Fluorouracil and dose-dense chemotherapy in adjuvant treatment of patients with early-stage breast cancer: an open-label, 2 × 2 factorial, randomised phase 3 trial

Lucia Del Mastro, Sabino De Placido*, Paolo Bruzzi, Michele De Laurentiis, Corrado Boni, Giovanna Cavazzini, Antonio Durando, Anna Turletti, Cecilia Nisticò, Enrichetta Valle, Ornella Garrone, Fabio Puglisi, Filippo Montemurro, Sandro Barni, Andrea Ardizzoni, Teresa Gamucci, Giuseppe Colantuoni, Mario Giuliano, Adriano Gravina, Paola Papaldo, Claudia Bighin, Giancarlo Bisagni, Valeria Forestieri, Francesco Cognetti, for the Gruppo Italiano Mammella (GIM) investigators†*

Summary

Background Whether addition of fluorouracil to epirubicin, cyclophosphamide, and paclitaxel (EC-P) is favourable in adjuvant treatment of patients with node-positive breast cancer is controversial, as is the benefit of increased density of dosing. We aimed to address these questions in terms of improvements in disease-free survival.

Methods In this 2×2 factorial, open-label, phase 3 trial, we enrolled patients aged 18–70 years with operable, node positive, early-stage breast cancer from 81 Italian centres. Eligible patients were randomly allocated in a 1:1:1:1 ratio with a centralised, interactive online system to receive either dose-dense chemotherapy (administered intravenously every 2 weeks with pegfilgrastim support) with fluorouracil plus EC-P (FEC-P) or EC-P or to receive standard-interval chemotherapy (administered intravenously every 3 weeks) with FEC-P or EC-P. The primary study endpoint was disease-free survival, assessed with the Kaplan-Meier method in the intention-to-treat population. Our primary comparisons were between dose schedule (every 2 weeks *vs* every 3 weeks) and dose type (FEC-P *vs* EC-P). This study is registered with ClinicalTrials.gov, number NCT00433420.

Findings Between April 24, 2003, and July 3, 2006, we recruited 2091 patients. 88 patients were enrolled in centres that only provided standard-intensity dosing. After a median follow-up of 7·0 years (interquartile range [IQR] 4·5–6·3), 140 (26%) of 545 patients given EC-P every 3 weeks, 157 (29%) of 544 patients given FEC-P every 3 weeks, 111 (22%) of 502 patients given EC-P every 2 weeks, and 113 (23%) of 500 patients given FEC-P every 2 weeks had a disease-free survival event. For the dose-density comparison, disease-free survival at 5 years was 81% (95% CI 79–84) in patients treated every 2 weeks and 76% (74–79) in patients treated every 3 weeks (HR 0·77, 95% CI 0·65–0·92; p=0·004); overall survival rates at 5 years were 94% (93–96) and 89% (87–91; HR 0·65, 0·51–0·84; p=0·001) and for the chemotherapy-type comparison, disease-free survival at 5 years was 78% (75–81) in the FEC-P groups and 79% (76–82) in the EC-P groups (HR 1·06, 0·89–1·25; p=0·561); overall survival rates at 5 years were 91% (89–93) and 92% (90–94; 1·16, 0·91–1·46; p=0·234). Compared with 3 week dosing, chemotherapy every 2 weeks was associated with increased rate of grade 3–4 of anaemia (14 [1·4%] of 988 patients *vs* two [0·2%] of 984 patients; p=0·002); transaminitis (19 [1·9%] *vs* four [0·4%]; p=0·001), and myalgias (31 [3·1%] *vs* 16 [1·6%]; p=0·019), and decreased rates of grade 3–4 neutropenia (147 [14·9%] *vs* 433 [44·0%]; p<0·0001). Addition of fluorouracil led to increased rates of grade 3–4 neutropenia (354 [34·5%] of 1025 patients on FEC-P *vs* 250 [24·2%] of 1032 patients on EC-P; p<0·0001), fever (nine [0·9%] *vs* two [0·2%]), nausea (47 [4·6%] *vs* 28 [2·7%]), and vomiting (32 [3·1%] *vs* 15 [1·4%]).

Interpretation In patients with node-positive early breast cancer, dose-dense adjuvant chemotherapy improved disease-free survival compared with standard interval chemotherapy. Addition of fluorouracil to a sequential EC-P regimen was not associated with an improved disease-free survival outcome.

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Introduction

Chemotherapy regimens that combine anthracyclines and taxanes improve the outcome of patients with early-stage breast cancer.[1] The most widely used anthracycline-based regimens in sequential combinations with taxanes are doxorubicin and cyclophosphamide (AC) or epirubicin and cyclophosphamide (EC) and fluorouracil, doxorubicin, and cyclophosphamide (FAC) or fluorouracil, epirubicin, and cyclophosphamide (FEC). The contribution of fluorouracil to the anthracycline-cyclophospamide regimens (AC or EC) has not been defined.

Various randomised trials[2–5] attempted to assess the role of dose-dense chemotherapy in patients with early-stage breast cancer. Most of these trials had important differences between the dose-dense and control groups in terms of number of cycles, dose size, type of drug, and total dose, thus rendering interpretation of which variable contributed to the observed results difficult. The few randomised trials with an adequate study design,[6–8]

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*Contributed equally

†Members listed in the appendix

Department of Medical Oncology (L Del Mastro MD, C Bighin MD) **and Epidemiology Unit** (P Bruzzi MD), **IRCCS AOU San Martino–IST, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy; Medical Oncology, Azienda Ospedaliera Universitaria Federico II, Napoli, Italy** (Prof S De Placido MD, M Giuliano MD, V Forestieri MD); **Medical Oncology, Istituto Nazionale Tumori–IRCCS Fondazione Pascale, Napoli, Italy** (M De Laurentiis MD, A Gravina MD); **Medical Oncology, IRCCS Arcispedale S Maria Nuova, Reggio Emilia, Italy** (C Boni MD, G Bisagni MD); **Medical Oncology, Azienda Ospedaliera Carlo Poma, Mantova, Italy** (G Cavazzini MD); **Breast Unit, Azienda Ospedaliera Universitaria Città della Saute e delle Scienze, Torino, Italy** (A Durando MD); **Medical Oncology, ASLTO1, Torino, Italy** (A Turletti MD); **Department of Medical Oncology, Istituto Regina Elena per lo Studio e la Cura dei Tumori, Roma, Italy** (C Nisticò MD, P Papaldo MD, F Cognetti MD); **Medical Oncology, ASL8-Ospedale Oncologico, A Businco, Cagliari, Italy** (E Valle MD); **Department of Medical Oncology, Ospedale di Insegnamento S Croce e Carle, Cuneo, Italy** (O Garrone MD); **Department of Oncology, Azienda Ospedaliera di Udine, Udine, Italy**

