Brief Report

Dynamics of cytogenetic aberrations in Philadelphia chromosome positive and negative hematopoiesis during dasatinib therapy of chronic myeloid leukemia patients after imatinib failure

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ABSTRACT

Clonal cytogenetic aberrations of the Philadelphia chromosome (Ph) positive hematopoiesis have been associated with the natural evolution of chronic myeloid leukemia (CML) to advanced disease. Clonal aberrations of Ph negative metaphases have been described after treatment with interferon or imatinib. This study evaluates the effect of dasatinib on Ph positive clones with additional cytogenetic aberrations and the frequency of novel aberrations in Ph positive and negative metaphases. Seventy-one patients treated with dasatinib after imatinib failure for a median of nine months were evaluated. Novel aberrations within Ph positive and negative clones appeared in six and three patients, respectively.

Key words: chronic myeloid leukemia, cytogenetics, clonal evolution, tyrosine kinase inhibitors, dasatinib.

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Chronic myeloid leukemia (CML) is a clonal hematologic disorder characterized by the translocation t(9;22)(q34;q11), resulting in the Philadelphia chromosome (Ph) and the *bcr-abl* fusion gene. This gene encodes a chimeric BCR-ABL fusion protein with deregulated tyrosine kinase activity that is required for maintaining the malignant phenotype.[1] The use of targeted therapies has significantly improved the treatment of CML. Imatinib (Glivec®), a selective inhibitor of the BCR-ABL tyrosine kinase achieved major or complete cytogenetic remissions (MCR or CCR) in 60% of CML patients who were intolerant or refractory to prior IFN therapy and up to 87% in newly diagnosed patients.[2,3] But the accumulation of point mutations in the ABL kinase domain of BCR-ABL leading to clinical resistance to the drug has become a major problem.[4] Clonal cytogenetic aberrations of the Ph positive hematopoiesis have been associated with the natural evolution of CML to advanced disease and also seem to play a role in imatinib resistance in CML patients. Clonal changes of Ph negative metaphases have been described after treatment with interferon alpha or imatinib in a minority of patients with cytogenetic response. Conflicting data suggest the selection of pre-existing clones indicating that residual Ph negative hematopoiesis co-exists with the malignant clone vs. induction of aneuploidy by tyrosine kinase inhibitors.[5,6] Therefore, the prognostic impact of aberrations in the Ph negative hematopoiesis must still be clarified. Dasatinib (formerly BMS-354825) is an ATP-competitive dual SRC/ABL kinase inhibitor showing antiproliferative activity against hematologic tumor cell lines that can bind to both active and inactive conformations of the respective kinase domains. It is, therefore, effective against most imatinib-resistant forms of BCR-ABL. Inhibition of other SRC-family members and activity against c-KIT and PDGFRβ were also demonstrated.[7,8] The efficacy and safety of dasatinib has been demonstrated in patients with Ph positive CML after failure of imatinib therapy.[9-11] However, little is known about the long-term efficacy and toxicity of dasatinib treatment. In contrast to patient treated with imatinib or IFN[12] no clonal evolution in Ph positive

Table 1. Patient's data.

Patient characteristics	*n=71*
Clinical data	
Median age (years) at start of dasatinib (range)	58 (28-78)
% male	56.3
Previous CML therapy, n (%)	
Imatinib	8 (11.3)
Imatinib, AraC	1 (1.4)
Imatinib, HU	11 (15.5)
Imatinib, IFN	11 (15.5)
Imatinib, HU, IFN	30 (42.3)
Imatinib, HU, IFN, AraC	5 (7)
Imatinib, HU, IFN, AraC , Nilotinib	2 (2.8)
Imatinib, IFN, AraC	1 (1.4)
Imatinib, HU, AraC	2 (2.8)
Median follow-up of all patients, months (range)	73 (14-231)
Median follow-up of dasatinib therapy, months (range)	8.6 (1-16)
Time from diagnosis to dasatinib, months (range)	59 (6-216)

Cytogenetic response	*CP (n=50) (%)*	*AP (n=6) (%)*	*BC (n=15) (%)*
Complete	22 (44)	0	4 (27)
Major	7 (14)	0	0
Normal karyotype prior to dasatinib	4 (8)	0	3 (20)
t(9;22) as sole change prior to dasatinib	38 (76)	1 (17)	3 (20)
Additional changes in Ph+ cells prior to dasatinib	8 (16)	5 (83)	9 (60)
Additional changes in Ph- cells prior to dasatinib	2 (4)	0	0
Clonal evolution in Ph+ cells on dasatinib	4 (8)	0	2 (13)
Clonal aberration in Ph- cells on dasatinib	3 (6)	0	0

n: number; AraC: cytosine arabinoside; HU: hydroxyurea; IFN: interferon α; CP: chronic phase; AP: accelerated phase; BC: blast crisis; CML: chronic myeloid leukemia.

