Fluorouracil and dose-dense adjuvant chemotherapy in patients with early-stage breast cancer (GIM2): end-of-study results from a randomised, phase 3 trial

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Summary

Background Previous analyses of the GIM (Gruppo Italiano Mammella) 2 study showed that addition of fluorouracil to epirubicin, cyclophosphamide, and paclitaxel in patients with node-positive early breast cancer does not improve outcome, whereas dose-dense chemotherapy induces a significant improvement in both disease-free survival and overall survival as compared with a standard schedule. Here, we present long-term results of the study.

Methods In this 2×2 factorial, open-label, randomised, phase 3 trial, we enrolled patients aged 18–70 years with operable, node-positive, breast cancer with Eastern Cooperative Oncology Group performance status of 0–1 from 81 hospitals in Italy. Eligible patients were randomly allocated (1:1:1:1) to one of the four following study groups: four cycles of standard-interval intravenous EC (epirubicin 90 mg/m² and 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 (q3EC-P group); four cycles of intravenous FEC (fluorouracil 600 mg/m², epirubicin 90 mg/m², and 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 (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, with stratification by centre, with permuted blocks of size 12, was done with a centralised, interactive, internet-based system that randomly generated the treatment allocation. The primary endpoint was disease-free survival in the intention-to-treat population, comparing different chemotherapy schedule (dose-dense *vs* standard-dose intervals) and regimen (FEC-P *vs* EC-P). Safety population included all patients that received at least one dose of any study drug according to the treatment received. This trial is registered with ClinicalTrials.gov, NCT00433420, and is now closed.

Findings Between April 24, 2003, and July 3, 2006, 2091 patients were randomly assigned to treatment: 545 to q3EC-P, 544 to q3FEC-P, 502 to q2EC-P, and 500 to q2FEC-P. 88 patients were enrolled in centres providing only standard interval schedule and were assigned only to q3FEC-P and q3EC-P; thus, 2091 patients were included in the intention-to-treat analysis for the comparison of EC-P (1047 patients) versus FEC-P (1044 patients) and 2003 patients were included in the intention-to-treat analysis for the comparison of dose-dense (1002 patients) versus standard interval analysis (1001 patients). After a median follow-up of 15·1 years (IQR 8·4–16·3), median disease-free survival was not significantly different between FEC-P and EC-P groups (17·09 years [95% CI 15·51–not reached] *vs* not reached [17·54–not reached]; unadjusted hazard ratio 1·12 [95% CI 0·98–1·29]; log-rank p=0·11). Median disease-free survival was significantly higher in the dose-dense interval group than the standard-interval group (not reached [95% CI 17·45–not reached] *vs* 16·52 [14·24–17·54]; 0·77 [95% CI 0·67–0·89]; p=0·0004). The most common grade 3–4 adverse events were neutropenia (200 [37%] of 536 patients in the q3EC-P group *vs* 257 [48%] of 533 in the q3FEC-P group *vs* 50 [10%] of 496 q2EC-P *vs* 97 [20%] of 492) and alopecia (238 [44%] *vs* 249 [47%] *vs* 228 [46%] *vs* 235 [48%]). During extended follow-up, no further grade 3–4 adverse events or deaths related to toxic-effects were reported. Treatment-related serious adverse events were reported in nine (2%) patients in the q3EC-P group, seven (1%) in the q3FEC-P group, nine (2%) in the q2EC-P group, and nine (2%) in the q2FEC-P group. No treatment-related deaths occurred.

Interpretation Updated results from the GIM2 study support that optimal adjuvant chemotherapy for patients with high-risk early breast cancer should not include fluorouracil and should use a dose-dense schedule.

Funding Bristol-Myers Squibb, Pharmacia, Dompè Biotec Italy, Italian Ministry of Health, Fondazione Italiana per la Ricerca sul Cancro, and Alliance Against Cancer.

Lancet Oncol 2022; 23: 1571–82

Published Online November 9, 2022 https://doi.org/10.1016/S1470-2045(22)00632-5

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See Online for appendix

Research in context

Evidence before this study

Anthracycline-based and taxane-based regimens are the most active treatments for patients with breast cancer who are candidates for adjuvant chemotherapy. At the time of the study design in 2003, the role of the addition of fluorouracil and the benefit of dose-dense schedules was controversial. We searched PubMed for articles on dose-dense regimens and fluorouracil in the adjuvant treatment of breast cancer without language or date restriction between March 15, 2015, to March 15, 2022, using the search terms "(dose dense OR adjuvant chemotherapy OR fluorouracil) AND breast cancer". Additionally, we searched the abstracts of major oncology congresses and relevant articles were cross-referenced. The Early Breast Cancer Trialists' Cooperative Group individual patient-level meta-analysis showed that chemotherapy regimens with an increased dose-density moderately reduced risk of recurrence and death from breast cancer without increasing mortality from other causes. Most studies included in this meta-analysis varied both in the chemotherapy combinations and in the scheduling, thus making interpretation of which variable contributed to the observed results complicated. Previous results of the phase 3 GIM2 study, at a median follow-up of 7·0 years, showed that addition of fluorouracil to a sequential epirubicin and cyclphosphamide plus paclitaxel regimen did not improve disease-free survival and overall survival, but that dose-dense chemotherapy on a 2-week schedule significantly improved outcomes compared with a 3-week schedule.

Added value of this study

To our knowledge, this is the only study comparing two anthracycline-based and taxane-based regimens differing only in the presence or not of fluorouracil. Our study robustly showed that fluorouracil should not be added because it increases toxicity without improving outcomes. The long-term follow-up of the GIM2 trial continues to support the increased efficacy of a dose-dense schedule.

Implications of all the available evidence

Some clinical practice guidelines (ie, Cancer Care Ontario and American Society of Clinical Oncology guidelines) state that fluorouracil-containing regimens, such as FAC (fluorouracil, doxorubicin, and cyclophosphamide) or FEC (fluorouracil, epirubicin, and cyclophosphamide), used in sequential combination with taxanes are acceptable adjuvant chemotherapy regimens. Notwithstanding the evidence of its superiority compared with standard interval regimens, dose-dense chemotherapy is not widely used and, also in modern clinical trials, regimens every 3 weeks are considered as standard treatment. Despite GIM2 being an old study that was not planned according to the modern concept of breast cancer subtypes, its long-term results strongly support that fluorouracil should not be included in anthracycline-taxane regimens, whereas dose-dense chemotherapy should be the preferred schedule of treatment for patients with high-risk early breast cancer.

Introduction

Anthracycline-containing and taxane-containing regimens are the most active treatments for patients with early breast cancer who are candidates for adjuvant chemotherapy.[1] In sequential combination with taxanes, both fluorouracil-containing regimens, such as FAC (fluorouracil, doxorubicin, and cyclophosphamide) and FEC (fluorouracil, epirubicin, and cyclophosphamide) and regimens without fluorouracil, such as AC (doxorubicin and cyclophosphamide) or EC (epirubicin and cyclophosphamide), are considered acceptable adjuvant chemotherapy regimens for patients with high-risk early breast cancer.[2,3] Despite the findings that dose-dense chemotherapy is able to reduce both recurrence and breast cancer mortality,[4] it is not widely used and a once every 3-weeks (thrice-weekly) treatment schedule is still considered standard in modern clinical trials.[5]

Previous results of the randomised, phase 3 Gruppo Italiano Mammella (GIM) 2 study, at a median follow-up of 7·0 years, showed that, in node-positive patients, addition of fluorouracil to a sequential EC followed by paclitaxel (EC-P) regimen does not improve disease-free survival or overall survival, and that twice-weekly dose-dense chemotherapy significantly improves both disease-free survival and overall survival compared with a thrice-weekly schedule.[6] To assess the long-term results of both the role of fluorouracil and dose-dense schedule, here, we report the end-of-study results of the GIM2 trial at a median follow-up of 15·1 years.

