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SPOTLIGHT

Successful peripheral blood stem cell mobilisation with filgrastim in patients with chronic myeloid leukaemia achieving complete cytogenetic response with imatinib, without increasing disease burden as measured by quantitative real-time PCR

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Imatinib mesylate (Glivec) is a selective inhibitor of *bcr-abl* tyrosine kinase, the product of the Philadelphia chromosome, which is the hallmark of chronic myeloid leukaemia (CML). With imatinib, complete cytogenetic response (CCR) can be achieved in over 70% of newly diagnosed patients with CML. However, the optimal long-term management of patients who achieve CCR after imatinib is unknown. With longer follow-up, it is anticipated that some patients are likely to progress and become candidates for autologous transplantation. We studied filgrastim (r-metHuG-CSF) mobilisation of peripheral blood stem cells (PBSC) in 32 patients who have achieved CCR with imatinib. Our data demonstrate that (1) the target CD34$^+$ cell yields of $\geqslant 2.0 \times 10^6$/kg were attained with filgrastim 10 μg/kg/day, in 9/18 (50%) of patients during uninterrupted imatinib therapy, and in 10/14 (70%) when imatinib was temporarily withheld. The median CD34$^+$ cell yield per aphaeresis was 0.70×10^6/kg (range 0.14–2.18) and 2.90×10^6/kg (range 0.15–8.71) in the two groups, respectively ($P < 0.005$). (2) The cell yields did not correlate with the duration of imatinib administration. (3) There was no impact of the mobilisation procedure on the level of leukaemia as measured by serial blood *bcr-abl* levels using real-time quantitative PCR with either protocol. (4) *bcr-abl* remained detectable at low levels in the harvests in most but not all patients. In conclusion, filgrastim can safely be used to mobilise PBSC in patients who have achieved CCR with imatinib, but CD34$^+$ cell yields are significantly improved when imatinib is temporarily withheld.

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Introduction

The treatment goal in patients with chronic myeloid leukaemia (CML) is the elimination of the Philadelphia (Ph) positive clone and restoration of Ph-negative haematopoiesis. To date, allogeneic stem cell transplantation is the only therapy with the established capacity for cure. For patients without an appropriate allogeneic donor, standard therapy has been with interferon-alpha with or without cytarabine. Complete cytogenetic response (CCR) can be achieved in 6–25%, which was associated with improved survival but rendered the collection of marrow stem cells difficult. Although associated with a high relapse rate,[1] autologous transplantation after *in vivo* selection by intensive chemotherapy has demonstrated the ability to re-establish Ph-negative haematopoiesis and potentially improve survival.[2] Other strategies of *ex vivo* purging such as long-term marrow culture, purging with cyclophosphamide derivatives, antisense oligodeoxynucleotides or interferon had also been attempted (reviewed by Bhatia *et al*[3] and Carella *et al*[4]). However, autologous stem cell transplantation would be a more attractive option if an effective, nontoxic means of reducing or even eliminating the leukaemic clone from the reinfused stem cell product was available.[5,6]

In the last 3 years, the treatment of CML patients has seen rapid progress with the availability of imatinib (Glivec, Novartis, Basel, Switzerland), formerly known as STI-571, which blocks the tyrosine kinase activity of the *bcr-abl* oncoprotein.[7,8] Clinical studies have demonstrated its efficacy in the successful induction of haematological remission, CCR[9–12] and dramatic molecular reduction of the *bcr-abl* transcript as shown by serial quantitative reverse transcriptase polymerase chain reaction (RT-qPCR).[13,14] In the setting of newly diagnosed chronic phase CML, imatinib therapy achieved CCR in 68% of patients compared with 7% with interferon plus cytarabine after 12 months of therapy.[15] In addition, the tolerability and toxicity profile of imatinib were markedly superior to the interferon–cytarabine combination. Imatinib has therefore been rapidly incorporated into the treatment algorithms of CML patients.[16–19]

There are no clinical data available, however, to determine the duration of response to imatinib or the long-term survival. Minimal residual disease (MRD) levels assayed by RT-qPCR in responding patients usually reach a plateau or continue to slowly fall, but few patients reach PCR negativity.[14,20] In addition, the development of resistance to imatinib is well documented, which may arise from various mechanisms including mutations within the *abl*-kinase domain, over-expression of *bcr-abl*, *bcr-abl* oncogene amplification and clonal cytogenetic evolution.[21–24] It is unclear therefore that imatinib alone will prove to be curative, and initial responders may eventually lose imatinib responsiveness.

