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Imatinib plus Granulocyte Colony-Stimulating Factor in Chronic Myeloid Leukemia Patients Who Have Achieved Partial or Complete Cytogenetic Response while on Imatinib

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Key Words
Chronic myeloid leukemia · Imatinib · Granulocyte colony-stimulating factor

Abstract
Background: The BCR/ABL tyrosine kinase inhibitor imatinib is highly effective in the treatment of chronic myeloid leukemia (CML) but fails to eliminate all leukemia cells. In this study, we investigated whether the addition of granulocyte colony-stimulating factor (G-CSF) could reduce the level of residual disease in patients with Ph-positive CML who appeared to have achieved a suboptimal response to imatinib alone.
Methods: Eleven patients with CML who had achieved ≥35% Ph-negativity on imatinib were enrolled. The starting dose of imatinib was 400 mg or 600 mg orally daily, and of G-CSF 5 μg/kg s.c. daily. The administration of G-CSF was postponed or interrupted in the event of leukocytosis (≥30 $\times 10^9$ leukocytes/l) until the white blood cell count fell below 20 $\times$ 10^9/l. Efficacy was assessed by serial monitoring of blood levels of BCR-ABL transcripts.
Results: Of 11 evaluable patients, 9 had an appreciable decline in BCR-ABL transcript levels; in 7 cases the reduction was greater than 1 log.
Conclusions: We conclude that the addition of G-CSF should be considered for patients on imatinib who fail to obtain optimal response to imatinib alone and that this approach deserves further evaluation as frontline therapy for newly diagnosed CML.

Baijun Fang and Ling Mai are equal contributors.

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Introduction

Chronic myeloid leukemia (CML) is a clonal disease of hemopoietic stem cell origin characterized by the t(9;22) chromosomal translocation that generates the BCR/ABL oncogene [1, 2]. Imatinib (Gleevec or Glivec; Novartis Pharmaceuticals, Basel, Switzerland), a small-molecule inhibitor of the BCR/ABL tyrosine kinase, has proven to be highly effective for the treatment of CML. Imatinib induces rapid hematologic and complete cytogenetic responses (CCRs) in most chronic-phase CML patients (82% at 30 months) but rarely eradicates the BCR-ABL^{+} clone [3–5]. The persistence in most imatinib-treated patients of a small but molecularly detectable population of leukemic cells is of concern as they represent a potential reservoir from which mutant imatinib-resistant CML cells may emerge [6]. Moreover, it is likely that the treatment of patients with imatinib is less effective on the most primitive, quiescent leukemic cells that display stem cell properties, as experienced in immunodeficient mice [7]. In vitro studies have further shown that imatinib exerts an antiproliferative effect on these primitive quiescent CML cells that reduces their rate of elimination [8, 9].

Recently, in vitro studies have shown that intermittent exposure to granulocyte colony-stimulating factor (G-CSF) can enhance the effect of imatinib on CML cells by specifically targeting the primitive quiescent leukemic elements [10, 11].

Taken together, these observations prompted us to look for a treatment that might enhance the rate of entry into the cycle of primitive quiescent CML cells and thereby improve responsiveness to imatinib. For these reasons, a pilot study was designed to involve the addition of G-CSF to imatinib for patients with CML who had achieved a partial cytogenetic response or a CCR with an apparent plateau of BCR-ABL transcript numbers at suboptimal levels. These patients were classified as partially sensitive to imatinib such that they might benefit from the addition of growth factor stimulation.