Methods

Study design and participants

Details of the GIM2 study have been previously reported.[6] Briefly, GIM2 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 a regimen with EC-P, and the role of the dose-dense schedule in the adjuvant chemotherapy of patients with node-positive early breast cancer. This study was done in 81 hospitals in Italy by the GIM group (appendix pp 3–5).

Women aged 18–70 years were eligible if they had histologically confirmed breast cancer with at least one axillary positive lymph node or one involved internal mammary node, had primary surgery with lumpectomy or total mastectomy plus axillary nodal dissection, did not have radiological evidence of distant metastases, had Eastern Cooperative Oncology Group performance status of 0–1, and had normal organ and bone marrow function. Participants had to be randomly assigned within 7 weeks after the date of surgery. We collected data on tumour size and nodal status according to the American Joint Committee on Cancer TNM of breast

cancer in the sixth edition of the Cancer Staging manual. Oestrogen and progesterone receptor-positive tumours were defined by a finding of at least 10% of positive cells by immunohistochemistry. 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.

We excluded men; patients with previous or concomitant malignancy, except adequately treated basal or squamous cell carcinoma of the skin or adequately treated cone-biopsied in situ carcinoma of the cervix; patients with symptomatic peripheral neuropathy greater than grade 2 according to the National Cancer Institute (NCI) Common Toxicity Criteria; and patients with recent acute myocardial infarction, congestive heart failure, or serious arrhythmia.

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.

Randomisation and masking

Eligible patients were randomly allocated (1:1:1:1) to one of the following study groups: standard-interval EC-P once every 3 weeks (q3EC-P); standard-interval FEC-P once every 3 weeks (q3FEC-P); dose-dense EC-P every 2 weeks (q2EC-P); and dose-dense FEC-P every 2 weeks (q2FEC-P). Five centres refused to randomly assign patients to the dose-dense treatment group, and so allocations were only to two groups in a 1:1 ratio (q3EC-P *vs* q3FEC-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 the treatment centre; within each centre, permuted blocks of size 12 in random sequence were used (block size was four in the five centres that only randomly assigned participants to FEC-P *vs* EC-P).

Procedures

Patients enrolled in the q3EC-P group received four cycles of intravenous EC (epirubicin 90 mg/m² and cyclophosphamide 600 mg/m²) once every 3 weeks on day 1, followed by four cycles of intravenous paclitaxel (175 mg/m²) once every 3 weeks on day 1. Participants in the q3FEC-P group received four cycles of intravenous FEC (fluorouracil 600 mg/m², epirubicin 90 mg/m², and cyclophosphamide 600 mg/m²) once every 3 weeks on day 1, followed by four cycles of intravenous paclitaxel (175 mg/m²) on day 1. Participants assigned to the dose-dense EC-P regimen received the same doses and drugs as the q3EC-P group, but administered every 2 weeks; and participants in the dose-dense FEC-P regimen received the same doses and drugs as the q3FEC-P group, but given every 2 weeks. Patients enrolled in the dose-dense groups received subcutaneous pegfilgrastim (6 mg) 24 h after chemotherapy.[7] Because of the occurrence of early leukocytosis (white blood cells >50 cells per mL), an amendment on March 18, 2004, required the provision of pegfilgrastim 72 h after chemotherapy. Patients treated with a standard-interval schedule who had febrile neutropenia could receive prophylactic administration of granulocyte colony-stimulating factor in subsequent cycles. After completion of chemotherapy, patients with hormone-receptor positive tumours received endocrine therapy. After the approval of adjuvant trastuzumab in Europe, an amendment, on April 10, 2006, required trastuzumab treatment for 1 year after the completion of chemotherapy for all patients with HER2-positive tumours. Chemotherapy dose reductions and delays for clinically significant grade 3 or 4 toxic-effects were done according to protocol-defined criteria. Patients were removed from the protocol therapy in case of disease progression, unacceptable toxicity, patient's decision, physician decision, and patients not compliant with the protocol requirements. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0.[8] Adverse events were assessed clinically and by hematological and biochemical measurements at each cycle of chemotherapy. Patients were followed up with physical examination once every 3–4 months for 2 years, then every 6 months for 3 years and every 12 months thereafter. A bilateral mammogram and blood chemistry were required every 12 months at each participating centre. No other radiographic assessments were required. No centralisation of samples or biopsies were performed.

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

Outcomes

The primary study endpoint was disease-free survival; secondary endpoints were overall survival and safety. Disease-free survival was calculated from the date of randomisation to the date of local recurrence, distant metastases, contralateral or ipsilateral breast tumour (excluding ductal carcinoma in situ), second primary malignancy, or death from any cause, whichever came first.[9] Overall survival was calculated from the day of randomisation to the date of death from any cause. Disease-free survival and overall survival of patients without an event when lost to follow-up or at the time of study closure were censored on the date of the last contact.

Statistical analysis

The two primary comparisons were between FEC-P and EC-P, and between dose-dense and standard-interval chemotherapy. Details on sample size and statistics have been previously reported.[6] Briefly, to detect a 20% relative reduction in the hazard of relapse in either comparison with 80% power and a two-sided 5% significance, 635 disease-free survival events had to be observed, and 2000 patients had to be enrolled. The present analysis reports results after the observation of