(F Puglisi MD); **Investigative Clinical Oncology, Fondazione del Piemonte per l'Oncologia/ Candiolo Cancer Center (IRCCS), Candiolo, Torino, Italy** (F Montemurro MD); **Medical Oncology, Azienda Ospedaliera, Caravaggio, Treviglio, Bergamo, Italy** (S Barni MD); **Medical Oncology, Azienda Ospedaliera Universitaria, Parma, Italy** (A Ardizzoni MD); **Medical Oncology, ASL Frosinone, Ospedale SS, Trinità, Sora, Frosinone, Italy** (T Gamucci MD); **and Medical Oncology, Azienda Ospedaliera S Giuseppe Moscati, Avellino, Italy** (G Colantuoni MD)

Correspondence to: Dr Lucia Del Mastro, Department of Oncology, IRCCS AOU San Martino–IST, Istituto Nazionale per la Ricerca sul Cancro, 16132 Genova, Italy **lucia.delmastro@hsanmartino.it**

See Online for appendix

that is, trials that assessed the same drugs and doses in the dose-dense and control groups, reported conflicting results. Therefore, the role of dose-dense adjuvant chemotherapy in patients with early-stage breast cancer is still controversial.[9,10] We aimed to establish whether an FEC followed by paclitaxel (FEC-P) regimen would improve disease-free survival compared with the same regimen without fluorouracil (EC-P) and whether the dose-dense schedule would improve disease-free survival compared with a standard-interval schedule.

Methods

Study design and patients

This study was a multicentre, open-label, randomised phase 3 trial, with a 2×2 factorial design aiming to address both the role of the addition of fluorouracil to the chemotherapy with epirubicin, cyclophosphamide, and paclitaxel and the role of the dose-dense schedule in the adjuvant treatment of patients with node-positive breast cancer. This study was done in 81 Italian centres by the Gruppo Italiano Mammella (GIM). Women were eligible if they met the following criteria: histologically proven unilateral operable invasive breast cancer confined to the breast and ipsilateral axilla, primary surgery with lumpectomy or total mastectomy plus axillary nodal dissection, histological evidence of tumour in at least one axillary lymph node, age 18 to 70 years, Eastern Cooperative Oncology Group performance status of 1 or lower, normal organ and bone marrow functions, and use of adequate contraception methods for potentially fertile women. Patients had to be randomly assigned within 5 weeks of the date of their last surgery. Oestrogen and progesterone receptor (ER/PR) positive tumours were defined by a finding of at least 10% of positive cells by immunohistochemical analysis. HER2 positive tumours were defined by a finding of at least 10% of tumour cells with HER2 protein expression assessed by an immunohistochemistry assay or by a positivity of an in-situ hybridisation assay.

The study was coordinated by GIM group, who were responsible for the study design, randomisation, collection and management of data, medical review, data analysis, and reporting. The full study protocol is available online. The study was approved by ethics committees of all participating institutions. Written informed consent was obtained from all patients before study entry.

For the **protocol** see http://www.oncotech.org/gim2

Randomisation and masking

Eligible patients were randomly allocated in a 1:1:1:1 ratio to one of the four study groups. In five centres, which refused the randomisation to the dose-dense treatment group, allocations were only to two groups in a 1:1 ratio (standard interval EC-P and standard interval FEC-P). The four study groups were the following (figure 1): four cycles of standard-interval intravenous EC (epirubicin 90 mg/m², cyclophosphamide 600 mg/m², on day 1, every 3 weeks [standard-interval chemotherapy]) followed by four cycles of intravenous paclitaxel 175 mg/m² on day 1, every 3 weeks (q3EC-P group); four cycles of standard-interval intravenous FEC (fluorouracil 600 mg/m², epirubicin 90 mg/m², cyclophosphamide 600 mg/m², on day 1, every 3 weeks) followed by four cycles of intravenous paclitaxel 175 mg/m² on day 1, every 3 weeks, (q3FEC-P group); dose-dense EC-P regimen, with the same doses and drugs as the q3EC-P group, but administered every 2 weeks (dose-dense chemotherapy; q2EC-P group); and the dose-dense FEC-P regimen, with the same doses and drugs as the q3FEC-P group, but given every 2 weeks (q2FEC-P). Randomisation was done by a centralised, interactive internet-based system that after a summary check of patient's eligibility, generated the random allocation. The only stratification factor was centre: within each centre, permuted blocks of size 12 in random sequence were used (block size was four in the five centres that randomised only to FEC-P *vs* EC-P). We used an open-label design because the procedures needed to mask the differences between the treatment groups in the timing of treatment administration (every 2 *vs* every 3 weeks) were not deemed acceptable from an ethical viewpoint.

Procedures

Patients enrolled in the q2 groups receiving treatment every 2 weeks also received subcutaneous pegfilgrastim (6 mg) 24 h after chemotherapy. Because of the occurrence of early leucocytosis (white blood cells in the blood >50 cells per mL), an amendment in March, 2004, needed the provision of pegfilgrastim 72 h after chemotherapy. Chemotherapy dose reductions and delays for clinically significant grade 3 or 4 haematological and non-haematological toxic effects were done according to protocol-defined criteria. After completion of chemotherapy, patients with hormone-receptor-positive tumours received endocrine therapy. After the approval of adjuvant trastuzumab for HER2 positive early breast cancer, an amendment, in April, 2006, needed trastuzumab treatment for 1 year after the completion of chemotherapy for all patients with HER2 positive tumours. Radiation therapy after completion of chemotherapy was mandatory for patients who had a lumpectomy. For patients who had a mastectomy, radiation therapy was done according to each participating institution guidelines.

Outcomes

The two primary comparisons were between FEC-P and EC-P, and between dose-dense and standard-interval chemotherapy. The primary study endpoint was disease-free survival; secondary endpoints included overall survival and safety.