Given this possibility, it may be prudent to collect autologous stem cells in patients treated with imatinib if cytogenetic or molecular tests reveal a low level of detectable leukaemia. The optimal technique for such stem cell collection is uncertain and several strategies are possible. Granulocyte-colony-stimulating factor (G-CSF) is the most frequently used cytokine for stem cell mobilisation. However, the effect of continuous administration

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of imatinib on the ability to mobilise $CD34^+$ stem cells is a potential concern in view of the uncertain downstream effect of c-kit inhibition,[25] and its effect on other kinases involved in cell signalling. Little data are available on the feasibility and safety of peripheral blood stem cell (PBSC) mobilisation after imatinib.[26]

We have investigated the efficacy and safety of mobilisation with filgrastim in CML patients, who achieved CCR with imatinib by determining the cell yields and blood *bcr-abl* levels postmobilisation in two cohorts of patients – the first group with uninterrupted imatinib and a second group with temporary cessation of imatinib.

Materials and methods

Patients

In all, 32 consecutive CML patients treated with imatinib who achieved CCR were recruited from five centres and consented to the study. Approval of the project was obtained from the hospital ethics committees, where such procedures were not considered part of standard management. Most patients had autologous blood cells collected at diagnosis as back-up storage.

PBSC mobilisation protocol and aphaeresis

There were two nonrandom cohorts of patients with the difference being the continuation or temporary discontinuation of imatinib with PBSC mobilisation that was incidental to the way these patients were treated at the various centres. The first cohort of 18 patients received filgrastim (Amgen, Thousand Oaks, USA) at a total dose of 10 μg/kg/day s.c., while oral imatinib therapy was continued daily at the previously established dose. The second cohort of 14 patients received the same dose of filgrastim, but had imatinib withheld 5–7 days prior to PBSC collection and until the completion of aphaeresis.

Autologous PBSC collection was performed from days 4 to 5 of filgrastim injections onwards, if adequate peripheral blood $CD34^+$ levels were achieved, using a continuous flow cell separator (Fenwall CS3000 Plus, Baxter, IL, USA; or COBE Spectra, Gambro BCT, Lakewood, CO, USA). The recommended threshold level of blood $CD34^+$ cells to commence aphaeresis was 5/μl. The target of aphaeresis was 2.0×10^6/kg $CD34^+$ cells with a maximum of five daily aphaeresis procedures. Harvested peripheral blood mononuclear cells (PBMNCs) were analysed for the percentage $CD34^+$ cells and cryopreserved using standard protocols.

Enumeration of $CD34^+$ cells

Aphaeresis product (100 μl) containing approximately 5×10^5 PBMNCs was immuno-stained with 10 μl each of anti-CD34 antibody (HPCA-2-PE; Becton Dickinson, Mountain View, CA, USA) and CD45 antibody (KC56-FITC; Coulter Electronics, Miami, FL, USA or 2D1-PerCP; Becton Dickinson). The cells were washed and suspended in FACS fixative solution. A mouse antibody IgG_1/phycoerythrin (PE) (Dakopatts or Becton Dickinson) was used in control samples. Analysis was performed using a Coulter Profile II flow cytometer (Coulter) or FACSCalibur (Becton Dickinson) with 10^5 events analysed per sample as per standard protocol.[27]

Blood and aphaeresis samples for bcr-abl transcript levels

EDTA blood specimens (20 ml) were collected before commencement of filgrastim and after mobilisation (at about the second and fourth week, and at third and sixth month in the first cohort). Cells were lysed in Trizol for the measurement of blood *bcr-abl* levels. For cryopreserved PBMNCs, aliquots containing 4×10^8 cells in 2 ml were thawed and their *bcr-abl* transcript levels determined by RT-qPCR.

