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Clinical trial

Timely withdrawal of G-CSF reduces the occurrence of thrombocytopenia during dose-dense chemotherapy

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Summary

Background. Post chemotherapy Granulocyte colony stimulating factor (G-CSF) reduces leucopenia, while G-CSF priming shortly before chemotherapy increases myelotoxicity. We performed a trial with a two-schedule crossover design to determine the optimal G-CSF schedule for densified 2-weekly chemotherapy.

Methods. During 2-weekly chemotherapy days 1 and 2, G-CSF was given on days 3–10, with a G-CSF-free interval before the next chemotherapy cycle of 5 days, or on days 3–13, with a G-CSF-free interval of 2 days. In schedule A, cycle II was preceded by a 5 days, cycle III and IV by a 2 days and cycle V by a 5 days G-CSF free interval. In schedule B, this was 2, 5, 5, and 2 days, respectively.

Results. Intra-patient comparison for cycles II versus III and cycles IV versus V showed that platelet (PLT) nadir count was significantly lower for cycles preceded by a 2-days compared to a 5-days G-CSF free interval: mean difference 45.7×10^9/l (95% CI 33.2–58.2, $p = 0.0001$). Neutrophil count did not differ significantly ($p = 0.85$).

Conclusion. Timely withdrawal of G-CSF in dose-dense chemotherapy reduces chemotherapy-related thrombocytopenia. Leucopenia was not aggravated, reflecting a protective effect of post-chemotherapy G-CSF.

Background

Granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF) have shown to be effective in reducing both the severity and duration of chemotherapy-induced neutropenia [1]. The availability of these growth factors has facilitated the study on dose-escalation and dose-densification of chemotherapy [2, 3]. In general, it is advised to give G(M)-CSF for an average of 10 days, starting 24 h after the end of chemotherapy [4].

Upfront administration of hematopoietic growth factors has also been studied. This is based on the idea of expanding the bone marrow progenitor pool before the administration of cytotoxic medication [5]. However, GM-CSF and G-CSF have different 'priming'-effects. While both growth factors result in an increase in numbers of bone marrow progenitor cells, there is an abrupt decline in number of progenitor cells in S-phase after discontinuing GM-CSF administration, while the progenitor cells are still rapidly proliferating for 2–4 days after cessation of G-CSF [6–8]. This explains the clinical observation, that upfront priming with GM-CSF – before the administration of the first chemotherapy cycle – is myeloprotective [9, 10], while upfront administration of G-CSF – till 48 h before the delivery of chemotherapy – only aggravates the myelosuppression [5, 11, 12].

These observations have not fully been appreciated in the design of dose-densification studies or guidelines on the use of hematopoietic growth factors. In the reported 2-weekly chemotherapy regimens G-CSF was continued until shortly before the next chemotherapy cycle, since post chemotherapy G-CSF was administered for the usual 10 days. And, in some of these studies myelotoxicity was increased in these densified treatment arms [13–16]. We hypothesized that the negative 'priming' effect of post chemotherapy G-CSF in the prior cycle may have caused the excess myelotoxicity on the subsequent cycle. In case we would be able to proof this hypothesis, the clinical benefit of post chemotherapy G-CSF can be enhanced by increasing the window of time between interruption of the G-CSF and re-initiation of the next chemotherapy cycle to 5 days, that is beyond the aforementioned 2–4 days of G-CSF-induced bone marrow proliferation. Therefore, the present

study was undertaken to determine whether a shortened regimen of daily G-CSF administration in relation to 2-weekly chemotherapy was beneficial.

Patients and methods

Study design

The here reported study on the optimal G-CSF schedule during 2-weekly chemotherapy was part of a larger phase II study in which the efficacy of a 2-weekly chemotherapy regimen, delivered on days 1 and 2 of each cycle, will be determined.

