



## Mobilization of peripheral blood progenitor cells after induction chemotherapy (THP-doxorubicin-vinorelbine-cyclophosphamide-fluorouracil) and granulocyte colony-stimulating factor in breast cancer

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### Summary:

In order to evaluate the mobilization of peripheral blood progenitor cells (PBPC) after an effective induction regimen in breast cancer, we performed a study on 15 breast cancer patients. Between January 1995 and June 1996, these patients received TNCF (THP-doxorubicin, vinorelbine, cyclophosphamide, fluorouracil for four days, every 21 days) with G-CSF support (5 µg/kg for 10 days after chemotherapy) to reduce aplasia. This regimen is known to result in a complete pathological response in 30% of patients. Between two cycles of TNCF treatment, hematological recovery was observed. Progenitor cells (CFU-GM and CD34<sup>+</sup> cells) and mononuclear cells in DNA synthesis (MCDS) counts were performed daily, between the 12th and 17th post-chemotherapy days (81 samples). The results showed a similarity for hematological recovery and PBPC mobilization kinetics depending on the number of treatment cycles. The three methods used for PBPC evaluation were well correlated ( $P < 0.01$ ) with an optimal mean PBPC recruitment by the last day of G-CSF administration: respectively, 11 520 (1729–26 539) CFU-GM/ml of blood, 249 (14–1160) CD34<sup>+</sup> cells/µl of blood and 211 (21–554) MCDS/µl of blood. These results suggested that a daily injection of G-CSF after one or two TNCF cycles will produce an effective PBPC mobilization in comparison with currently used regimens.

**Keywords:** breast cancer; TNCF induction chemotherapy; hematological recovery; PBPC mobilization

Since 1990, in order to increase the response rate, and based on our previous AVCF (doxorubicin, vincristine, cyclophosphamide, fluorouracil),<sup>6</sup> we developed a new cytotoxic regimen,<sup>7</sup> using a combination of THP-doxorubicin, vinorelbine, cyclophosphamide and fluorouracil (TNCF). Considering its lower cardiac toxicity, the analogue THP-doxorubicin replaced the mother compound<sup>8</sup> and vinorelbine replaced vincristine because of its high response rate in metastatic breast cancer<sup>9</sup> (40% response as first-line treatment). This schedule was applied at the maximally tolerated dose with colony-stimulating factor (CSF) support to reduce the anticipated neutropenia. For the first 50 patients treated in the primary setting this regimen induced a severe, but not life-threatening, hematological toxicity as expected, but also resulted in a high complete clinical (51%) and pathological response (30% vs <10% with most standard protocols<sup>10,11</sup>). This protocol was also used in young metastatic patients for its efficacy.

We postulate that this efficient TNCF regimen could reach optimal efficacy with an increasing dose. However, in this case, isolated CSF administration would not be sufficient for hematological recovery and further support by autologous peripheral blood progenitor cells (PBPC) would be necessary.<sup>12–14</sup>

Chemotherapy, with or without the addition of CSF, has been used successfully to mobilize progenitor cells into the peripheral blood of cancer patients.<sup>15–17</sup> The most frequently used drugs to recruit PBPC were cyclophosphamide plus or minus etoposide and cisplatin.<sup>18,19</sup> Etoposide is not a classical drug for breast cancer. Therefore, it appeared interesting to mobilize with TNCF treatment, whose efficacy and specificity in breast cancer has been demonstrated, although a study of its mobilization capacity has not been done. Here, we performed a study of the kinetics of mobilization of PBPC and the regeneration of more mature cells in peripheral blood after TNCF. We also studied whether PBPC mobilization was sufficient to allow subsequent autografts.