Cytogenetic analysis

Chromosomal preparations of bone marrow cells were performed according to standard procedure and metaphase cells were subjected to trypsin-Giemsa banding. Cytogenetic analysis was performed on a minimum of 30 cells and karyotype was described according to the ISCN (1995).

Quantitative RT-PCR

Reverse transcription real-time quantitative PCR was based on the 5′ nuclease assay[28] and used TaqMan dual-labelled fluorescent hybridisation probes as described previously.[29] In brief, total leucocyte RNA was extracted by the Trizol method (Invitrogen Life Technologies, Carlsbad, CA, USA) and cDNA synthesis using the Superscript™ II RNAse H^- Reverse Transcriptase kit (Life Technologies, Gaithersburg, MD, USA) according to the manufacturers' instructions. Standards were prepared by cloning PCR products of the two *bcr-abl* transcripts and of normal *bcr* from cell lines. Primer Express software (PE Applied Biosystems, Foster City, CA, USA) was used to design appropriate fluorescent hybridisation probe and primer pairs. The probes were synthesised by PE Applied Biosystems with a 5′ FAM reported dye and a 3′ TAMRA quencher dye. The amplification and assay were performed in the ABI/Prism 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA) that incorporated a 96-well thermal cycler, fluorescence detector and software to calculate quantitation results. Normal *bcr* mRNA was quantitated to control for RNA degradation and for differences in the efficiency of the reverse transcription step. The number of transcripts for b2a2, b3a2 and *bcr* in the controls and patient samples were quantitated in duplicate. Results were expressed as a percentage of *bcr-abl*/*bcr* with a sensitivity of 10^{-5}. Precision analysis of samples with a low level of *bcr-abl* showed a coefficient of variation (cv) of 24% intraassays and 48% interassays. The assay could thus accurately distinguish a two- to three-fold change in consecutive patient results.

Statistics

Correlation between cell yield and the duration of CML or imatinib therapy was studied using the bivariate Pearson equation. The cell yields were compared using samples *t*-test.

Results

Between September 2001 and August 2002, 32 patients were enrolled. There were 20 males and 12 females with a median age of 53 years (range 21–67) (Tables 1 and 2). The median duration of CML prior to mobilisation was 21 months (range

Table 1 Blood stem cell yields in patients with CML who achieved CCR to imatinib therapy