Disease-free survival was computed from the date of randomisation to the date of local recurrence, distant metastases, contralateral or ipsilateral breast tumour

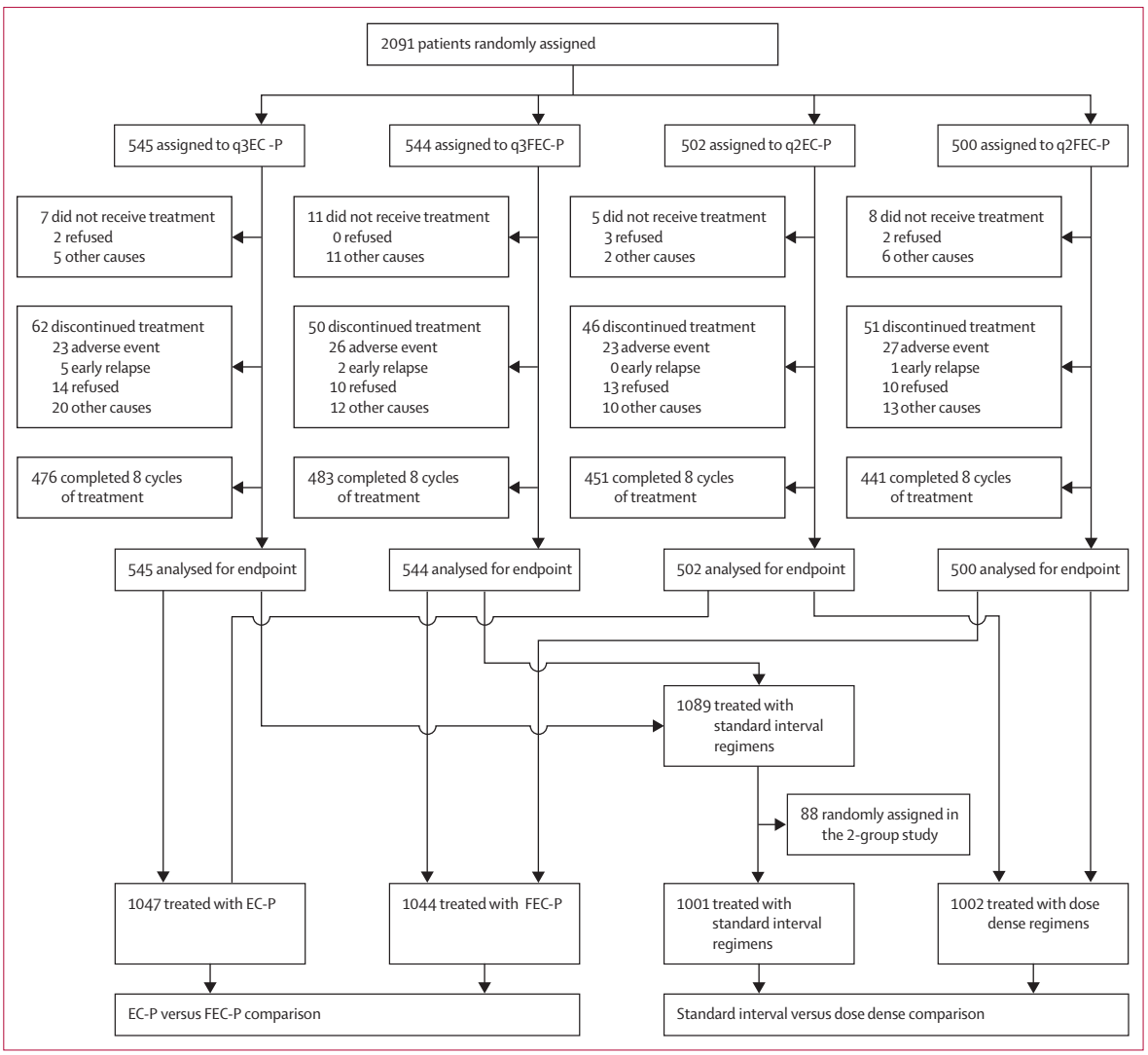

Figure 1: **Trial profile**
q3=standard interval chemotherapy. q2=dose-dense chemotherapy. EC-P= epirubicin, and cyclophosphamide, followed by paclitaxel. FEC-P=fluorouracil, epirubicin, and cyclophosphamide, followed by paclitaxel.

(excluding ductal carcinoma in situ), second primary malignancy, death from any cause, and loss to follow-up or end of study, whichever came first. Disease-free survival times of patients without a disease-free survival event when lost to follow-up or at the time of study closure were censored on the date of the last contact. The definition of disease-free survival corresponds to the Invasive Disease Free Survival defined by Hudis and colleagues.[11] Similarly, overall survival was computed from the day of randomisation to the date of death from any cause, loss to follow-up, or the end of study.

Adverse events were assessed clinically and by haematological and biochemical measurements throughout chemotherapy. Adverse events were graded according to the National Cancer Institute common toxicity criteria version 2.0.[12]

Statistical analysis

Our power calculations, based on previous data,[13] assumed a 5 year disease-free survival of about 75% in the control group (q3EC-P). Furthermore, we assumed that the minimum therapeutic effect worth detecting in this study, in view of the increased toxic effects associated with the experimental treatments, was a 20% relative reduction in disease-free survival (hazard ratio [HR]=0·80), corresponding to a 4·4% absolute increase in 5-year disease-free survival. To detect with an 80% power and a significance 5% (two-sided), a 20% relative reduction in the risk of relapse in either comparison (EC-P *vs* FEC-P and dose-dense *vs* standard interval chemotherapy), 635 disease-free survival events had to be observed. We estimated that to observe 635 events, 1500 patients needed to be enrolled, with an average

follow-up of 7–8 years, or 2000 patients with an average follow-up of 5·5–6 years. We planned the final analysis once we had observed 635 events. No formal intermediate analyses were planned. However, in March, 2013, 10 years after the enrolment of the first patient, the Steering Committee of the study, in view of the fact that the study had enrolled 2091 patients that had been followed for a median of 7·0 years, and that, so far, the total number of disease-free survival events was still close to 500, decided to proceed with the final analysis of the study and to publish its results, because to wait the 2 or more years that would have been necessary to achieve the target number of 635 events was not deemed appropriate. This decision was based only on information about the event rates in the four treatment groups combined, and was entirely masked to treatment-specific data and results, thereby avoiding any bias related to an unplanned interim analysis. On the basis of the final number of events recorded at the closing date (521 events; May 9, 2013), the power to detect the target HR of 0·80 was decreased to 72%, whereas an 80% power was available for a HR of 0·785.

