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Dasatinib in imatinib-resistant or -intolerant CML patients: data from the clinical practice of 6 hematological centers in the Czech Republic

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Dasatinib is effective second line treatment for patients with chronic myeloid leukemia (CML) resistant or intolerant to imatinib. We report here the first experiences with dasatinib therapy in 71 CML patients resistant or intolerant to imatinib from the real clinical practice of 6 hematological centers in the Czech Republic. Dose 100 mg daily and 70 mg twice daily was administered to patients with chronic phase (CP) and advanced phases (AP) CML.

In chronic phase (n=46), complete hematological reponse (CHR) was achieved in 97%, major cytogenetic reponse (MCgR) in 77% and complete cytogenetic response (CCgR) in 67%. Major molecular reponse (MMR) was achieved in 19/31 patients in median of 10 months. In advanced phase (n=25), CHR was attained in 77%, MCgR in 39%, CCgR in 33% and MMR in 2/18 patients. Eleven different baseline mutations were followed up in 15 patients. Dasatinib eliminated mutations in most of the patients, but 3 patients acquired a new one. Novel mutations were detected under dasatinib therapy in 2 patients. Dasatinib was well tolerated, cytopenias were common and was managed by dose modification.

The estimated progression free survival (PFS) at 12 months was 97±3% in CP and 62±21% in AP. The median time to treatment failure was 605 days in AP while it was not reached in CP patients. Our clinical experiences, described here, confirmed that dasatinib is associated with high response rates especially in imatinib resistant or intolerant CML patients in chronic phase.

Key words: chronic myeloid leukemia, dasatinib, cytogenetic response, mutation

Contemporary treatment of chronic myeloid leukemia (CML) is based on tyrosine kinase inhibitors (TKIs) targeted against the BCR-ABL fusion protein [1]. Imatinib (IM) was the first TKI approved for the first-line standard therapy for patients with chronic phase (CP) CML treatment on the basis of the randomized IRIS trial (imatinib versus interferon alpha study) [2]. The most impending problems of imatinib therapy are resistance and intolerance. These should be resolved by second generation TKIs [3].

Dasatinib (Sprycel®, formerly BMS-354825) is an orally active dual inhibitor targeting BCR-ABL/Src-family kinases that exhibits 325-fold higher potency than imatinib and 16-fold higher potency than nilotinib, as shown *in vitro.* Throughout a series of phase II and III trials, dasatinib has demonstrated durable efficacy in patients with resistance, suboptimal response and intolerance to imatinib. Dasatinib was initially approved based on data from START program (SRC/ABL Tyrosine kinase inhibition Activity: Research Trials of dasatinib) derived from a series of multicenter open-label phase II clinical trials in imatinib-resistant or intolerant patients with CML or Ph+ALL (Acute Lymphoblastic Leukemia). In the START-C, 59% of patients with imatinib-resistant CP-CML achieved MCgR (Major Cytogenetic Response) while 49% achieved CCgR (Complete Cytogenetic Response) after dasatinib treatment during a mean follow-up of 15.2 months [4]. Patients in accelerated phase (AP) evaluated in the START-A showed CHR (Complete Hematological Response) in 39%, MCgR in 33% and CCgR in 24%. Patients in blast crisis (BC) undergoing START-B achieved hematological response (HR) in 33%. MCgR was achieved in 33% of myeloid and in 52%

of lymphoid transformations and CCgR in 26% and 46%, respectively [5].

One of the major mechanisms of resistance to TKIs is point mutations within the ABL kinase domain of the BCR-ABL fusion protein. Mueller et al. gathered 1780 blood samples of 202 patients examined for mutations within the frames of START-C. They recorded 34 different BCR-ABL mutations in 85/202 patients prior to dasatinib therapy [6]. They found the emergence of new mutations in 17/85 imatinib-resistant patients (e.g. T315I, n=2; F317L, n=6) during dasatinib therapy.

Here we report the first experiences with dasatinib therapy of CML patients from the practice of 6 hematological centers in the Czech Republic. We evaluated the hematological, cytogenetic and molecular responses and adverse events on dasatinib in CP (n=46) and AP (n=25) CML patients. Our data are discussed in terms of the data from START studies. In our study, 52 patients were examined for baseline mutations. The efficacy of dasatinib in 15 patients with mutation is described here.