For this purpose, in 15 patients treated by TNCF regimen, PBPC mobilization was studied for six days, beginning when leukocytes were higher than  $1.0 \times 10^9/l$  of blood. Analysis included leukocyte, red blood cell and platelet counts. PBPC were evaluated by the colony-forming units granulocyte-macrophage assay (CFU-GM), CD34<sup>+</sup>

Over the past two decades, systemic chemotherapy has been used as primary treatment in locally advanced or inflammatory breast cancer making breast-conserving surgery possible.<sup>1,2</sup> Furthermore, complete clinical or pathological response after induction treatment results in a better individual prognosis in terms of disease-free and overall survival.<sup>3–5</sup>

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cell and mononuclear cell in DNA synthesis (MCDS) counts.

## Patients and methods

### Patients

Between January 1995 and June 1996, 15 breast cancer patients with poor prognosis were included in this study. Twelve had primary (neo)adjuvant treatment and three were metastatic. Patient characteristics are shown in Table 1. The median age of all patients was 42 years (range 33–62).

### Treatment schedule

Patients were treated by 3-week cycles of TNCF (THP-doxorubicin 20 mg/m<sup>2</sup> day one to day three, vinorelbine 25 mg/m<sup>2</sup> day one and day four, cyclophosphamide 300 mg/m<sup>2</sup> and fluorouracil 400 mg/m<sup>2</sup> day one to day four) and received colony-stimulating factor support (r-metHuG-CSF, filgrastim; Amgen (Thousand Oaks, CA, USA) or glycosylated rHuG-CSF, lenograstim; Rhône-Poulenc Rorer, Anthony, France), 5 µg/kg once daily, for an average of 10 days from the end of chemotherapy until the end of aplasia (>10 × 10<sup>9</sup> leukocytes/l of blood). Treatment management is defined in Table 1. With regard to chemotherapy given to patients during this study, the dose intensity was 97% for THP-doxorubicin, 97% for vinorelbine, 96% for cyclophosphamide and 97% for 5-fluorouracil. Filgrastim was given to 11 patients and lenograstim to four patients. The median duration of G-CSF administration was 10 days (range 10–15).

**Table 1** Patient characteristics and treatment management

	No. patients	Percentage
Clinical characteristics		
Breast cancer stage		
2a	2	13
2b	7	47
3a	1	7
3b	2	13
4	3	20
Scarff–Bloom–Richardson grading		
II	6	40
III	7	47
not done	2	13
Treatment management		
TNCF chemotherapy for		
Adjuvant treatment	1	7
Neoadjuvant treatment	11	73
Metastatic disease	3	20
Duration of G-CSF administration		
10 days	9	60
11 days	5	33
15 days	1	7
PBPC evaluation after TNCF regimen		
first cycle	11	73
second cycle	2	13.5
fifth or sixth cycle	2	13.5

*Mature cells and PBPC evaluations:* Leukocyte, red blood cell (RBC) and platelet counts were performed before chemotherapy and daily from the start of aplasia until the next cycle. When white blood cells (WBC) reached  $1.0 \times 10^9/l$  of blood, CFU-GM, CD34<sup>+</sup> cells and MCDS were evaluated daily until two days after the last G-CSF injection. Median time of PBPC evaluation was 5 days (range 4–7). Mononuclear cell (MNC) samples were taken after Ficoll–Hypaque density gradient centrifugation ( $d = 1.077$ ). Eighty-one samples from 15 patients were analyzed.

*Colony-forming units granulocyte–macrophage assay:* 20 000 MNC in defined culture medium (Myelocult-StemCell Technology, Vancouver, Canada) were cultured in triplicate in semi-solid medium (Methocult-StemCell Technology, Vancouver, Canada) supplemented with rhGM-CSF (100 ng/ml; molgramostim, Novartis, Rueil Malmaison, France), rhG-CSF (100 ng/ml, filgrastim, Amgen) and rhIL-3 (100 ng/ml, SDZ ILE 964, Novartis). Plates were incubated for 14 days in a humidified atmosphere (37°C, 5% CO<sub>2</sub> in air). CFU-GM were scored using an inverted microscope. The count of circulating progenitor cells per milliliter of blood was determined by multiplying their frequency in culture (for  $20 \times 10^3$  MNC) with the absolute MNC count in the same sample of peripheral blood.