To evaluate the effect of two different G-CSF schedules on hematological recovery, post chemotherapy G-CSF was started at day 3 and administered for a total of 8 days (days 3–10) or for a total of 11 days (days 3–13). Patients acted as their own control, and were treated either according to schedule A or according to schedule B (Figure 1). So, there were no comparisons between different patients. In schedule A, the duration of G-CSF administration for cycle I through V was: 8, 11, 11, 8 and 8 days, respectively, with a G-CSF free interval preceding cycle II through V of 5, 2, 2 and 5 days, respectively. In schedule B, the duration of G-CSF administration for cycle I through V was: 11, 8, 8, 11 and 11 days, respectively, with a G-CSF free interval preceding cycle II through V of 2, 5, 5 and 2 days, respectively. Intra-patient comparisons on peripheral blood cell counts were done for cycle II versus cycle III and for cycle IV versus cycle V. Of note, for these coupled cycles the duration of G-CSF treatment was the same, while the G-CSF free interval before the chemotherapy administration was the only difference: 5 versus 2 days or 2 versus 5 days.

Twenty-six patients were registered to participate in the G-CSF-scheduling-part of the study. To be assessable, patients had to receive at least 3 chemotherapy cycles with at least 2 comparator cycles (cycles II and III and ideally also IV and V), provided that the time frame was precisely followed (2-weekly chemotherapy and G-CSF according schedule A or B) and provided that the comparator cycles were delivered at the same dosage. As a result, 11 patients were not assessable: 1 patient went off study after only 2 cycles, 1 patient died after 1 cycle, 4 patients did not receive G-CSF according to schedule (doctor's mistake) and in the remaining 5 patients none of the cycles were comparable due to dose-reduction and/or delay for several reasons.

Patient selection

Patients with locally advanced or metastatic breast cancer aged between 18 and 70 years with a life expectancy of over 3 months and an ECOG performance status 0–2 were considered eligible. Prior hormonal treatment was allowed as well as radiotherapy, if recovered from acute toxicity and no more than 20% of the total bone marrow compartment was involved. Prior adjuvant chemotherapy was allowed (in case of adjuvant classical CMF at least 6 months ago), but prior chemotherapy for advanced disease was not allowed. Inclusion required a white blood cell (WBC) count $\geq 3.0 \times 10^9$/l, platelet (PLT) count $\geq 100 \times 10^9$/l, creatinine-clearance (60 ml/min (Cockroft), and bilirubin $\leq$ 25 micromol/l. During the study no other anti-tumor treatment or concomitant therapy with non-steroidal anti-inflammatory drugs, tetracyclines, phenytoin, sulphonamides or high dose vitamin C were permitted. Written informed consent was obtained from all patients. The hospital ethical review board approved the protocol.

Treatment plan

Chemotherapy

Chemotherapy consisted of cyclophosphamide 700 mg/m^2, methotrexate 40 mg/m^2 and 5-fluorouracil 600 mg/m^2 on days 1 and 2 intravenously every two weeks. Folinic acid started 24 h after the last administration of methotrexate in a total dose of 120 mg (8×15 mg orally every 6 h). A total of six cycles was planned for each patient, unless serious complications or progressive disease prohibited continuation of treatment.

G-CSF

To facilitate the delivery of 2-weekly CMF chemotherapy, G-CSF (filgastrim, Amgen B.V. Breda, the