Table 1 Baseline characteristics of the patients

Characteristics	Phases of CML		
	Chronic	Advanced	Total
No. Patients	46	25	71
Age, years median (range)	53 (21-69)	63 (34-70)	58 (21-70)
Male sex, %	46	32	44
Months from diagnosis to dasatinib starting median (range)	29 (3-125)	64 (7-276)	39 (3-276)
Imatinib therapy, months median (range)	32 (2 – 76)	27 (6 – 62)	36 (3-76)
IFN therapy, % Median (range) months	52 12 (3 – 95)	56 15 (3 – 48)	54 17 (3-95)
Chemotheraphy, %	15	24	18

Abbreviations: IFN- interferon

Patients and methods

Patients' data were collected within the Czech CML registries INFINITY (n=52) and CAMELIA (n=19) [7, 8]. The reasons for dasatinib initiation were primary or secondary resistance, disease progression and intolerance to imatinib therapy. The study was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

Patients were examined physically and blood and marrow aspirate were taken to examine hematological response. Cytogenetics, BCR-ABL transcript level and kinase domain mutational status were analyzed at intervals recommended by the group of experts of European Leukemia Net (ELN). ELN criteria were also used for definitions of hematological, cytogenetic and molecular responses [9]. Hematological response was assessed by complete blood counts and cytogenetic response was monitored in bone marrow (BM) aspirates based on the proportion of Ph+ metaphases among BM mitotic cells. Molecular response was evaluated based on BCR-ABL transcript level that was quantified by real-time polymerase chain reaction (RT-PCR). The BCR-ABL quantifications by real-time PCRs applied in this study were standardized within the frames of the international standardization project EUTOS for CML (ELN). Mutational analysis was performed using direct sequencing, adopting the protocol of Brandford et al. [10].

Adverse events were graded according to the National Cancer Institute Common Terminology Criteria (NCI CTC) Version 3.0 (grade 1- 4). Progression-free survival and overall survival were estimated using the Kaplan-Meier method.

Results

Patient demographics and disease characteristics. By September 2008, 84 patients were treated with dasatinib, 71 of them were evaluated in this study. Thirty one men and 40 women with a median age of 58 years (range 21-70) were enrolled in this study (Table 1). Forty six patients were in CP and twenty five patients were in advanced phases (both accelerated and blast crisis). Median time from CML diagnosis to the first administration of dasatinib in the whole cohort (CP+AP) was 39 months (range 3-276 months). Prior to dasatinib, imatinib treatment was carried out in 71 patients in a median duration of 36 months (range 2-76 months). Prior to imatinib, INF was administered to 38 patients (54%) in a median duration of 17 months (range 3-95 months). The starting dose of dasatinib was 100 mg once daily in CP and 140 mg daily in advanced phases. The duration of treatment of CP and AP patients lasted from 4 weeks to 30 months, with a median follow-up of 8 months. Dose escalation was applied in the cases of the lack of initial response, dose reduction due to toxicity.

Hematological, cytogenetic and molecular responses. In chronic phase CML with a median follow-up of 9 months (treatment duration 1-30 months) a complete hematological response was achieved in 97% (45/46) (Table 2). The time from dasatinib administration to CHR was 15-38 days (median 21 days). MCgR was observed in 77% (24/31) with median follow-up of 5 months. The cytogenetic remission was complete in 67% (27/31). The frequency of MMR was observed in 61% (19/31) of CP treated with dasatinib for a median of 10 months (range 4-24 months).

In advanced phases, in a median follow-up of 8 months (range 3-29 months) hematological response was induced by dasatinib in 77% (19/25). MCgR was achieved in 39% (7/18) (median 6 months) and CCgR was achieved by 33% (6/18) of patients in a median of 10 months. Major molecular response was confirmed in 2/18 patients (11%).