cells and no clonal abnormalities in BCR-ABL negative cells are documented so far. This study, therefore, describes the effect of dasatinib on Ph positive clones with additional cytogenetic aberrations and the frequency of novel aberrations in Ph positive and Ph negative metaphases during therapy. In primary human cells dasatinib induced centrosome defects and delay of spindle formations.[13] The influence on the hematopoietic cells of CML patients was, therefore, of major interest.

Design and Methods

Patient characteristics

Seventy-one patients with Ph and BCR-ABL positive CML after imatinib failure were investigated. Median age was 58 years (range 28-78), medium time from diagnosis 73 months (range 14-231) and time from diagnosis to dasatinib treatment 59 months (range 6-216). Patients were in chronic phase (CP, n=50), accelerated phase (AP, n=6), myeloid (my, n=8) or lymphoid (ly, n=7) blast crisis (BC) according to standard definitions.[4] Dasatinib therapy was started at a dose of 100-140 mg/day (2×50 mg/day or 2×70 mg/day). Pre-treatment for the 71 patients consisted of imatinib, HU (n=48), IFN (n=49), cytosine arabinoside (AraC, n=11) and nilotinib (n=2) alone or in combination. Median duration of dasatinib therapy was nine months (range 1-16). Dasatinib was stopped due to progressive disease in four, and because of hematologic toxicity in three patients. Five patients received allogeneic stem cell transplantation (SCT). One died after SCT, four patients developed resistance, and five died while receiving dasatinib (from myBC) (Table 1).

Cytogenetic analysis

Cytogenetic analyses of bone marrow (BM) were made and interpreted according to the International System for Human Cytogenetic Nomenclature.[13-15] Data at onset of dasatinib treatment and at least one follow-up were available for each patient. Complete cytogenetic response (CCR) was defined as 0% major cytogenetic response (MCR) as 1-35%, minor cytogenetic response as 36-95%, and no response as 96-100% of Ph positive metaphases. Prior to dasatinib therapy, 41 patients showed a standard, and one a variant, Ph translocation (59%). Twenty-two patients (31%) demonstrated additional chromosomal aberrations in the Ph positive clone. In seven patients, a normal karyotype was observed prior to dasatinib treatment (Table 1). Two Ph positive patients (3%) had trisomy 8 as an aberration of the Ph negative clone at baseline (Patients #6 and #20, Table 2). Clinical and cytogenetic data of 30 CML patients with additional chromosomal changes in Ph positive and Ph negative metaphases prior to and during dasatinib therapy are presented in Table 2. Medium time from diagnosis to dasatinib therapy was 72 months (range 10-216). Patients with no cytogenetic evolution at all (41 patients) are not shown.

Results and Discussion

Cytogenetics

During dasatinib therapy, seven patients (10%) achieved a major cytogenetic remission and 26 (37%) a CCR. Out of the 22 patients with clonal evolution, one (5%) achieved major cytogenetic remissions and two (9%) a CCR.

Clonal chromosomal evolution

Novel aberrations of the Ph positive clone appeared during dasatinib treatment in six patients (8%) between one to 16 months after start of therapy including one case with t(3;22)(q21;q11), one with +idic(22)(p11) and two cases with +8. Two cases with two or more aberrations were observed (inv(3)(q21q26), monosomy 7 and i(17)(q10) in the first and +4,+12,+13,+21 in the second). In patients #22 and #23 the proportion of Ph positive cells with additional aberrations increased with time and patient #22 developed a myeloid BC under treatment. The remaining four patients showed no change or reduction of aberrant cells over time. No or only minor cytogenetic response was observed (Table 2). Time from diagno-

Table 2. Clinical and cytogenetic data of chronic myeloid leukemia patients with additional chromosomal changes in Ph+ and Ph- metaphases prior to and during dasatinib (D) therapy.

