2008. Among patients with invasive ductal carcinoma, 11,438 (58%) received endocrine therapy alone and 8,171 received combined chemo-endocrine therapy; and, similarly, 2,170 patients with invasive lobular carcinoma received endocrine therapy alone (59%), while 1,515 received chemo-endocrine therapy [310]. Chemo-endocrine therapy significantly reduced mortality compared with endocrine therapy alone in patients with ductal carcinomas (HR=0.71; 95% CI 0.65 to 0.78), but not in patients with lobular carcinomas (HR=0.90; 95% CI 0.76 to 1.06); and although a heterogeneity was apparent ( $P_{interaction}=0.014$ ), it does not rule out a small benefit in lobular carcinoma.



mas [310]. Tubular, mucinous and medullary carcinomas are rare subtypes that, depending on the definitions used, constitute about 5% of breast cancers and are associated with a favourable prognosis [210, 311, 312]. The potential benefit from chemotherapy has not been clarified in patients with these rare histological subtypes, and in particular it is controversial whether these patients should be recommended chemotherapy for ER- and HER2-negative tumours.

Histological grade combines scores of tubule formation (glandular differentiation), nuclear pleomorphism, and mitotic counting and was standardised for its use in the Nottingham Prognostic Index by Elston and Ellis [212, 213]. Nuclear pleomorphism expresses the variation in cellular DNA content, and mitotic count is a direct measure of proliferation [313]. A close correlation between histological grade and chromosomal instability has been demonstrated in breast cancers by FISH and flow cytometry [314-317]. It has been proposed that high chromosomal instability might predict sensitivity to alkylating agents and anthracyclines, while low instability might predict benefit from taxanes [318]. However, individual clinical trials have not been able to demonstrate such a relationship.

Most chemotherapeutic agents specifically target proliferation, e.g. ribonucleotide depletion by DNA synthesis inhibitors, stabilisation of microtubules by taxanes and stabilisation of topo-II-DNA complex by anthracyclines. Consequently, it has been hypothesised that Ki67 might potentially be a general marker for combination chemotherapy and in particular for taxanes. Ki67 has been evaluated retrospectively in several trials, and possessed independent prognostic information but was not associated with a benefit from CMF (DBCG 82C and IBCSG VII/IX; Table 1) or an incremental benefit from epirubicin (NEAT/BR9601; Table 4) [5,227,319]. Conflicting results emerged for docetaxel as high Ki67 was associated with a differential benefit from docetaxel in the WSG-AGO trial ( $P_{\text{interaction}}=0.001$ ), but not in the BCIRG 001 and the PACS04 (Table 7-8) [120, 270].

With 12 years of follow-up, the NSABP B20 continued to demonstrate that CMF adds benefit to tamoxifen in node-negative and ER-positive breast cancer (Table 1) [320]. When patients were grouped according to low- (10-49 fmol/mg) against high-content ER (>49 fmol/mg), a benefit was observed in both groups, but there was a trend towards a greater benefit in women with low ER content. The IBCSG collaborators randomised 349 node-positive breast cancer patients younger than 65 years in the Ludwig III trial to one year of tamoxifen plus prednisone (pTAM), pTAM plus twelve cycles of classical CMF, or no systemic therapy [70]. DFS survival was significantly longer in patients allocated to pTAM+CMF than in patients receiving pTAM ( $P=0.02$ ) or no systemic therapy ( $P<0.0001$ ). Among patients with ER-positive cancers, there was no significant difference in DFS between chemoendocrine- and endocrine-treated groups. In the Ludwig III trial, this comparison was based on 67 patients, but this was further explored by the IBCSG in trial IX (Table 1); and at a median 13-year follow-up, IBCSG IX confirmed no significant improvement in DFS from three cycles of classic CMF in postmenopausal patients with ER-positive cancers (Table 1) [321]. In the IBCSG IX, tumour tissue for central re-evaluation of ER was available from 1,339 (80%) patients, and the effect of CMF on DFS but not OS was significantly different according to central ER in a test of interaction ( $P=0.002$ ) [322]. In the IBCSG IX, the impact of ER expression on DFS was further explored in a Subpopulation Treatment Effect Pattern Plot (STEPP), and the 17% ER-absent patients formed the first subpopulation and only patients with absent or very low expression of ER seem to benefit from CMF. In

contrast to the NSABP B20, no difference in clinical benefit was observed in the IBCSG IX between patients with low and high ER expression [322].

