The use of nilotinib or dasatinib after failure to 2 prior tyrosine kinase inhibitors: long-term follow-up

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Responses can be achieved with dasatinib or nilotinib after failure of 2 prior tyrosine kinase inhibitors (TKIs). We report on 48 chronic myeloid leukemia patients sequentially treated with 3 TKIs: 34 with dasatinib after imatinib/nilotinib failure and 14 with nilotinib after imatinib/dasatinib failure. Before the third TKI, 25 patients were in chronic phase (CP), 10 in accelerated phase (AP), and 13 in blast phase (BP). Best response to third TKI in CP was 5 major molecular responses (MMR), 3 complete cytogenetic (CCyR), 2 partial cytogenetic (PCyR), 3 minor cytogenetic (mCyR), 6 complete hematologic responses (CHR), and 6 with no response (NR). In AP, 1 patient achieved MMR, 1 CCyR, 2 PCyR, 1 mCyR, 4 CHR, and 1 NR. In BP, 1 achieved MMR, 2 CCyR, 1 PCyR, 1 mCyR, 2 returned to CP, and 6 NR. Median CCyR duration was 16.3 months; 3 CP patients achieving CCyR had a response more than 12 months. Median failure-free survival was 20 months for patients in CP, 5 months in AP, and 3 months in BP. Use of second-generation TKI after failure to 2 TKIs may induce responses, but these are usually not durable except in some CP patients. New treatment options are needed. (Blood. 2009;114:4361-4368)

Introduction

Most patients with chronic-phase chronic myeloid leukemia (CP CML) have a sustained response to imatinib. However, some can develop resistance to imatinib via various mechanisms, including point mutations in the Abl kinase domain and overexpression of Bcr-Abl.[1,2] Mutations have been reported in many different amino acids, each conferring different levels of resistance.[3,4] In addition, Src-related kinases are up-regulated in some cases of imatinib resistance, a phenomenon that is thought to contribute to leukemogenesis.[5-8]

Second-generation tyrosine kinase inhibitors (TKI), such as nilotinib and dasatinib, have shown increased inhibitory potency against Bcr-Abl kinase and have shown efficacy in treating patients with many of the Bcr-Abl kinase domain mutations that develop on imatinib; T315I is the one mutation clearly resistant to second-generation TKI.[9-13] Nilotinib (Tasigna, AMN107; Novartis) is structurally related to imatinib but has 30-fold higher potency and increased selectivity against Bcr-Abl.[9] Dasatinib (Sprycel, BMS-354825; Bristol-Myers Squibb) has 300-fold increased potency against Bcr-Abl compared with imatinib and also has Src-inhibitory activity.[14] Both nilotinib and dasatinib have been approved for the treatment of patients with CML after imatinib failure.

With the availability of imatinib, nilotinib, and dasatinib, a scenario seen with increasing frequency is that of patients who have failed imatinib and one of the second-generation TKIs. The other second-generation TKIs are usually considered viable alternatives for therapy, and preliminary results suggest that some patients may indeed respond to a second-generation TKI used as third-line therapy.[15,16] However, the long-term benefit of such an approach is largely unknown. In this study, we report the response rates and long-term results of using a second-generation TKI after failure to imatinib and another second-generation TKI.

Methods

Study group

Patients with CML who were sequentially treated with 3 different TKIs at M. D. Anderson Cancer Center between September 2004 and July 2008 were included in this analysis. Doses were adjusted for toxicity as previously described.[12] Patients were followed with complete blood counts, cytogenetic analysis, bone marrow aspirations, and real-time reverse transcription-polymerase chain reaction every 3 months. Mutational analysis by direct sequencing was performed on each patient after imatinib failure and before the start of both second and third TKIs.

Patients were switched to second- or third-line TKI when they had a treatment failure. Treatment failure was defined as failure to achieve a complete hematologic response (CHR), (CP only), or any hematologic response (accelerated phase [AP] or blast phase [BP]) after 3 months of therapy, persistence of 100% Philadelphia chromosome (Ph)–positive metaphases after 6 months of therapy, or 35% or more after 12 months, transformation to AP or BP, or loss of cytogenetic response or CHR at any time during the course of therapy.[17] Patients who were unable to continue therapy because of toxicity (ie, intolerant) were recorded as having treatment failure.

All patients were registered in studies approved by the M. D. Anderson Cancer Center Institutional Review Board, and informed consent was provided in accordance with the Declaration of Helsinki.