Patients and Methods

Patients

A total of 11 patients were registered in this study between January 2005 and March 2008 (**table 1**). Patients were eligible if they met all the following criteria: (1) treatment with imatinib at a minimum dose of 400 mg per day for at least 2 years; (2) the achievement of at least a minor cytogenetic response (defined as at least 35% of Ph-negative marrow metaphases); (3) achievement of a plateau in BCR-ABL transcripts defined by measuring BCR-ABL transcripts on at least 4 occasions over a minimum period of 1 year, with the latest value not lower than the previous minimum value. Patients were excluded from the study if their imatinib dosage had been modified over the preceding 12 months. Other eligibility criteria included age ≥18 years, bilirubin <1.5 × upper limit of normal value, serum creatinine <2.0 mg/dl, aspartate aminotransferase <3.0 × upper limit of normal value, left ventricular ejection fraction >40%, and a prediction of pulmonary function forced expiratory volume at 1s >50%. Pregnancy and active infection were exclusion criteria. All patients gave signed informed consent indicating that they were aware of the investigational nature of this study, and the protocol was approved by the Institutional Review Board at Henan Tumor Hospital. The definitions of chronic and accelerated phases of CML, the classification of cytogenetic responses, and criteria for failure of interferon-α were based on previous publications [4, 12].

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Treatment Schedule

For these registered patients, the starting dose of imatinib was 400 or 600 mg orally daily, and of G-CSF 5 μg/kg s.c. daily. The administration of G-CSF was postponed or interrupted in the event of leukocytosis (≥30 $\times 10^9$ leukocytes/l) until the white blood cell count fell below 20 $\times 10^9$/l. Treatment with G-CSF was discontinued if a patient did not achieve a reduction in the transcript level of at least 0.5 log after 6 months. For patients whose BCR-ABL transcript levels continued to decline but who had not yet reached molecular remission, treatment was designed to continue for 1–6 months. The management of tumor lysis syndrome was performed under the guidelines of our institute.

Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction

The numbers of BCR-ABL transcripts in the peripheral blood were measured serially while imatinib was administered as a single agent, at the time of starting G-CSF, and serially thereafter as Cross et al. described [13]. Expression of the *ABL* gene was used as control and results were expressed as the BCR-ABL/ABL ratio percentage. Occasional blood specimens were invalidated for technical reasons; for example, samples with control <1 $\times 10^4$ ABL transcripts were considered suboptimal and were excluded from the analysis. In cases where BCR-ABL transcripts were undetectable by quantitative real-time reverse transcription polymerase chain reaction (PCR), the results were confirmed by nested primer PCR.

Results

Response

Patients' responses are shown in table 1. After G-CSF was added, the transcript levels declined by >0.5 log in 9 cases and by >1 log in 7 cases. The 5 patients not in CCR prior to G-CSF therapy achieved CCR (patients 1, 3, 5, 7 and 8). Patients 5 and 8 had developed a trisomy 8 in addition to the Ph chromosome in all metaphases when they were receiving interferon. Before enrollment in the study, they had been treated with imatinib, first 400 mg and then 600 mg for 19 and 21 months, respectively, but failed to gain a major cytogenetic response. On G-CSF, both achieved a CCR; their marrow metaphases contained neither Ph chromosome nor trisomy 8. Two patients achieved complete molecular responses (patients 2 and 9). These molecular responses lasted for 7 months (patient 2) and 5 months (patient 9), respectively, after discontinuation of the G-CSF; transcript numbers had, by then, risen to levels similar to their respective baseline levels.

Toxicity

The most frequent grade 1–2 nonhematologic toxicities experienced in the study were nausea (n = 6) and fatigue (n = 8). In the majority of cases, these side effects were mild and did not prevent the administration of G-CSF. Grade 3–4 nonhematologic side effects consisted of myalgias (n = 1) and fatigue (n = 2). All patients had reactions at the site of injection (grade I), which needed 4–8 days to resolve. No bleeding episodes occurred. No patient discontinued therapy because of toxicity and there were no treatment-related deaths.