Tumour tissue was available for central assessment of ER from 969 (67%) of the 1,445 patients randomised in the DBCG trial 82C, and although tissue was more often available from patients randomised to tamoxifen plus CMF, the assessable cohort had a similar potential follow-up for OS, a similar DFS and a similar relative treatment effect as the total study cohort [5]. No evidence of heterogeneity in treatment efficacy was observed in the two treatment groups according to ER. In the DBCG 82C, the pattern of treatment effect was also explored by STEPP analyses according to ER expression in terms of ten-year DFS. Results from the DBCG 82C confirmed that patients with low (<10%) ER expression appear to benefit from chemotherapy, but in contrast to the NSABP B20 and the IBCSG IX, it showed that patients with a very high ER expression ( $\geq 90\%$ ) may benefit also from chemotherapy [5]. Three distinct groups of patients were eligible for participation in the DBCG 89D. Group A: premenopausal node-negative patients with a ductal carcinoma  $\leq 5$  cm and malignancy of grade II or III; Group B: premenopausal patients with ER-negative and PgR-negative or unknown tumours; Group C: postmenopausal patients with hormone receptor-negative tumours, and either node-positive or a tumour > 5 cm. No significant differences were observed when comparing the adjusted per protocol treatment effects in the three strata (A, B, and C) with respect to DFS ( $P_{\text{interaction}}=0.93$ ) or OS ( $P_{\text{interaction}}=0.16$ ). Patients with ER-positive and -negative cancers appear to derive a similar benefit in DFS and OS from substituting methotrexate with epirubicin [2]. No differential benefit was observed among 2,280 participants in the NEAT trial according to ER status regarding RFS ( $P_{\text{interaction}}=0.39$ ) or OS ( $P_{\text{interaction}}=0.31$ ) from sequential epirubicin-CMF as compared with CMF [95]. Unfortunately, no attempt was made to report a differential benefit according ER status in the INT 0102, a 4-arm trial evaluating CAF against CMF as well as tamoxifen against control [88].

Patients with tumours classified as PR-negative in the Intergroup trial E 2197 experienced a more favourable outcome following doxorubicin and docetaxel than following AC ( $P_{\text{interaction}} 0.02$  for ER-negative and  $<0.01$  for ER-positive) [131]. Patients with ER-positive and -negative cancers seemed to derive a similar benefit from docetaxel in the UK TACT, in the BCIRG001 and in the PACS01 trials, irrespective of whether the latter two were analysed separately or jointly [119, 121, 129, 323]. The CALGB 9344 showed incremental benefit from adding paclitaxel to AC, and an unplanned exploratory analysis suggested that a particular benefit from paclitaxel was obtained by patients with ER-negative or unknown status (HR 0.72), but a formal test of interaction was not reported (Table 6) [111]. In contrast, no evidence of a differential benefit of paclitaxel according to hormone receptor status was observed in the NSABP B-28 ( $P_{\text{interaction}} 0.30$  for DFS and 0.82 for OS) [112]. A pooled analysis of three consecutive CALGB trials (8541, 9344, and 9741) analysed the possible predictive value of ER status for chemotherapy benefit and reported an overall greater benefit in patients with ER-negative tumours, but did not report results according to individual chemotherapies [324]. A differential outcome according to HER2 status was suggested in standard versus lower dose of CAF in the CALGB 8869, but the assay used for HER2 expression was not standardised and different cut-offs were used [325]. Previous studies had suggested that patients with HER2-positive tumours did not benefit from adjuvant CMF, and together these studies formed a hypothesis regarding anthracyclin benefit in HER2-positive patients [326, 327].

A significant interaction between HER2 status and benefit from an anthracycline (see Table 2-4) was shown in the NSABP B11 and the MA.5, while the NSABP B15, Brussels, the DBCG 89D, the MA.5 and the NEAT-BR9601 at best showed a trend and only if TOP2A was unaccounted for [9, 95, 328-332]. Four of the taxane trials listed in Table 6 and 7 have published results by HER2 status. In the CALGB 9344, tumour tissue was collected retrospectively from 1,322 of the 3,121 participants and HER2 positivity was associated with a differential benefit of adding paclitaxel to AC ( $P_{\text{interaction}}=0.01$  for DFS and 0.01 for OS) independently of ER status [333]. In contrast, no evidence of differential benefit from docetaxel according to HER2 status was demonstrated in the PACS 01, the UK TACT or the WSG-AGO trials [118-121, 334].