Patient	*Sex/age*	*Duration of CML (months)*	*Prior treatment (duration in months)*	*Disease status at commencement of imatinib*	*Duration of imatinib prior to aphaeresis (months)*	*Dose of imatinib at aphaeresis (mg)*	*Runs of aphaeresis*	*Total MNC yield (10^8/kg)*	*MNC yield per aphaeresis*	*Total CD34$^+$ yield (10^6/kg)*	*CD34 yield per aphaeresis*
Cohort 1											
1	M/21	38	HU	CP1	18	400	3	13.44	4.48	2.99	1.00
2	M/54	12	HU	CP1	11	400	3	9.37	3.12	4.15	1.38
3	M/67	18	HU	CP1	17	400	5	8.11	1.62	1.73	0.35
4	M/36	17	HU	CP1	14	400	2	4.97	2.49	0.64	0.32
5	M/35	23	HU, IFN (8)	CP1	13	400	2	6.22	3.11	4.35	2.18
6	F/42	16	HU, IFN (7)	CP1	6	400	4	8.64	2.16	3.46	0.87
7	M/61	32	HU, IFN	CP1	6	400	3	8.61	2.87	0.43	0.14
8	F/51	17	HU, IFN	CP1	9	400	4	14.09	3.52	0.87	0.22
9	M/43	25	HU, IFN (7)	CP1	16	600	0	ND	ND	ND	ND
10	F/30	11	IFN (6)	CP1	5	400	3	18.38	6.13	5.23	1.74
11	F/58	34	IFN (25), Ara-c (1)	CP1	11	400	1	5.70	5.70	0.70	0.70
12	F/52	141	HU,IFN (60), Ara-c, autograft, IFN (36)	CP	7	400	4	15.74	3.94	3.50	0.88
13	F/60	80	HU, Cy, IFN (36), autograft,	CP	6	400	2	6.94	3.47	0.66	0.33
14	M/43	252	Bu (48), HU, combination chemotherapy (ADE)	CP2 with prior BC	16	600	4	40.62	10.16	3.58	0.90
15	M/40	6	HU	AP	6	600	3	12.20	4.07	2.18	0.73
16	F/58	8	HU	AP	8	600	4	13.91	3.48	2.35	0.59
17	F/56	21	HU, IFN (24), Ara-c (12)	AP	4	600	3	8.66	2.89	1.83	0.61
18	F/67	28	HU, IFN, combination chemotherapy	BC then CP2	9	400	3	6.96	2.32	0.67	0.22
Cohort 2											
19	F/23	13		CP1	13	400 (I)	2	14.58	7.29	3.70	1.85
20	M/57	21	Nil	CP1	5	400 (I)	1	9.44	9.44	8.71	8.71
21	M/58	17	Nil	CP1	14	400 (I)	1	13.41	13.41	5.66	5.66
22	M/52	18	HU	CP1	15	400 (I)	2	36.37	18.19	1.67	0.84
23	M/59	58	HU, IFN	CP1	8.5	400 (I)	1	11.33	11.33	5.40	5.40
24	F/48	20	HU, IFN	CP1	9	400 (I)	1	9.84	9.84	1.59	1.59
25	M/52	63	HU, IFN	CP1	17	400 (I)	1	8.91	8.91	5.18	5.18
26	M/61	30	IFN (11), Ara-c (8)	CP1	15	400 (I)	1	2.70	2.70	2.30	2.30
27	M/57	9	HU, IFN (2)	AP	7	600 (I)	1	13.34	13.34	4.95	4.95
28	M/64	22	IFN (9), Ara-c (2)	AP	10	600 (I)	1	4.70	4.70	2.90	2.90
29	M/49	84	HU, IFN, autograft	CP2 with prior BC	7	400 (I)	2	18.22	9.11	2.27	1.14
30	F/56	20	Combination therapy	CP2 with prior BC	11	400 (I)	0	ND	ND	ND	ND
31	M/54	4	Combination chemotherapy (LaLa)	BC	4	400 (I)	1	12.00	12.00	5.78	5.78
32	M/42	96	HU, IFN, autograft, Ara-c	AP with prior BC	19	400 (I)	1	5.51	5.51	0.15	0.15

ND=not done in view of very low blood CD34 $<5/\mu$l.
MNC, mononuclear cell; (I), interrupted; HU, hydroxyurea; IFN, interferon; Ara-c, cytarabine; Cy, cyclophosphamide; Bu, busulphan; CP, chronic phase; AP, acceleration phase; BC, blastic crisis; ADE, 7:3:7 with AraC/Doxorubicin/Etopside; LaLa, the French LALA induction for ALL.
Patients 1–18 (cohort 1) had filgrastim with continuing imatinib while patients 19-32 (cohort 2) had filgrastim but with imatinib interrupted (I).

Table 2 Comparison of the demographics of the two cohorts of CML patients in CCR with imatinib who underwent filgrastim mobilisation. The first group continued imatinib throughout mobilisation while the second group had imatinib interrupted 5–7 days prior to collection till completion of aphaeresis

	Continuing imatinib (n=*18*)	*Interrupted imatinib* (n=*14*)
Male:female	9:9	11:3
Median age (range)	51.5 years (21–67)	55 years (23–64)
Median duration of CML (range)	22 months (6–252)	20.5 months (4–96)
Disease status at commencement of imatnib		
Chronic phase (1st and 2nd)	14	10
Acceleration phase	3	3
Blastic transformation	1	1
Treatment prior to imatinib		
Nil	0	2
HU alone	6	2
HU, IFN	7	5
HU, IFN, Ara-c	3	3
Induction chemotherapy	2	2
Prior autograft	2	2
Median duration of imatinib (range)	9 months (4–18)	10.5 months (4–19)
Dose of imatinib before aphaeresis		
400 mg	13	12
600 mg	5	2