Definition of response

Response criteria were as previously described.[18] CHR was defined as a normal white blood cell count with normal differential and platelet count

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Table 1. Patient characteristics (N = 48)

	Third-line TKI	
Variable	**Dasatinib (n = 34)**	**Nilotinib (n = 14)**
Median age, y (range)	53 (18-70)	49 (19-70)
Stage at start of imatinib		
CP	29 (85%)	10 (72%)
AP	5 (15%)	3 (21%)
BP	0	1 (7%)
Time on imatinib, mo	30.2	43
Best response to imatinib		
MMR	4	
CCyR	4	
mCyR	5	5
CHR	7	3
NR		1
NA	14	5
Failure to imatinib		
Resistance	34	13
Intolerance		1
Imatinib dose, mg		
400	26 (76%)	11 (79%)
More than or equal to 600 mg	8 (24%)	3 (21%)
Stage at start of second-line TKI		
CP	17 (50%)	8 (57.2%)
AP	10 (29%)	3 (21.4%)
BP	7 (21%)	3 (21.4%)
Mutations (second TKI)	20	4
Time on second TKI, mo	7.7	10.3
Best response to second-line TKI		
MMR	5	
CCyR	3	2
PCyR	4	1
mCyR	11	5
CHR	10	4
NR	1	2
Failure to second TKI		
Resistance	30 (88%)	9 (64%)
Intolerance	4 (12%)	5 (36%)
Stage at third-line TKI		
CP	16 (47%)	9 (64.3%)
AP	8 (24%)	2 (14.3%)
BP	10 (29%)	3 (21.4%)
Mutation at third TKI	24 (71%)	8 (57%)
Stage at start of third-line therapy		
CP	16 (47%)	9 (64%)
AP	8 (26%)	2 (14%)
BP	10 (29%)	3 (21%)
Interval treatment (between second- and third-line TKI)		
Not treated	25 (74%)	7 (50%)
Treated	9 (26%)*	7 (50%)†
Starting dose of third-line TKI		
QD dosing	140 mg, 9 (26%), 100 mg, 3 (9%), 50 mg, 1 (3%)	800 mg, 2 (14%), 400 mg, 1 (7%)
BID dosing	70 mg, 15 (44%), 50 mg, 5 (15%), 120 mg, 1 (3%)	400 mg, 11 (79%)

MUD indicates matched unrelated donor transplantation; QD, once daily; BID, twice daily; NA, not applicable; and TKI, tyrosine kinase inhibitors.
*In dasatinib third-line group, interval treatments included 6 imatinib, 2 HyperCVAD, 1 MUD, 1 INNO-406, 1 KOS-953, and 1 MK-0457.
†In nilotinib third-line group, interval treatments included 4 SKI-606, 1 INNO-406, 1 Ara-C/idarubicin/imatinib, and 1 imatinib.

less than 10×10^9/L, and no signs or symptoms of leukemia, including resolution of splenomegaly. Cytogenetic response assessment was based on karyotype analysis of at least 20 metaphases and defined as complete (CCyR, 0% Ph^+), partial (PCyR, 1%-35%, Ph^+), and minor (mCyR, 36%-95% Ph^+). A major cytogenetic response (MCyR) included CCyR and PCyR (ie, $\leq$ 35% Ph^+). Molecular response was assessed by real-time TaqMan-based quantitative polymerase chain reaction as previously described. Major molecular response (MMR) was defined as bcr-abl/abl ratio of less than or equal to 0.05%[19]

Statistical analysis

Event-free survival was considered from the time the third TKI was started to loss of major hematologic response, loss of cytogenetic response, transformation to AP or BP phase, or death. Failure-free survival was considered from the start of the third TKI to development of an event as defined for event-free survival, or loss of CCyR, or discontinuation because of toxicity. Overall survival was considered from the start of the third TKI to death.

Table 2. Mutation status of patients before second- and third-line TKI therapy and the treatment response