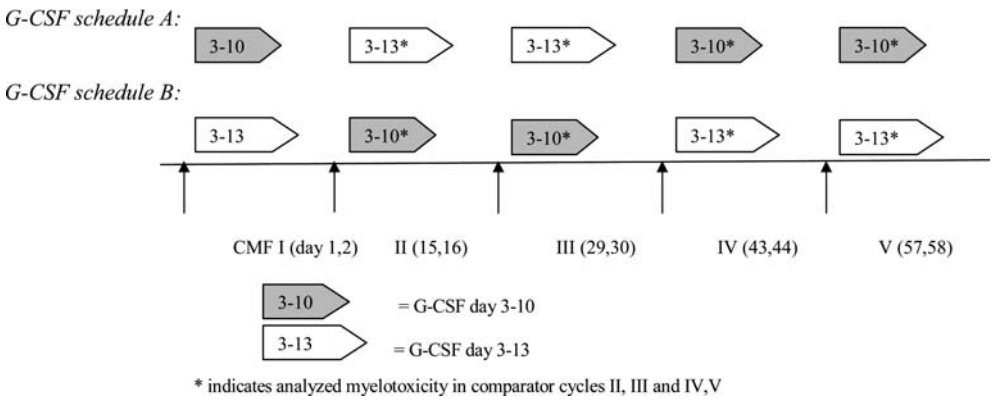

Figure 1. Schematic representation of G-CSF schedules.

Netherlands) was administered subcutaneously once a day, starting 24 h after each CMF cycle (on day 3) in a dose of 300 μg (weight ≤ 75 kg) or 480 μg (weight > 75 kg), according to schedule A or B (Figure 1).

Myelotoxicity

To evaluate bone marrow suppression, peripheral blood cell counts and differentiation were measured on days 1, 8, 10, 12 and 15 of each 2-weekly cycle.

Also clinical events related to myelotoxicity as infection with or without neutropenia, need of red blood cell or PLT transfusion and haemorrhage were documented.

Dose-modifications

Chemotherapy was delayed for 1 week or longer as necessary, if WBC counts were (3.0×10^9/l and/or PLT counts (100×10^9/l. When the delay was more than 2 weeks, the patient went off study. Dose adjustments were recommended in case of absolute neutrophil count (ANC) < 0.5×10^9/l for more than 7 days, ANC < 0.5×10^9/l complicated by fever > 38.5 °C lasting > 2 days, sepsis or PLT count < 25×10^9/l. Dose adjustments were also recommended in case of non-haematological toxicity. Note that dose modifications could lead to exclusion of the involved cycles or of the patient from analysis of myelotoxicity.

Statistical analysis

The G-CSF part of the trial was designed as a four-period, two-schedule crossover design (Figure 1). Patients acted as their own control. Due to the four-period design the only variable was the G-CSF free interval in the preceding cycle, since compared cycles were identical in chemotherapy dose, interval between cycles and post chemotherapy G-CSF duration. A cross-over design was used to ensure that both a long followed by a short G-CSF free interval (schedule A cycle II and III) opposed to a short followed by a long G-CSF free interval (schedule B cycle II and III) could be assessed in order to exclude a bias from a period effect (cumulative chemotherapy-dose). In case patients would only be treated according to schedule A and myelotoxicity was shown to be more severe in cycle III than in II, it is not possible to determine whether this increased toxicity in cycle III would be due to the preceding short G-CSF free interval or to the higher cumulative total chemotherapy dose. Using a crossover design, it is actually possible to calculate both the size of the impact of G-CSF schedule and the period effect of two successive chemotherapy cycles.

Based on the previous study, it was calculated that 44 cycles were needed to demonstrate a significant difference (power of 90%, one-sided significance level of 5%) at a G-CSF free-interval effect on nadir ANC count of the next cycle of 0.5×10^9/l assuming a residual standard deviation of 0.8×10^9/l. This last assumption was based on within- patient estimates.

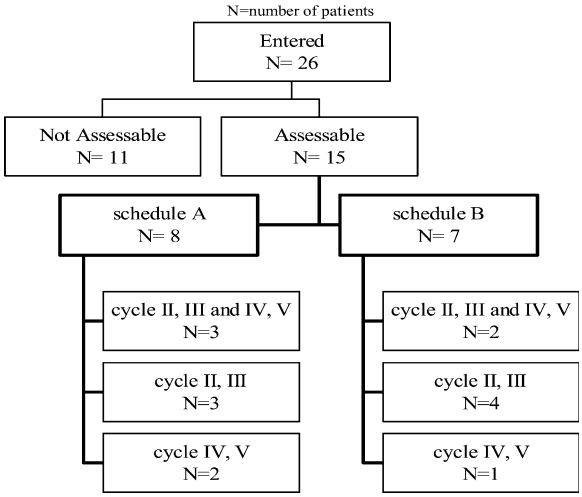

Figure 2. Patient inclusion and assessed cycles.