Table 3 Summary of the mononuclear and $CD34^+$ cell yields between the two cohorts of CML patients. The first cohort of patients with continuing imatinib has significantly lower cell yields compared to the second cohort with imatinib interrupted

	Continuing imatinib (n=*18*)	*Interrupted imatinib* (n=*14*)
Median number of aphaeresis	3	1
Total mononuclear cell yields (10^8/kg)[a]	8.65 (4.97–40.62)	11.33 (2.70–36.37)
Mononuclear cell yield per aphaeresis*	3.47 (1.62–10.16)	9.44 (2.70–18.19)
Total $CD34^+$ cell yield (10^6/kg)**	2.18 (0.43–5.23)	3.70 (0.15–8.71)
$CD34^+$ cell yield per aphaeresis*	0.70 (0.14–2.18)	2.90 (0.15–8.71)
Number of patients with $CD34^+$ yield per aphaeresis $>1.0 \times 10^6$/kg	4 (22%)	11 (92%)
Number of patients with $CD34^+$ yield per aphaeresis $>2.0 \times 10^6$/kg	1 (6%)	8 (67%)
Number of patients reaching a target $CD34^+$ yield $>2.0 \times 10^6$/kg	9 (50%)	10 (71%)

[a]Nonsignificant.
*$P<0.005$.
**$P<0.05$ (independent samples *t*-test).
Results are expressed as medians (range).

4–252 months). Five patients had prior accelerated phase while six had blastic transformation prior to imatinib. All patients had achieved CCR following imatinib treatment at daily doses of 400–600 mg prior to mobilisation. The median duration of imatinib therapy prior to aphaeresis was 9.5 months (range 4–19 months). The administration of filgrastim and PBSC mobilisation procedures were well tolerated by all patients with no significant adverse effects noted.

Efficacy of $CD34^+$ cell yields

Table 1 shows the cell yields of all patients in both cohorts. One patient in each cohort (patients nine and 30) did not undergo aphaeresis as the blood CD34 counts were persistently below 5/μl. The median total $CD34^+$ cell yield was 2.63×10^6/kg body weight (bw) (range 0.15–8.71) with a median $CD34^+$ cell yield per aphaeresis of 0.95×10^6/kg bw (range 0.14–8.71). The MNC yield per aphaeresis showed an overall fair correlation with the $CD34^+$ yields ($r=0.56$, $P=0.01$).

With continuing imatinib, seven of 18 patients (39%) had an inadequate collection (less than 1.0×10^6/kg), while two had a borderline collection (between 1.7 and 2.0×10^6/kg). Nine patients (50%) obtained a total cell dose above 2.0×10^6/kg $CD34^+$ cells. In comparison, among the 14 patients with imatinib temporary interrupted, two (14%) were inadequate, two borderline and 10 (71%) achieved the target cell dose. Overall, 53% (17 of 32) patients achieved the target cell dose and by a median of three *vs* one aphaeresis session in the two groups of patients. Table 3 contrasts the cell yields between the two cohorts. The median $CD34^+$ yield per aphaeresis for the first cohort was 0.70×10^6/kg bw (range 0.14–2.18) compared to 2.90×10^6/kg bw (range 0.15–8.71) for the second cohort. The difference in $CD34^+$ yield per aphaeresis, as well as the MNC yield per aphaeresis, is highly significant, with the group of continuing imatinib having inferior cell yields ($P<0.005$).