	q3EC-P group (n=545)	q3FEC-P group (n=544)	q2EC-P group (n=502)	q2FEC-P group (n=500)
Age at study entry, years	51 (43–60)	53 (45–61)	53 (44–59)	51 (44–59)
Menopausal status				
Premenopausal	281 (52%)	245 (45%)	232 (46%)	263 (53%)
Postmenopausal	264 (48%)	299 (55%)	270 (54%)	237 (47%)
Type of surgery				
Lumpectomy	338 (62%)	320 (59%)	298 (59%)	313 (63%)
Mastectomy	207 (38%)	224 (41%)	204 (41%)	187 (37%)
Tumour size				
T1	283 (52%)	262 (48%)	262 (52%)	253 (51%)
T2	218 (40%)	233 (43%)	202 (40%)	208 (42%)
T3	21 (4%)	25 (5%)	25 (5%)	29 (6%)
T4	19 (3%)	23 (4%)	10 (2%)	9 (2%)
Unknown	4 (1%)	1 (<1%)	3 (1%)	1 (<1%)
Number of positive nodes				
1–3	327 (60%)	319 (59%)	319 (64%)	284 (57%)
4–9	135 (25%)	136 (25%)	116 (23%)	135 (27%)
≥10	83 (15%)	89 (16%)	67 (13%)	81 (16%)
Unknown	1 (<1%)	1 (<1%)	0	0
Tumour grade				
G1	30 (5%)	21 (4%)	35 (7%)	30 (6%)
G2	236 (43%)	238 (44%)	225 (45%)	240 (48%)
G3	270 (49%)	266 (49%)	229 (46%)	214 (43%)
Unknown	9 (2%)	19 (3%)	13 (3%)	16 (3%)
Expression of HER2 protein				
Positive	123 (23%)	131 (24%)	105 (21%)	121 (24%)
Negative	344 (63%)	332 (61%)	318 (63%)	299 (60%)
Unknown	78 (14%)	81 (15%)	79 (16%)	80 (16%)
Expression of hormone receptors				
ER or PR positive	420 (77%)	442 (81%)	407 (81%)	401 (80%)
ER and PR negative	103 (19%)	88 (16%)	83 (16%)	85 (17%)
Unknown	22 (4%)	14 (3%)	12 (2%)	14 (3%)
KI67 value (% of positive cells)				
0–14	120 (22%)	113 (21%)	142 (28%)	132 (26%)
15–20	33 (6%)	51 (9%)	44 (9%)	41 (8%)
>20	273 (50%)	269 (49%)	214 (43%)	232 (46%)
Unknown	119 (22%)	111 (20%)	102 (20%)	95 (19%)

Data are median (IQR) or n (%). q3EC-P=standard-interval chemotherapy with epirubicin and cyclophosphamide, followed by paclitaxel. q3FEC-P=standard-interval chemotherapy with fluorouracil, epirubicin, and cyclophosphamide, followed by paclitaxel. q2EC-P=dose-dense chemotherapy with epirubicin and cyclophosphamide, followed by paclitaxel. q2FEC-P=dose-dense chemotherapy with fluorouracil, epirubicin, and cyclophosphamide, followed by paclitaxel. HER2=human epidermal growth factor receptor 2. ER=oestrogen receptor. PR=progesterone receptor. Ki67=Ki67 labelling index.

Table 1: **Baseline characteristics**

The two primary comparisons were between FEC-P and EC-P, and between dose-dense and standard-interval chemotherapy. All statistical analyses were done on an intention-to-treat basis and all protocol violators were analysed within their randomised groups.

The presence of an interaction between the two study factors (FEC-P *vs* EC-P) and dosing every 2 weeks *vs* every 3 weeks) was assessed by fitting two multivariate Cox models to the data, one for disease-free survival and the other for overall survival. Only patients enrolled in centres that randomised to all four treatment groups were included in these analyses. In each of these two models, only three factors were included, the two treatment assignments (dosing type and dosing frequency) and the interaction term between them. The significance of the interaction between the two treatments was assessed by means of a backward procedure, based on the likelihood ratio test.

The two primary study hypotheses were tested independently by comparison of disease-free survival in the groups of patients assigned to EC-P with those assigned to FEC-P, and comparison of those in the 2 weeks group with those in the 3 weeks group. We used a stratified log-rank test to assess the significance of the differences between the disease-free survival curves, where the stratification factor for the EC-P versus the FEC-P comparison was the assignment to 2 week or 3 week dosing, whereas for the comparison between dose-dense versus standard-interval chemotherapy, the stratification factor was assignment to EC-P or FEC-P. In all analyses focused on dose-density, we excluded patients enrolled to the five centres that randomised only to standard-interval EC-P and standard-interval FEC-P and did not randomise to dose schedule. The same procedures were used in the analyses of overall survival.

Kaplan-Meier product-limit estimators were plotted to describe disease-free survival and overall survival in EC-P and FEC-P groups, adding together patients in the dose schedule groups, and for q2 and q3 groups, adding together patients in the two drug groups.

To provide estimates of treatment effects adjusted for potential confounding factors, and to assess the consistency of these estimates across strata of these factors (subgroup analyses), we fitted several multivariate Cox

disease-free survival and overall survival models to the data. These analyses were done separately for the FEC-P versus EC-P and for the 3 week and 2 week comparisons.

The following covariates were included in the models: age, menopausal status, type of surgery, histological type, tumour size, nodal status, grade, HER2 status, and hormonal receptors. In a substantial proportion of patients (57 [3%] patients) the information about the grade of the primary tumour was missing. To avoid the exclusion from all multivariate analyses, the grade of these patients was reclassified, according to the procedure reported in the appendix.

For subgroup analyses, the interaction terms between the treatment group and each of the prognostic factors were introduced in the model one at a time. The statistical significance of the interaction term was then assessed by a backward procedure based on the likelihood ratio test. Proportionality was tested with Schoenfeld residuals.

The same procedures were repeated for the subgroup analyses of overall survival. Because of the absence of any correction for multiple testing, and the fact that no subgroup analysis was anticipated in the study protocol, the results of these analyses (ie, tests for interactions) are to be thought of as merely exploratory and any significant results must be considered with caution. Forest plots were used to summarise the results of various subgroup analyses. All reported p values are two-sided. We used SPSS version 20.0 for all statistical analyses. This trial is registered with ClinicalTrials.gov, number NCT00433420.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. LDM, SDP, and the statistician (PB) had full access to all of the data and had final responsibility for the decision to submit the manuscript for publication.

Results

Between April 24, 2003 and July 3, 2006, 2091 patients entered the study. Of these, 2003 patients were randomly assigned to four treatment groups, while 88 were randomly assigned only to q3FEC-P and q3EC-P (figure 1). The treatment groups were well balanced with respect to demographic and tumour characteristics (table 1).

The planned number of chemotherapy cycles was completed by almost 90% of patients in every group (figure 1). The relative dose-intensity was 99% in the q3EC-P group, 98% in the q3FEC-P group, 98% in the q2EC-Pgroup, and 98% in the q2FEC-P group (a complete summary of treatment compliance and dose-intensity is provided in the appendix). Patients with HER2-positive disease who were enrolled after the approval of adjuvant trastuzumab in April, 2006, received trastuzumab for 1 year; overall 130 (27%) of 480 patients with HER2-positive tumours received trastuzumab: 40 (33%) patients in the q3EC-P group, 32 (24%) in the q3FEC-P group, 26 (25%) in the q2EC-P group, and 32 (26%) in the q2FEC-P group.

