Dose-dense adjuvant chemotherapy, treatment-induced amenorrhea and overall survival in premenopausal breast cancer patients.
A pooled analysis of the MIG1 and GIM2 studies

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STUDY BACKGROUND

Breast cancer is the more common tumor type arising in women of reproductive age and accounts for more than one quarter of all malignant tumors diagnosed in young patients: every year, more than 25,000 new cases of invasive breast carcinoma are diagnosed in patients under the age of 45 years in the United States [1].

Since young women with breast cancer has an increased risk of presenting with biologically aggressive types of tumors, the majority of young women with early-stage breast cancer are candidates to receive an anticancer treatment that includes the use of chemotherapy [2]. However, to date, there is no evidence to recommend a specific chemotherapy regimen for premenopausal women requiring adjuvant chemotherapy [2]. According to the European Society of Breast Cancer Specialists (EUSOMA) recommendations for the management of young women with breast cancer, the identification of the optimal chemotherapy regimen for young women regarding efficacy and long-term tolerance should be considered a research priority [3].

A possible side effect of chemotherapy in premenopausal patients is the occurrence of treatment-induced amenorrhea [4]. The effects of chemotherapy on ovarian function are variable and are strongly affected by patients’ age at the time of treatment, type and dose of chemotherapy [4]. The most common chemotherapy regimens used in the adjuvant or neoadjuvant treatment of breast cancer are associated with an intermediate risk of developing treatment-induced amenorrhea (40% to 60%), with a higher risk in older women and a lower risk in younger patients [4].

Although still controversial, the development of treatment-induced amenorrhea seems to be associated with improved survival outcomes in premenopausal breast cancer patients [5]. However, the loss of ovarian function negatively impacts on global health of young breast cancer survivors being associated with several side effects, such as hot flashes, sweats, breast pain or sensitivity, vaginal dryness, vaginal discharge, lack of sexual desire, and weight gain [6]. Moreover, strongly associated with the loss of ovarian function is the risk of infertility: fertility issues represent a major concern for young breast cancer patients and can also influence their treatment decisions [7].

Dose-dense adjuvant chemotherapy showed to be associated with improved survival outcomes in high risk breast cancer patients [8,9]. However, no specific data exist on the efficacy of this
schedule in premenopausal women. To date, very limited data, mainly based on small retrospective studies, exist on the impact of dose-dense chemotherapy on the risk of developing treatment-induced amenorrhea [10,11].

Using individual patient data from all the premenopausal patients enrolled in the MIG1 (Mammella InterGruppo 1) and GIM2 (Gruppo Italiano Mammella 2) dose-dense adjuvant trials [12,13], the present pooled analysis aims to better investigate the efficacy of dose-dense schedule in premenopausal women and its impact on the risk of developing treatment-induced amenorrhea.
STUDY AIMS

Primary Objectives:

• To evaluate the efficacy of dose-dense chemotherapy in premenopausal patients.
• To evaluate the impact of dose-dense chemotherapy on the risk of developing treatment-induced amenorrhea.

Secondary Objective:

• To evaluate the prognostic impact of treatment-induced amenorrhea.
PATIENTS AND METHODS

MIG1 study

The MIG1 study is an open-label phase III randomized study conducted within the Gruppo Oncologico Nord Ovest-Mammella InterGruppo (GONO-MIG), from November, 1992 and June, 1997 [12]. In this trial, node-positive or high-risk node negative early breast cancer patients were randomly assigned to treatment arms with the same chemotherapy regimen administered at the same dose (i.e., fluorouracil, epirubicin, and cyclophosphamide [FEC]) but with different intervals between the treatment cycles (i.e., 2 weeks [the FEC-14 arm] or 3 weeks [the FEC-21 arm]).