No.	*Sex*	*Age [years]*	*Treatment prior to D*	*Disease stage*	*D treatment [mo]*	*Ph+ cells with and without additional aberrations and normal metaphases prior to D [No of metaphases]*	*Ph- cells prior to D [No of metaphases]*	*New add aberrations during D in Ph+ cells {No of cells} [emergence mo]*	*New add changes during D in Ph- cells {No of cells} [emergence mo]*	*Cytogenetic response*
1	M	66	IFN,I	AP	14	46,XY,t(9;22)[3]/46,XY,inv(3),t(9;22)[22]		No	No	No
2	F	69	IFN/HU/I	AP	12	46,XX,t(9;22)[7]/47,XX,t(9;22),+der(22)[18]		No	No	No
3	F	65	HU/I	myBC	9	46,XX,t(9;22)[5]/47,XX,+8,t(9;22)[16]		No	No	No
4	M	65	IFN/HU/I	myBC	12	47,XY,+8,t(9;22),i(17q)[13]		No	No	No
5	F	61	HU/IFN/I	CP	16	46,XX,der(9)t(9;22),ider(22)t(9;22)[1]/46,XX[24]		No	No	CO
6	M	41	HU/I/IFN	CP	15	46,XY,t(9;22)[12]/46,XY[11]	+8[2]	No	No	CO
7	M	47	IFN/I	CP	13	46,XY,t(9;22)[5]/47,XY,t(9;22),+der(22)[11]/46,XY[9]		No	No	MIN
8	M	31	I	lyBC	14	46,XY,t(9;22)[7]/47,XY,t(9;22),der(19)t(8;19),+der(22)[5]/50,XY,+8,t(9;22),+15,+19,del(20q),+der(22)[3]/46,XY[10]		No	No	CO
9	M	67	HU/I	CP	7	46,XY,t(9;22)[10]/46,XY,t(9;22),dup(16p)[15]		No	No	MAJ
10	F	62	HU/I/AraC	CP	6	46,XX,t(9;22)[18]/47,XX,+8,t(9;22)[1]/46,XX[1]		No	No	MIN
11	M	63	HU/IFN/I	CP	4	46,XY,t(9;22)[24]/47,XX,+8,t(9;22)[1]		No	No	MIN
12	M	47	I	CP	2	46,XY,t(9;22)[16]/47,XY,+4,t(9;22)[3]/46,XY[1]		No	No	No
13	F	38	HU/IFN/I	myBC	3	46,XX,t(9;22)[3]/46,XX,t(3;21),t(9;22)[1]		No	No	No
14	F	76	HU/IFN/I	myBC	4	46,XX,t(9;22)[22]/46,XX,dup(1q),t(9;22)[2]		No	No	No
15	M	69	HU/IFN/I/AraC	AP	2	46,XY,t(9;22)[17]/47,XY,t(9;22),+der(22)[3]		No	No	No
16	F	55	I	AP	5	46,XX,der(9)t(9;22)del(9q)del(22q),der(22)t(9;22)[1]/46,XX,idem,ins(5;12)[8]/46,XX[2]		No	No	No
17	M	36	IFN/HU/I	AP	3	47,XY,t(9;22),+der(22)[6]47,XX,+8,t(9;22)[1]		No	No	No
18	M	72	HU/IFN/I	CP	3	46,XY,t(9;22)[22]/48,XY,+8,t(9;22),+der(22)[3]		No	No	No
19	F	73	IFN/HU/I	myBC	3	46,XX,t(9;22)[9]/48,X,-X,+6,-7,t(9;22),+11,+17,+der(22)[4]		No	No	No
20	M	52	I	CP	4	46,XY,t(9;22)[10]/45,X,-Y,t(9;22)[8]/46,XY[2]	+8[2]	No	No	MIN
21	M	58	I	BC	3	50,XY,t(3;21),t(9;22),+8,+8,+12,+der(22)[5]/51-52,XY,idem,-12,+9,+der(22)[13]/46,XY[1]		No	No	No
22	M	44	HU/I	CP	11	46,XY,t(9;22)[20]/46,XY[5]		inv(3),-7,i(17){12}[6]	No	No
23	M	64	HU/IFN/I	CP	15	46,XY,t(9;22)[23]		t(3;22){3}[6]	No	MIN
24	M	58	HU/I/AraC	myBC	3	49,XY,+8,t(9;22;12),i(17q),+19,+der(22)[14]/50,XY,idem,+8[7]			+8{7}[1]	No
No										
25	M	47	IFN/HU/I/AraC/N	lyBC	3	46,XY,t(9;22)[15]/47,XY,+9,t(9;22)[5]		+4,+12,+13,+21{8}[1]	No	No
26	F	55	IFN/HU/I/AraC	CP	16	46,XX,t(9;22)[25]		+8{2}[16]	No	No
27	M	59	HU/IFN/I	CP	12	46,XY,t(9;22)[25]		+idic(22){10}[2]	No	No
28	F	66	IFN/HU/I	CP	16	46,XX,t(9;22)[25]		No	+8{1}[2]	CO
29	M	61	IFN/I	CP	14	46,XY,t(9;22)[25]		No	+8{10}[12]	MAJ
30	M	46	HU/I	CP	6	46,XY,t(9;22)[23]/46,XY[2]		No	+8{1}[3]	MAJ