*MNC expressing the surface CD34 antigen:* CD34 was identified by direct and indirect immunofluorescence. Direct immunofluorescence used QBEnd10 (IOM-34-FITC, Immunotech, Marseille, France) and a mixture of QBEnd10, Immu-133 and Immu-409 (POOL-34-RPE, Immunotech) monoclonal antibodies (Mabs). Indirect immunofluorescence used MY10 (HPCA-1, Becton Dickinson, Le Pont de Chaix, France), 8G12 (HPCA-2, Becton Dickinson), TUK-3 (TUK-3, Dako, Trappes, France) MAb. CD34<sup>+</sup> cells were the percent of CD45<sup>+</sup> cells using Immu-19.2 (CD45) and RMO52 (CD14) Mabs (CD45-FITC/CD14-PE Opticlone; Immunotech) and compared to isotype control stains. Mouse IgG1 and IgG2b (Becton Dickinson) were used as appropriate isotypic controls. Cells were stained according to a previously published procedure.<sup>19</sup> After incubation, labeled cells were analyzed by flow cytometry.

*Cell cycle analysis:* As previously described,<sup>19</sup> cell cycle analysis for MCDS determination was done after propidium iodide labeling of mononuclear cells.

*Flow cytometry analysis:* Flow cytometry analysis was performed using an EPICS XL analyzer (Coulter, Miami, FL, USA). Fluorescence attributable to FITC, PE and PI was determined using excitation by an argon laser operating at 488 nm. The acquisition gate included the entire mononuclear cell population and excluded the polymorphonuclear population. A minimum of 50 000 events was acquired in list mode for each sample.

Data analysis for CD45 and CD34 evaluations used Immuno four software. The percent of staining was calculated in comparison to the appropriate isotype control. For each DNA histogram, analysis of the cell cycle distribution used the Multicycle Software program (Phoenix, Flow Sys-

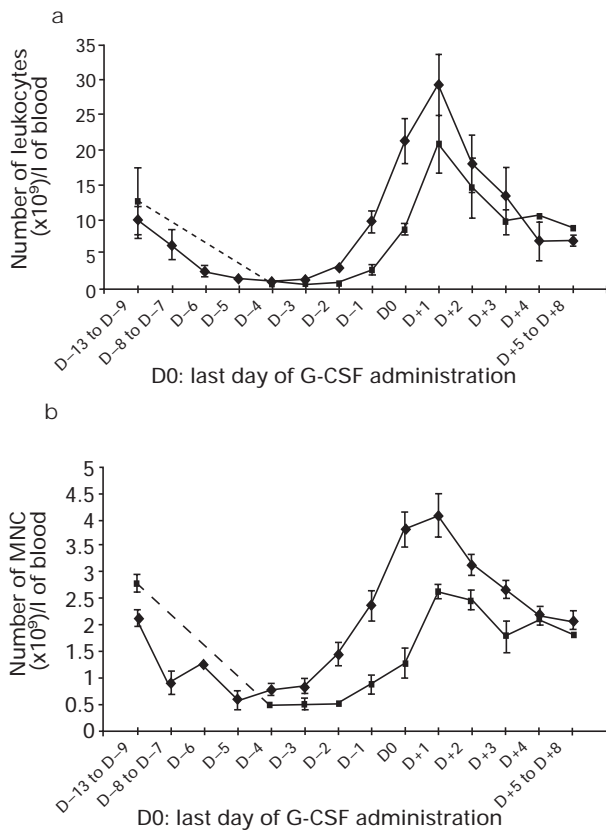
tems, San Diego, CA, USA). Circulating progenitor cells (CD34<sup>+</sup> cells and mononuclear cells in cycle) per microliter of blood was determined by multiplying their frequency by the MNC count in the same blood sample. Circulating CD34<sup>+</sup> cells, MCDS and CFU-GM were expressed as an absolute count per  $\mu\text{l}$  or ml of peripheral blood.