	Second-line				Third-line		
Patient no.	Mutation status	Phase	Best response	Outcome	Mutation status	Phase	Best response
Nilotinib second-line, dasatinib third-line							
1	H396R	CP	MMR	Intolerance	H396R	CP	MMR
2	E355G	AP	mCyR	Resistance	None	CP	CHR
3	G250E	CP	PCyR	Resistance	G250E	CP	mCyR
4	F359V	CP	CHR	Resistance	F359V	CP	mCyR
5	T315I	CP	mCyR	Resistance	T315I	CP	CHR
6	None	CP	mCyR	Intolerance	None	CP	CHR
7	None	CP	mCyR	Resistance	H396P	CP	CCyR
8	None	CP	CHR	Resistance	None	CP	NR
9	G250E	AP	CHR	Resistance	G250E	AP	CHR
10	A433T	CP	CCyR	Resistance	None	CP	PCyR
11	E355G	AP	PCyR	Resistance	E355G	AP	mCyR
12	T315I	CP	CHR	Resistance	T315I	CP	NR
13	Y253F	CP	mCyR	Intolerance	Y253F	CP	CHR
14	E255K	AP	mCyR	Resistance	E255K	BP	mCyR
15	E255K	AP	CHR	Resistance	E255K	AP	PCyR
16	G250E	AP	mCyR	Resistance	G250E	CP	CCyR
17	None	CP	PCyR	Resistance	None	BP	NR
18	F359V	CP	mCyR	Resistance	F359V	AP	CCyR
19	None	CP	mCyR	Resistance	None	CP	mCyR
20	None	AP	CHR	Resistance	Y253F	AP	CHR
21	None	AP	CHR	Resistance	F311L	AP	CHR
22	T315I	BP	CHR	Resistance	T315I	BP	NR
23	None	BP	NR	Resistance	None	BP	NR
24	None	BP	mCyR	Resistance	None	AP	NR
25	H396R	BP	CHR	Resistance	H396R	BP	RCP
26	None	BP	CCyR	Resistance	E255V	BP	CCyR
27	None	AP	MMR	Resistance	None	BP	NR
28	Q252H	CP	mCyR	Resistance	Q252H	BP	NR
29	None	BP	PCyR	Resistance	Y253H	BP	PCyR
30	None	CP	CCyR	Resistance	F359C	CP	NR
31	E355G	AP	MMR	Resistance	A276G	AP	MMR
32	E459G	CP	MMR	Intolerance	E459G	CP	CCyR
33	None	CP	MMR	Resistance	None	BP	MMR
34	G250E	BP	CHR	Resistance	G250E	CP	MMR
Dasatinib second line, nilotinib third line							
1	F317L	CP	CHR	Intolerance	F317L	CP	NR
2	G250E	CP	CCyR	Intolerance	Y253H	CP	MMR
3	None	CP	NR	Resistance	None	AP	CHR
4	None	CP	CHR	Intolerance	None	AP	PCyR
5	None	BP	mCyR	Resistance	None	BP	RCP
6	None	BP	NR	Resistance	none	BP	NR
7	E355G	BP	PCyR	Resistance	E355G	BP	CCyR
8	L248V	CP	CHR	Intolerance	Del248-275	CP	PCyR
9	None	CP	mCyR	Intolerance	None	CP	mCyR
10	None	AP	mCyR	Resistance	F359V, F311L	CP	NR
11	None	CP	mCyR	Resistance	F317L	CP	MMR
12	None	AP	CHR	Resistance	None	CP	CHR
13	None	CP	CCyR	Resistance	V299L, F486S	CP	MMR
14	None	AP	mCyR	Resistance	F317L	CP	NR

RCP indicates return to chronic phase.

Results

Patient characteristics

A total of 48 patients were treated with sequential TKI: 34 with dasatinib after imatinib and nilotinib failure, and 14 with nilotinib after imatinib and dasatinib failure (Table 1). The median age was 52 years (range, 18-70 years). Before starting imatinib, 27 (56%) patients received interferon-α, 5 (10%) received homoharringtonine (HHT), 14 (29%) received single-agent cytarabine, and 2 (4%) had undergone an allogeneic stem cell transplantation (SCT). Twenty patients (42%) received imatinib as first-line therapy. At the start of imatinib, 39 (81%) patients were in CP, 8 (17%) in AP, and 1 (2%) in BP; 5 in AP in the group who went on to nilotinib as second-line therapy and 3 AP/1 BP in the group who went on to dasatinib as second-line therapy. The median time on imatinib was 36.3 months, and the median dose was 400 mg daily. Best response to imatinib was 4 MMR, 4 CCyR, 10 mCyR, 10 CHR, and 1 no response (NR); 19 patients did not have data available as they presented to our hospital after having failed imatinib. Forty-seven

Table 3. Cumulative response by phase on third-line TKI

Response	Dasatinib, no. (%)	Nilotinib, no. (%)	Overall, no. (%)
Chronic phase	n = 16	n = 9	n = 25
Hematologic			
CHR	13 (81)	6 (67)	19 (76)
Cytogenetic			
mCyR	4 (25)	2 (22)	6 (24)
CCyR	5 (31)	1 (11)	6 (24)
PCyR	1 (6)	1 (11)	2 (8)
Molecular			
MMR	2 (13)	3 (33)	5 (20)
Accelerated phase	n = 8	n = 2	n = 10
Hematologic			
CHR	7 (88)	2 (100)	9 (90)
Cytogenetic			
mCyR	1 (13)	0 (0)	1 (10)
CCyR	2 (25)	0 (0)	2 (20)
PCyR	1 (13)	1 (50)	2 (20)
Molecular			
MMR	1 (13)	0 (0)	1 (10)
Blast phase	n = 10	n = 3	n = 13
Hematologic			
CHR/RCP	4 (40)	2 (67)	6 (46)
Cytogenetic			
mCyR	1 (10)	1 (33)	2 (15)
CCyR	2 (20)	1 (33)	3 (23)
PCyR	1 (10)	0 (0)	1 (8)
Molecular			
MMR	1 (10)	0 (0)	1 (8)

If the same patient achieved both a MMR and CCyR, it was recorded for both responses in the table.

patients (98%) were taken off of imatinib because of resistance and 1 (2%) because of intolerance (grade 3 rash). Twenty-three patients (48%) were in either AP or BP on starting a second TKI.