The nadir blood cell counts were analyzed using a linear mixed model. In this model the within-patient comparison was accomplished by assuming a subject-specific random effect. Furthermore, as independent fixed effects, a period effect and the effect of G-CSF-free interval (5 or 2 days) on nadir blood cell counts of the next cycle were added to the model. 95% Confidence intervals (CI) were calculated for the effect of G-CSF free interval on nadir blood counts of the next cycle. All analyses were done within the framework of linear mixed models for repeated measurements using procedure Mixed of the SAS package.

Results

Patient characteristics

In total, 40 chemotherapy cycles were evaluated (Figure 2). Five patients received 4 comparator cycles and ten patients 2 comparator cycles. Eight patients were treated according to schedule A and 7 according to schedule B.

The patient characteristics are summarized in Table 1. Ten patients had metastatic disease of which 5 received prior adjuvant chemotherapy (CMF or FEC

Table 1. Patient characteristics at baseline of all 15 assessable patients

Median Age (range, years)	48 (37–67)
ECOG Performance status (N)	
0	5
1	10
Locally advanced (N)	5
Metastatic (N)	10
Prior adjuvant chemotherapy[a] (N)	5
Median WBC (range, $\times 10^9$/l)	6.8 (4–11.4)
Median PLT count (range, $\times 10^9$/l)	318 (178–504)
Median Hb count (range, mmol/l)	7.9 (6.4–9.1)
Median creatinine clearance (range, ml/min)	86 (59–143)

[a]Interval at least 6 months in all patients.

Table 2. Mean nadir PLT count $\times 10^9$/l per cycle

Schedule	Mean PLT nadir count $\times 10^9$/l (95%CI)							
	Comparing cycles II versus III				Comparing cycles IV versus V			
	G-CSF free (days)	Cycle II	G-CSF free (days)	Cycle III	G-CSF free (days)	Cycle IV	G-CSF free (days)	Cycle V
A[a]	5	133.0 (109–157)	2	38.6 (21–46)	2	63.2 (47–79)	5	96.4 (56–136)
B[b]	2	68.7 (45–92)	5	81.0 (53–109)	5	91.3 (84–98)	2	54.7 (52–56)

[a]Based on respectively 6, 6, 5, 5 cycles for schedule A and [b] 6, 6, 3, 3 cycles for schedule B.

(5-fluorouracil, epirubicine and cyclophosphamide). Interval to prior chemotherapy ranged from 6 months to 15 years.

PLT nadir count

In schedule A, mean PLT nadir counts in cycles II and V were higher than in their respective counterparts, cycles III and IV, and the opposite occurred in schedule B. Results are summarized in Table 2 and Figure 3. PLT nadir count in cycles preceded by a 5-days G-CSF-free interval was significantly higher compared to cycles preceded by a 2-days G-CSF-free interval (mean difference of 45.7×10^9/l; 95% CI 33.2–58.2, Table 3) (Table 4). A PLT nadir count less than 100×10^9/l was seen in 20 cycles after a 2-days G-CSF – free interval compared to only 11 cycles after a 5-days G-CSF – free interval. A PLT nadir count less than 25×10^9/l was observed in 4 cycles, all of them preceded by a 2-days G-CSF-free interval. Median PLT nadir count occurred on day 10 (range day 8–15) regardless of the preceding G-CSF-free interval.