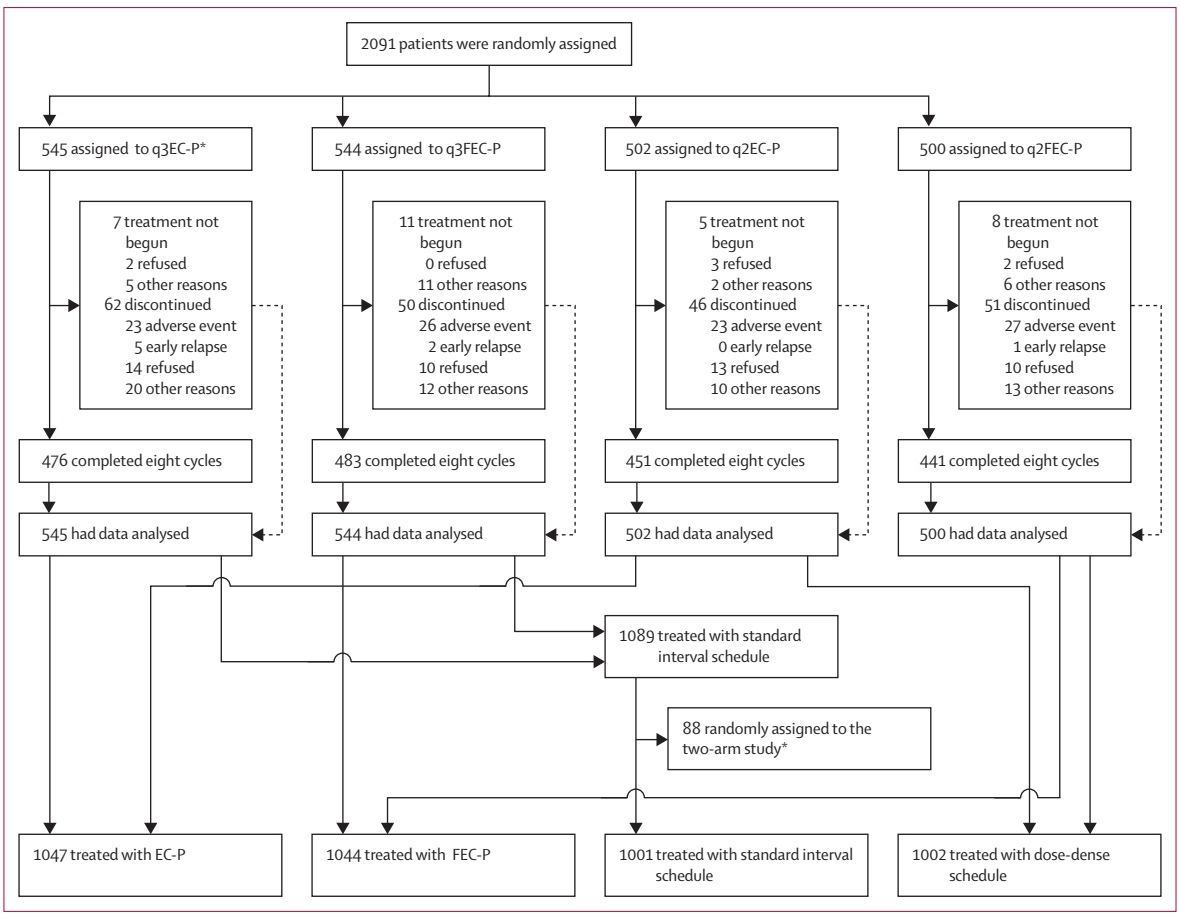


Figure 1: **Trial profile**
q3EC-P=standard-interval chemotherapy with epirubicin and cyclophosphamide followed by paclitaxel. q3FEC-P=standard-interval chemotherapy with fluorouracil, epirubicin, and cyclophosphamide followed by paclitaxel. q2EC-P=dose-dense chemotherapy with epirubicin and cyclophosphamide followed by paclitaxel. q2FEC-P=dose-dense chemotherapy with fluorouracil, epirubicin, and cyclophosphamide followed by paclitaxel. *Five of 81 centres chose to only randomly assign patients to a two-group study (q3FEC *vs* q3EC).

the planned number of at least 635 disease-free survival events.

All statistical analyses were done on an intention-to-treat basis. Safety population included all patients that received at least one dose of any study drug. 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 as previously described.[6] Briefly, the interaction term was included in a Cox model with the two treatment assignments variables and its statistical significance was tested with 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. A stratified log-rank test was used to assess the significance of the differences between the disease-free survival curves. For the comparison between FEC-P versus EC-P, the stratification factor was assignment to 2-week or 3-week dosing, whereas the stratification factor for the dose-dense versus the standard-interval comparison was assignment to EC-P or FEC-P. In the analyses of the comparison between dose-dense and standard-interval schedule, patients enrolled by the five centres that randomly assigned participants only to standard-interval EC-P and standard-interval FEC-P were excluded. Based on median follow-up (exceeding 15 years), disease-free survival and overall survival estimates at 15 years are reported descriptively.

We computed and plotted disease-free survival and overall survival estimates in EC-P and FEC-P groups (adding together patients in the dose schedule groups) and disease-free survival and overall survival estimates in the dose schedule groups (adding together patients in the two drug groups) using the Kaplan-Meier method. Cox models were used to provide unadjusted and adjusted estimates of treatment effects on disease-free survival and overall survival. The proportional hazards assumptions were assessed by visual inspection of the plots of Schoenfeld residuals and by means of the Grambsch-Therneau test. In the unadjusted Cox model, as well as in

the subgroup analyses, stratification for the other comparison was made. The following covariates were included in the multivariable models: age, menopausal status, type of surgery, histological type, tumour size, nodal status, tumour grade, HER2 status, and hormone receptors. Tumour grading was recoded according to the procedure previously described[6] to avoid the exclusion of patients with missing information about the grade of the primary tumours from all multivariable analyses. Analyses were done separately for the FEC-P versus EC-P and for the 2-week and 3-week comparisons.

In the current updated analysis, we did a post-hoc evaluation of invasive breast cancer-free survival, calculated from the date of randomisation to the date of local recurrence, distant metastases, controlateral or ipsilateral breast tumour excluding ductal carcinoma in situ, or death from any cause.[10] Post-hoc subgroup analyses were done according to the interaction test and were based on well known baseline prognostic factors, and no correction for multiple testing was done. For this reason, the results of these analyses are exploratory and any significant results must be considered with caution. Forest plots were used to summarise the results of various subgroup analyses. Post-hoc disease-free survival and overall survival estimates for dose schedule groups according to hormone receptor status were computed and plotted using the Kaplan-Meier method.

To analyse the data according to the modern concept of breast cancer subtypes, we did a post-hoc analysis according to three different subgroups: hormone receptor-positive and HER2-negative, HER2-positive, and triple-negative breast cancer. Moreover, to better identify hormone receptor-positive patients who had the greatest benefit from dose-dense chemotherapy, we did a post-hoc exploratory analysis in this subgroup according to nodal status. A post-hoc analysis was done to evaluate interaction between trastuzumab therapy and the dose-dense effect.

No interim analyses were planned. All reported p values are two-sided and values equal to or less than 0·05 were considered significant. Statistical analyses were done using SAS 9.2. This trial is registered with ClinicalTrials.gov, 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.

Results

Between April 24, 2003, and July 3, 2006, 2091 patients were randomly assigned to treatment (figure 1): 545 to q3EC-P, 544 to q3FEC-P, 502 to q2EC-P, and 500 to q2FEC-P. Of these, 88 (4%) patients were enrolled in the five centres providing only standard interval schedule and then were assigned only to q3FEC-P and q3EC-P. The planned number of chemotherapy cycles was completed by 476 (87%) patients in the q3EC-P group, 483 (89%) in the q3FEC-P group, 451 (90%) in the q2EC-P group, and 441 (88%) in the q2FEC-P group (figure 1). Dose reduction was necessary in 15 (3%) patients in the q3EC-P group, 16 (3%) in the q3FEC-P group, 19 (4%) in the q2EC-P group, and 21 (4%) in the q2FEC-P group; treatment was discontinued for grade 3 or worse toxicity in 23 (4%) patients