Mutations in BCR-ABL kinase domain. Mutation analysis was completed altogether in 52 patients. Mutations were detected in 9 patients in CP (25%, 9/36) and in 6 patients

Table 2 Best responses to dasatinib

	Phases of CML	
	Chronic	**Advanced**
Hematological response, % (n)	100 (46)	77 (19/25)
Complete, % (n)	97 (45/46)	56 (14/25)
Cytogenetic response, % (n/n)	87 (27/31)	45 (8/18)
Major, % (n)	77 (24/31)	39 (7/18)
Complete, % (n)	67 (21/31)	33 (6/18)
Partial, % (n)	10 (3/31)	6 (1/18)
Major molecular response, % (n)	61 (19/31)	11 (2/18)
Median time to MCgR, months (range)	5 (5-15)	6 (3-12)
Median time to CCgR, months (range)	6 (3-18)	10 (9-18)
Median time to MMR, months (range)	10 (4-24)	12 (6-24)
Dasatinib therapy, months (range)	9 (1-30)	8 (3-29)

Abbreviations: CCgR- complete cytogenetic response, MCgR- major cytogenetic response, MMR- major molecular response, AP- advanced phase– accelerated phase and blast phase CML

in accelerated phases (37%, 6/16) prior to dasatinib therapy. Overall 11 different mutations were found in those 15 patients as baseline.

The most frequent were mutations Y253H (n=3), F311I (n=2), H396R (n=2) and F359V (n=2). Dasatinib therapy eradicated mutations in 7 patients (median follow-up 4 months), while 3 patients maintained their mutations (F311I, M244V, E453V). Three patients lost the original mutation but acquired a new one: T315A, F317L and K271R. Novel mutations were detected during dasatinib treatment in 2 patients: F317L, T315I.

Adverse events related to dasatinib. Non-hematological toxicity of any grade in CP was reported in 42% (17/41) of patients; the most frequent were diarrhea, dyspnoea and nausea (Table 3). More serious toxicity grade 3/4 was registered in 7% (3/41); pleural effusion appeared in 2 patients. All grades of hematological cytopenia were reported in 66% (27/41) of CP, grade 3/4 neutropenia and thrombocytopenia in 28% (11/41). In AP, any grade of cytopenia was reported in 74% (17/23), grade 3/4 in 62% (14/23), and grade 3/4 non-hematological

Table 3 Adverse events related to dasatinib (toxicity)

	Phases of CML	
	Chronic	**Advanced**
Hematological toxicities, % (n)		
All grades	66 (27/41)	74 (17/23)
Grades 3/4	28 (11/41)	62 (14/23)
Non-hematological toxicities, % (n)		
All grades	42 (17/41)	43 (10/23)
Grades 3/4	7 (3/41)	33 (8/23)
Dasatinib discontinuation % (n)	15 (6/41)	26 (6/23)
Alive, % (n)	97,8 (45/46)*	60 (15/25)

*1 death not of CML progress

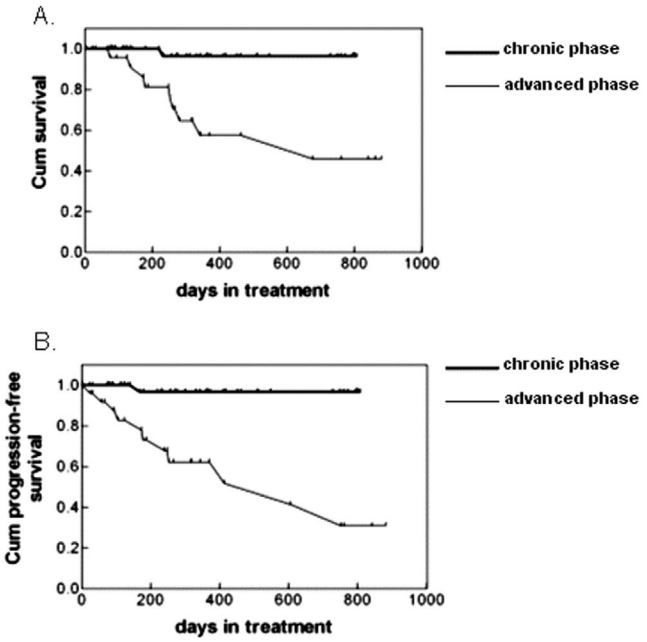

Figure 1 Kaplan-Meier analysis of dasatinib-treated patients with chronic-phase and advanced phases CML. A. Overall survival; B. Progression free-survival.

toxicity in 33% (8/23) of patients. Discontinuation of therapy was necessary in 15% (6/41) of CP and 26% (6/23) of AP patients due to drug toxicity.

The estimated overall survival (OS) at 12 months was 97±3% in CP (1 patient death not of CML) and 58±23% in advanaced phases (AP). The median OS in CP was not achieved, in AP it was 676 days (22,3 months) (Figure 1a).

