Adding Hydroxyurea to Imatinib is Effective in Patients with Chronic Myelogenous Leukemia Resistant to Imatinib Alone

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Abstract: From 11/02 to 11/03, 11 patients in chronic phase (6 males and 5 females, median age 52.9 years, range 29.9-67.9 years) with persistance of >66% Ph+ cells after both standard and increased dose of Imatinib alone, were considered resistant and added Hydroxyurea (HU) to Imatinib. Seven patients were pretreated with IFN before Imatinib, median times from diagnosis and from Imatinib treatment to HU addition were 51 months (range 23-151) and 13 months (range 9-31), respectively. Four patients achieved. Complete Cytogenetic Response (CCR) after 3, 7, 12 and 32 months and 2 patients achieved a Major Cytogenetic Response (MCR) after 3 and 7 months, the remaining 5 patients were resistant, with persistence of Ph+ 100%. One patient in CCR achieved also Complete Molecular Response (CMolR) and is still responsive after 23 months. The other 3 patients in CCR as well as the 2 patients in MCR relapsed after 4, 4, 4, 10 and 12 months. In conclusion, this study provides the 1st *in vivo* evidence of an additive activity of HU and Imatinib; this association seems capable to induce cytogenetic response in at least one third of patients resistant to Imatinib alone, with minimal toxicity: a longer follow-up and a comparison with other associations is needed to evaluate the quality and duration of such response.

Keywords: Chronic myelogenous leukemia, imatinib resistance, hydroxyurea.

INTRODUCTION

The use of Imatinib in the treatment of Chronic Myelogenous Leukemia (CML) leads to the achievement of Complete Cytogenetic Response (CCR) in 70%-90% of patients, with 10%-30% showing persistence of Ph+ cells after standard (400 mg/day) or even high (600-800 mg/day) doses of the drug. These patients are thus cytogenetically resistant to Imatinib alone [1, 2].

Before the advent of 2nd generation tyrosine-kinase (TK) inhibitors, treatment of resistant patients was uncertain and mainly based on the transplant procedures, unfortunately, in almost half of the cases, this approach was hampered by patient age or donor lack [3]. The association of Imatinib with other drugs, such as low-dose cytarabine, was tested *in vitro* [4-6] and *in vivo* [7, 8] as alternative therapy, but results were conflicting.

Hydroxyurea (HU) has been employed in CML setting for more than 40 years and its efficacy in managing leukocytosis as well as other disease-related symptoms is well known; in addition, its toxicity profile is very favorable.

Thus, before the introduction of new TK inhibitors, we considered HU a good candidate to be added to Imatinib in patients resistant to this drug alone and report herein our experience on this association.

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METHODS

Imatinib resistance in patients, receiving the drug at the standard dosage of 400 mg/day was defined according to the following criteria:

a) lack of any cytogenetic response after 6 months, or

b) lack of major cytogenetic response (MCR) after 12 months

Cytogenetic analyses were performed on bone marrow (BM) aspirates from direct or short term (24-48h) cultures, with or without colcemid exposure. These had been carried out at diagnosis, before starting imatinib, after 3, 6, and 12 months of therapy with Imatinib, before adding HU, after 3, 6 and 12 months of HU and Imatinib association and thereafter every six months. Metaphases were examined after GAG banding, according to standard methods. Cytogenetic response was categorized according to the standard criteria. CCR was defined as the presence of 100% Ph- metaphases, MCR as the presence of > 66% Ph- metaphases.

Qualitative RT-nested PCR was carried out according to the standardized RT-PCR analysis of fusion gene transcripts, as previously described in the report of the BIOMED-16 [9].

RESULTS

Baseline Clinical Characteristics

From 11/2002 to 11/2003, 11 patients with CML in chronic phase (6 males and 5 females, median age 52.9 years, range 29.9-67.9 years) showing persistence of > 66% Ph+ cells after Imatinib treatment were considered cytogenetically resistant to this drug alone and added HU.

