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

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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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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.

bone marrow cells were re-tested to assess the *KIT* mutational status.

3. Results

3.1. Patients with KIT kinase mutations

Case no. 1: A 58-year-old woman, with a history of breast cancer, was diagnosed with AML(M4Eo) and D816V *KIT* mutation on June 2003. After a standard induction chemotherapy (idarubicin and cytarabine), she received two consolidation courses (idarubicin and High Dose-cytarabine; High Dose-cytarabine) but relapsed 3 months thereafter. Treatment with Imatinib was started 8 days after a single dose of cytarabine 100 mg/m^2 as continuous infusion and 24 h after hydroxiurea 1500 mg per day orally for 3 consecutive days. After 15 days of treatment, Imatinib was discontinued for the evidence of a progressive leukemic re-growth. Despite a transient response to an additional chemotherapy (mitoxantrone, cytarabine and etoposide), the patient died 115 days after Imatinib withdrawal, for disease progression.

Case no. 2: The patient, a 54-year-old man, in second relapse of de novo CBF-leukemia (AML-M2) with D816Y *KIT* mutation was enrolled in this study in July 2003. The patient achieved the first complete remission with a standard induction regimen (idarubicin, cytarabine and etoposide); postremission therapy consisted of five consolidation courses, including three cycles with High Dose-cytarabine. At first relapse, additional chemotherapy with Ida-FLAG (idarubicin, fludarabine, High Dose-cytarabine and G-CSF), FLANG (fludarabine, cytarabine, mitoxantrone and G-CSF) and High Dose-cytarabine resulted in a second CR lasting 2 months. Treatment with Imatinib was started on day 40 after a second course of FLANG, while the WBC were 25×10^9/L (97% blasts). The drug was stopped after 9 days of treatment because the patient experienced a rapid increase of peripheral blood blasts and a leukemic skin infiltrate. In spite of the administration of additional chemotherapy (daunorubicin and carboplatin), the patient died for leukemia 5 days later.

3.2. Patient with extracellular juxtamembrane mutation

Case no. 3: A 70-year-old man was enrolled in this study in August 2003, in first relapse of AML with exon 8 in-frame deletion plus insertion *KIT* mutation, occurring 7 months after the diagnosis. Previous chemotherapy consisted of an induction with FLAG followed by two consolidation courses (daunoxome and High Dose-cytarabine; cytarabine). At relapse, the peripheral blood counts were WBC 7×10^9/L (differential count: neutrophils 6%, lymphocytes 37%, eosinophils 6%, atypical monocytes 23%, blasts 28%),