The estimated progression-free survival (PFS) at 12 months was 97±3% in CP and 62±21% in AP. The median time to treatment failure was 605 days (20,1 months) in AP while it was not reached in CP patients (Figure 1b). The difference between the two treatment groups was highly statistically significant (P=0,0001).

Discussion

Reported data reflect the responses and disease status of 71 patients with CML in chronic and advanced phases from 6 hematological centers in the Czech Republic. In all patients we followed up the hematological, cytogenetic and molecular responses. Mutations in BCR-ABL kinase domain were monitored in 52 patients before and during dasatinib therapy.

When comparing our results in patients with CP CML to the data from the START-C study, the rates of hematological responses were fairly identical, but a higher rate of cytogenetic responses was achieved in our study; MCgR 77% and CCgR 67%, vs. 51% and 49% in START-C. One possible explanation may be the fact that our patients were treated with dasatinib

outside the clinical trials, thus enabling individual treatment approaches such as dose adjustment and temporal treatment interruption; it is of note that 60% of these patients were pretreated with IFN. In comparison to START-C we observed a lower number of patients suffering from hematological toxicity grade 3/4. This may be explained by the dosage of 100 mg of dasatinib once daily used in the majority of our patients. The low toxicity of 100 mg dose once daily as compared to 50 mg BID has been confirmed by the data from the clinical study CA1800034 [11]. We did not find any major difference in the intensity or in the pattern when comparing non-hematological toxicity (diarrhea, nausea and dyspnoea).

Results comparable with those of START-A and START-B were found in patients in AP concerning the number of hematological and cytogenetic responses [5]. However, the occurrence of adverse events in the first months of dasatinib treatment, especially in patients in blast crisis, was higher in our group. The thrombocytopenia and neutropenia grade 3/4 was prevalent. This might be explained by the presence of a small proportion of non-leukemic hemopoesis present in AP; also the duration of CML before start dasatinib therapy and proportion of patients with severe cytopenia before the initiation of dasatinib therapy might contribute. Out of the non-hematological side effects we would like to emphasize an affliction from pleural effusion grade 3/4, which was observed in 2 patients. Dasatinib treatment was interrupted in both patients with administration of diuretics, corticosteroids for a short time, and life-saving fluidothorax evacuation in one of them. Dasatinib treatment was terminated due to toxicity, both hematological and non-hematological, in 15% of CP and 26% of AP patients.

Mutational analysis and its monitoring in 52 patients confirmed the presence of mutations in BCR-ABL kinase domain prior to dasatinib treatment initiation in 15 of them. More frequent mutations were Y253H, F311I, H396R and F359V. While all CP patients analyzed for mutation expressed a single mutation, some patients in AP carried 2 types. In some patients, original mutated BCR-ABL clone had disappeared and a new one has appeared. These findings correspond to the hypothesis of a clone selection under the targeted therapy [12]. As described by Mueller et al. [6], similarly as in our study the newly developed mutations on dasatinib were those in positions 315 and 317, resistant or expressing intermediate resistance to dasatinib, respectively.

It was possible to evaluate the frequency of MMR in 31 patients in CP. MMR (<0.1% BCR-ABL^{IS}; IS = international scale) was observed in 61% of CP patients treated with dasatinib in a median of 10 months (range 4-24 months). The follow-up of BCR-ABL transcript level was quite short (median 10 months, range 3-25). The number of patients with MMR is expected to be higher because a decreasing trend of BCR-ABL transcript level continues in some patients. Also patients with mutations F359V, Y253H, E255K responded to dasatinib with MMR. It was proved that MMR, achieved on dasatinib at any time, positively influences PFS.

We confirmed that dasatinib is an effective drug for patients with non-mutated and mutated BCR-ABL, especially in those with CP CML. The response rate of CP patients with mutations seems to be comparable to the whole cohort treated with dasatinib. The efficacy of dasatinib in mutation-negative patients showed that dasatinib was potent in overcoming other mechanisms of imatinib resistance [13]. Our results contributed to the observation that dasatinib is associated with a high rate response (CHR and MCgR) in patients with imatinib-resistant or intolerant CP-CML and is also a very potent drug for AP patients. Duration of response in some AP patients does not seem to be prolonged with continuation of dasatinib therapy; however, it may facilitate the preparation of allogeneic stem cell transplantation. Dasatinib represents an effective treatment option for patients with resistance and intolerance to imatinib in all phases of CML.

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