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Clinical characteristics of patients at diagnosis and before HU addition are shown in Table **1**. Before Imatinib, 7 patients had received IFN and 2 HU, only 2 patients were given Imatinib from diagnosis as front-line treatment. All patients received at least 6 months of Imatinib at the standard dosage of 400 mg/day. In 9 patients, this dose was increased to 600 mg/day for at least 3 additional months, while 2 patients, who developed severe neutropenia during standard dosage of Imatinib, remained on the same 400 mg/day dosage. Median time of Imatinib treatment before HU addition was 13 months (range 9-31), and the median time from diagnosis to HU addition was 51 months (range 23-151).

Treatment Results

The initial standard dosage of HU given in addition to Imatinib was 1000 mg/day: thereafter, the HU dosage was adjusted to maintain WBC levels < 5 x 10^9/l. All patients continued Imatinib with the same dose received before HU addition (600 mg/day in 9 patients and 400 mg/day in 2 patients).

Clinical patient's characteristics and response to Imatinib + HU combination are shown in Table **2**. With HU addition to Imatinib, 4 patients achieved CCR after 3, 7, 12 and 32 months, respectively, one of the patients achieved a complete molecular response (CMolR) after a further 6-month period of combined treatment. In addition, 2 patients achieved MCR after 3 and 7 months, respectively, the remaining 5 patients were resistant, with unmodified persistence of Ph+ 100% all along the combined treatment.

Both hematological and extra-hematological toxicity were mild and no patient discontinued treatment, 3 patients had granulocytopenia and/or thrombocytopenia of WHO grade 3, which promptly resolved with Imatinib dose reduction (from 600 to 400 mg/day in 2 patients and from 400 to 300 mg/day in 1 patient). Only 1 patient had a severe infectious complication (bronhopneumonia), which required hospitalization and resolved under iv antibiotic treatment.

Mutational status before HU addition was studied in only 5/11 patients. There were 2 unmutated patients and 3 mutated patients, one of the unmutated patients and the patient with F317L mutation responded to HU addition, as shown in Table **2**.

Follow-Up

Follow-up of all enrolled patients is shown in Table **2**. The patient who achieved a CMolR is still responsive after 23 months. The other 3 patients who obtained CCR relapsed after 4, 4 and 12 months, respectively; of them, 1 patient (#5) developed a blastic phase (BP) 3 months after the relapse and died during intensive chemotherapy, 1 (#1) was treated with Nilotinib and achieved a persisting 2nd CCR, and 1 (#6) was resistant to Nilotinib and is waiting for haploidentical marrow transplantation. Both patients who obtained MCR relapsed after 4 and 10 months respectively; one of them (#8) achieved a persisting 2nd CCR with Nilotinib and 1 (#10) was resistant to Nilotinib with appearance of T315I mutation and underwent a successful marrow transplantation from unrelated donor. Among the 5 resistant patients, 1 evolved to BP and died from progressive disease while 4 received salvage treatment with Nilotinib, 2 of them (#3, #9) achieved a persisting CCR and 2 (#4, #7) were resistant and are still alive in chronic phase.

DISCUSSION

Imatinib has profoundly changed the prognosis of CML, and at present the vast majority of patients can achieve a CCR and possibly a complete disappearance of BCR/ABL hybrid gene, however, patients who do not achieve CCR after 12-18 months of Imatinib treatment must be considered resistant and are candidate to alternative therapies.