As of May 9, 2013, after a median follow-up of 7·0 years (IQR 4·5–6·3), 521 (25% of patients) disease-free survival events occurred in the overall study population. 266 (13%) deaths occurred in total (table 2). No interaction was seen between the effect of two randomisation variables (FEC-P *vs* EC-P and dense *vs* standard-interval dosing) on disease-free survival ($p_{interaction}$=0·628) or overall survival ($p_{interaction}$=0·889), making it possible to analyse the two study factors independently.

270 disease-free survival events occurred in the two FEC-P groups combined and 251 occurred in the two EC-P groups combined. The estimated rates of disease-free survival at 5 years were 78% (95% CI 75–81) in the FEC-P group and 79% (76–82) in the EC-P group (p=0·561; figure 2). Overall survival rates at 5 years were 91% (89–93) in the FEC-P group and 92% (90–94) EC-P group (p=0·234; figure 2). The similar outcome between patients assigned to FEC-P and EC-P was confirmed in multivariate analyses, where the comparisons between FEC-P and EC-P were adjusted for prognostic factors (HR for disease-free survival 1·02, 95% CI 0·86–1·22, p=0·812 and for overall survival (1·07; 0·84–1·37, p=0·578; data not shown).

In subgroup analyses (appendix), no evidence of interaction was observed between FEC-P or EC-P and any factor, with the exception of nodal status, for which a significant interaction was seen (p=0·011) for disease-free survival only. However, the interaction was attributable to the difference in favour of the EC-P group observed in patients with 4–9 affected lymph nodes, without any discernible trend of increasing or decreasing HR.

224 disease-free survival events occurred in the two groups given chemotherapy every 2 weeks and 270 in the two groups given chemotherapy every 3 weeks. The estimated rates of disease-free survival at 5 years were 81% (95% CI 79–84) in the dose dense group and 76% (74–79) in the standard interval groups (p=0·004; figure 2).

	q3EC-P group (n=545)	q3FEC-P group (n=544)	q2EC-P group (n=502)	q2FEC-P group (n=500)
Disease-free survival events*	140 (26%)	157 (29%)	111 (22%)	113 (23%)
Breast cancer relapse	121 (22%)	138 (25%)	94 (19%)	93 (19%)
Locoregional alone	13 (2%)	18 (3%)	10 (2%)	12 (2%)
Distant	93 (17%)	102 (19%)	74 (15%)	74 (15%)
Concurrent distant and locoregional	11 (2%)	13 (2%)	8 (2%)	5 (1%)
Unknown site	4 (1%)	5 (1%)	2 (<1%)	2 (<1%)
Second primary malignancy	19 (3%)	15 (3%)	12 (2%)	16 (3%)
Primary breast cancer	7 (1%)	11 (2%)	4 (1%)	6 (1%)
Second primary tumour, non-breast	12 (2%)	4 (1%)	8 (2%)	10 (2%)
Death without evidence of relapse	0	4 (1%)	5 (1%)	4 (1%)
Overall survival events†	75 (14%)	88 (16%)	48 (10%)	55 (11%)

Data are n (%). *Only first events are considered. †Overall survival events denote death from any cause.

Table 2: **Disease-free survival and overall survival events in the intention-to-treat population**