Participants in the DBCG 89D trial were randomised to CEF against CMF (see Table 2), and HER2 and TOP2A were assessed retrospectively in 767 of the 980 Danish patients [9, 225]. In 89D, an incremental benefit from substituting methotrexate with epirubicin was observed for RFS among patients with TOP2A CNV ( $P_{\text{interaction}}=0.02$ ). When analysed separately, patients with TOP2A amplification obtained a 61% relative reduction in the risk of an RFS event ( $P<0.001$ ) from CEF and a 52% reduction in mortality ( $P=0.01$ ). Among patients with TOP2A deletions, a non-significant trend was observed for RFS and OS. The Canadian MA.5 trial (Table 2) had a similar design and reported a significant interaction between TOP2A status and treatment for OS ( $P_{\text{interaction}}=0.02$ ) but not for RFS ( $P_{\text{interaction}}=0.09$ ) [226]. Following optimisation of the cut-point for topo2 protein in an exploratory analysis of the MA.5, the investigators found no advantage of using topo-2 expression in favour of TOP2A copy number [335]. The expression of topo-2 reflects cell cycle in malignant as wells and normal human cells, and is restricted to the S and G2/M phase in the latter [336]. Hence, topo2 expression is linked to cell proliferation and Ki67 levels [337]. NEAT and BR9601 (Table 4) were analysed in conjunction and reported a significant interaction between TOP2A status and treatment for RFS ( $P_{\text{interaction}}=0.008$ ), but not for OS ( $P_{\text{interaction}}=0.69$ ) [95]. The BCIRG 006 randomised patients with HER2-positive breast cancer to paclitaxel, doxorubicin and cyclophosphamide (PAC), PAC plus trastuzumab, or docetaxel, carboplatin (DC) and trastuzumab. Among patients randomised to the PAC arm, those with amplified TOP2A had a lower mortality than patients with TOP2A-normal tumours. In contrast, patients randomised to PAC plus trastuzumab had a similar outcome irrespective of TOP2A status [228]. Taken together, the BCIRG 006 trial suggests that patients with joint amplification of HER2 and TOP2A could be spared either trastuzumab or doxorubicin.

A significant interaction between CEP17 alteration and treatment was observed independently from TOP2A/CEN17 ratio in the NEAT/BR9601 for RFS ( $P=0.005$ ) and OS ( $P=0.02$ ), but not in the MA.5 ( $P=0.09$  for RFS and 0.13 for OS) or in the DBCG 89D ( $P=0.39$  for RFS and 0.67 for OS) [95, 338, 339]. Cross trial comparisons are, however, difficult due to lack of standardisation [338, 340]. High ploidy levels may reflect faulty DNA repair, and these cancer cells may become immunogenic following accumulation of calreticulin of the cell surface, leading to a TOP2A-independent anthracycline sensitivity [341, 342]. In a prospectively planned individual-patient pooled analysis of participants from the DBCG 89D, the MA.5, NEAT, the BR9601 and a small Belgian trial, a heterogeneity of treatment effect of epirubicin according to CEP17 or TOP2A aberration versus normal was highly significant for RFS ( $P_{\text{interaction}}=0.001$ ) and OS ( $P_{\text{interaction}}=0.001$ ) [10]. Preclinically, intrinsic TIMP1 has been associated with chemosensitivity and response to anthracyclins [343]. The DBCG collaborators in trial 89D compared nine cycles of CEF with CMF (Table 2), and tumour

tissue was available from 623 participants for retrospective analyses. Among the available tumours, 154 lacked TIMP1 immunoreactivity, 188 were HER2-positive and 139 had a copy number change of TOP2A. As previously reported, a significant interaction was shown between presence of TOP2A aberrations and benefit from CEF, while no significant interaction was shown between HER2 or TIMP1 status and benefit from CEF [9, 344]. By combining lack of TIMP1 expression and/or presence of TOP2A aberration, 269 (43%) were classified as anthracycline-responsive, and a highly statistically significant interaction was shown with incremental benefit from CEF ( $P_{\text{interaction}}<0.0001$  for DFS and 0.004 for OS) [11]. For the combination of TIMP1 and HER2, the result was less clear. The heterogeneity in treatment effect of CEF versus CMF according to the combination of TOP2A and TIMP1 was validated in the MA.5 trial (Table 2) for OS, but not for RFS which was the primary endpoint [345].

### Profiles

A high Oncotype Dx score was associated with benefit from adding CMF to 5 years of tamoxifen in 651 patients with node-negative and ER-positive breast cancer who participated in the NSABP B20 [346]. Similarly, a high Oncotype Dx score was associated with an additional benefit from CAF followed by tamoxifen as compared with tamoxifen alone in 367 patients from the SWOG 8814 [347]. The WSG-AGO for DFS ( $P_{\text{interaction}}=0.01$ ) found an association between the luminal-B like IHC subtype and benefit from sequential docetaxel [120].