Eligible patients were women with histologically confirmed breast cancer who had undergone radical mastectomy or breast-conserving surgery in addition to full ipsilateral axillary lymph node dissection, and who had lymph node-positive disease with no more than 10 involved axillary lymph nodes or if they had no involved lymph nodes but did have a high risk of recurrence. A high risk of recurrence was defined as the presence of one or more of the following criteria: age ≤ 35 years, negative hormone receptor status, tumor size ≥ 2 cm, poor histologic grade, and/or a high proliferative rate. Hormone receptor negative status was defined as less than 10 fmol of receptor per milligram of protein or less than 10% positive cells by immunohistochemical analysis; proliferative rate was determined by a [3H]thymidine labeling index or by an S-phase fraction obtained with flow cytometry. Exclusion criteria were: age ≥ 71 years, prior chemotherapy, clinical or radiologic evidence of distant metastases, an inadequate bone marrow, hepatic and renal function, surgery performed ≥ 5 weeks before randomization.

Eligible patients were randomly allocated to receive either six courses of intravenous FEC-21 (5-fluorouracil at 600 mg/m2, epirubicin at 60 mg/m2, and cyclophosphamide at 600 mg/m2 on day 1, with 21 days between cycles) or six courses of intravenous FEC-14 (the same drugs at the same doses as in FEC 21 arm but with 14 days between cycles and with the support of filgrastim). Filgrastim was self administered by patients subcutaneously, at a dose of 5 µg/kg of body weight/day, from day 4 through day 11 of each cycle.

Patients with hormone receptor positive tumors received tamoxifen at 20 mg/day for 5 years.
Postoperative regional radiotherapy limited to the remaining breast was given to patients who received breast-conserving surgery.

Premenopausal status was defined by the presence of active menses in the prior 6 months before random assignment. Chemotherapy-induced amenorrhea was defined by the absence of menstrual activity for at least 3 months during chemotherapy or within 3 months after the end of chemotherapy.

The study was conducted at 21 Italian centers in accordance with the International Good Clinical Practice principles and local ethical and regulatory requirements. The trial was approved by the internal review board of the coordinating center, the National Cancer Research Institute in Genoa, Italy. Written informed consent was obtained from all patients before enrollment.

**GIM2 study**

The GIM2 study is a multicenter, open-label, randomized phase III trial, with a 2x2 factorial design conducted within the Gruppo Italiano Mammella (GIM), from April, 2003 to July, 2006 [13]. The study was conducted in women with node positive early breast cancer who were randomly assigned to 4 treatment arms with the aim to investigate the role of the addition of fluorouracil to the same anthracycline- and taxane-based chemotherapy regimen administered at the same dose (i.e., epirubicin and cyclophosphamide [EC] followed by 3-weekly paclitaxel [P]) and of a different interval between the treatment cycles (i.e., 2 weeks [dose-dense] or 3 weeks [standard-interval]).

Eligible women were those who 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 between 18 and 70 years, Eastern Cooperative Oncology Group performance status ≤ 1, normal organ and bone marrow functions, and use of adequate contraception methods for potentially fertile women. Exclusion criteria were: male patients, clinical or radiologic evidence of distant metastases, inflammatory carcinoma, metastasis in ipsilateral supraclavicular lymph nodes, past or current history of ipsilateral or controlateral
invasive breast carcinoma, pregnant or lactating women or women of pregnancy potential, previous malignancies at other sites (with exception of adequately treated cone-biopsied in situ carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin), symptomatic peripheral neuropathy > grade 2 according to the National Cancer Institute Common Toxicity Criteria, recent acute myocardial infarction, congestive heart failure or serious arrhythmia. Patients had to be randomly assigned within 5 weeks of the date of their last surgery. Hormone receptor positive tumors were defined by a finding of at least 10% of positive cells by immunohistochemical analysis. HER2 positive tumors were defined by a finding of at least 10% of tumor cells with HER2 protein expression assessed by an immunohistochemistry assay or by a positivity of an in-situ hybridisation assay.

Eligible patients were randomly allocated 1:1:1:1 to one of the following 4 study arms: 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); 2) 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); 3) 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); 4) dose-dense FEC-P regimen, with the same doses and drugs as the q3FEC-P group, but administered every 2 weeks (q2FEC-P). Patients enrolled in the dose-dense arms also received subcutaneous pegfilgrastim (6 mg) 24-72 hours after chemotherapy.