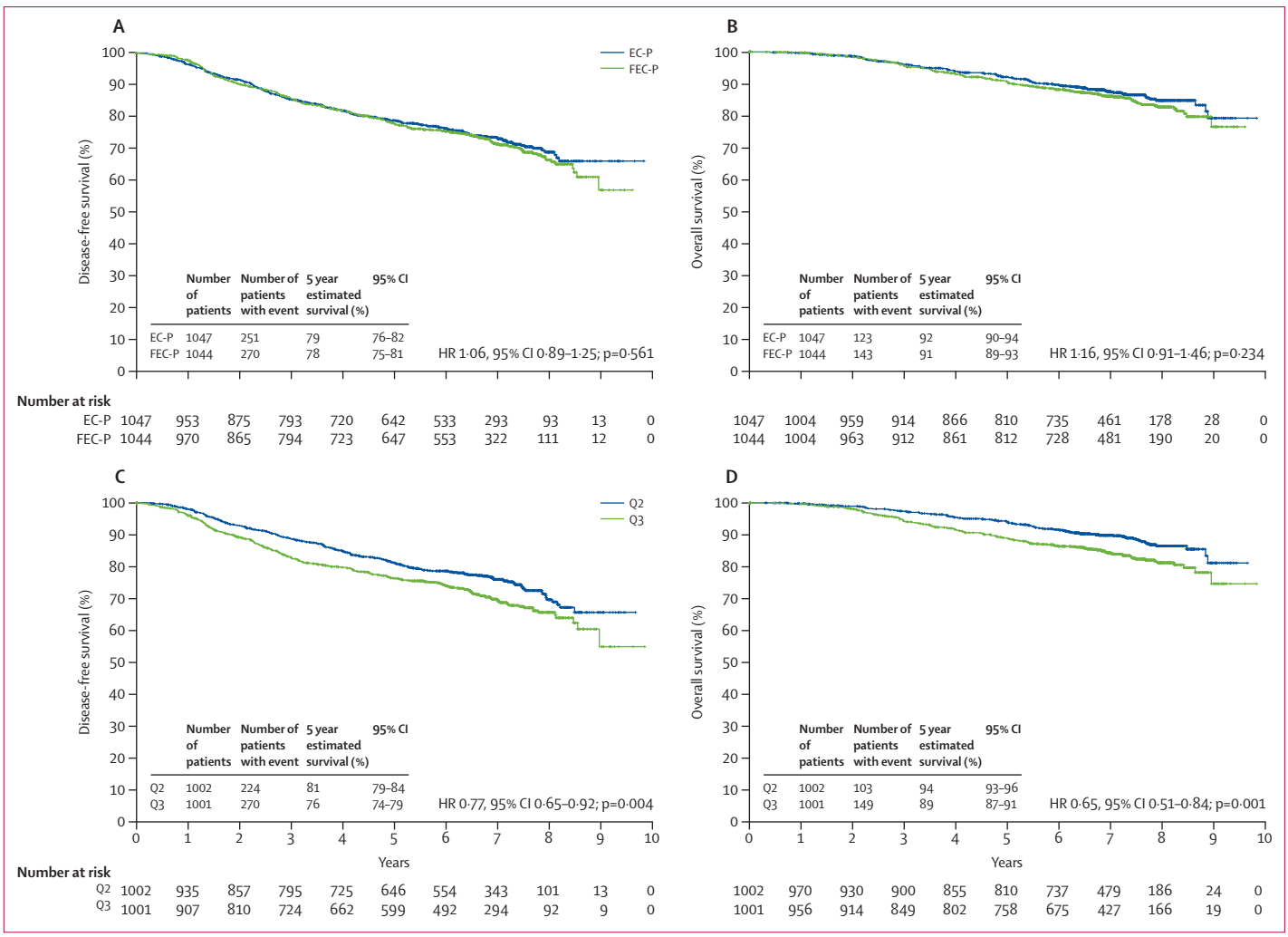

Figure 2: **Disease-free survival and overall survival**

Kaplan-Meier estimates of disease-free survival (A) and overall survival (B) in EC-P and FEC-P groups. Kaplan-Meler estimates of disease-free survival (C) and overall survival (D) in dose-dense and standard interval chemotherapy groups. EC-P=epirubicin, and cyclophosphamide, followed by paclitaxel. FEC-P=fluorouracil, epirubicin, and cyclophosphamide, followed by paclitaxel.

Overall estimated survival rates at 5 years were 94% (93–96) in the dose dense group and 89% (87–91) in the standard interval group (p=0·001; figure 2). These differences were confirmed in multivariate analyses, in which the comparisons between dose times were adjusted for prognostic factors (HR for disease-free survival 0·78; 0·65–0·94, p=0·008 and HR for overall survival 0·66; 0·51–0·85, p=0·002; data not shown).

A remarkable consistency in the difference between 2 week and 3 week dosing was observed in subgroup analyses of disease-free survival (appendix). No significant interaction was seen between type of regimen and any of the prognostic factors. In subgroup analyses of overall survival (appendix), a significant interaction was observed between treatment assignment and tumour size (p=0·043), with no effect seen in patients with pT1 tumours (HR 0·98). No noteworthy variation in the HRs was observed in other subgroups considered.

The effect of 2 week or 3 week dosing on disease-free survival did not differ according to hormone-receptor status (HR=0·80, 95% CI 0·65–0·98 in hormone-receptor-positive patients; HR=0·69, 0·48–0·99 in hormone-receptor-negative patients; $p_{interaction}$=0·43; appendix). For hormone-receptor positive patients, the 5 year disease-free survival was 83% (81–86) with the dose dense schedule and 80% (77–83; p=0·033) with the standard interval schedule; and for hormone-receptor negative patients, 71% (64–78) with the dose dense schedule and 61% (53–69; p=0·046) in with the standard interval schedule (figure 3). The effect of the schedule on overall survival was similar in hormone-receptor-positive (HR 0·69, 0·51–0·94) and hormone-receptor-negative patients (HR 0·55, 0·34–0·90; $p_{interaction}$=0·423).

No significant interaction occurred between tras tuzumab therapy and the effect of the dose-dense schedule ($p_{interaction}$=0·728 for disease-free survival and $p_{interaction}$=0·679

for overall survival) even though, in the subgroup of patients with HER2-positive tumours treated with trastuzumab, the effect of dose dense therapy was smaller (HR 0·99; 95% CI 0·49–1·99 for disease-free survival and HR 0·93 0·35–2·54 for overall survival). Subgroup analyses according to HER2 status and treatment with trastuzumab are shown in the appendix.

Table 3 shows major (occurring in at least 5% of the population) toxic effects that occurred in the four groups. Grade 3–4 toxic effects that occurred in the standard interval and dose-dense groups are reported in the appendix. Patients receiving dose-dense chemotherapy, compared with those treated with q3 regimens, more frequently had grade 3–4 anaemia (14 [1·4%] of 988 patients *vs* two [0·2%] of 984 patients; p=0·002), transaminitis (19 [1·9%] *vs* four [0·4%]; p=0·001), and myalgias (31 [3·1%] *vs* 16 [1·6%], p=0·019), and less frequently had grade 3–4 neutropenia (147 [14·9%] *vs* 433 [44·0%]; p<0·0001). The appendix shows grade 3–4 toxic effects that occurred in the EC-P and FEC-P groups. Grade 3–4 neutropenia, fever, nausea, and vomiting were significantly more frequent in patients treated with FEC-P than in patients treated with EC-P. We noted one case of acute myeloid leukaemia and one case of myelodysplastic syndrome, both in the q2EC-P arm.

No treatment-related deaths occurred. Five patients (two patients in the q3EC-P group, one in the q3FEC-P group, one in the q2EC-P group, and one in the q2FEC-P group) died from early tumour recurrence within 1 year of randomisation.

Discussion

This trial shows that, in women with node-positive breast cancer, the sequential treatment regimen FEC-P did not improve disease-free survival compared with the same regimen without fluorouracil (EC-P), whereas the dose-dense regimen was associated with a significant reduction in the risk of recurrence and death compared with standard-interval chemotherapy.

Both AC/EC and FAC/FEC[14] are widely used in the sequential treatment regimens with anthracycline-containing chemotherapy and taxanes, and no data are available so far to support the choice between the treatment with or without fluorouracil. The findings of this trial suggest that, at least in sequential regimens with anthracyclines and paclitaxel, the addition of fluorouracil to the EC-P regimen was associated with an increase in toxic effects (grade 3–4 neutropenia, fever, nausea, and vomiting) whereas we observed no additional outcome benefits. Although, the role of fluorouracil in the adjuvant treatment of early breast cancer cannot be completely discarded, the lack of efficacy noted in our study raises a general question on the opportunity to include it in modern adjuvant chemotherapy regimens, especially if the increase in toxic effects associated with its use is taken into account.

An important finding of this study is that the provision of dose-dense chemotherapy was associated with a significant and clinically relevant disease-free survival and overall survival benefit compared with standard interval chemotherapy: the observed reduction from 24% to 19%, in the cumulative 5-year risk of invasive disease or death, corresponds to a hazard ratio of 0·78. Even more impressive was the effect on overall survival, for which we observed a reduction in 5-year cumulative mortality from 11% to 6%, for a hazard ratio of 0·66.