**Expression of results and statistical analysis:** Due to a different timing of recovery, the last day of G-CSF administration was dependent on medical prescription with a median of 10 days. Results were expressed according to the last day of G-CSF administration, namely D0 (day 0). The relationships between CFU-GM, CD34<sup>+</sup> cells and MCDS values were evaluated by their correlation coefficient using the EXCEL software program.

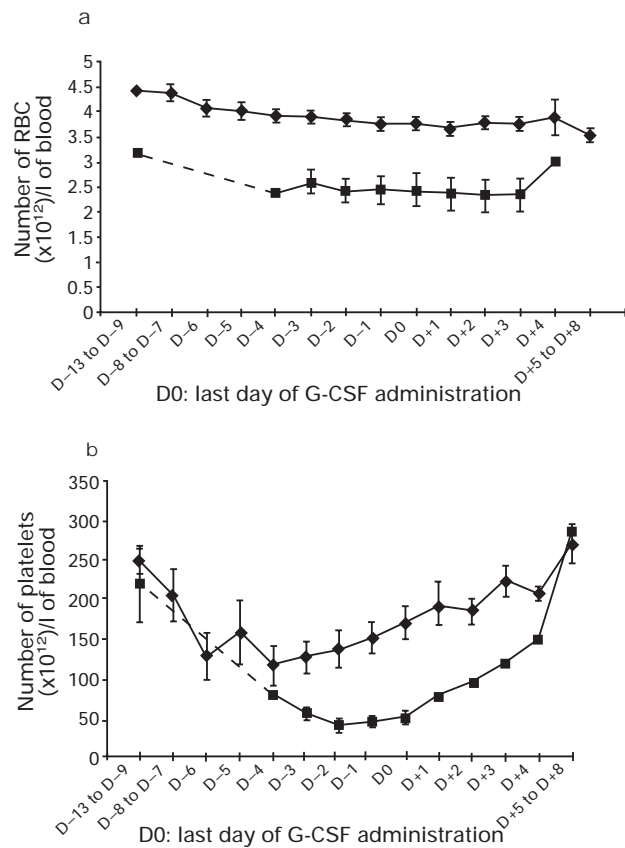
## Results

### Hematological recovery

In this study, we evaluated 15 patients (Figures 1 and 2): 11 patients after the first cycle of TNCF regimen, two patients after the second cycle, and two patients after the 5th or 6th cycle of chemotherapy (Table 1). For all patients,



**Figure 1** Hematological (leukocyte (a) and mononuclear cell (b)) recovery after TNCF cycle according to the last day of G-CSF administration (mean  $\pm$  standard error of the mean). ◆, one or two TNCF cycles; ■, five or six TNCF cycles, (D - 13 to D - 9): the start of TNCF cycle, (D0): the last day of G-CSF administration and (D + 5 to D + 8): the next start of TNCF cycle.



**Figure 2** Hematological (red blood cell (a) and platelet (b)) recovery after TNCF cycle according to the last day of G-CSF administration (mean  $\pm$  standard error of the mean). ◆, one or two TNCF cycles; ■, five or six TNCF cycles, (D - 13 to D - 9): the start of TNCF cycle, (D0): the last day of G-CSF administration and (D + 5 to D + 8): the next start of TNCF cycle.

WBC nadir occurred systematically 11 days after the initiation of chemotherapy (generally three days before the last G-CSF administration or D - 3) and its maximal duration was usually 3 days (range 2-6). The median value of WBC nadir was  $0.7 \times 10^9/\text{l}$  of blood and did not differ according to the number of TNCF cycles. For all patients, WBC recovery  $>1.0 \times 10^9/\text{l}$  of blood occurred on median day 12 (range 12-13) after the initiation of chemotherapy (ie two days before the last G-CSF administration or day - 2). The maximum mean value of WBC ( $28.2 (10.6-63.1) \times 10^9/\text{l}$  of blood) (Table 2) was obtained 15 days after the initiation of chemotherapy (ie 1 day after the last administration of G-CSF or D + 1). Afterwards, WBC values progressively decreased until the first day of the next cycle of TNCF chemotherapy ( $6.9 (3.0-10.5) \times 10^9/\text{l}$  of blood). The kinetics of recovery slowed with increasing TNCF cycles such that for the two patients studied after five or six cycles, the maximum WBC mean value obtained on D + 1 was  $20.8 (16.6-25.0) \times 10^9/\text{l}$  of blood.