Figure 1 depicts the serial rise in blood $CD34^+$ counts in 11 patients of the first cohort using filgrastim and continuing imatinib therapy. The steady-state blood $CD34^+$ cell levels rose from undetectable level to more than 20/μl by days 4–5 in seven of 11 patients and peaked at days 5–6. We did not have the

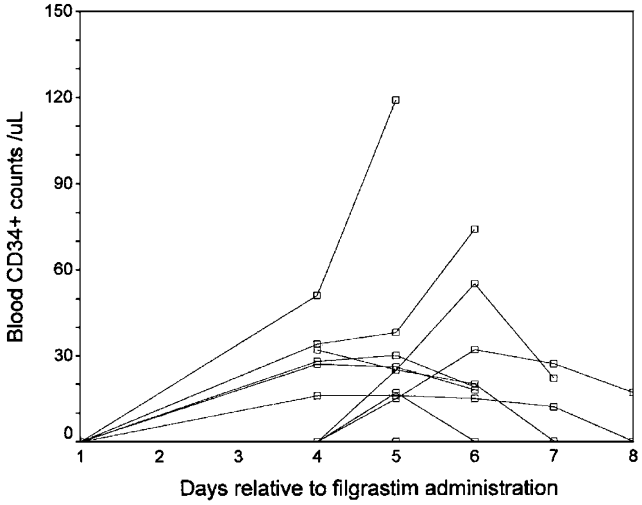

Figure 1 Serial blood CD34$^+$ cell counts in CCR CML patients given filgrastim 10 μg/kg/day subcutaneously and uninterrupted imatinib. The CD34$^+$ cell counts usually rose from undetectable baseline steady-state levels to $>20/\mu$l by days 4–5 when we performed PBSC harvest.

serial blood CD34$^+$ counts in the second cohort of patients, but a count of more than 20/μl was noted in nine of 14 patients on day 5.

Correlation of cell yields with clinical variables

The duration of imatinib therapy did not correlate with the CD34$^+$ yield per aphaeresis ($r=-0.15$, $P=0.44$). Similarly, the duration of CML had no significant correlation with this parameter ($r=-0.16$, $P=0.40$). The means of CD34$^+$ yield per aphaeresis did not differ significantly ($P=0.77$) between patients on 400 or 600 mg daily doses of imatinib. Prior interferon therapy and prior advanced disease status did not appear to affect adversely the cell yield.

Blood bcr-abl *transcript levels after filgrastim mobilisation*

Table 4 shows the serial measurements of blood *bcr-abl* fusion transcripts by RT-qPCR.

In 14 of the cohort of patients with continuing imatinib, the median blood *bcr-abl* level was 0.04% for measurements taken just prior to filgrastim mobilisation (range 0.00–0.95%), compared with a median 0.02% for all measurements taken postmobilisation (range 0.00–0.80%). At a median follow-up of 26.5 weeks (range 8–35.5 weeks), none of the 14 patients had shown any significant changes in *bcr-abl* levels nor exhibited any clinical signs of disease transformation.

RT-qPCR findings of the harvests, generally day 1 (or blood samples on the day 1 of aphaeresis) are tabulated in Table 4. These showed no significant difference from those measured before mobilisation ($P=0.18$). PCR showed very low levels of *bcr-abl* transcription (<1.00%) in all but one case (patient 18) with *bcr-abl* negative harvest in one of 11 evaluable patients. The level of transcription was below the level of detection of the assay (PCR negative) in two patients throughout the whole study period (patients 12 and 13).

The full serial blood *bcr-abl* quantitation was not available with the second cohort of patients with interrupted imatinib.

Table 4 Blood *bcr-a*bl fusion transcript levels by real-time quantitative PCR before and after filgrastim mobilisation in the two cohorts of patients. Results are expressed as percentage *bcr-abl/bcr*. Quantitative PCR for residual disease studies of the aphaeresed harvests were also tabulated