The role of dose-dense chemotherapy, as adjuvant treatment for patients with breast cancer, was assessed in various phase 3 studies, but the most of these trials compared dose-dense chemotherapy with regimens that use standard intervals but with different drugs or dose in the treatment groups, thus making difficult to extrapolate the true role of a dose-dense strategy.[2–5] Few studies[6–8] compared the same chemotherapy regimen administered

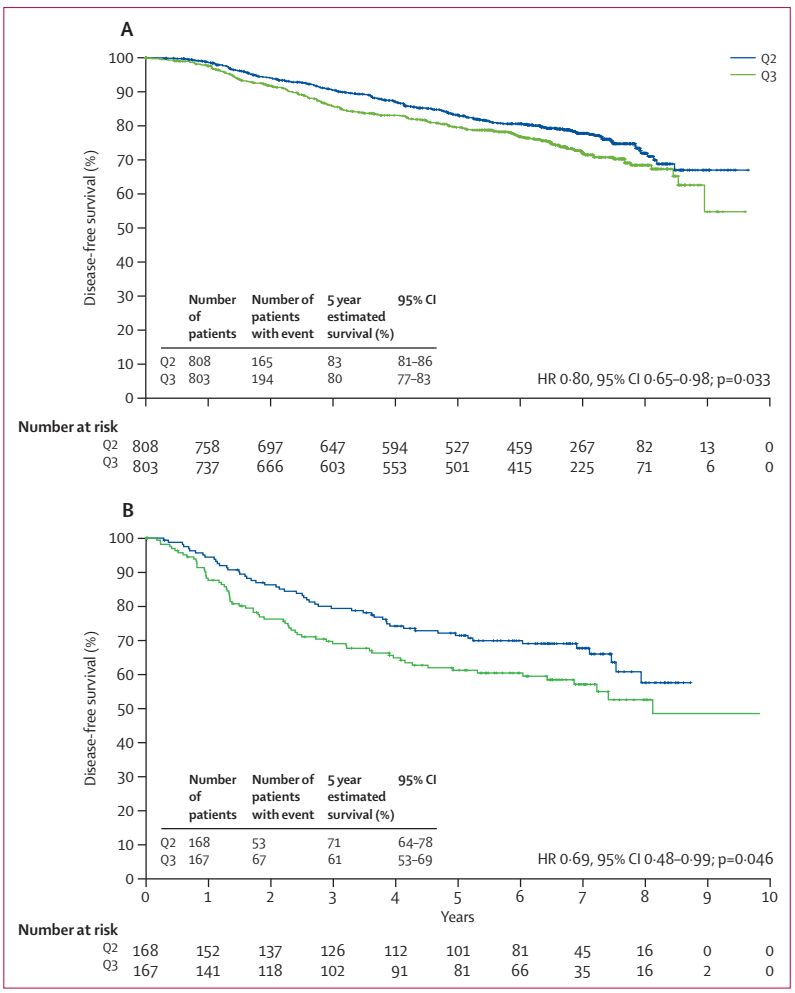

***Figure 3*: Disease-free survival according to hormone-receptor status**
(A) Kaplan-Meier estimates of disease-free survival in hormone-receptor positive patients treated with dose-dense chemotherapy or standard-interval chemotherapy. (B) Kaplan-Meier estimates of disease-free survival in hormone-receptor negative patients treated with dose-dense chemotherapy or standard-interval chemotherapy. EC-P=epirubicin, and cyclophosphamide, followed by paclitaxel. FEC-P=fluorouracil, epirubicin, and cyclophosphamide, followed by paclitaxel.

	q3EC-P group (n=536)		q3FEC-P group (n=533)		q2EC-P group (n=496)		q2FEC-P group (n=492)	
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
Anaemia	264 (49%)	0	263 (49%)	2 (<1%)	329 (66%)	6 (1%)	321 (65%)	8 (2%)
Neutropenia	153 (29%)	200 (37%)	103 (19%)	257 (48%)	69 (14%)	50 (10%)	61 (12%)	97 (20%)
Thrombocytopenia	29 (5%)	2 (<1%)	40 (8%)	2 (<1%)	57 (11%)	1 (<1%)	86 (17%)	5 (1%)
Asthenia	275 (51%)	5 (1%)	286 (54%)	12 (2%)	294 (59%)	13 (3%)	294 (60%)	15 (3%)
Diarrhoea	79 (15%)	1 (<1%)	69 (13%)	2 (<1%)	77 (16%)	2 (<1%)	94 (19%)	3 (1%)
Bone pain	200 (37%)	10 (2%)	198 (37%)	11 (2%)	263 (53%)	11 (2%)	234 (48%)	20 (4%)
Fever	91 (17%)	1 (<1%)	103 (19%)	5 (1%)	131 (26%)	1 (<1%)	127 (26%)	4 (1%)
Myalgia	248 (46%)	9 (2%)	242 (45%)	8 (2%)	237 (48%)	15 (3%)	236 (48%)	16 (3%)
Stomatitis	164 (31%)	0	208 (39%)	3 (1%)	180 (36%)	4 (1%)	189 (38%)	5 (1%)
Nausea	390 (73%)	13 (2%)	374 (70%)	22 (4%)	365 (74%)	15 (3%)	345 (70%)	25 (5%)
Vomiting	199 (37%)	8 (1%)	197 (37%)	12 (2%)	203 (41%)	7 (1%)	210 (43%)	20 (4%)
Neuropathy	269 (50%)	16 (3%)	269 (50%)	12 (2%)	232 (47%)	19 (4%)	233 (47%)	16 (3%)
Transaminase elevation	130 (24%)	3 (1%)	159 (30%)	2 (<1%)	174 (35%)	11 (2%)	196 (40%)	8 (2%)

Comparison between EC-P and FEC-P in terms of grade 3–4 toxicity is reported in the appendix. Comparison between dose dense and standard interval regimens in terms of grade 3–4 toxic effects is reported in the appendix.

Table 3: **Adverse events occurring in at least 5% of any one group**

Panel: **Research in context**

Systematic review

We searched PubMed for articles about dose-dense regimens and fluorouracil in the adjuvant treatment of breast cancer without language or date restriction. Additionally, we searched the abstract of major oncology congresses. A pooled analysis[10] of randomised trials showed that chemotherapy regimens with an increased dose-density are associated with an improved overall survival and disease-free survival. However, in most studies differences were reported between the dose-dense and control groups in terms of number of cycles, dose size, type of drugs and total dose, thus making interpretation of which variable contributed to the observed results difficult.[2–5] Of the few randomised studies with an adequate design to specifically address the role of dose-dense, the CALGB B9741 study[6] showed a benefit of dose-dense chemotherapy, mainly restricted to hormone-receptor-negative tumours;[15] the GONO-MIG trial[7] showed a trend toward a benefit of dose-dense adjuvant chemotherapy, and, conversely, results from the UK TACT2 trial[8] showed no benefit of this chemotherapy schedule. The GIM2 trial, beside assessment of the role of dose-density, was unique in addressing the role of the addition of fluorouracil to the chemotherapy regimen with anthracycline-cyclophosphamide followed by paclitaxel.