WHO grade three thrombocytopenia occurred in six of 15 patients (40%). Anemia was less frequent, only one patient had a WHO grade three toxicity.

**Table 2** Hematological recovery and mobilization of PBPC evaluated by CD34 expression (anti-HPCA-1, anti-HPCA-2, anti-TUK-3, anti-IOM-34, anti-POOL34 Mabs), MCDS and CFU-GM according to the last day of G-CSF administration after TNCF chemotherapy

<i>D0: last day of G-CSF administration</i>	<i>D - 2</i>	<i>D - 1</i>	<i>D 0</i>	<i>D + 1</i>	<i>D + 2</i>	<i>D + 3</i>
Median post-chemotherapy day (range)	D12 (D12–D17)	D13 (D13–D18)	D14 (D14–D19)	D15 (D15–D20)	D16 (D16–D17)	D17 (D17–D18)
Leukocytes ( $\times 10^9/l$ blood)	3.31 2.90 (1.10–6.30)	9.52 8.00 (2.00–18.60)	19.55 16.20 (4.20–45.60)	28.23 25.00 (10.60–63.10)	17.46 14.15 (8.40–53.40)	13.70 9.60 (5.80–40.40)
MNC ( $\times 10^9/l$ of blood)	1.50 1.48 (0.54–2.56)	2.31 2.37 (1.00–4.19)	3.45 3.55 (0.91–6.35)	3.87 3.29 (2.18–7.33)	2.99 2.89 (2.27–4.15)	2.423 2.276 (1.482–3.636)
HPCA-1 CD34 <sup>+</sup> cells/ $\mu l$ blood	30 21 (2–95)	108 52 (2–460)	230 126 (12–1259)	200 158 (32–546)	126 91 (32–310)	85 48 (4–291)
HPCA 2 CD34 <sup>+</sup> cells/ $\mu l$ blood	33 24 (2–110)	103 49 (4–385)	249 142 (11–1341)	219 162 (20–600)	142 111 (19–315)	96 57 (6–302)
TUK-3 CD34 <sup>+</sup> cells/ $\mu l$ blood	27 22 (1–80)	97 42 (4–402)	227 127 (8–1259)	186 149 (44–469)	130 102 (19–321)	94 68 (8–309)
IOM-34 CD34 <sup>+</sup> cells/ $\mu l$ blood	25 14 (1–120)	97 43 (1–498)	255 133 (12–1083)	162 105 (14–523)	116 87 (19–351)	90 59 (13–269)
POOL-34 CD34 <sup>+</sup> cells/ $\mu l$ blood	30 20 (1–117)	115 52 (6–515)	249 97 (14–1160)	195 157 (37–463)	128 113 (5–310)	78 61 (6–240)
MCDS/ $\mu l$ blood	37 26 (9–101)	98 76 (9–465)	211 145 (21–554)	188 133 (33–452)	106 84 (18–223)	69 30 (16–171)
CFU-GM/ml blood	3591 3971 (48–7871)	8229 5928 (300–30 767)	11 520 9461 (1729–26 539)	11 232 9309 (1973–42 610)	7717 5453 (1814–18 720)	6291 4438 (653–18 907)

Results (mean, median, range) are expressed as an absolute count/l,  $\mu l$  or ml of blood.