Several adjuvant chemotherapy trials have used molecular selection markers, and the MINDACT, the TAILORx, the RxPonder and the ADAPT trials used these markers to select an intermediate or low-risk group for randomisation to chemotherapy against control. The READ trial randomised TOP2A-normal patients to an anthracycline-containing regimen versus a regimen with no anthracycline, and German investigators used uPA/PAI1 to select a high-risk but node-negative group for randomisation to a docetaxel-containing regimen versus a regimen with no docetaxel [348].

Two large trials, the MINDACT (microarray in node negative disease may avoid chemotherapy) and the TAILORx, are currently investigating the benefit of chemotherapy in patients with a low or intermediate risk of relapse by genetic profile [349-350]. In 2008, the MINDACT trial was amended and thereafter included patients with less than four positive nodes. The TAILORx trial has enrolled more than 11,000 patients with ER-positive, HER2-negative and node-negative breast cancer. Patients with a recurrence score (RS) of 11 through 25 are offered randomisation, while patients with a score  $< 11$  are recommended endocrine therapy and those with a score  $> 25$  are recommended chemotherapy followed by endocrine therapy [350]. The RxPONDER or SWOG S1007 trial plans to screen 9,400 ER-positive and HER2-negative patients in order to randomise 4,000 patients with a RS  $< 25$  and 1-3 positive nodes to endocrine therapy versus chemotherapy followed by endocrine therapy [351]. The nCounter version of the PAM50 ROR is prospectively compared with Oncotype DX in the RxPONDER trial.

### CONCURRENT SYSTEMIC ANTI-NEOPLASTIC THERAPY

Conflicting results emerged from preclinical research in the early 1980s as Osborne reported that chemotherapy was antagonised by the G1-S blockade induced by tamoxifen, while others observed a synergism between tamoxifen and fluorouracil or anthracyclins [352-354]. This was analysed in the three-armed

SWOG 8814 trial were the addition of CAF to tamoxifen resulted in a significant improvement of DFS and OS, and suggested a differential benefit in DFS from CAF followed by tamoxifen as compared with CAF given concurrently with tamoxifen (HR=0,84; 95% CI 0.70 to 1.01 and P=0.055) [77]. Other randomised trials have addressed this important question, but no differential benefit was observed in DFS or OS when tamoxifen and chemotherapy were administered sequentially as compared to concurrently to 474 postmenopausal node-positive breast cancer patients in the GEICAM trial 9401 [80], to 1096 hormone receptor-positive patients in a joint analysis of the Italian GONO-MIG 1 and 5 trials [81], or to 225 node-positive patients in a small Genoese trial [79]. In the CALGB 9344 (N=3121), tamoxifen was recommended for ER-positive tumours following completion of chemotherapy, while concurrent tamoxifen was recommended in NSABP B-28 (N=3,060) to patients aged 50 years or older and to younger patients with ER-positive tumours [111, 112]. In both trials, patients with ER-positive tumours appeared to have less benefit from the addition of paclitaxel to AC even without definitive evidence of interaction between tamoxifen and chemotherapy. Although the benefits of adjuvant trastuzumab are only justified when combined with chemotherapy, questions remain as to whether a specific chemotherapy should be given preference and as to sequence. The BCIRG006 compared adjuvant AC followed by docetaxel to the same chemotherapy with trastuzumab and to docetaxel plus carboplatin with trastuzumab. No significant difference was shown in DFS or OS between the two chemotherapy regimens, and the BCIRG006 was not powered to detect equivalence [355]. The N9831 is the only trial assessing concurrent against sequential administration of trastuzumab and chemotherapy, and it pointed towards a better outcome from concomitant administration although it was unable to provide a definitive answer [356].

## SUMMARY

With long-term follow-up, the DBCG 77B trial demonstrates that oral single-agent cyclophosphamide significantly reduces the risk of recurrence and mortality as compared with no systemic therapy in pre-menopausal patients with high-risk early breast cancer. DBCG 77B is the only randomised trial assessing single-agent cyclophosphamide; and a second comparison suggests that its benefits are comparable to what may be achieved by classic CMF [1]. The lack of benefits from adding methotrexate and fluorouracil to cyclophosphamide paved the way for combining cyclophosphamide with anthracyclines and later taxanes. DBCG 89D showed an incremental benefit in DFS and OS from substituting methotrexate with epirubicin [2]. The advantage of anthracycline-containing 3-drug combinations over CMF was confirmed by others and in the individual-patient EBCTCG meta-analysis, while standard AC or EC for four cycles not was superior to classic CMF [3]. A further reduction in breast cancer mortality appeared in the EBCTCG meta-analysis from the addition of a taxane to a standard AC, while the substitution of cycles or drugs with a taxane was not associated with a reduction in mortality [3].