	Patient	*Premobilisation*	*Aphaeresed harvests*	*Postmobilisation*
Cohort 1	2	0.02	0.10	0.04
	3	0.02	0.12	0.01
	4	0.04	0.13	0.04
	5	0.18	0.42	0.48
	6	0.10	0.05	0.01
	7	0.08	0.01	0.00
	8	0.03	0.02	0.00
	9	0.95	–	0.29
	12	0.00	–	0.00
	13	0.00	0.00	0.00
	15	0.20	0.13	0.02
	16	0.03	0.06	0.02
	17	0.03	–	0.00
	18	0.60	1.20	0.47
Cohort 2	19	0.00		0.01
	20	0.00		0.01
	21	–		0.01
	22	0.05		0.03
	24	0.02		0.00
	25	–		0.13
	26	0.00		0.01
	27	–		0.02
	28	0.00		0.00
	29	–		0.04
	30	–		0.00
	32	–		0.18

–, results not available.

However, the transcript levels postmobilisation at a median follow-up of 13.3 weeks (range 4–52 weeks) available in 12 patients showed a median 0.01% (range 0.00–0.18%) with no patient exhibiting any signs of disease transformation (Table 4).

Remobilisation with filgrastim and interruption of imatinib in six patients who failed first mobilisation with filgrastim and continuing imatinib

Six patients (patients 4, 7, 8, 9, 13 and 18) in the first cohort, while on the same dose of imatinib, underwent remobilisation using the same filgrastim regimen following at least 4 months' interval (range 17–54 weeks) from the first mobilisation. In the second mobilisation, imatinib was withheld 2 days prior to the commencement of filgrastim until completion of the PBSC harvest to reach the target of total 2.0×10^6/kg CD34$^+$ cells. Table 5 compares the cell yields from the two mobilisations in these patients. There was a significant increase in cell yields per aphaeresis ($P=0.04$) when imatinib was temporarily withheld, without any significant difference in the leukaemic levels of the harvests obtained by the two protocols.

Discussion

We have demonstrated that standard doses of filgrastim with continuing imatinib treatment permitted a collection of PBSC of $\geqslant2.0\times10^6$/kg in the majority of our patients who achieved

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Table 5 Comparison of cell yields (10^6/kg) in six patients who underwent remobilisation. In the second attempt, filgrastim at the same dose regimen (10 μg/kg daily) was given but with imatinib withheld 2 days prior to the commencement of filgrastim and until the completion of PBSC harvests

	First mobilisation with continuing imatinib				*Second mobilisation with imatinib withheld*			
Patient	*No. of runs of aphaeresis*	*Total CD34$^+$ yield*	*CD34 yield per aphaeresis*	*q-PCR of aphaeresed harvest*	*No. of runs of aphaerises*	*Total CD34$^+$ yield**	*CD34 yield per aphaeresis***	*q-PCR of aphaeresed harvest ****
4	2	0.64	0.32	0.13 (day 1)	3	3.07	1.02	0.03 (day 1)
7	3	0.43	0.14	0.01 (day 3)	5	1.97	0.39	0.00 (day 1)
8	4	0.87	0.22	0.02 (day 4)	3	1.15	0.38	0.00 (day 1)
9	ND	ND	ND	ND (1.60 from blood on day of planned apheresis)	4	1.83	0.46	3.80 (day 1)
13	2	0.66	0.33	0.00 (day 1)	3	2.40	0.80	0.00 (day 3)
18	3	0.67	0.22	1.20 (day 1)	3	1.02	0.34	1.40 (day 1)

*P=0.04.
**P=0.04.
***P=0.35 by paired samples t-test, when the means of the CD34$^+$ cell yields and *bcr-abl* level of aphaeresed harvests between the first and second mobilisation were compared.
ND, not done.

CCR, without an adverse impact on disease control. The yield may be improved with temporary interruption of imatinib, a manoeuvre that was also successful in individuals who failed mobilisation with prior continuing imatinib/filgrastim.

The efficacy of G-CSF mobilisation in the context of continuing imatinib appears suboptimal when compared with most reported series of PBSC harvests from other CML patients using various chemotherapy–GCSF regimens[30,31] or an interferon–G-CSF combination.[32] Our decision to continue imatinib therapy during the cytokine mobilisation in a first cohort of patients was based on the absence of reported clinical data to support temporary interruption, and a postulated benefit that leukaemic stem cells stimulated into cell cycle may be rendered more susceptible to cellular inhibition induced by the continuous presence of imatinib.