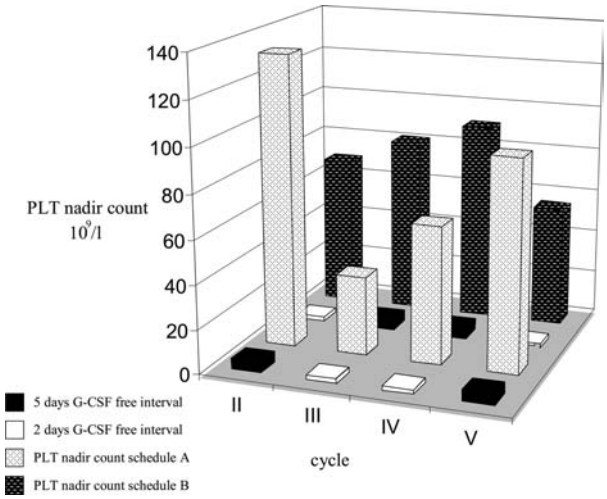

Figure 3. Mean PLT nadir count per cycle. For patients treated by schedule A, cycle II was preceded by a 5-days G-CSF free interval, cycle III and IV by a 2-days G-CSF free interval, and cycle V again by a 5-days G-CSF free interval. For patients treated by schedule B, cycle II was preceded by a 2-days G-CSF free interval, cycle III and IV by a 5-days G-CSF free interval, and cycle V again by a 2-days G-CSF free interval. Comparison of blood cell counts for cycles II versus III and cycles IV versus V shows that PLT nadir count is significantly lower for cycles preceded by a short 2-days G-CSF free interval (for 95% CI see Table 2).

Table 3. Differences in mean nadir counts in cycles after 5 versus 2 days G-CSF-free interval

	Difference 5 days versus 2 days (95% CI)	*p*-value
PLT $\times 10^9$/l	45.7 (33.2–58.2)	0.0001
WBC $\times 10^9$/l	0.29 (−0.21–0.80)	0.24
ANC $\times 10^9$/l	0.06 (−0.60–0.73)	0.85
Hb mmol/l	0.16 (−0.05–0.38)	0.12

Over the entire treatment period the mean nadir values of PLT count dropped. The mean PLT nadir was 33.8×10^9/l higher in cycle II compared to cycle V (when schedule A and B are combined, $p = 0.0003$). There was no significant difference in mean PLT count on day 15 of the next cycle (data not shown).

WBC and ANC nadir counts

WBC and ANC count were not significantly different for cycles preceded by a 2-days versus a 5-days G-CSF-free interval (1.3 versus 1.6×10^9/l, $p = 0.24$, and 0.81 versus 0.87×10^9/l, $p = 0.85$, respectively, see also Table 3), nor was the moment on which the nadir count occurred (median on day 10). Over the entire treatment period, there was no significant effect seen on WBC and ANC nadir values when respectively cycle II, III or IV were compared to cycle V ($p = 0.33$).

Hemoglobin nadir counts

Hemoglobin (Hb) nadir counts (5.9 and 5.8 mmol/l respectively, $p = 0.12$,see also Table 3) and moment of nadir (median on day 10) were not significantly different for cycles preceded by a 2-days versus a 5-days G-CSF-free interval. Over the entire treatment period, the mean nadir values of Hb level in cycle V significantly dropped respectively by 1.24 mmol/l compared to cycle II, 0.53 mmol/l compared to cycle III and 0.41 mmol/l compared to cycle IV ($p = 0.0001$).

Clinical events

In none of the assessed cycles clinical overt hemorrhage requiring transfusion or episodes of febrile neutropenia were reported.

Table 4. Period effect: impact of cumulative chemotherapy dose

	Mean difference in nadir count compared to cycle V#			
	PLT $\times 10^9$/l (±SEM)	WBC $\times 10^9$/l (±SEM)	ANC $\times 10^9$/l (±SEM)	Hb mmol/l (±SEM)
Cycle II versus V#	33.81 (±9.2)	0.28 (±0.39)	0.21 (±0.45)	1.24 (±0.16)
Cycle III versus V#	−6.97 (±9.0)	0.62 (±0.38)	0.66 (±0.45)	0.53 (±0.15)
Cycle IV versus V#	4.07 (±9.2)	−0.01 (±0.37)	−0.24 (±0.46)	0.41 (±0.16)
p-value	0.0003	0.33	0.35	0.0001

Cycles of schedule A and B collectively. SEM = standard error of mean.