	q3EC-P (n=545)	q3FEC-P (n=544)	q2EC-P (n=502)	q2FEC-P (n=500)
Age at randomisation, 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				
Mastectomy	207 (38%)	224 (41%)	204 (41%)	187 (37%)
Lumpectomy	338 (62%)	320 (59%)	298 (59%)	313 (63%)
Histological type				
Ductal	456 (84%)	443 (81%)	389 (77%)	404 (81%)
Lobular	53 (10%)	76 (14%)	65 (13%)	64 (13%)
Other	36 (7%)	25 (5%)	48 (10%)	32 (6%)
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 (2%)	1 (<1%)
Nodal status				
N1	327 (60%)	319 (59%)	319 (64%)	284 (57%)
N2	135 (25%)	136 (25%)	116 (23%)	135 (27%)
N3	83 (15%)	89 (16%)	67 (13%)	81 (16%)
Tumour grade				
G1	30 (6%)	21 (4%)	35 (7%)	30 (6%)
G2	236 (43%)	238 (44%)	225 (45%)	240 (48%)
G3	270 (50%)	266 (49%)	229 (46%)	214 (43%)
Unknown	9 (2%)	19 (3%)	13 (3%)	16 (3%)
HER2 status				
Negative	344 (63%)	332 (61%)	318 (63%)	299 (60%)
Positive	123 (23%)	131 (24%)	105 (21%)	121 (24%)
Unknown	78 (14%)	81 (15%)	79 (16%)	80 (16%)
Hormone receptor status				
Negative	103 (19%)	88 (16%)	83 (17%)	85 (17%)
Positive	420 (77%)	442 (81%)	407 (81%)	401 (80%)
Unknown	22 (4%)	14 (3%)	12 (2%)	14 (3%)
Ki67*				
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 n (%) or median (IQR). 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. *Measured using the Ki67 labelling index.

Table 1: **Baseline characteristics**

in the q3EC-P group, 26 (5%) in the q3FEC-P group, 23 (5%) in the q2EC-P group, and 27 (5%) in the q2FEC-P group. 57 (3%) patients had missing information about the grade of the primary tumours.

2091 patients were included in the intention-to-treat analysis for the comparison of EC-P (1047 patients) versus FEC-P (1044 patients) and 2003 patients were included in the intention-to-treat analysis for the comparison of dose-dense (1002 patients) versus standard interval analysis (1001 patients). Baseline demographic and tumour characteristics are in table 1. Ethnicity data were not collected. Trastuzumab was given to 130 (27%) out of 480 patients with HER2-positive cancer: 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.

The type of endocrine therapy received by hormone receptor-positive patients is reported in the appendix (p 6). As of March 23, 2022, after a median follow-up of 15·1 years (IQR 8·4–16·3), 786 patients (37·6 %) had had a disease-free survival event and 473 (22·6%) had died (table 2). No interaction was seen between the effect of the two randomisation variables (FEC-P *vs* EC-P and dose-dense *vs* standard-interval dosing) on disease-free survival ($p_{interaction}$=0·52) or overall survival ($p_{interaction}$=0·70), making it possible to analyse the two study factors independently.

	q3EC-P (n=545)	q3FEC-P (n=544)	q2EC-P (n=502)	q2FEC-P (n=500)
Disease-free survival events*	205 (38%)	238 (44%)	169 (34%)	174 (35%)
Locoregional relapse	26 (5%)	25 (5%)	21 (4%)	15 (3%)
Distant relapse	116 (21%)	139 (26%)	91 (18%)	93 (19%)
Concurrent locoregional and distant relapse	5 (1%)	7 (1%)	5 (1%)	6 (1%)
Unknown site of relapse	3 (1%)	0	3 (1%)	0
Second primary breast cancer	12 (2%)	27 (5%)	12 (2%)	14 (3%)
Second primary, non-breast cancer	21 (4%)	15 (3%)	15 (3%)	27 (5%)
Death without relapse	22 (4%)	25 (5%)	22 (4%)	19 (4%)
Overall survival events	126 (23%)	150 (28%)	97 (19%)	100 (20%)

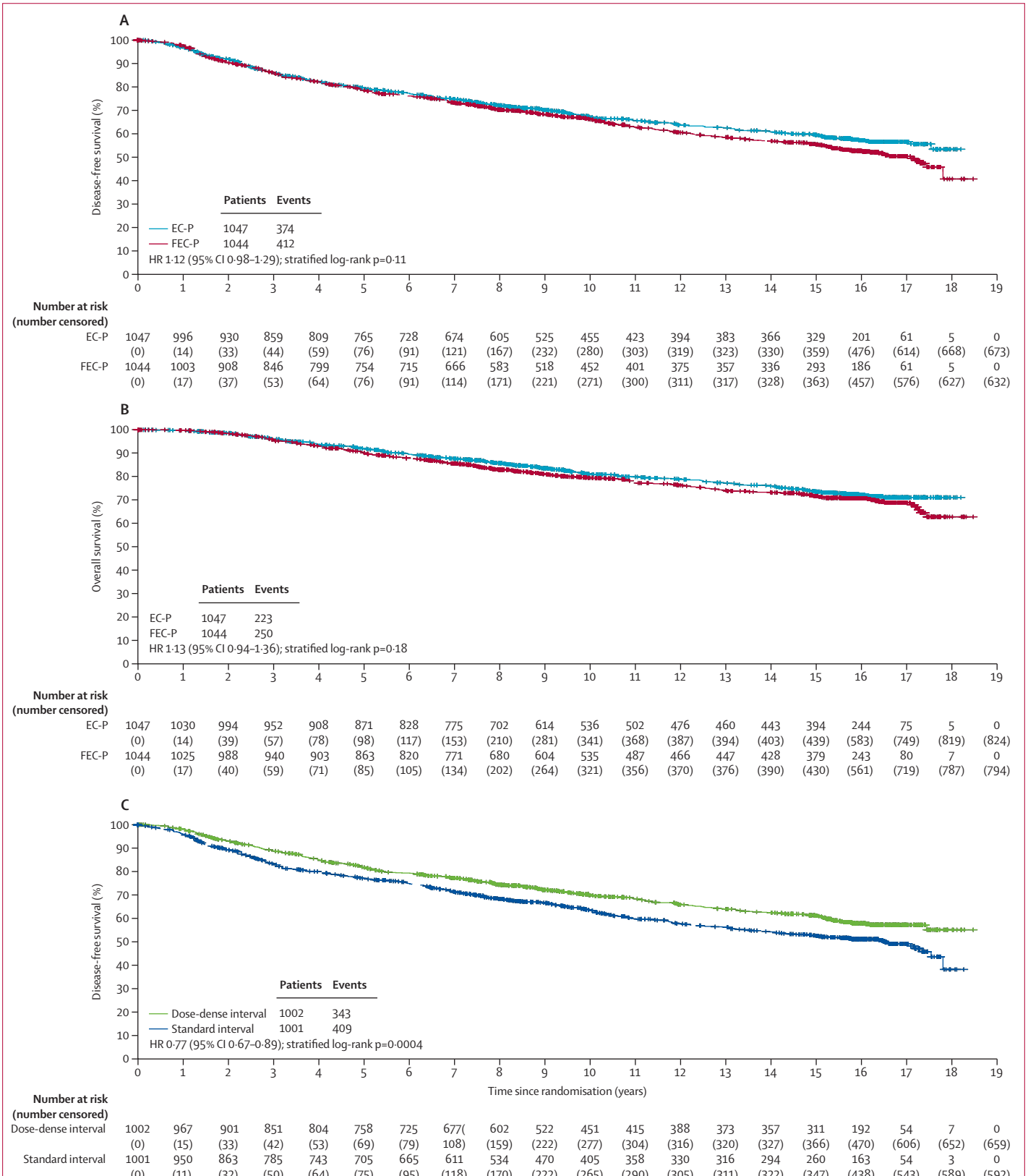
Data are n (%). 17 disease-free survival events in the q3EC-P group and 17 disease-free survival events in the q3FEC-P group were observed among the 88 patients excluded from the ITT analysis for the comparison of dose-dense versus standard-interval chemotherapy. 10 overall survival events in the q3EC-P group and 12 overall survival events in the q3FEC-P group were observed among the 88 patients excluded from the ITT analysis for the comparison of dose-dense versus standard-interval chemotherapy. ITT=intention-to-treat. 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. *Only first events are considered.

