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Leukemia Research 29 (2005) 397–400
Leukemia Research
www.elsevier.com/locate/leukres

Imatinib mesylate in the treatment of Core Binding Factor leukemias with *KIT* mutations A report of three cases

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Received 14 July 2004; accepted 14 October 2004
Available online 12 January 2005

Abstract

Aim of this study is to investigate the capability of Imatinib to induce an anti-leukemic effect in Core Binding Factor (CBF)-leukemia patients presenting either with extracellular juxtamembrane or kinase *KIT* mutations. On the basis of a screening analysis for *KIT* mutations, two patients with a kinase mutation and one with extracellular juxtamembrane mutation, in first or subsequent leukemic relapse, received 400 mg Imatinib twice daily for 30 days. After Imatinib discontinuation, bone marrow cells were re-tested to assess the *KIT* mutational status and the chromosomal set. In our experience, none of the treated patients had a response by standard criteria; in particular, we did not observe any activity against acute myeloid leukemia (AML) associated with *KIT* kinase mutations. However, in the patient with extracellular juxtamembrane mutation, Imatinib seems to have some clinical beneficial effect and, most important, is able to abrogate the leukemic subclone carrying the mutation. Whether Imatinib, in combination with other agents, may play a role in the treatment of AML with more sensitive extracellular juxtamembrane *KIT* mutation remains to be determined.

Keywords: Imatinib; CBF-leukemias; *KIT* mutations

1. Introduction

It has been recently documented that the incidence of *KIT* point mutations ranges from 5% to 40% of newly diagnosed acute myeloid leukemias (AML) with *t*(8;21) (q22;q22) and inv(16) (p13;q22) [1,2]. Mutations may affect either the juxtamembrane domain proposed to regulate the activity of an otherwise normal enzymatic site of the *KIT* receptor, such as insertion or deletions of exon 8 or 11 of the *KIT* gene, or may affect the structure of the tyrosine kinase domains I and II (kinase domain mutations) as in cases with single amino acid substitution at codon 816 (D816 mutants). These types of mutations lead to a gain-of-function of the *KIT* receptor and induce a *KIT*-dependent proliferation. Recently, investigators at the Columbia University reported that Imatinib is able to inhibit *KIT*-dependent phosphorylation in a human mast cell leukemia cell line subclone (HMC1.1), which expresses only the Val560Gly juxtamembrane mutation, but failed to suppress constitutive phosphorylation of *KIT* in the HMC1.2 subclone, which expresses both the Val560Gly and kinase mutations (Asp816Val), establishing a general rule whereby classification of mutations may be useful in predicting tumour sensitivity to inhibitory drugs [3].

Aim of the present study is to investigate the capability of Imatinib to induce an anti-leukemic effect in Core Binding

Abbreviations: AML, acute myeloid leukemia; HMC, human mast cell leukemia; CBF, Core Binding Factor

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0145-2126/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.leukres.2004.10.005

Factor (CBF)-leukemia patients presenting either with extracellular juxtamembrane or kinase *KIT* mutations.

2. Patients and methods

2.1. Mutational analysis and patients selection

2.1.1. Mutational analysis

Exon 17 mutations in the *KIT* gene were identified by sequencing and by more sensitive assays as HinfI assay for Asp816Val as previously described [1] and amplification refractory mutation system (ARMS) PCR for Asp816Tyr. Briefly, 168 bp (mutated) ARMS PCR products of *KIT* exon 17 were generated using the following primers: 17A 5′-AGTTTTCACTCTTTACAAG-3′ and 17B 5′-TTAGAATCATTCTTGATGTA-3′. Denaturing, annealing and extension steps were performed at 94 °C for 30 s, 48 °C for 20 s, 72 °C for 20 s, and a final extension step at 72 °C for 4 min.

Products were resolved on 1.8% agarose-gel and visualized by using Typhoon 9200 FluorImager system (Amersham Pharmacia).

Semiquantitative mutation analysis of exon 8 of *KIT* gene was obtained after capillary electrophoresis of PCR products using the primers 8A and 8B, as previously described [2].

The mutational screening showed the presence of a gain-of-function *KIT* mutation in 24 out of 52 (46.1%) newly diagnosed CBF-leukemias [4]. Two patients with a kinase mutation and one with extracellular juxtamembrane mutation, in first or subsequent leukemic relapse, were enrolled in this study. Patients and disease characteristics are summarised in Table 1.

2.2. Study design

This was a multi-centre, open-label, single arm trial. AML patients with *KIT* mutation were eligible if they had recurrence after chemotherapy or had not been candidates for intensive treatments because of concomitant medical problems or their refusal to receive chemotherapy.

The enrolled patients received 400 mg Imatinib twice daily for 30 days. Responding patients were scheduled to receive further treatment in cases in which the investigators judged that prolongation of therapy was of clinical benefit. The concomitant use of other anti-cancer drugs or radiation therapy was not permitted. Treatment was interrupted in cases of disease progression and was reduced in response to hematologic or non-hematologic toxicity, graded according to the WHO common toxicity criteria. All patients gave written consent to participate in the study and the protocol was reviewed and approved by a local ethics review committee.

2.3. Efficacy assessment

Patients were evaluated for hematologic response at enrolment, at week 4 and whenever it was clinically necessary. Furthermore, at enrolment and at Imatinib discontinuation,

Table 1
Imatinib mesylate in the treatment of CBF-leukemia with *KIT* mutations: patients' characteristics and outcome

	Patient no.		
	1	2	3
Age/sex	58/F	54/M	70/M
FAB	M4Eo	M2	M4Eo
Blasts phenotype	CD34+, CD117+, CD13+, CD33+; CD56−, CD19−	CD34+, CD117+, CD13+, CD33+, CD56+; CD19−	CD34+, CD117+, CD13+, CD33+; CD56−, CD19−
Type of *KIT* mutation	D816V	D816Y	Exon 8 insertion
Disease status at enrolment	1st relapse	2nd refractory relapse	1st relapse, hematological and extramedullary
Treatment duration (days)	15	9	35
WBC $\times 10^9$/L (%blasts)			
At enrolment	8 (17)	25 (97)	7 (28)
After Imatinib	16 (35)	49 (98)	16.6 (45)
Platelet count $\times 10^9$/L			
At enrolment	9	6	11
After Imatinib	11	6	14
Cytogenetic			
At enrolment	46XX, inv(16) (q13;q22) [50]	46XY, *t*(8;21) (q22;q22) [20]	46XY, inv(16) (q13;q22) [20]
After Imatinib	46XX, inv(16) (q13;q22) [20]	46XY, del(7) (q22), *t*(8;21) (q22;q22) [11]; 46XY, del(3) (p24), del(7) (q22), t(8;21) (q22;q22) [9]	46XY, inv(16) (q13;q22) [20]
KIT mutation[a]			
At enrolment	+	+	+
After Imatinib	+	+	−

The number in brackets [] indicates the number of metaphases.
[a] Presence (+) or absence (−) of *KIT* mutation.