

Table 1 Patient and treatment characteristics

Patient no	1	2	3	4	5	6
Age (years)	20	22	40	44	52	75
Sex	M	F	F	F	F	F
CML date of diagnosis	9/96	1/99	3/99	11/97	3/94	5/96
CML phase at study entry (date)	Acceler. 3/01	Chronic 6/01	Chronic 12/00	Chronic 1/00	Acceler. 11/00	Acceler. 10/00
Best response	CCR	MCR	CHR	CHR	CHR	RTC
Current status	Alive, CCR	Alive, MCR	Alive, cP	Alive, cP	Alive, CHR	Died
Time to grade 3 or 4 neutropenia (days)	34	28	28	12	41	27
Imatinib breaks for neutropenia	3	0	1	1	1	4
Duration of G-CSF therapy (days)	173	37	450	840	180	84
G-CSF dose (MIU)/week max	7 × 48	3 × 30	3 × 30	7 × 30	3 × 15	3 × 30
G-CSF dose (MIU)/week min	1 × 20	1 × 15	2 × 30	2 × 15	2 × 15	3 × 30
Current Imatinib mg/d	500	400	600	400	300–400	NA
G-CSF MIU	30/12d	0	2 × 30/w	2 × 15/w	0	
ANC (× 10 ⁹ /l)	1.4	0.76	3.7	3.4	1.0	

CCR: complete cytogenetic response (Philadelphia chromosome negative), MCR: major cytogenetic response (1–35% Ph positive), cP: chronic phase CML, CHR: complete hematological response, RTC: return to chronic Phase, MIU: million international units, NA: not applicable.

was 0.5 MIU/kg body weight three times per week with the exception of one patient who received 0.5 MIU/kg body weight per day because of profound neutropenia and concurrent sepsis. The G-CSF dose was reduced after resolution of neutropenia to NCI grade 1 level (> 1.5 × 10⁹/l) and maintained at the lowest dose level that kept the neutrophils at or above NCI grade 1 level.

Five of six patients are alive after a median follow-up of 15.5 months (range 11–28 months) after start of G-CSF. All five patients are continuously treated with Imatinib. Two patients achieved a major cytogenetic response. Three patients had a CHR as their best response. Three of the patients still receive G-CSF simultaneously with Imatinib.

A 20-year-old male patient (Table 1, patient no. 1) was enrolled in the STI trial 114 having relapsed after allogeneic hematopoietic stem cell transplantation and progressed to accelerated phase CML. After 4 weeks on Imatinib he achieved a CHR. He was treated with a donor lymphocyte infusion (DLI) from the original matched unrelated donor. He achieved a complete cytogenetic response and a complete hematological chimerism could be demonstrated. Since the *bcr/abl* transcript was still detectable with the nested PCR method, Imatinib therapy was continued. This patient had three breaks in the Imatinib therapy because of recurring grade 3 and 4 neutropenias. After the third break we started G-CSF until the ANC reached normal values. G-CSF was stopped again and Imatinib was reintroduced at a dose of 300 mg/day. At 3 days after the restart of the Imatinib therapy, the dose had to be reduced further to 200 mg/day for grade 3 neutropenia. We then started him again on G-CSF and increased the Imatinib dose simultaneously to 400 mg/day. This patient is currently on 500 mg Imatinib per day. With 30 MIU of G-CSF every 12 days the ANC stays over 1.5 × 10⁹/l.

One patient (Table 1, patient no. 2) showed a minor cytogenetic response (46% Ph-positive metaphases) 3 months after start of the Imatinib therapy and reached a partial cytogenetic response (4% Ph positive) after 6 months. From the 2nd to the 6th month on Imatinib she received G-CSF for grade 4 neutropenia. She was then taken off G-CSF and for the remaining observation period she had ANCs between 0.5 and 1.0 × 10⁹/l on 400 mg Imatinib.

Two patients (patients nos. 3 and 4) had one break of the Imatinib therapy before the growth factor was given for recurring neutropenia after restart of the study drug. Both patients did not achieve a cytogenetic response. Attempts to stop G-CSF failed because of recurrence of neutropenia.

Patient no. 5 (Table 1) with accelerated phase CML had a therapy break 5 weeks after the start of the Imatinib therapy at a dose of

600 mg per day. After the toxicity resolved therapy was resumed with 400 mg per day. The ANC again dropped to less than 1.0 × 10⁹/l and G-CSF was added. The Imatinib/G-CSF combination therapy was carried on for 6 months. We then stopped G-CSF and reduced the Imatinib dose to 300 mg alternating with 400 mg per day. With this regimen the ANC is stable at 1.0 × 10⁹/l without G-CSF.

Patient no. 6 (Table 1) with accelerated phase CML had four breaks in the Imatinib therapy, two for grade 4 thrombopenia and two for grade 4 neutropenia. After the fourth break of the Imatinib therapy, the CML evolved into blast crisis. We started Imatinib 600 mg per day and G-CSF simultaneously when the ANC was still in the normal range. This patient died 2 months after the diagnosis of CML blast crisis.

No serious adverse events owing to the use of G-CSF did occur in any of the six treated patients.

These data show that Imatinib induced neutropenia in CML can be overcome by concomitant stimulation of myelopoiesis with G-CSF. This allows for continued Imatinib therapy.