Table 2: **Disease-free survival and overall survival events in the ITT population**

412 (40%) disease-free survival events occurred in the two FEC-P groups combined and 374 (36%) occurred in the two EC-P groups combined. No differences were observed in median disease-free survival between the two groups: 17·09 (95% CI 15·51–not reached [NR]) in the FEC-P *vs* NR [17·54–NR] in the EC-P group; unadjusted HR 1·12 [95% CI 0·98–1·29]; log-rank p=0·11; figure 2A). The estimated rates of disease-free survival at 15 years were 55·4% (95% CI 51·8–58·8) in the FEC-P group and 59·4% (56·0–62·8) in the EC-P group. Plot of Schoenfeld residuals is shown in the appendix (p 10). Similarly, no differences were observed in median overall survival (NR [95% CI NR–NR] in the FEC-P *vs* NR [NR–NR] in the EC-P group; figure 2B). The similar outcome between patients assigned to FEC-P and EC-P was supported by post-hoc multivariable analyses, in which the comparisons between FEC-P and EC-P were adjusted for prognostic factors (disease-free survival adjusted HR 1·08 [95% CI 0·94–1·25], p=0·28; overall survival adjusted 1·11 [95% CI 0·92–1·34], p=0·27). The post-hoc analysis for invasive breast cancer-free survival showed no differences between the two groups (appendix p 11). In subgroup analyses, no evidence of interaction was observed between FEC-P or EC-P and any factor, except for nodal status, for which a significant interaction was seen (p=0·01) for disease-free survival only (appendix p 12).

343 (34%) disease-free survival events occurred in the two groups given dose-dense interval chemotherapy and 409 (41%) in the two groups given standard-interval chemotherapy. Median disease-free survival was significantly higher in the dose-dense interval group than the standard-interval group (NR [95% CI 17·45–NR] in the dose-dense interval *vs* 16·52 [14·24–17·54] in the standard interval group]; HR 0·77 [95% CI 0·67–0·89]; p=0·0004; figure 2C). The estimated rates of disease-free survival at 15 years were 61·1% (95% CI 57·5–64·5) in the dose-dense group and 52·5% (48·8–56·0) in the standard interval group. Plot of Schoenfeld residuals is shown in the appendix (p 10). Median overall survival was significantly better in the dose-dense group compared with the standard interval group (NR [95% CI NR–NR] in the dose-dense interval chemotherapy *vs* NR [NR–NR] in the standard interval chemotherapy group; figure 2D). Similar results were observed in the post-hoc multivariable analyses, in which the comparisons between dose-dense and standard-interval schedules were adjusted for prognostic factors (disease-free survival adjusted HR 0·79 [95% CI 0·69–0·92]; p=0·0021; overall survival 0·74 [95% CI 0·61–0·89]; p=0·0018). Results were similar in terms of the post-hoc invasive breast cancer-free survival analysis (appendix p 13).

A similar difference between 2-week and 3-week dosing was observed in subgroup analyses of disease-free survival (figure 3). No significant interaction was



(Figure 2 continues on next page)

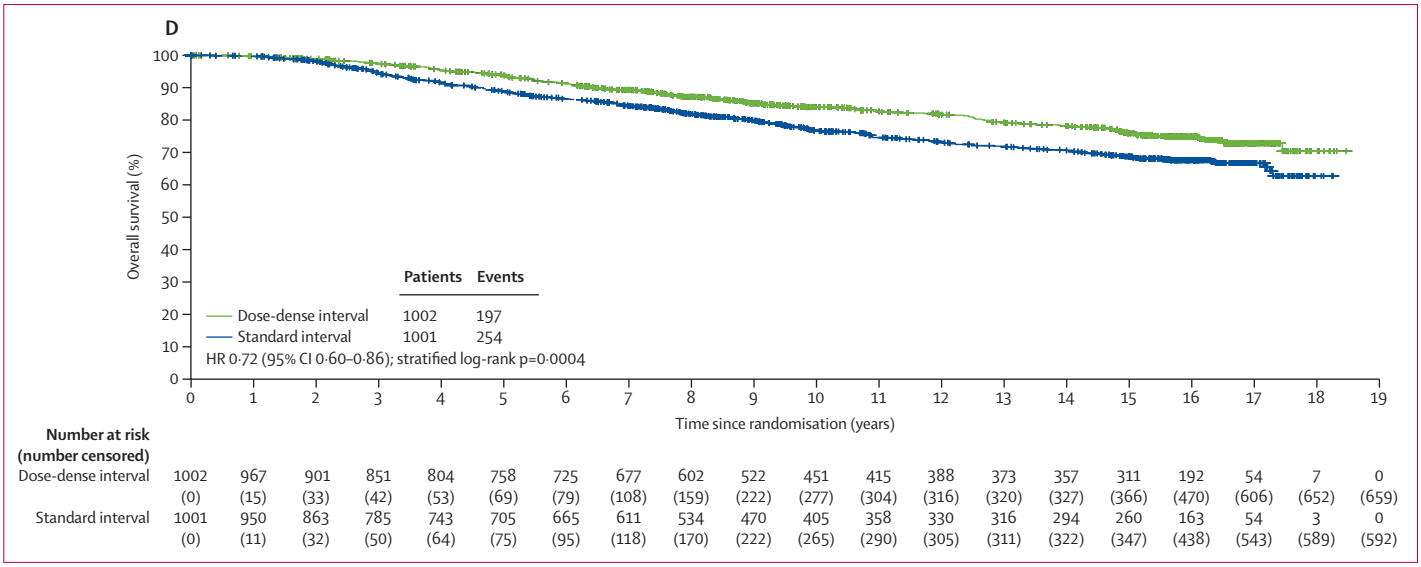


***Figure 2*: Kaplan-Meier estimates of disease-free survival (A) and overall survival (B) in FEC-P and EC-P groups and disease-free survival (C) and overall survival (D) in dose-dense and standard interval chemotherapy groups**

For p value, a stratified log-rank test was used. HR=hazard ratio. EC-P=epirubicin and cyclophosphamide, followed by paclitaxel. FEC-P=fluorouracil, epirubicin, and cyclophosphamide, followed by paclitaxel.

seen between type of regimen and any of the prognostic factors. In a post-hoc analysis, no significant interaction occurred between trastuzumab therapy and the effect of the dose-dense schedule (appendix p 14).

In a post-hoc analysis, we found the effect of a 2-week or 3-week schedule did not differ according to hormone receptor status in terms of disease-free survival (figure 3; appendix p 15). Results of the effect of 2-week or 3-week schedule according to hormone receptor status adjusted for baseline prognostic factors provided similar results (data not shown).

Post-hoc overall survival comparisons according to hormone receptor status are reported in the appendix (p 16). In the post-hoc analyses in the three subtypes defined according to hormone receptor and HER2 status, 319 patients (15%) were excluded due to the unknown status of HER2 or oestrogen and progesterone receptor (appendix p 6). No interaction between schedule regimens (FEC-P *vs* EC-P and 2-weeks *vs* 3-weeks) and tumour subtype was observed (appendix p 7). The analyses of disease-free survival according to the three different subtypes is reported in the appendix (pp 17–22). In the hormone receptor-positive and HER2-negative patients, the effect of 2-week or 3-week schedule according to nodal status is reported in the appendix (p 7).