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Discussion

Imatinib treatment results in a significant inhibition of CML progenitor cell proliferation but only a modest increase in apoptosis [8, 9, 14]. Apoptosis is restricted to dividing cells, whereas nondividing cells resist apoptosis [8, 14]. Imatinib-induced inhibition of CML progenitor proliferation together with the resistance of nondividing CML progenitors to imatinib-mediated apoptosis likely contributes to incomplete elimination of malignant progenitor cells in patients otherwise responding well to this agent. Undivided CML progenitors remaining after imatinib treatment represent either dormant, noncycling cells, or cells that are inhibited from entering the cell cycle by the antiproliferative effects of imatinib. The antiproliferative effect of imatinib enhances this population of nondividing cells and potentially interferes with the elimination of malignant progenitors through apoptosis. Holtz et al. [14] have shown that the undivided population is BCR/ABL positive, is not enriched for BCR/ABL-negative cells, and expresses the BCR/ABL gene. In addition, the nondividing CML $CD34^{+}$ cell fraction is also resistant to elimination following treatment with several therapeutic agents.

Recently, in vitro studies have shown that G-CSF stimulation could activate CML progenitors into cell cycle and reduce the number of viable undivided CML progenitors that persist after imatinib treatment [10, 11]. In addition, G-CSF has been safely and successfully used for peripheral blood stem cell mobilization in healthy donors and in CML patients treated with imatinib with no significant increase in *BCR-ABL* transcript levels by quantitative reverse transcription-PCR [15, 16]. G-CSF is currently being used in patients with CML to overcome imatinib-induced neutropenia, as myelosuppression during imatinib therapy has been found to be associated with a poorer cytogenetic response [17, 18]. In this setting, it has been postulated that the improved cytogenetic responses observed result from an increased exposure to imatinib [19–21]. However, another effect of pharmacologic doses of G-CSF given to CML patients might be to stimulate the entry of their quiescent CML stem cells into cycle and, hence, to increase the sensitivity of these cells to imatinib [10, 21, 22].

Recently, we demonstrated the existence of a population of rare, primitive cells with full characteristics of leukemic stem cells in bone marrow of CML patients [23], and further studies suggested that the G-CSF could significantly enhance the effect of imatinib on CML cells by specifically targeting these primitive quiescent leukemic elements in vitro [unpublished observations].

Conclusion

The results of the current study provide a strong rationale for further clinical evaluation of the effectiveness of G-GSF in reducing residual disease in CML patients treated with imatinib. We suggest that the addition of G-CSF should be considered for patients on imatinib who fail to obtain optimal response to imatinib alone and that this approach deserves further evaluation as a frontline therapy for newly diagnosed CML. However, the mechanism of partial sensitivity to imatinib was not evaluated in this study. This could be an important factor in determining which patients might respond to the addition of G-CSF, and this should be addressed in future studies.

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Disclosure Statement

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Table 1. Patients' characteristics and responses

Patient	Age (sex)	Clinical status at the onset of imatinib	Dose of imatinib before G-CSF (mg/day)	Time from onset of imatinib to G-CSF therapy (m)	Baseline Q-PCR before G-CSF therapy	Minimal Q-PCR before G-CSF therapy	Q-PCR on G-CSF 6 m later	Log reduction from baseline on G-CSF 6 m later
1	39 (F)	newly diagnosed	400	32.4	17.2	4.8	0.58	>1
2*	19 (M)	IFN-α failure	400	27.6	0.16	0.09	0	>1
3	27 (F)	IFN-α failure	400	28.2	16.2	4.3	0.6	>1
4	41 (M)	IFN-α failure	400	31	8.98	2.56	3.75	<0.5 lack of efficacy
5	39 (F)	IFN-α failure	600	25.4	27	12.8	0.8	>1
6	52 (M)	IFN-α failure	400	42.6	0.42	0.19	0.087	>0.5
7	32 (M)	AP	600	27	26.5	11.8	0.56	>1
8	19 (M)	IFN-α failure	600	27.2	25.1	15.4	0.52	>1
9*	30 (M)	IFN-α failure	400	28	0.03	0.02	0	>1
10	26 (F)	IFN-α failure	400	26.5	4.5	3.2	3.8	<0.5 lack of efficacy
11	38 (M)	newly diagnosed	400	38.3	4.6	2.18	1.2	>0.5

* Patients who achieved complete molecular responses on G-CSF 6 months later. IFN-α = Interferon-alpha; AP = accelerated phase; m = monthsQ-PCR = quantitative real-time reverse transcription PCR.

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