### Kinetics of PBPC mobilization

CD34<sup>+</sup> cells were evaluated by five antibodies (Table 2), each recognizing a different class of CD34 antigen. The values of CD34<sup>+</sup> cells from two days before (D - 2) to three days after (D + 3) the last day of G-CSF administration were similar no matter which antibody was used, and were positively correlated ( $r = 0.92$  to  $0.99$ ,  $P < 0.01$ ). Consequently, in the following results, the selected antibody for CD34<sup>+</sup> expression was the anti-Pool CD34 antibody, which recognized the three classes of CD34 antigen.

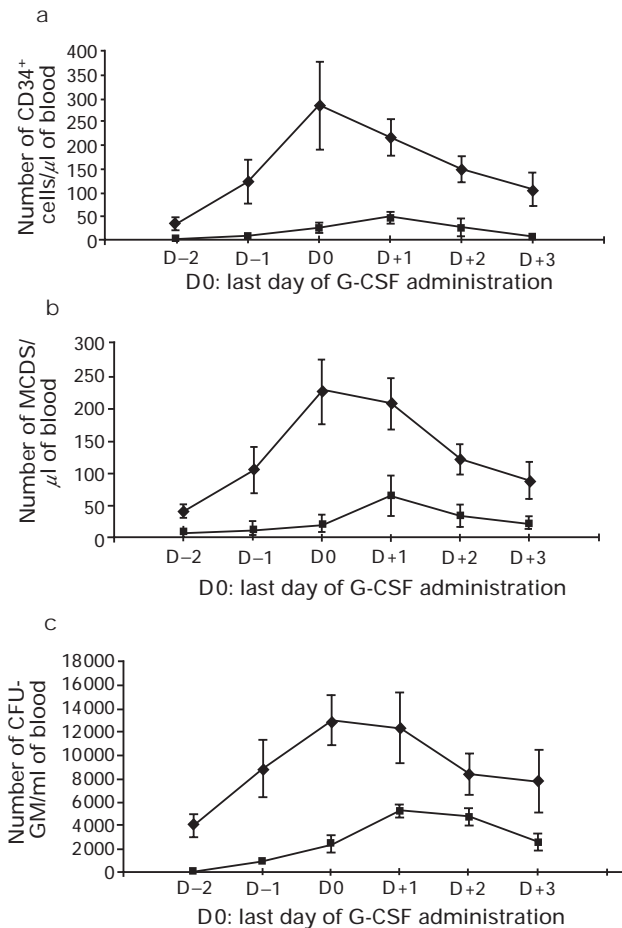
We evaluated the PBPC recruitment after TNCF chemotherapy by quantity of CD34<sup>+</sup> cells, mononuclear cells in DNA synthesis phase and CFU-GM. The profile of PBPC kinetics from D - 2 until D + 3 was similar with the different methods of evaluation. A positive correlation ( $P < 0.01$ ) was observed between these three indicators. Thirteen out of 15 patients were studied after one or two TNCF cycles. On D - 2, the numbers of CFU-GM, CD34<sup>+</sup> cells and MCDS were respectively 4026 (48–7871)/ml of blood, 34 (4–117)/ $\mu l$  of blood and 41 (17–101)/ $\mu l$  of blood. These values increased a median three- and eight-fold over the D - 2 baseline values, on D - 1 and D0. The maximum value of progenitor cells (Figure 3) was obtained the last day of G-CSF administration (ie median 14 post-chemo-

therapy day, range 14–19): 12 943 (2822–26 539) CFU-GM/ml of blood, 219 (28–1160) CD34<sup>+</sup> cells/ $\mu l$  of blood and 226 (26–554) mononuclear cells in DNA synthesis/ $\mu l$  of blood. As soon as the PBPC stimulation by G-CSF stopped, a progressive decrease in PBPC was observed until the end of the study. However, as shown in Figure 3, the recruitment of PBPC was strongly dependent on the number of TNCF cycles whatever the PBPC indicator. Indeed, the PBPC recruitment was about 10-fold less in patients receiving five or six TNCF cycles compared to the patients receiving one or two courses of mobilizing chemotherapy.