As previously reported, the most common grade 3–4 adverse events were neutropenia (200 [37%] of 536 patients in the q3EC-P group *vs* 257 [48%] of 533 in the q3FEC-P group *vs* 50 [10%] of 496 q2EC-P *vs* 97 [20%] of 492) and alopecia (238 [44%] *vs* 249 [47%] *vs* 228 [46%] *vs* 235 [48%]; appendix p 8).[6]

Treatment-related serious adverse events were reported in nine (2%) patients in the q3EC-P group (five hospitalised for infection, one paroxysmal supraventricular tachycardia, and three severe allergic reaction), in seven (1%) in the q3FEC-P group (three hospitalised for infection, two severe allergic reaction, one hospitalised for gastrointestinal toxicity, and one hospitalised for viral infection), in nine (2%) in the q2EC-P group (five hospitalised for infection, two severe allergic reaction, one thrombotic event, and one hyperglycemia), and nine (2%) in the q2FEC-P group (four hospitalised for infection, three severe allergic reaction, one paroxysmal tachycardia, and one extravasation of epirubicin). No treatment-related deaths occurred; during extended follow-up, no further grade 3–4 adverse events or deaths related to toxic-effects were reported. 88 patients (11%) died without relapse (table 2). A second primary breast cancer occurred in 65 (3%) patients and a second non-breast tumour in 78 patients (4%; table 2). The type of second primary non-breast cancers is reported in the appendix (p 9).

Discussion

The long-term results of the GIM2 study support findings that, in women with node-positive breast cancer, the addition of fluorouracil to the EC-P regimen does not improve disease-free survival or overall survival, whereas dose-dense chemotherapy significantly improves both disease-free survival and overall survival compared with standard-interval chemotherapy. To our knowledge, GIM2 has the longest follow-up of clinical trials evaluating these two issues, and the findings suggest that fluorouracil should not be included in adjuvant regimens and that dose-dense is highly efficacious in patients with early node-positive breast cancer.

	Dose-dense interval		Standard interval		HR (95% CI)	p value
	Events/patients (%)	15-year disease-free survival (95% CI)	Events/patients (%)	15-year disease-free survival (95% CI)		
Age group, years						0·49
<40	47/128 (36·7%)	56·9 (46·4–60·1)	67/141 (47·5%)	45·8 (36·3–54·8)	0·65 (0·45–0·94)	
40–49	90/305 (29·5%)	68·5 (62·2–73·9)	94/306 (30·7%)	63·2 (56·4–69·2)	0·88 (0·66–1·17)	
50–59	116/343 (33·8%)	60·7 (54·4–66·4)	126/308(40·9%)	54·2 (47·6–60·3)	0·83 (0·64–1·07)	
≥60	90/226 (39·8%)	53·8 (45·9–61·2)	122/246 (49·6%)	41·1 (34·0–48·7)	0·70 (0·53–0·92)	
Menopausal status						0·41
Postmenopausal	196/507 (38·7%)	55·0 (49·8–60·0)	232/507 (45·8%)	47·4 (42·2–52·3)	0·82 (0·68–0·99)	
Premenopausal	147/495 (29·7%)	67·1 (62·2–71·5)	177/494 (35·8%)	58·2 (53·0–63·1)	0·72 (0·58–0·90)	
Type of surgery						0·63
Lumpectomy	194/611 (31·8%)	63·6 (59·0–67·8)	235/614 (38·3%)	54·6 (49·8–59·1)	0·75 (0·62–0·91)	
Mastectomy	149/391 (38·1%)	57·1 (51·2–62·5)	174/387 (45·0%)	49·1 (43·2–54·7)	0·80 (0·65–1·00)	
Histological type						0·96
Ductal	270/793 (34·0%)	60·9 (56·9–64·7)	332/825 (40·2%)	53·1 (49·0–57·0)	0·78 (0·66–0·92)	
Lobular	51/129 (39·5%)	57·3 (47·0–66·4)	57/120 (47·5%)	45·1 (34·5–55·1)	0·77 (0·53–1·12)	
Other	22/80 (27·7%)	68·6 (55·4–78·7)	20/56 (35·7%)	60·0 (43·5–73·1)	0·71 (0·39–1·31)	
Tumour size						0·99
T1	145/515 (28·2%)	67·3 (62·4–71·8)	177/508 (34·8%)	58·9 (53·7–63·7)	0·77 (0·61–0·95)	
T2	164/410 (40·0%)	55·3 (49·7–60·6)	190/414 (45·9%)	47·0 (41·3–52·6)	0·77 (0·62–0·95)	
T3–T4	33/73 (45·2%)	48·7 (34·9–61·2)	42/74 (56·8%)	36·9 (24·7–49·2)	0·74 (0·47–1·17)	
Nodal status						0·47
N1	166/603 (27·5%)	68·3 (63·8–72·4)	187/598 (31·3%)	62·4 (57·6–66·8)	0·84 (0·68–1·03)	
N2	98/251 (39·0%)	54·9 (47·5–61·7)	124/247 (50·2%)	43·3 (36·1–50·3)	0·68 (0·52–0·89)	
N3	79/148 (53·4%)	41·6 (32·5–50·3)	98/156 (62·8%)	28·5 (20·4–37·2)	0·74 (0·55–1·00)	
Tumour grade						0·32
G1	14/77 (18·2%)	73·5 (58·4–83·8)	14/56 (25·0%)	7·0·2 (53·7–81·7)	0·83 (0·40–1·74)	
G2	156/465 (33·5%)	62·0 (56·6–66·8)	163/439 (37·1%)	56·8 (51·1–62·1)	0·88 (0·70–1·09)	
G3	170/453 (37·5%)	58·3 (53·0–63·2)	230/501 (45·9%)	46·6 (41·4–51·6)	0·70 (0·57–0·85)	
HER2 status						0·77
Negative	204/617 (33·1%)	61·9 (57·2–66·2)	256/626 (40·9%)	51·8 (47·1–56·3)	0·75 (0·62–0·90)	
Positive	81/226 (35·8%)	60·7 (53·3–67·3)	91/226 (40·3%)	54·9 (47·2–62·0)	0·85 (0·63–1·15)	
Unknown	58/159 (36·5%)	58·7 (49·6–66·7)	62/149 (41·6%)	51·8 (41·7–60·9)	0·75 (0·52–1·07)	
Hormone receptor status						0·22
Negative	64/168 (38·1%)	58·3 (49·7–66·0)	79/167 (47·3%)	45·5 (36·4–54·1)	0·65 (0·46–0·90)	
Positive	271/808 (33·5%)	61·7 (57·7–65·4)	312/803 (38·9%)	54·6 (50·5–58·6)	0·82 (0·69–0·96)	
Overall	**343/1002 (34·2%)**	**61·1 (57·5–64·5)**	**409/1001 (40·9%)**	**52·5 (48·8–56·0)**	**0·77 (0·67–0·89)**	