Add: additional; No: number of patients; F: female; M: male; CP: chronic phase; AP: accelerated phase; my/lyBC: myeloid or lymphoid blast crisis; HU: hydroxyurea; IFN: interferon α; AraC: cytosine arabinoside; I: imatinib; D: dasatinib; N: nilotinib; mo.: month; Ph+ /Ph-: Philadelphia positive/negative clone. CO: complete; MIN: minor; MAJ: major.

sis to dasatinib treatment was 81 months (range 22-124). Ph chromosome independent clonal evolution was identified in three patients (4%), two with major (patients #29 and #30) and one with complete cytogenetic response (patient #28) (Table 2). All patients showed trisomy 8 in the Ph negative metaphases and did not show any other chromosomal aberrations other than t(9;22).

Two Ph positive patients displayed trisomy 8 in Ph negative metaphases after imatinib failure prior to dasatinib treatment. No increase in Ph negative clones on dasatinib was observed after periods of four or 15 months treatment.

In total, 5 out of 71 patients (7%) showed clonal aberrations of Ph negative metaphases after consecutive imatinib/dasatinib therapies. None of these patients had morphological evidence for secondary neoplastic changes. After a median follow-up of nine months, four dasatinib treated patients showed resistance and three were intolerant to the drug. Six patients died during therapy, five of them in BC and one in CP after SCT.

In seventy-one dasatinib treated CML patients CCR of 37% and MCR of 10% were achieved after a median treatment duration of nine months. Dasatinib showed a benefit for patients resistant to or intolerant of a prior imatinib therapy.[9-11] Moreover, dasatinib was efficacious in patients with clonal cytogenetic aberrations that concur with BCR-ABL independent imatinib resistance.

New additional chromosome aberrations in Ph positive metaphases were observed in six patients (8.5%). None of them achieved any cytogenetic response. The frequency of additional aberrations on dasatinib is comparable to those of patients treated with imatinib (9.3 %)[12] and with untreated patients (8.8%).[16] The observed abnormalities were common secondary changes frequently observed in CML during CP, AP, or BC after treatment with imatinib or IFN or even in untreated patients 12 and could be due to the natural history of the disease.

Furthermore, a significant minority of patients (4.2%) demonstrated novel aberrations in Ph negative cells on dasatinib treatment during the observation period. Three

patients pre-treated with hydroxyurea, imatinib, and IFN up-front showed trisomy 8 in their Ph negative cells (patients #28, #29, and #30). The incidence is comparable to that after IFN or imatinib therapies (2-17%).[5] Since clonal chromosome aberrations in Ph negative cells are mainly numerical, at least after imatinib treatment,[17] these findings suggest a causative role of imatinib or dasatinib or prior chemotherapy in the emergence of clonal evolution or reflect genomic instability of the hematopoietic cells of CML patients. Ph negative evolution could also be due to selective pressure by imatinib or dasatinib on Ph positive clones, allowing pre-existing Ph negative clones to predominate. However, only a few cases with trisomy 8 in Ph positive and negative metaphases have been reported so far.[18,19] In conclusion, dasatinib is efficient in patients resistant to or intolerant of imatinib. The incidence and quality of aberrations occurring in Ph positive cells during dasatinib treatment are comparable to those observed in clonal evolution during the natural course of CML or during treatment with other drugs. The prevalence of aberrations in Ph negative cells under dasatinib is low. Despite the inhibition of both ABL and SRC tyrosine kinases, no specific cytogenetic *drug pattern* for dasatinib could be detected and no secondary neoplastic diseases were observed after a short observation period.

Since numerical aberrations are frequent and recurrent in myeloid diseases, cytogenetic monitoring of patients with chromosomal changes in Ph negative cells is suggested.[20]

However, the wide variety of cases in regards to phases and types of disease, length of time from diagnosis to dasatinib treatment and short duration of dasatinib therapy allows only reduced valuation.

Authors' Contributions

AF: principal investigator with primary responsibility for the publication. CH, MCM, PE, TL, MG, OF, WS, RH and AH: contributed to the design of the study, to the work and to the interpretation of the results. All authors checked the final version of the manuscript.

Conflict of interest

The author reported no potential conflicts of interest.

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