### Discussion

PBPC have been used with increasing frequency as an alternative to autologous bone marrow, and more recently in addition with CSF, to support hematopoietic recovery after high-dose chemotherapy.<sup>20</sup> The advantages of this modality were fewer malignant and contaminating cells,<sup>21</sup> and a more rapid recovery of leukocytes and platelets.<sup>22,23</sup>

We describe here a new approach to recruit PBPC with TNCF induction chemotherapy. TNCF has been found to be efficient in breast cancer therapy, although it induces



**Figure 3** PBPC (CD34<sup>+</sup> cells (a), MCDS (b), CFU-GM (c)) mobilization after TNCF cycle according to the last day of G-CSF administration (mean ± standard error of the mean). ◆, one or two TNCF cycles; ■, five or six TNCF cycles.

severe myelotoxicity. In a previous study, TNCF neoadjuvant treatment has been evaluated in 50 breast cancer patients.<sup>7</sup> An objective clinical response was observed for 43 patients with 26 complete (51%) and 18 partial (37%) responses. After pathological review, 11 patients (22%) were devoid of any tumor cells and four others (8%) had only *in situ* carcinoma; whereas neoadjuvant chemotherapy generally results in tumor disappearance on pathological examination, in less than 10% of patients.<sup>10,11</sup> For this treatment, growth factor administration has been particularly effective to reduce hematological toxicity.<sup>7</sup> From 253 completely evaluated cycles, WHO grade IV toxicity occurred in 167 cycles (66%) for leukopenia, 17 cycles (7%) for thrombocytopenia and 10 cycles (4%) for anemia. Neutropenia was associated with febrile episodes (without septic shock or toxic death) in 54% of patients during cycle 1. Therefore, the TNCF regimen induced a severe but not life-threatening hematological toxicity and resulted in a good pathological complete response rate (30%) compared to the standard protocols.<sup>10,11</sup> This higher complete pathological response resulted in a better survival with 72% disease-free survival and 90% overall survival at five years. There is a trend towards a better outcome in patients whose tumors showed a complete pathological response.<sup>3-5</sup>

In order to obtain better efficacy of this regimen, the dose of TNCF drugs could be increased. However, since the limiting factor of TNCF treatment is the hematological toxicity, PBPC support would be required. Several protocols exist for PBPC recruitment, mainly cyclophosphamide (or ifosfamide)<sup>24</sup> and etoposide<sup>19,25</sup> and/or cisplatin<sup>18</sup> plus G-CSF, but induction chemotherapy can likewise be used in order to obtain both mobilizing and specific cytotoxic effects at the same time. To test this idea, Van der Wall<sup>26</sup> and D'Hont<sup>27</sup> mobilized PBPC with fluorouracil, epidoxorubicin and cyclophosphamide (FEC) chemotherapy associated with G-CSF in high-risk breast cancer.

In our study, we wanted to know the PBPC mobilization capacity of TNCF treatment which has excellent clinical efficacy. To reach this goal, we had to evaluate PBPC mobilization after TNCF chemotherapy and G-CSF administration by CFU-GM assay, CD34<sup>+</sup> cells and MCDS counts.

For all our patients, a short period of aplasia (generally three days) was followed by a rapid hematological reconstitution, with a PBPC rebound occurring on median day 14 after the start of chemotherapy. Kinetics of PBPC mobilization were studied from two days before, to three days after, the last G-CSF administration and showed a maximum median level of PBPC on the last day of G-CSF injection: 9461 CFU-GM/ml of blood and 97 CD34<sup>+</sup> cells/μl of blood (Table 2). These results were similar to other protocols of PBPC mobilization (Table 3).<sup>13,28,29</sup>

We found that the magnitude of the PBPC peak seemed to be related to the number of TNCF cycles. For the two patients studied after five or six TNCF cycles, the PBPC recruitment was 10-fold lower than for patients evaluated after one or two cycles of TNCF chemotherapy. Consequently, since several TNCF cycles led to a somewhat exhausting effect on the bone marrow, it would be better to collect PBPC after the first or second cycle of the TNCF regimen. This finding is in line with Venturini *et al*<sup>29</sup> showing a decrease of PBPC after multiple cycles of chemotherapy.