Dose reduction or delay in all 26 patients

Considering all 26 initially registered patients, 51 of 133 cycles were dose-reduced or delayed. In 30 cycles (59%) this was PLT-related, that is in 5 cycles a PLT nadir count of less than 25×10^9/l necessitated dose reduction and in 25 cycles a PLT count of less than 100×10^9/l on the planned day of start necessitated postponement of the next chemotherapy cycle. In 63% of these 30 cycles the preceding G-CSF-free interval was 2-days, and in 37% of cycles the G-CSF-free interval was 5 days.

Discussion

This is the first study ever reported, in which the effect of two different G-CSF schedules was evaluated in order to determine a preferential G-CSF schedule during 2-weekly chemotherapy. Based on prior reports, the clinical benefit of post chemotherapy G-CSF during dose-densified chemotherapy may theoretically be enhanced by increasing the time between interruption of G-CSF and re-initiation of chemotherapy beyond 2–4 days. For this purpose, G-CSF was given during 2-weekly chemotherapy either on days 3–10, with a G-CSF-free interval before the next chemotherapy cycle of 5 days, or on days 3–13 with a G-CSF-free interval before the next chemotherapy cycle of 2 days. The G-CSF schedule appeared to have a large impact on the degree of thrombocytopenia of the next chemotherapy cycle. The mean PLT nadir count was 46×10^9/l lower following a preceding 'short' 2-days G-CSF-free interval compared to a 'long' 5-days G-CSF-free interval.

Previously, we reported on the results of a study in twelve patients with relapsed small cell lung carcinoma. [11] Patients were treated with two *4-weekly* chemotherapy cycles and 6 days G-CSF priming was given till 48 h before the first chemotherapy cycle only or till 48 h before the second cycle only with patients acting as their own control. G-CSF priming was shown to increase both the chemotherapy-associated leucopenia and thrombocytopenia. In that study no post-chemotherapy G-CSF was given. The results of the current study suggest that a possible negative effect on WBC nadir count of the short G-CSF free interval before the next chemotherapy cycle can indeed be compensated for by the use of post-chemotherapy G-CSF. But, post-chemotherapy G-CSF during the next cycle cannot protect against the negative priming effect on PLT nadir counts.

Thrombocytopenia is often reported as an important side effect of dose dense chemotherapy schedules and hypothesized to be a consequence of cumulative chemotherapy dose.[14, 13] Our results do confirm a significant effect of cumulative dose on Hb and PLT nadir counts: the mean Hb nadir was 1.24 mmol/l higher in cycle II versus cycle V ($p = 0.0001$) and the mean PLT nadir count was 33.8×10^9/l higher in cycle II versus cycle V ($p = 0.0003$). However, mean PLT nadir count did not decline gradually during the course of treatment: it was higher in cycle V than in cycle III and IV for schedule A for example (Table 2). The double cross over design permitted to demonstrate that the scheduling of G-CSF affects thrombocytopenia for an even larger degree (difference in mean PLT nadir count 46×10^9/l, 95% CI 33.2–58.2, $p = 0.0001$). Although the number of patients and cycles assessed in this study is only modest, the 95% CI permits to conclude that the duration of G-CSF-free interval is of importance.