Interpretation

This GIM2 trial supports the increased efficacy of dose-dense chemotherapy and suggests that the benefit is present in both hormone-receptor-negative and hormone-receptor-positive tumours. Our results first show that the addition of fluorouracil to anthracycline and cyclophosphamide followed by paclitaxel is not associated with an improved outcome compared with the same treatment without fluorouracil.

every 2 or every 3 weeks. A dose-dense chemotherapy approach using concurrent doxorubicin and cyclophosphamide followed by paclitaxel was assessed in the Cancer and Leukaemia Group B 9741 trial,[6] which showed a significant improvement in disease-free survival and overall survival for the dose-dense chemotherapy group. The Italian Gruppo Oncologico Nord Ovest-Mammella Intergruppo Trial assessed the dose-dense approach in a non-taxane-based regimen. Although numerically improved outcome in the dose-dense group was observed, the difference was not statistically significant.[7] The UK TACT2 trial,[8] so far presented only in abstract form, compared standard chemotherapy with epirubicin followed by CMF (cyclophosphamide, methotrexate, fluorouracil) or capecitabine to dose-dense epirubicin and, at a median follow-up of 49 months, showed no difference in disease-free survival between the two treatment strategies. The pooled analysis of randomised trials showed that dose-dense chemotherapy results in better overall and disease-free survival.[10] However, because of the paucity of randomised controlled trials with an adequate design, additional data from randomised trials were claimed to be necessary to better clarify the role of dose-dense chemotherapy. Our findings underline the results of the CALGB B9741 trial and support the clinically relevant benefit associated with the dose-dense strategy in regimens based on the sequential provision of anthracycline-containing chemotherapy followed by paclitaxel. In the CALBGB trial, dose-dense chemotherapy was particularly effective in patients with hormone-receptor-negative breast cancer, whereas the benefit in hormone-receptor-positive patients was smaller and not significantly different from that observed with standard-interval chemotherapy.[15] Our results, instead, show that the benefits of dose-dense chemotherapy are present in hormone-receptor-positive patients as well. However, because of the underrepresentation of hormone receptor-negative patients (only 17% of the overall study population) in our study, the statistical power of the test for interaction between hormone receptor status and dose-density was limited, and then a difference in the magnitude of benefit of dose-dense chemotherapy in the two groups of patients (hormone receptor-positive and hormone receptor-negative) cannot be completely ruled out.

The outcome improvement observed in hormone-

receptor-positive patients might be due to the longer follow-up (median 7·0 years [IQR 4·5–6·3]) of our study compared than in other studies.[15] In fact, the pattern of risks over time is different in hormone-receptor-negative and hormone-receptor-positive patients: in the first few years after surgery the risk of relapse is high in hormone-receptor-negative patients and very low in hormone-receptor-positive patients, in whom a substantial number of events are observed after the fifth year. This pattern of recurrence suggests that a long follow-up is necessary to observe the potential benefit of adjuvant treatment in hormone-receptor-positive patients.

Because of the small proportion of patients with HER2-positive tumours treated with trastuzumab, no significant interaction was seen between trastuzumab therapy and the effect of the dose-dense schedule. However, the lack of a clear effect in patients with HER2-positive tumours treated with trastuzumab suggests that the efficacy of dose-dense chemotherapy might be less noticeable or absent in this subgroup of patients.

The main limitation to the generalisability of the dose-dense regimen tested in the present study might be the schedule of paclitaxel. At the time the study was planned, paclitaxel at the dose of 175 mg/m² every 3 weeks, was the standard schedule in the adjuvant treatment of breast cancer. The demonstration that weekly paclitaxel is more effective than the every 3 weeks schedule, only came in 2008, 2 years after the end of recruitment in our trial.[16] Since then, the 3 weekly paclitaxel is no longer a standard treatment and has been replaced by 12 cycles of weekly paclitaxel. Nevertheless, recent results from the SWOG S0221 phase 3 trial showed that dose-dense paclitaxel, given every 2 weeks, is similar, in terms of both disease-free survival and overall survival, to weekly paclitaxel and then, that either dose-dense and weekly regimen are acceptable schedules of paclitaxel administration.[17] Yet, in that study, grade 3–4 toxic effects were higher with the dose-dense administration than with weekly administration of paclitaxel, thus suggesting that weekly paclitaxel might be a better option than dose-dense paclitaxel.

An additional limitation of our trial is that the study design does not allow separation between the effect of dose-dense (F)EC *vs* dose-dense paclitaxel, leaving the possibility that the intensification of either (F)EC or paclitaxel might be sufficient to obtain the full benefit observed with the whole dose-dense regimen. Moreover, no correction for multiple testing was planned to account fo the 2 primary analyses conducted in this trial (FEC-P *vs* EC-P and q2 *vs* q3), since this correction is not considered necessary in factorial trials.[18] At any rate, the use of a stricter (eg, p=0·0253 using the Bonferroni correction) criterion for statistical significance would not change the interpretation of either comparison.

Dose-dense chemotherapy was confirmed to be more toxic than standard-interval chemotherapy, with the exception of neutropenia, that, in view of the provision of pegfilgrastim, was less frequent in the dose-dense group. The use of pegfilgrastim is still considered investigational in the setting of dose-dense chemotherapy.[19] Our data showing that pegfilgrastim, given after 72 h, is safe and feasible in dose-dense regimens,[20] are supported by findings from a prospectively randomised study,[21] which showed that pegfilgrastim after 72 h has a similar efficacy of pegfilgrastim after 24 h and is associated with a lower incidence of grade 3–4 leucopenia. The observed incidence of acute myeloid leukaemia and myelodisplastic syndrome, two cases in 1002 patients (0·19%) treated with dose-dense chemotherapy, is similar to that generally reported in dose-dense studies[22] and should be considered in view of the 52 fewer deaths observed in the dose-dense groups than in the standard interval groups (panel).

Contributors

LDM, PB, SDP, FC, and MDL designed the study and analysed, interpreted, and collected data. LDM and PB wrote and approved the final report. SDP, MDL, C, GC, AD, AT, CN, EV, OG, FP, FM, SB, AA, TG, GC, MG, AG, PP, CB, GB, VF, and FC were involved in data collection, report revision, and final approval of the report.

Declaration of interests

LDM reports personal fees from Takeda, Roche, Novartis, GlaxoSmithKline, Amgen, and Teva outside the submitted work. SDP reports speaker's honoraria from Roche, EISAI, and Celgene outside the submitted work. FC reports personal fees from Astellas Oncology, outside the submitted work. MDL reports grants and personal fees from Amgen, outside the submitted work. TG reports grants from EISAI and Glaxo, outside the submitted work. All other authors declare no competing interests.

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