After completion of chemotherapy, patients with hormone-receptor-positive tumors received endocrine therapy. After the approval of adjuvant trastuzumab for HER2 positive early breast cancer, an amendment, in April, 2006, required trastuzumab treatment for 1 year after the completion of chemotherapy for all patients with HER2 positive disease. 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.

Premenopausal status was defined in the protocol as regular menses in the 12 months prior to randomization, or premenopausal FSH levels (≤ 35 mIU/ml) in patients undergoing hormone
replacement therapy. The same definition of post-menopausal status specified in the protocol was used to define chemotherapy induced-amenorrhea (i.e. amenorrhea ≥ 12 consecutive months after the end of chemotherapy).

The study was conducted at 81 Italian centers in accordance with the International Good Clinical Practice principles and local ethical and regulatory requirements. The GIM group coordinated the study and was responsible for the study design, randomisation, collection and management of data, medical review, data analysis, and reporting. The trial was approved by ethics committees of all participating institutions. Written informed consent was obtained from all patients before enrollment.

**Present analysis (pooled analysis of the MIG1 and GIM2 studies)**

For the purpose of the present analysis, individual patient data from all premenopausal patients enrolled in the MIG1 and GIM2 studies will be retrieved.

In the MIG1 study, a total of 1,214 patients were enrolled, 604 in the dose-dense arm and 610 in the standard-interval arm. A total 528 (43.5%) women were premenopausal at study entry and will be included in the present analysis: 267 (44.2%) in the dose-dense arm and 261 (42.8%) in the standard-interval arm.

In the GIM2 study, for the purpose of the present analysis, dose-dense FEC-P and dose-dense EC-P arms will be considered together (i.e. “dose-dense arm”), as well as standard-interval FEC-P and standard-interval EC-P (i.e. standard-interval arm”). A total of 2,091 patients were entered, 1,002 in the dose-dense arm and 1089 in the standard-interval arm. A total of 1,021 (48.8%) women were premenopausal at study entry and will be included in the present analysis: 495 (49.4%) in the dose-dense arms and 526 (48.3%) in the standard-interval arms.
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STATISTICAL METHODS

MIG1 study

The primary study endpoint was overall survival. Secondary endpoints included event-free survival and toxicity. Overall survival was estimated from the date of randomization to the date of last contact or death from any cause; for event-free survival, the events were local relapse, distant relapse, second primary cancer, or death from any cause, whichever came first. Adverse events were graded according to World Health Organization (WHO) criteria [14].

The primary hypothesis of the study was that dose-dense chemotherapy (FEC-14) would be associated with a 20% relative reduction in the hazard of death. This reduction corresponded to a 5-6% absolute increase in 5-year survival, which was estimated to be between 65-70% in the control group. Thus, it was estimated that for a type I error level of 0.05 and 80% power, a total of 700 patients per arm over a 4-year period were required to be enrolled. All analyses were conducted according to the intention-to-treat principle.

GIM2 study

The two primary comparisons were between FEC-P and EC-P, and between dose-dense and standard-interval chemotherapy. The primary 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 (excluding ductal carcinoma in situ), second primary malignancy, death from any cause, and loss to follow-up or end of study, whichever came first. 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 graded according to the National Cancer Institute common toxicity criteria version 2.0 [15].

The power calculations was based on the assumption of a 5 year disease-free survival of about
75% in the control group (q3EC-P); with the experimental treatments (either comparison: EC-P vs FEC-P and dose-dense vs standard-interval chemotherapy), it was assumed to gain a 20% relative reduction in disease-free survival, corresponding to a 4.4% absolute increase in 5-year disease-free survival. To detect this difference with an 80% power and a type I error level of 0.05 (two-sided), 2,000 patients with an average follow-up of 5.5–6 years were planned to be enrolled with a final analysis after 635 events. In March, 2013, 10 years after the enrollment of the first patient, in view of the fact that the total number of disease-free survival events was less than 500, the Steering Committee of the study, decided to proceed with the final analysis of the study. On the basis of the final number of events observed at the closing date (521 events; May 9, 2013), the power to detect the target 20% relative reduction in disease-free survival was decreased to 72%, whereas an 80% power was available for a hazard ratio of 0.785. All analyses were conducted according to the intention-to-treat principle.