No apparent benefit was observed in an early analysis of the DBCG 82C evaluating the addition of CMF to tamoxifen in postmenopausal high-risk breast cancer patients [4]. Apart from menopausal status, the two trials had identical selection criteria, and the differences in outcome warranted a long-term follow-up of the 82C trial. After 10 years of follow-up, CMF in the DBCG 82C was associated with a significant improvement in DFS; but even

with 24 years of follow-up, mortality was not significantly improved [5]. The diversity in outcome from the 77C and the 82B trials triggered further studies. The 77B trial used classic CMF with oral cyclophosphamide, while a four-weekly intravenous CMF regimen was used in the 82B and C trials, and a three-weekly CMF regimen was used in the succeeding 89B and D trials. The outcome following these CMF regimens has not been compared within the context of a randomised trial. Shifting from the 77B's classic CMF regimen to the 82B four-weekly i.v. regimen or the 89B three-weekly i.v. regimen was associated with a 30% increased risk of a DFS event in a multivariate analysis of a population-based cohort study [6]. Furthermore, the four-weekly regimen used in 82B was associated with a 40% increase in mortality. The strengths of the design include identical selection criteria, uniform and prospective registration of treatment, tumour and patient characteristics. Caution is still required due to the non-experimental design of the comparison. Another finding was a substantial difference in the risk of amenorrhoea; and while 15% of patients aged 40 or younger in 77B had regular menses throughout chemotherapy, the corresponding percentage was 37% in 82B and 47% in 89B. The DBCG in collaboration with a Swedish and a Dutch centre participating in the DBCG trial 89B compared CMF with ovarian ablation in premenopausal high-risk breast cancer patients with ER-positive tumours. No significant differences were found in DFS or OS in the preplanned analysis, suggesting that the benefits of CMF may, at least in part, be explained by ovarian suppression in premenopausal patients with ER-positive tumours [7, 8]. However, these results are not clinically useful by themselves as other chemotherapy regimens have been more efficacious, and knowledge is still lacking regarding the benefits from adding ovarian suppression to chemotherapy plus tamoxifen. The results from the DBCG 77B and 82C are in accordance with other large adjuvant trials and the EBCTCG meta-analyses [3].

The benefits obtained with any individual anticancer drug are largely determined by the cancer (somatic) genome; and by being a molecular target of anthracyclines, TOP2A aberrations could obviously be associated with cancer drug benefits. In the DBCG 89D, a significant heterogeneity was observed between a beneficial effect on DFS and OS of epirubicin and the presence of TOP2A, but not the presence of HER2 aberrations [9]. The results obtained in the 89D trial regarding TOP2A have been reproduced by others, but not consistently. However, a recent individual-patient pooled analysis of five adjuvant trials demonstrated that patients with either TOP2A or centromere 17 aberrations, but not with HER2 amplification, benefit from anthracycline-containing adjuvant chemotherapy [10]. Anthracyclines have additional distinct biological mechanisms; and results from the DBCG 89D suggested that tumours with normal TOP2A were only non-responsive to anthracyclines if they were TIMP1 immunoreactive [11]. The DBCG READ trial (N=2,015) prospectively included patients without TOP2A-aberrated breast cancers, and its results are awaited for prospective confirmation of the results from the DBCG 89D and the individual-patient pooled analysis. Adjuvant chemotherapy substantially reduces the risk of recurrence and mortality of breast cancer, but is also associated with significant toxicity [3]. However, according to a large cohort study from DBCG, chemotherapy can safely be withheld in one fourth of postmenopausal patients who will be without excess mortality following sufficient adjuvant endocrine therapy for ER positive breast cancer. A prognostic standard mortality rate index (PSI) was constructed using regression coefficients obtained in a multivariate fractional polynomials model, and most accurately identi-

fied those who could be spared chemotherapy. In addition to age, tumour size, nodal status, histological type and malignancy grade, the PSI also includes ER level addressed as a continuous variable in the MFP model [12, 13]. In the MFP model, absence of LVI was sufficient to counteract the impact of other risk factors, while that could not be achieved with a categorical multivariate model in a prior study. An evaluation of whether the addition of results from a molecular assay may improve the clinical utility of the PSI is ongoing, but when used alone evidence from such assays has been insufficient.

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