We observed that the majority of PLT-related dose reductions or delays occurred in cycles following a short G-CSF-free interval (19 out of 30 cycles), so one may hypothesize that earlier discontinuation of G-CSF before the (re-)start of chemotherapy can contribute to maintain the delivery of planned chemotherapy dose and dose-intensity. As mentioned before, in our study dose and dose-intensity in the assessed cycles are by definition the same. But, de Wit et al randomized 36 breast cancer patients both to receive pre- and post chemotherapy G-CSF or only post chemotherapy G-CSF [12]. They found that in patients receiving G-CSF until 48 h before chemotherapy more frequent dose reductions because of thrombocytopenia were applied than in those patients who did not receive pre-chemotherapy G-CSF. Also in children with neuroblastoma treated with multiple cycles of strongly myelosuppressive alkylator-based combination chemotherapy, prophylactic use of G-CSF hastened ANC recovery but did not result in augmented dose intensity and was associated with prolonged thrombocytopenia (compared to a historical control group) [17].

The results reported here show, that there is an impact of the duration of the G-CSF-free interval before the next chemotherapy cycle on peripheral blood counts,

but the underlying mechanism(s) for the effect of G-CSF schedule on PLT count is (are) not entirely clear. After 2–4 days cessation of G-CSF treatment the bone marrow progenitor cells are still rapidly proliferating with progenitor cells in S-phase ranging from 38 ± 5 to 63 ± 8% compared with 26 ± 9 to 39 ± 8% before G-CSF treatment [6, 7]. This means that after a 2 days G-CSF-free interval compared to a 5 days interval more progenitor cells are still proliferating and this might explain in part the observed impact of G-CSF schedule on myelotoxicity. A functional G-CSF receptor on PLT's has been identified [18] and there is some in vivo evidence that G-CSF has a suppressive effect on the maturation of mouse bone marrow megakaryocytes when monitored by the DNA polyploidy [19]. When G-CSF is given to healthy donors a drop in PLT count is seen by day 8 and an even lower than pre-treatment PLT level by day 10 [20, 21]. Some authors hypothesize that splenic enlargement due to G-CSF induced extra-medullar hematopoiesis contributes to thrombocytopenia [21], but this is contradicted by the observation in splenectomized mice that circulating platelets decreased after 5FU-treatment followed by G-CSF (whereas granulopoietic recovery was accelerated and all stages of bone marrow megakaryocytopoiesis were decreased) [22]. Also redirection of hematopoiesis towards neutrophil recovery and subsequent suppression of other cell lines is often held responsible. Again in splenectomized mice the delayed start of G-CSF treatment for more than 5 days after chemotherapy (5FU) showed no longer an impact on granulopoiesis but PLT count was still significantly reduced. This indicates that neither recruitment nor competition between different hematopoietic cell lines are critical events in the cause of decreased PLT counts [22].

The importance of G-CSF schedule on myelotoxicity might be of special concern since a long acting pegylated G-CSF recently has been introduced. For patients a major practical advantage of pegylated G-CSF is that administration is needed only once a week. But the variable and sometimes long half life indicates that the effect in an individual patient cannot be predicted and may interfere with a safe interval to the next cycle, especially during dose dense chemotherapy. Recently, the results of a randomized phase III trial showed that a single fixed dose (6 mg) of pegylated G-CSF could be administered effectively and safely in a 3-weekly chemotherapy schedule [23]. But, no published data are available in dose dense chemotherapy yet. In another trial with pegylated G-CSF, thrombocytopenia was more frequent after chemotherapy in the highest dosage group of 100 (g/kg compared to 20 and 60 (g/kg pegfilgrastim or daily filgrastim [24]. These findings and our results stress the importance of carefully determining the optimal dose and timing of (peg)filgrastim in relation to the planned chemotherapy regimen.

In conclusion, during 2-weekly densified chemotherapy daily post chemotherapy G-CSF until 2 days before the next cycle compared to post chemotherapy G-CSF until 5 days before the next chemotherapy cycle significantly worsened the degree of thrombocytopenia. There was no impact of G-SCF schedule on the degree of leukopenia, reflecting a counterbalancing protective effect of post chemotherapy G-CSF in both the 8 day and 10 day schedule. Timely withdrawal of G-CSF during 2-weekly chemotherapy reduces chemotherapy-related thrombocytopenia without jeopardizing ANC recovery and therefore, may realize planned dose-intensity increase.

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