

***In Vivo* Biological Effects of Pegfilgrastim after Myelosuppressive Chemotherapy in Breast Cancer**

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Abstract. *Background:* No exhaustive data are available on the *in vivo* biological effects of pegfilgrastim utilized in dose-dense chemotherapy (CT). The cytokinetic effects exerted in a multicyclic CT program by this cytokine on CD34+/38+ peripheral blood (PB) progenitor cells was the focus of this study. *Patients and Methods:* PB samples from 19 breast cancer patients treated with 4 courses of docetaxel and epirubicin followed by pegfilgrastim were studied. The absolute number of CD34+/38+ circulating progenitor cells (CPCs) along with the percentage undergoing G0/G1, S and G2-M phases of the cell cycle or showing apoptotic features, were evaluated at baseline, after the first and before the fourth CT course using a dedicated flow cytometric technique. *Results and Conclusion:* Pegfilgrastim, after CT, exerted stimulatory effects on the cell cycle status of PB CD34+/38+ CPCs, at the same time protecting them from apoptosis. This was particularly evident 7 days after administration and tended to decrease one week later, without additional cytokinetic changes during the subsequent CT courses.

The dose and schedule of chemotherapy (CT) play a crucial role in the outcome of patients with chemosensitive tumors, affecting the success of the treatment (1, 2). In breast cancer, shortening the time interval between each cycle while maintaining CT agents at full dose resulted in significant improvements in disease-free and overall survival in patients with node-positive disease (3, 4). The dose-dense regimens were made feasible with the primary use of colony stimulating factors (CSFs), which prevent or reduce the incidence of febrile neutropenia (FN) and FN-related complications.

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The development of pegfilgrastim (a recombinant granulocyte colony-stimulating factor, G-CSF, engineered by attaching a polyethylene glycol molecule to the G-CSF protein and characterized by a unique pharmacokinetic and pharmacodynamic profile) and its validation in clinical trials, have shown the efficacy of this long-lasting new cytokine in supporting dose-dense CT programs. In particular, once-per-cycle dosing of pegfilgrastim reduced the incidence and duration of severe neutropenia related to myelosuppressive regimens, allowing planned dosing and timing of CT in breast cancer patients (5).

The most appropriate way to schedule and combine the administration of the hematopoietic cytokines with CT is critical for obtaining the most effective clinical result. Hematopoietic precursors are recruited into a proliferative state when a CSF is utilized after CT and, being rapidly proliferating at the beginning of the next CT course, could become sensitive to cycle-active agents. At the same time, several CT drugs are able to induce progressive apoptosis in target cells which leads to a deficit of functioning.

To date, no detailed information is available on the proportion of circulating progenitor cells (CPCs) that proliferate, remain quiescent or undergo apoptosis after *in vivo* treatment with CT followed by pegfilgrastim.

From a biological standpoint, one of the concerns of the present study was to better understand and define the possible cytokinetic sensitivity of the CD34+/38+ peripheral blood (PB) progenitor cell subsets, as well as the possible protection from CT-induced apoptosis by pegfilgrastim administration after myelosuppressive CT administered with a short cycle interval.

Patients and Methods

A biological study was performed in breast cancer patients not previously exposed to CT. The patients were treated with docetaxel (80 mg/m², day 1) + epirubicin (75 mg/m², day 1), followed by a single dose per cycle of pegfilgrastim (6 mg s.c. on day 2). This CT schedule was applied according to a clinical trial approved by the Ethical Committee of our Institution for patients with locally advanced disease and was repeated every 14 days for a maximum of

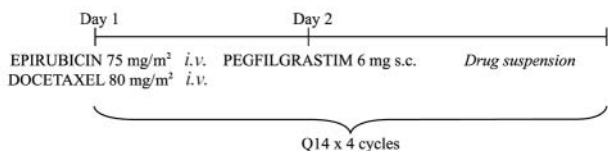


Figure 1. Dose and schedule of CT + pegfilgrastim administration.

4 courses (Figure 1). Each patient gave her informed consent according to our institutional guidelines.

The cell preparation and CD34+/38+ cell purification, as well as the cell cycle studies with DNA flow cytometry, were performed utilizing the methods detailed in our previous studies in which specific techniques were adapted in order to define the functional and kinetic characteristics of human hematopoietic progenitors mobilized in the blood by the association of CT drugs and CSFs (6-9).

A 10 cc PB sample, collected during the routine biochemical monitoring of each patient according to the clinical protocol. The mononuclear cells were isolated by a density gradient and the CD34+/38+ subset was separated by an immunomagnetic procedure (Milteny Biotech, Bergisch Gladbach, Germany). Annexin V expression was quantified at the single cell level and correlated with the cell cycle phases (DNA content on the flow cytometric (FCM), profile obtained after staining with propidium iodide) in this cell subset. The high resolution FCM analysis was then performed by a dual-colour assay using Coulter XL FCM equipment. The results were expressed as means ± SD and the Student's *t*-test for paired data was used to verify the probability of significant differences between the means.

Results

Starting from January 2004, 19 patients were enrolled in the clinical study and their clinical characteristics are displayed in Table I. The median age was 47 years (range 36-64); the majority of patients were in the IIIB stage and the WHO performance status was 0 in all patients.