In conclusion, after one or two cycles of TNCF, the level of progenitor cells in peripheral blood was sufficient to allow leukapheresis. Indeed, in a preliminary study, PBPC from another nine patients treated for metastatic breast disease with the same protocol were collected by leukapheresis. A total of 25 leukaphereses was performed at a median day 15 (range 13–18) after the first or second cycle of TNCF chemotherapy. Median levels of CD34<sup>+</sup> cells and CFU-GM harvested, respectively  $7 \times 10^6/\text{kg}$  and  $124 \times 10^4/\text{kg}$ , were similar to those obtained with different mobilization protocols (Table 4).<sup>24–28,30</sup> These prior results showed sufficient collection of PBPC for autologous transplantation.

Finally, our results suggest that daily subcutaneous injection of G-CSF after TNCF regimen will produce effective PBPC mobilization in comparison with standard regimen mobilization. However, it is uncertain that mobilization chemotherapy and G-CSF alone is adequate to mobilize 'stem cells' which reconstitute long-term hematopoiesis and immune functions. It remains to be seen whether after chemotherapy, a combination of different colony-stimulating factors, ie GM-CSF plus G-CSF would release the most effective stem cells.<sup>31,32</sup>

**Table 3** Results of CD34<sup>+</sup> cells and CFU-GM in peripheral blood with different mobilization regimens in breast cancer

Authors	Mobilization regimen	Patient number	CD34 <sup>+</sup> cells/ $\mu$ l of blood	CFU-GM/ml of blood
Vanàsek J <sup>28</sup>	Cyclophosphamide, epirubicin and G-CSF	16	644* (312–1344)	14 423* (1050–29 340)
Van Hoef M <sup>13</sup>	Fluorouracil, adriamycin, cyclophosphamide + G-CSF	24	29.82 (0.6–77.9)	2840 (53–21 436)
Venturini M <sup>29</sup>	Fluorouracil, epirubicin, cyclophosphamide + G-CSF	12	256 (45–951)	2223 (1935–2683)
Our institution, unpublished data	Cyclophosphamide, etoposide and G-CSF	20	76 105* (12.7–512)	3259 4720* (182–17 423)
Our institution	TNCF and G-CSF (D0)	15	97 249* (14–1160)	9461 11 520* (1729–26 539)

Values are median (\*mean) range.

**Table 4** Results of leukapheresis products with different mobilization regimens in breast cancer (median, range)

Authors	Mobilization regimen	Patient number	CD34 <sup>+</sup> cells $\times 10^6$ /kg	CFU-GM $\times 10^4$ /kg
Cameron DA <sup>24</sup>	Cyclophosphamide and G-CSF	28	Not done	48.5 (9–191)
Vanàsek J <sup>28</sup>	Cyclophosphamide, epirubicin and G-CSF	16	4.93 (0.36–10.54)	21.8 (0.7–42)
Richman C <sup>25</sup>	Cyclophosphamide, etoposide and G-CSF	6	3.6 (2.4–7.4)	155 (45–240)
Rosti G <sup>30</sup>	Epirubicin and G-CSF	29	12.9 (3.9–48.1)	117.7 (34.5–479)
Van der Wall E <sup>26</sup>	Fluorouracil, epirubicin, cyclophosphamide + G-CSF	31	10.2 (0.7–25.1)	110 (9–419)
D'Hont L <sup>27</sup>	Fluorouracil, epirubicin, cyclophosphamide + G-CSF	20	12.2 (0.9–44.6)	82 (6–403)
Our institution unpublished data	Cyclophosphamide, etoposide and G-CSF	20	3.6 (0.4–46.9)	110 (9–483)
Our institution unpublished data	TNCF and G-CSF (D0)	15	7.3 (2–20)	124 (44–684)

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