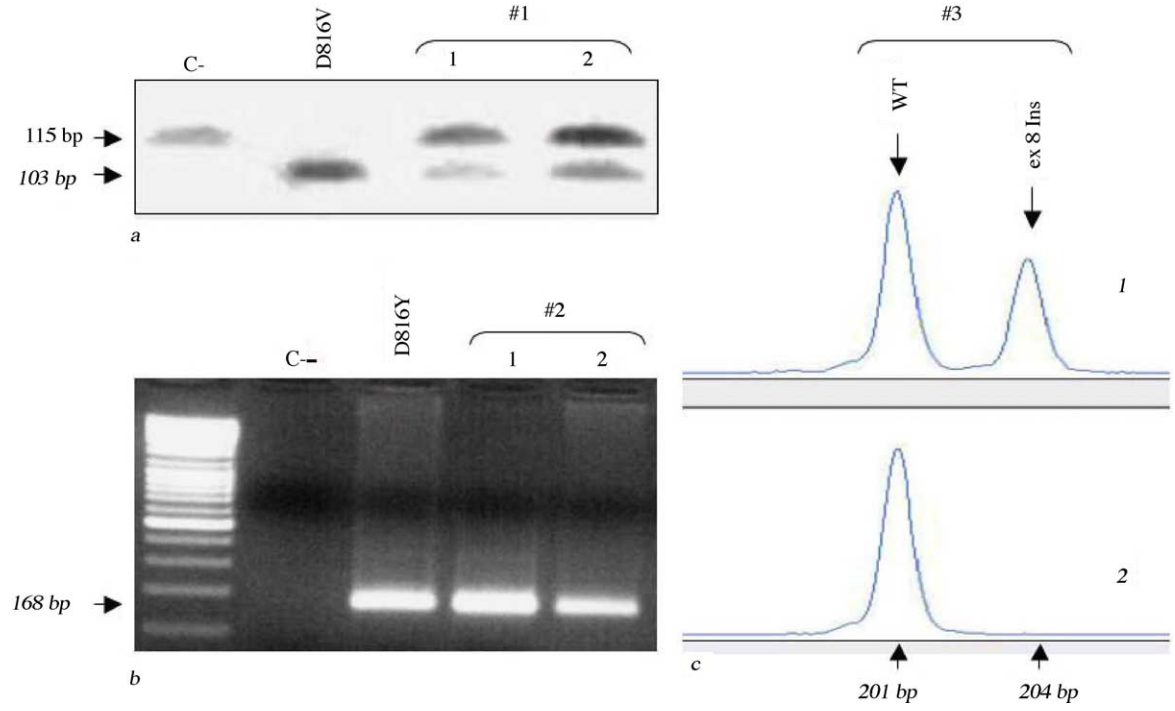

Fig. 1. Detection of *KIT* mutations in three CBFL patients at initial diagnosis and relapse (1) and after Imatinib mesylate treatment (2). D816V mutation was detected in CBFL patient no. 1; we amplified exon 17 by PCR and then digested it with HinfI endonuclease (a). When amplified products contain D816V mutation, two bands (115 bp and 103 bp) were obtained and visualized on agarose gel electrophoresis. (b) D816Y mutation was detected in CBFL patient no. 2. Amplification refractory mutation system (ARMS) PCR products of *KIT* exon 17 were generated and resolved on 1.8% agarose-gel. (c) *KIT* exon 8 in-frame deletion plus insertion mutation was detected in patient no. 3; abnormal exon 8 amplification products (204 bp) were resolved by capillary electrophoresis. In CBFL patient no. 3, whose leukemia cells had the *KIT* exon 8 in-frame deletion plus insertion mutation at the initial diagnosis and relapse, the mutation was lost after Imatinib mesylate treatment.

Hb 7.6 g/L and platelets 11×10^9/L; the morphological features of bone marrow and routine cytochemical reactions were consistent with acute myelomonocytic leukemia with eosinophilia (74% blasts) and an extramedullary leukemia was present in form of salivary gland tumour. Furthermore, a lumbar MRI was consistent with leukemic infiltration at L3 level leading to severe bone pain with radicular features. Imatinib was started 7 days after single dose of cytarabine 100 mg/m^2 as continuous infusion. During Imatinib therapy, the absolute neutrophils count rose from 0.3×10^9/L (baseline value) to 2.1×10^9/L (after 29 days of treatment) and declined in the following days; the blasts remained quite stable around the baseline until the day after 27 days (WBC 5.4×10^9/L, blasts 11%) and then rose quickly. There was no improvement in platelet count. After 35 days of treatment, Imatinib was stopped for leukemia progression (WBC 16.6×10^9/L, peripheral blood blasts 45%, bone marrow blasts 70%). During Imatinib therapy, a complete regression of the salivary gland tumour and a decrement of the radicular pain were noted. Treatment was well tolerated; the patient had only nausea and dispepsia leading to a 24% dose reduction. After 121 days from the Imatinib discontinuation, the patient experienced a meningeal leukemia, showed only a transient response to additional palliative therapies (etoposide, i.t. methotrexate, radiation therapy on lumbar spine) and died for disease progression.

3.3. Laboratory correlative studies

After Imatinib discontinuation, bone marrow cells were re-tested to assess the *KIT* mutational status and the chromosomal set: it is noteworthy that *KIT* mutation was still present in the two patients with kinase mutation but was not detectable in the patient with extracellular juxtamembrane mutation (case no. 3) (Fig. 1). Cytogenetic analysis showed no karyotypic change in two patients (case nos. 1 and 3) and multiple additional abnormalities in patient no. 2.

4. Discussion

Complete remission achieved upon administration of Imatinib in the treatment of patients with advanced leukemias has been rarely reported in the recent literature; furthermore, the biological basis of the observed responses remained unknown [5–7]. Aim of this work was to investigate the capability of Imatinib to induce an anti-leukemic effect in CBF-leukemia patients presenting either with kinase or extracellular juxtamembrane *KIT* mutations to validate, in vivo, the general paradigm whereby this categorisation of mutations is able to predict tumour sensitivity to inhibitory drugs [3]. In our experience, none of the treated patients had a response by standard criteria; in particular, we did not observe any activity against AML associated with *KIT* kinase mutations. However, in the patient with extracellular juxtamembrane mutation, Imatinib seemed to have some clinical beneficial effect and, most important, was 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.

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