All the patients completed treatment. Seventeen patients underwent surgery, 1 progressed (soft tissue), 1 refused surgery, while 3 had breast conserving surgery (15%). Objective response was obtained in 18/19 patients and a partial pathological response was achieved in 16/19 cases. A total of 77 treatment cycles were given, 4 per patient, except for one patient who received 5 cycles because surgery was not available at the necessary time.

There were no treatment delays or CT dose reductions.

Grade 3/4 neutropenia without fever occurred in two patients after the first cycle, no other grade 3/4 hematological toxicities were observed. Main non-hematological grade 2 toxicities were: nausea and vomiting (9/19 patients), fatigue (8/19 patients) mucositis (6/19 patients) and alopecia (all patients), no grade 3/4 non-hematological toxicity was recorded.

Table I. Clinical features of the 19 patients enrolled in the clinical study.

Age	
Median	47
Range	34-64
WHO Performance Status	
0	19
Menopausal status	
Premenopausal	11
Post/perimenopausal	8
Oestrogen receptor before treatment	
Positive	15
Negative	4
Progesterone receptor before treatment	
Positive	12
Negative	7
Clinical stage	
IIB	6
IIIA	2
IIIB	11
Histology	
Ductal	14
Inflammatory	1
Lobular	3
Other	1

For the flow cytometric analysis, PB samples from 18 patients before their first and fourth courses of CT were utilized.

At baseline (day 0), the mean number of CD34+/38+ cells/Bl was 47 (30-65), the percentage of the CD34+/38+ CPCs in S-phase was 8.9±4 while 3.1±3% of this cell subset showed apoptotic features.

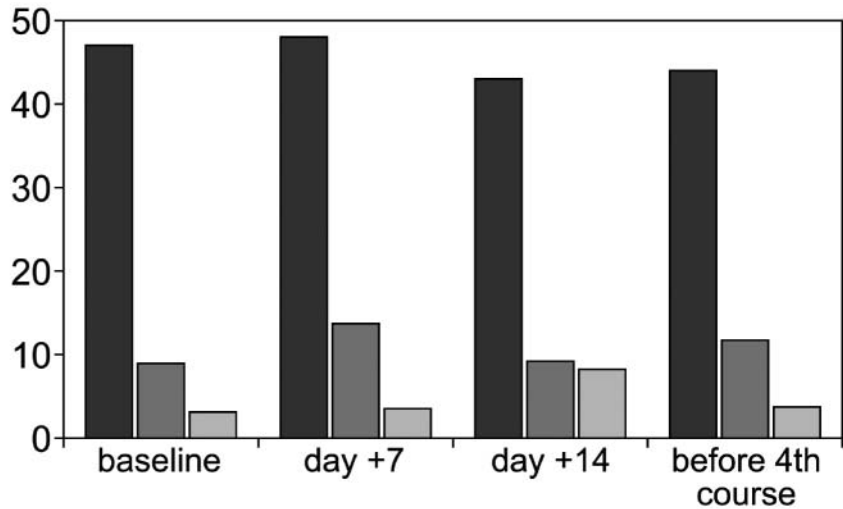
Seven days after CT + pegfilgrastim, the mean number of CD34+/38+ cells/Bl was 48 (25-78) while on day 14+ it was 43 (24-69) (*p* not significant). At the same time-point, the percentage of CD34+/38+ CPCs in S-phase was 13.7±4 while 3.5±3% of this cell subset showed apoptotic features.

One week later, at the time of the second CT course, these values were 9.2±3% (*p*<0.01) and 8.2±2%, (*p* not significant), respectively.

At the start of the fourth CT course, the mean number of CD34+/38+ cells/Bl was not significantly different: 44 (range: 23-68) (*p* not significant). At this time, the percentage of CD34+/38+ CPCs in S-phase was 11.7±3 (*p* not significant), while 3.7±2% (*p* not significant) of this cell subset showed apoptotic features (Figure 2).

Discussion

A rapid and functionally complete hematopoietic recovery is essential during multicyclic dose-dense CT programs and CPCs play a key role in this process. Whether the clinical



■ CD34+/38+ x 10 ³ /ml absolute number	47	48	43	44
■ Proliferative activity (S-phase %) of CD34+/38+ cells	8.9	13.7	9.2	11.7
■ % Apoptosis of CD34+/38+ cells	3.1	3.5	8.2	3.7

Figure 2. Cytokinetic results on CPCs (see text).

efficacy of CPCs alone could account for a quantitative restoration by itself or the different combination CT schedules followed by CSFs could induce relevant functional changes in the CPC population is still not completely clear (10).

In particular, one concern is that the proliferative status and the proportion of CPCs undergoing apoptosis in response to exposure to the combination of CT and CSFs (especially when utilized in a dose-dense approach) could affect the degree of hematopoietic recovery before the next planned CT course (11).

Our data indicate that the combination of docetaxel and epirubicin at standard dosages followed by pegfilgrastim exerted some stimulatory effects on the cell cycle status of PB-derived CD34+/38+ hematopoietic progenitors, protecting them at the same time from apoptosis. This was particularly evident 7 days after pegfilgrastim administration, tending to decrease one week later. No significant cumulative detrimental effects on these biological characteristics, in particular on the percentage of apoptotic progenitor cells, were evident during the whole course of CT.

These findings could be taken into account when different types of dose-dense combination CT programs are planned with pegfilgrastim support.

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