**Present analysis (pooled analysis of the MIG1 and GIM2 studies)**

The present analysis will be conducted according to the present protocol after approval of the members of the Steering Committee of both studies.

The primary objectives of the present pooled analysis are to investigate the efficacy of dose-dense chemotherapy in premenopausal patients and to assess the impact of dose-dense chemotherapy on the risk of developing treatment-induced amenorrhea. The primary study endpoints are overall survival and incidence of treatment-induced amenorrhea comparing between patients treated with dose-dense chemotherapy and women receiving standard-interval chemotherapy. The secondary objective is to determine the prognostic effect of the occurrence of treatment-induced amenorrhea among all premenopausal patients enrolled in the studies. The secondary study endpoint is overall survival comparing between patients who developed treatment-induced amenorrhea and women who did not develop treatment-induced amenorrhea, irrespectively of the study arm.

As the two studies were not designed to the same primary objectives and showed some peculiar characteristics (i.e. different median follow-up time, different types of adjuvant treatment administered [e.g. type and dose of chemotherapy, taxanes, trastuzumab, luteinizing hormone-
releasing hormone analogues and aromatase inhibitors] and treatment duration [e.g. 6 or 8 cycles of chemotherapy], although patients individual data are available, a meta-analysis approach without joining the two datasets will be used. This analysis would allow to assess the heterogeneity between studies and to consider whether it is proper to provide the weighted average of the parameters estimated from the two studies as summary indicators of effect.

Median period of follow-up and its interquartile range will be calculated in each trial for the entire study cohort according to the reverse Kaplan-Meier method. Overall survival will be computed as the time interval between the date of randomization and the date of death from any cause or loss-to follow-up. Observation times of patients known to be alive without events at the last follow-up will be censored. Overall survival probability will be computed according to the Kaplan-Meier method; confidence intervals of survival time probability will be calculated according to the log-log method. As estimates of treatment effect, hazard ratio with 95% confidence intervals will be calculated with the Cox proportional hazards model adjusting by age, number of lymph nodes, grading and hormone receptors.

The risk of developing treatment-induced amenorrhea will be computed by applying the logistic regression model to the binary variable presence/absence of amenorrhea. The definition of treatment-induced amenorrhea used in each of the two studies will be used. Chemotherapy-induced amenorrhea was defined by the absence of menstrual activity for at least 3 months during chemotherapy or within 3 months after the end of chemotherapy in the MIG1 study, and as amenorrhea ≥ 12 consecutive months after the end of chemotherapy in the GIM2 study. Odds ratio with 95% confidence intervals will be calculated to estimate the treatment effect adjusting by age, number of lymph nodes, grading and hormone receptor status. To adjust for guarantee-time bias, a conditional landmark analysis may be considered [16].

Individual patient data for all premenopausal women enrolled in the MIG1 and GIM2 will be used to calculate all the study endpoints separately in each of the two studies. A meta-analysis of the two studies will be then performed; the random effect model will be used to calculate the parameter estimates [17]. This is a special form of linear regression model,
where $\ln(ES_i) = \mu + \beta_i + \tau_i$

Subgroup analyses of overall survival will be performed through a meta-regression model and the consistency of the treatment effect on the outcome according to hormone receptor status (positive and negative) will be assessed by means of the t-test statistic with the modification suggested by Knapp and Hartung [18].

All reported statistical analyses will be based on the study intention-to-treat population. All statistical tests will be 2-sided, and p values less than 0.05 will be considered statistically significant.
REFERENCES


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