An international panel of experts recently published general guidelines for defining resistance to Imatinib [10]. These guidelines are now worldwide accepted, but they were not available when resistance to Imatinib was considered in the present study; nevertheless, all the patients included would

Table 1. Clinical Characteristics of Patients Before HU Addition

PTS	Sex	Age (at Diagnosis)	SOKAL	Pre-Imatinib Treatments	Months of Treatment with Imatinib 400 mg	Months of Treatment with Imatinib 600 mg
1	F	31.9	Int	IFN	10	4
2	M	63.3	High	IFN+HU	6	3
3	F	47.3	High	HU	6	3
4	M	52.2	Low	HU	6	3
5	F	23.5	Low	IFN	9	4
6	F	46.6	Int	None	21	NA
7	M	40.1	Low	IFN	6	3
8	M	50.6	Low	IFN+AraC	15	16
9	M	47.7	Low	IFN+AraC	10	4
10	M	39.1	Int	IFN+HU	10	NA
11	F	66.4	Int	None	12	5

Int = Intermediate; IFN = αInterferon; AraC = Cytarabine; NA=Not applicable.

Table 2. Clinical Characteristics at HU Addition and Response

PTS	AGE (at HU Addition)	Ph+ Cells at HU Addition	Mutational Status	Response	Time to Response (Months)	Time to Relapse (Months)	Follow-Up
1	35.9	100%	NA	CCR	32	4	Alive (Nilotinib→CCR)
2	66.6	100%	NA	Res	/	/	BC after 16 mos→ died
3	52.0	100%	M244V	Res	/	/	Alive (Nilotinib→CCR)
4	57.4	100%	NA	Res	/	/	Alive in CP
5	29.9	95%	NA	CCR	3	4	BC after 2 mos→ died
6	48.4	100%	F317L	CCR	12	12	Alive in CP
7	52.9	100%	L387F	Res	/	/	Alive in CP
8	54.1	80%	NA	MCR	7	4	Alive (Nilotinib→CCR)
9	57.4	66%	Unmutated	Res	/	/	Alive (Nilotinib→CCR)
10	41.1	100%	NA	MCR	3	10	Alive after BMT
11	67.9	75%	Unmutated	CCR	7	/	Alive in CMolR +23 mos

NA= Not assessed; CCR = Complete Cytogenetic Response; MCR = Major Cytogenetic Response; Res = Resistant; BP = Blastic Phase; CP = Chronic Phase; CMolR = Complete Molecular Response.

be classified as resistant to Imatinib also according to the present definition of resistance.

Along all this study period, new TK inhibitors (nilotinib and dasatinib) were not yet available for treatment of Imatinib resistant patients, the only chance we had at that time for patients not eligible for transplant procedures was the association with other "old" drugs, and it is conceivable that many resistant patients have been managed in such way. Some previous *in vitro* studies have shown conflicting results on reciprocal effects between Imatinib and HU [4-6], however, HU seemed us to be a reasonable association mainly due to its very low toxicity profile as compared with other drugs (i.e. low-dose cytarabine).

Our results in this small Imatinib resistant patient population show that association of HU and Imatinib is capable to induce a sustained cytogenetic response in at least one half of patients resistant to Imatinib alone, with minimal toxicity. The duration of such responses appears often short, but 2/7 of our patients achieved a long-lasting (> 12 months) CCR.

Mutational status of our patients was not routinely studied before HU addition, because in that period its role and significance were still unclear. As a matter of fact, only 5/11 patients were assessed for point mutations before HU addition, however, it is worth to note that a patient with F317L mutation, which is at present known to be resistant to Dasatinib [11], achieved a relatively long response with this association.

We are obviously aware that new TK inhibitors have a major role as salvage treatment in CML patients primarily resistant to Imatinib. However, there are at least 2 subsets of patients for which the addition of HU to Imatinib could represent a useful additional choice:

– very elderly patients (aged > 75 - 80 years) as 1st line treatment, as Imatinib very often must be employed at dosage < 400 mg/day and the association of HU could improve response rate without adding severe toxicity.

– patients resistant/intolerant to new TK inhibitors and not eligible for transplant procedures as 3rd line treatment.

Moreover, to the best of our knowledge, this study also provides the first *in vivo* evidence of an additive activity of HU and Imatinib, this evidence could suggest to combine HU and new TK inhibitors in patients resistant to new drugs given alone, particularly to face with some mutations known to be unresponsive to other approaches.

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