The observation that the CD34$^+$ cell yields did not correlate with either the duration of imatinib therapy or CML disease raises the concern that imatinib may actually impair the egress of stem cells from the marrow microenvironment into the peripheral blood. A dose-dependent relation cannot be demonstrated, which may either reflect the small number of study patients or an 'all-or-none' effect by imatinib. The significantly better cell yields in the second cohort of patients, as well as our experience of six patients undergoing remobilisation and acting as their own controls, when imatinib was withheld prior to filgrastim, provide strong clinical clues that imatinib may reversibly impair PBSC mobilisation. Until the mechanisms of PBSC mobilisation are fully defined, this question can only be definitely answered by a randomised study of a larger number of patients with and without temporary cessation of imatinib during mobilisation.

While it has been demonstrated recently that imatinib selectively suppresses primitive nonquiescent CML progenitors *in vitro* by reversing abnormally increased proliferation rather than by increased apoptosis,[33] the biological effects of imatinib on normal marrow stem cells and the interaction of imatinib with cytokines used for mobilisation are largely unknown. The events of progenitor cell proliferation and mobilisation mediated by cytokine are believed to be linked.[34,35] In our study, the proliferative effect of filgrastim may be counteracted by an antiproliferative effect of imatinib on CML and perhaps normal progenitors, resulting in suboptimal mobilisation of progenitors.

This hypothesis may explain the higher stem cells yields when imatinib was interrupted. It is also consistent with our observations that the mobilisation process did not appear to preferentially mobilise leukaemic progenitors, and that the leukaemic burden in our patients, as measured by serial quantitative PCR of blood *bcr-abl,* showed no significant change at a median follow-up of 6 months after filgrastim mobilisation.

Nonetheless, our data on MRD suggest the persistence of low levels of PCR positivity in 10/11 of the PBSC harvests, despite the absence of Ph-positive cells in prior marrows by conventional cytogenetics and persistently low blood *bcr-abl* levels prior to harvests. Should the more sensitive nested PCR be performed, it is possible that all our harvests will be shown to have residual *bcr-abl* positivity. Persistent MRD in steady-state blood and bone marrow in CCR responders to imatinib had similarly been reported,[14,20,36] analogous to the situation in CCR after interferon-alpha where a variable number of *bcr-abl* transcripts were found to persist in blood in all patients.[37] It has recently been reported that primitive, quiescent Ph-positive stem cells from CML patients are insensitive to imatinib and growth factors *in vitro*.[38] If imatinib cannot eradicate CML progenitors that are merely silenced into the G_o phase, these quiescent CML progenitors may be the source of *bcr-abl* transcripts that remain detectable postmobilisation.

The PBSC harvested with our protocols further pose two therapeutic questions. Firstly, whether the progenitors collected while on imatinib are truly long-term repopulating haemopoietic stem cells can only be addressed by long-term culture assays or transplantation studies. In animal models using syngeneic mice, it has at least been shown that continuous administration of imatinib has no adverse influence on bone marrow engraftment.[39] Secondly, the residual PCR positivity for *bcr-abl* in the harvests raises the question whether imatinib alone or combination therapy should be given after such autografts to prevent relapse. Studies using imatinib postautograft are preliminary[40] and it is not yet clear how well it will be tolerated and what impact it will have on leukaemic relapse.

In conclusion, adequate PBSC mobilisation using filgrastim in patients who achieved CCR with imatinib is feasible, and the

yield is significantly better with temporary cessation of imatinib. The procedure is safe without increasing disease burden, which provides the platform for further modifications to optimise the efficacy and safety of mobilisation strategies. The apparent beneficial increase in cell yields by interrupted imatinib requires confirmation in large studies, particularly in newly diagnosed chronic phase patients treated with imatinib alone. The profound effect of imatinib on mobilisation of normal stem cells warrants further investigation.

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