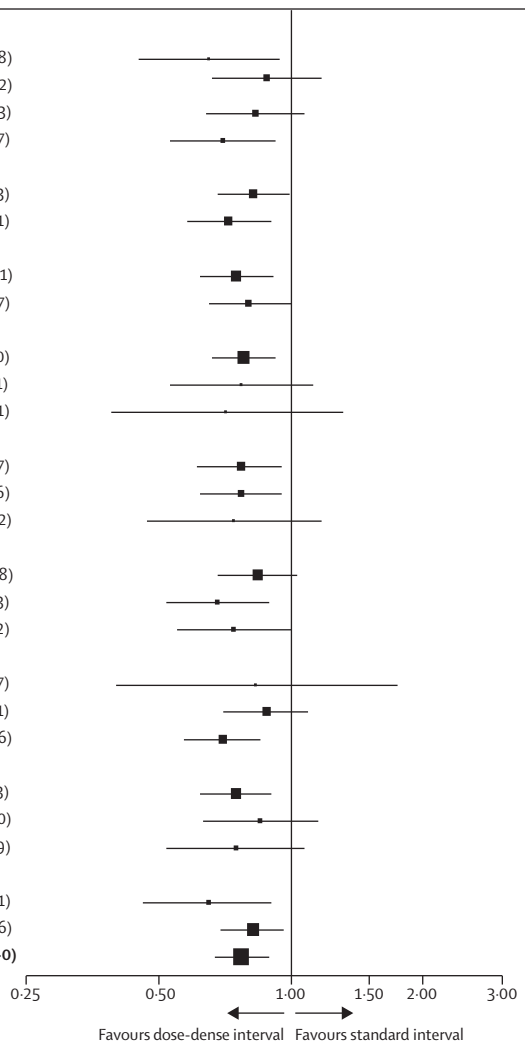


***Figure 3*: Disease-free survival subgroup analyses for dose-dense interval and standard-interval chemotherapy**
HR=hazard ratio. *p value is the test of interaction between treatment and each factor.

The role of fluorouracil in the adjuvant treatment of patients with breast cancer has been scarcely evaluated and both AC plus EC and FEC plus FAC are considered acceptable regimens. GIM2 is the only study comparing two regimens (FEC-P *vs* EC-P) differing only for the presence or not of fluorouracil and shows that fluorouracil should not be added to EC-P regimen because it does not improve efficacy and does increase toxicity.[6] This finding is consistent with the results of the studies evaluating the role of the capecitabine, the orally administered prodrug of fluorouracil. An individual patient-level meta-analysis[11] showed that the addition of capecitabine did not improve the outcome in hormone receptor-positive and HER2-negative breast cancer subgroups. A benefit was observed only in the subgroup of patients with triple-negative breast cancer receiving prolonged duration of treatment by adding sequential capecitabine after standard adjuvant chemotherapy. In our unplanned analysis, none of the breast cancer subtypes showed a benefit from FEC-P as compared with EC-P. Although our study showed that the addition of fluorouracil did not improve the efficacy of the EC-P regimen only, this result raises a general question about the opportunity to include it in other adjuvant chemotherapy regimens, such as FEC followed by docetaxel.[2,3]

The long-term results of our study support the clinically relevant benefit of dose-dense chemotherapy compared with standard-interval regimen: the 15-year disease-free

survival absolute benefit of nearly 9% was higher than that observed at 5 years (5%).[6] Similarly, the 15-year overall survival absolute benefit was 7%, as compared with the 5% benefit at 5 years.

The benefit of dose-dense regimen observed in GIM2 study was consistent with the findings of the Early Breast Cancer Trialists' Collaborative Group meta-analysis, although the magnitude of the benefit was different. In the meta-analysis, the 10-year absolute disease-free survival benefit was 4% in the overall population,[4] and 4% in hormone receptor-negative patients and 3% in the hormone receptor-positive patients. The difference in the benefit magnitude could be related to the differences in the length of the follow-up (7·4 years in the meta-analysis and 15·1 years in the GIM2 study) and in the study populations. Data from both the meta-analysis and GIM2 study also support dose-dense as the preferred regimen in patients with hormone receptor-positive and node-positive disease who are candidates for adjuvant chemotherapy. As previously reported, the appropriate selection of hormone receptor-positive patients who are candidates for dose-dense chemotherapy could be based on additional variables such as the number of lymph nodes, the tumour size, and the Ki-67 value.[12]

As expected, the incidence of second primary non-breast cancer malignancies has increased compared with our previous report (2% at a median follow-up of 7 years and 4% at the present update). This percentage is similar to that observed in the long-term results of the MIG1 study (3·5% at a median follow-up of 15·8 years).[13] Some population-based cancer registry studies[14,15] showed that breast cancer survivors had nearly a 20% increase in the risk of developing a new primary non-breast cancer in comparison with women without cancer. In those studies, a second primary non-breast cancer was observed in 6–7% of patients with an absolute risk of nearly 80 cases per 10 000 person-years. The lower absolute risk observed in the GIM2 study (39·4 cases per 10 000 person-years [data not shown]) is probably due to the loss of the registration of second primary malignancies occurring after breast cancer recurrence: in fact, only second primary malignancies observed as first disease-free survival events were reported. Compared with the general female population in Italy, in which the estimated cancer incidence is 33 cases per 10 000 person-years,[16] the incidence in our study is slightly higher because patients were followed up yearly with clinical and laboratory exams, which could have increased diagnosis of secondary malignancies. Despite these limitations and the potential risk of bias, our data show that some secondary neoplasms (eg, ovarian cancer observed in 6% of patients and pancreatic cancer in 5%) might be related to *BRCA1* or *BRAC2* mutations, although information about the BRCA mutation status was not available in our study. Lung cancer (14 cases [18%] of all second primary non-breast cancers) was the most frequent second cancer observed in our study population. This finding confirms the results of previous studies,[17] but contrasts with the results of a large SEER study in which a decrease in the risk of lung cancer in breast cancer survivors was reported.[18] These conflicting data indicate that longer follow-up of clinical trials are needed to better understand potential long-term consequences of adjuvant treatments, such as radiotherapy, which was hypothesised to be associated with the increased risk of some second malignancies (eg, lung cancer and angiosarcoma of the breast).[19]

Acute toxicity was different in 2-week and 3-week dosing interval chemotherapy, with an increased rate of grade 3–4 anaemia, myalgias, and transaminitis and a decreased rate of neutropenia in the 2-week schedule as compared to the 3-week regimen.[6] In terms of long-term side-effects, our data show similar number of deaths without recurrence and second primary non-breast cancer in all treatment groups. These reassuring findings are supported by the Early Breast Cancer Trialists' Collaborative Group meta-analysis, in which no increase in death without recurrence, cardiovascular mortality, or deaths from haematological malignancies were reported in patients treated with dose-dense chemotherapy.[4] Together with the efficacy data, the safety profile indicates that the balance of benefit versus toxicity appears to favour dose-dense chemotherapy. A further advantage of the 2-week schedule is the shorter duration of the treatment. Moreover, because dose-dense paclitaxel given every 2 weeks is similar in both disease-free survival and overall survival to weekly paclitaxel,[20] and it can be safely administered without pegfilgrastim,[21] it might be preferred to the weekly administration, especially if a reduction in the frequency of hospital admissions is required.[22]

There are some limitations to the generalisability of our long-term results to modern early breast cancer management, mainly based on different subtypes. Since the GIM2 study started almost 20 years ago, analyses were not planned according to the three different subtypes. Moreover, HER2 status might have been assessed by methods other than those currently considered standard. In fact, current international recommendations for HER2 testing were first released in 2007,[23] after the end of the GIM2 patient enrolment and, according to the recommendations available at the time of our study, HER2 status was assessed by institutional assay of any type.[24] Additionally, 318 patients, nearly 15%, had HER2 status unknown and only 130 (27%) out of 480 HER2-positive patients received adjuvant trastuzumab. Our unplanned analyses according to subtypes suggest a benefit of a dose-dense regimen both in patients with triple-negative and hormone receptor-positive or HER2-negative breast cancer; however, due to the limitations above reported, these results should be interpreted with caution. Another limitation of the generalisability of our results is the proportion of patients with four or more positive nodes

(pN≥2]; 842 [40%] of 2091) and the proportion of patients with grade 3 tumours (979 [47%] of 2091); both greater proportions than those in an unselected node-positive population.