The mechanisms by which CML affects normal hemopoiesis is only partly understood so far. Displacement through the expanding clone, microenvironmental factors or inhibitory cytokines are discussed as factors responsible for the suppression of normal hematopoiesis.⁵ Additional factors to displacement alone are required to explain the fact that none of the six patients regained adequate output of normal neutrophils after tumorload reduction. Even in remission, as evidenced by achieving a major cytogenetic response and/or a CHR, neutropenia was observed.

The early clinical trials with Imatinib showed that myelotoxicity was not only dose dependent, but primarily associated with disease stage. CML patients with advanced disease had significantly more frequently grade 3 or 4 myelotoxicity than patients with chronic phase CML.⁴ The percentage of peripheral blood or marrow blasts has been identified as the only significant variable for the development of grade 3 or 4 neutropenia for patients on Imatinib therapy.⁶ The presence of significant cytopenia after 6 months of Imatinib treatment is a bad prognostic factor. The development of grade 3 or 4 cytopenia in patients with chronic phase CML treated with Imatinib is associated with a significantly higher rate of progression to accelerated phase or blastic phase CML.⁷ We postulate that the treatment interruption at least might have been a contributing factor.

These observations motivate for continued Imatinib therapy especially in those patients at high risk for disease progression. A dose reduction of Imatinib or even a therapy break in these

situations should and can be avoided. G-CSF is a safe and effective drug to stimulate myelopoiesis and allows for continued Imatinib therapy in CML patients at risk for disease progression.

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Simultaneous occurrence of a t(9;22) (Ph) with a t(2;11) in a patient with CML and emergence of a new clone with the t(2;11) alone after imatinib mesylate treatment

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TO THE EDITOR

Chronic myeloid leukemia (CML) is characterized by the translocation t(9;22), called Philadelphia (Ph) chromosome. It is an acquired abnormality of hematopoietic stem cells, which is present in all dividing granulocytic, erythroid, and megakaryocytic cells in the marrow and also in some B and probably a minority of T lymphocytes. Fusion of the *ABL* gene on 9q34 to the *BCR* gene on 22q11 results in a constitutive activation of the ABL tyrosine kinase, which plays a major role in leukemogenesis.¹ Although a multistep model for the development of CML has been proposed with the acquisition of the Ph chromosome as a second event,^{2–4} the expression of *Bcr/Abl* as a sole oncogenic event in mice results in the development of leukemia, establishing *Bcr/Abl* as the molecular pathogenic event in CML.^{5,6} The tyrosine kinase activity of the fusion protein is critical for the transforming ability, making it an attractive target for antitumor drug development. The novel molecular, targeted anticancer agent imatinib mesylate, also called STI571 or Glivec (Novartis), specifically inhibits the p210^{BCR/ABL} tyrosine kinase activity, the *in vitro* growth of *BCR/ABL* positive cells, and eradicates *Bcr/Abl* positive tumors in nude mice.^{7,8} Phase I and II clinical trials of patients with chronic phase CML produced encouraging results with a complete hematologic response in a large proportion of patients (for a recent review, see Capdeville *et al.*⁸).

Here we describe a patient with a Ph⁺ CML in chronic phase, with a second translocation present in all cells at the time of the first cytogenetic analysis. The patient, a 63-year-old male, was initially treated with hydroxyurea (HU) for 7 weeks, and a cytoreduction from 93.8 leukocytes/nl to 8.9/nl with 4% metamyelocytes was achieved. Then, therapy with imatinib mesylate (400 mg/day) was started in the context of a randomized phase III study and a

complete hematological remission (CHR) was observed after 1 week, lasting until today (22 months of therapy).

The first cytogenetic analysis was performed before starting the imatinib mesylate therapy and during therapy every 3 months. At least 20–22 metaphases were fully karyotyped. Two translocations, t(2;11)(p21;q23) and t(9;22)(q34;q11), were found in all cells analyzed. To exclude a complex variant translocation involving all four chromosomes, we used whole-chromosome paints for these four chromosomes, confirming the presence of two independent reciprocal translocations. A FISH analysis using the 1S-FISH dual color translocation probe (ONCOR, Gaithersburg, MD, USA) was performed on meta- and interphases and a regular *BCR/ABL* rearrangement without amplification was apparent (not shown). As assessed by multiplex RT-PCR the patient had the b3a2 variant of the *BCR/ABL* mRNA.

During imatinib mesylate treatment, cells containing both translocations gradually decreased. After 2½ months of therapy, only four cells had both abnormalities and a clone with the t(2;11) as the sole abnormality became apparent. In the most recent analysis, at 22 months of imatinib mesylate therapy, both translocations were seen in 1/20 cells and the t(2;11) in 19/20 cells. Table 1 shows the results of the consecutive analyses. Quantitative real-time RT-PCR on peripheral blood (PB) was used to follow the treatment response on a molecular level. We found a continuous reduction of the *BCR/ABL:G6PDH* ratios from 3.53 to 0.0027% during treatment, indicating a good molecular response of the disease. This correlates with a CHR and a major cytogenetic response with 0/20 Ph⁺ metaphases at 17 and 1/20 at 22 months of therapy (Table 1).

As the t(2;11) was still present in a high number of the analyzed cells, it seems possible that the patient carried this translocation constitutionally. This was tested by the analysis of PB. No metaphases were obtained from unstimulated cells, but 18 out of 101 PHA stimulated cells carried the t(2;11), indicating the presence of this translocation in at least a precursor for T cells. We also analyzed fibroblasts from a skin biopsy of the patient, but the t(2;11) was not found in 200 cells. This suggests that he does not carry it constitutionally; however, a constitutional mosaicism limited to

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