Moreover, the current, widely-used schedule of paclitaxel is the weekly administration.[25] However, the dose-dense paclitaxel, administered every 2 weeks, is similar in terms of efficacy to weekly paclitaxel, and can still be considered an acceptable schedule.[3,20] The current standard of endocrine therapy for patients with hormone receptor-positive tumours has somewhat changed, compared with that used at the time of the study design. Therefore, it is not possible to estimate how improved long-term outcomes of dose-dense therapy might change by the use of modern adjuvant endocrine therapy.[26,27]

Cardiac toxicity is a potential late effect of anthracycline-containing chemotherapy that should be weighed against the long-term benefit. A limitation of our long-term data is that no cardiotoxicity data were collected, which hampers the assessment of the balance between costs and benefit of our chemotherapy regimens. Another limitation of our long-term results is the high number of censored patients. Despite the completeness of follow-up, which is important in randomised clinical trials, the equal distribution of censored patients in the treatment groups of our study limits the potential bias in the study results associated with patients lost to follow-up.

Long-term results of the GIM2 study show that fluorouracil should not be added to EC-P regimen because it does not increase the efficacy yet worsens toxicity, whereas dose-dense chemotherapy, as compared with the same regimen administered every 3 weeks, lead to a clinically significant, long-lasting benefit in terms of both disease-free survival and overall survival. Although these results are from an old study, which was not planned according to the modern concept of different breast cancer subtypes, these findings are still useful for optimising adjuvant chemotherapy regimen in patients with breast cancer at high risk of relapse.

Contributors

LDM, SDP, FC, MDL, and LB designed the study and analysed, interpreted, and collected the data. LDM and LB wrote and approved the final report. LDM, EB, and LB contributed to data analysis and interpretation. LDM, FPo, EB, and ML contributed to writing the manuscript. LDM obtained funding and supervised the study. All the authors contributed to data collection, critical revision of the manuscript, and material support. The study was coordinated by Gruppo Italiano Mammella (GIM) group, who were responsible for the study design, randomisation, collection and management of data, medical review, data analysis, and reporting. LDM, FPo, EB, and LB accessed and verified the data. All authors had full access to all the data and had final responsibility for the decision to submit the manuscript for publication.

Declaration of interests

LDM reports grants or contracts from Eli Lilly, Novartis, Roche, Daiichi Sankyo, and Seagen; fees or honoraria from Roche, Novartis, Pfizer, Eli Lilly, AstraZeneca, MSD, Seagen, Gilead, Pierre Fabre, Eisai, Exact Sciences, and Ipsen; support for attending meetings or travel from Roche, Pfizer, and Eisai; and participation on a data safety monitoring board or advisory board from Novartis, Roche, Eli Lilly, Pfizer, Daiichi Sankyo, Exact Sciences, Gilead, Pierre Fabre, Eisai, and AstraZeneca outside the submitted work. FPo reports support for attending meeting from Daichii Sankyo and Gilead; participation on advisory board from AstraZeneca; and fees or honoraria from Eli Lilly and Novartis outside the submitted work. SDP reports honoraria from Roche, Novartis, Pfizer, Celgene, Eli Lilly, AstraZeneca, Clovis, Seagen, Daichii Sankyo, and MSD outside the submitted work. MG reports fees or honoraria from Lilly, Novartis, Pfizer, Roche, Seagen, AstraZeneca, Daichii Sankyo, MSD, and Genomic Health outside the submitted work. MDL reports personal fees from Pfizer, Novartis, Roche, Celgene, AstraZeneca, Eisai, Eli Lilly, Amgen, Pierre Fabre, MSD, Genomic Health, Daiichi-Sankyo, Gilead, and Seagen outside the submitted work. FC reports consulting fees or honoraria from GlaxoSmithKline, Roche, Astrazeneca, Eli Lilly, Pierre Fabre, Pfizer, Seagen, and Dompè outside the submitted work. AF reports consulting fees or honoraria and participation on an advisory board from Roche, Novartis, Lilly, Pfizer, MSD, Dompè, Pierre Fabre, Eisai, Sophos, Epionpharma, Gilead, Seagen, AstraZeneca, and Exact Science outside the submitted work. OG reports consulting fees or honoraria and participation on an advisory board from Daiichi Sanko, AstraZeneca, Seagen, Gilead, Eli-Lilly, and Novartis. MM reports consulting fees or honoraria and participation on an advisory board from Roche, Novartis, Lilly, Pfizer, MSD, Gilead, Seagen, AstraZeneca, and Gentili outside the submitted work. CB reports fees or honoraria from Novartis, Roche, and Eli Lilly outside the submitted work. FPu reports consulting fees or honoraria and participation on an advisory board from Amgen, Astrazeneca, Daichii Sankyo, Celgene, Eisai, Eli Lilly, Gilead, Ipsen, MSD–Novartis, Pierre Fabre, Pfizer, Roche, Seagen, Takeda, and Viatris outside the submitted work; and research grants and support from AstraZeneca, Eisai, and Roche outside the submitted work. FM reports fees or honoraria and participation on an advisory board from AstraZeneca, Eli Lilly, Roche, Novartis, Seagen, Pfizer, MSD, and Daiichi Sankyo outside the submitted work. ML reports consulting fees or honoraria from Roche, AstraZeneca, Novartis, Eli Lilly, Sandoz, and Takeda outside the submitted work. All other authors declare no competing interests.

Data sharing

All of the individual participant data collected during the study, after de-identification, are already shared with Early Breast Cancer Trialists' Cooperative Group. Individual participant data that underlies the results reported in this Article, after de-identification will be available for further sharing. Data will be available beginning 9 months and ending 5 years following Article publication. Data will be shared with researchers who provide a methodologically sound proposal. The types of analyses allowed will be those able to achieve aims in the approved proposal. Proposal should be directed to lucia.delmastro@hsanmartino.it.

Acknowledgments

The GIM group received financial support for trial conduct from Bristol-Myers Squibb and Pharmacia. Bristol-Myers Squibb provided paclitaxel and Dompè Biotech, Italy provided filgrastim. We thank patients, physicians, nurses, and trial coordinators who participated in the GIM2 trial. We acknowledge Giovanni Cucchiara and Carlo Panzano of Clinical Research Technology, Salerno, Italy, for clinical record online management. Simona Pastorino and Annalisa Abate provided all the technical assistance and computer work for statistical analyses. This paper is dedicated to the loving memory of Marco Venturini, leader of the GIM.

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