Response to therapy with a second TKI

At the start of nilotinib as second-line TKI treatment, 17 patients were in CP (50%), 10 in AP (29%), and 7 in BP (21%). Fifteen patients (44%) received a starting dose of 400 mg twice daily. Other patients received 600 mg twice daily (n = 6), 400 mg once daily (n = 5), 800 mg once daily (n = 2), 100 mg once daily (n = 2), and 1 patient each 1200 mg once daily, 100 mg twice daily, 50 mg once daily, and 200 mg once daily. Twenty patients (59%) in this group had a mutation before starting nilotinib (Table 2). Twenty patients (59%) received no other treatment between the time of imatinib failure and the start of nilotinib, whereas 3 (9%) received anagrelide, 2 (6%) tipifarnib, 2 (6%) decitabine, 3 (9%) chemotherapy with cytarabine and idarubicin, and 1 patient each (3%) HHT, 6-mercaptopurine, topotecan, single-agent cytarabine, gemtuzumab ozogamicin, etoposide, mitoxantrone, all-trans retinoic acid, and allogeneic SCT. The best response to the second-line treatment with nilotinib was 5 MMR (15%), 3 CCyR (9%), 4 PCyR (12%), 11 mCyR (32%), 10 CHR (29%), and 1 (3%) NR. Thirty patients (88%) eventually failed nilotinib therapy because of resistance, whereas 4 (12%) were considered intolerant (dyspnea/pulmonary edema, gynecomastia, atrial fibrillation, and non–Q-wave myocardial infarction in a patient with a history of stent placement). Among the 30 patients who developed resistance to nilotinib, 17 (57%) had mutations identified before starting nilotinib. In 14 (82%), the same mutation persisted after failure to nilotinib, whereas in 1 (6%) a new mutation was observed. In 2 patients (12%), the baseline mutation disappeared (Table 2).

Table 4. Reason for discontinuation of treatment with third-line TKI

Treatment status	Dasatinib (n = 34), no. (%)	Nilotinib (n = 14), no. (%)
On treatment	6 (18)	7 (50)
Discontinued treatment	28 (82)	7 (50)
Side effects	7 (21)	0 (0)
Loss CHR	6 (18)	0 (0)
Died on therapy	4 (12)	1 (7)
No response	3 (9)	2 (14)
Allogeneic transplantation	1 (3)	2 (14)
Transformation	7 (21)	2 (14)

Among the 14 patients treated with dasatinib as second-line treatment, 8 patients were in CP (57%), 3 in AP (21%), and 3 in BP (21%). Seven patients (50%) received a starting dose of 70 mg twice daily. Other patients received doses of 50 mg twice daily (n = 3), 100 mg once daily (n = 2), 180 mg once daily (n = 1), and 50 mg once daily (n = 1). Four patients (29%) had baseline mutations before starting dasatinib (Table 2). Eleven patients (79%) did not receive interval treatment between the discontinuation of imatinib and start of dasatinib, whereas 1 patient received HyperCVAD followed by allogeneic SCT, 1 received anagrelide, and 1 received several sequential treatments, including cytarabine, interferon-α, decitabine, HHT, and anagrelide. The best response to dasatinib included 2 CCyR (14%), 1 PCyR (7%), 5 mCyR (36%), 4 CHR (29%), and 2 NR (14%). Nine patients (64%) eventually failed dasatinib treatment because of resistance and 5 (36%) were taken off because of intolerance (pleural effusion in 2 patients, thrombocytopenia in 2, and protein-losing enteropathy in 1). Of 9 (64%) patients who failed dasatinib because of resistance, 1 (11%) had a mutation identified before the start of therapy with this agent, and this mutation persisted at the time resistance developed (Table 2).

The median time on second-line TKI (dasatinib or nilotinib) was 8.3 months (range, 1.1-42 months). Nineteen of 34 patients (56%) in the nilotinib group and 5 of the 14 (36%) in the dasatinib group had either AP or BP before starting the third-line TKI (Table 2).

Response to therapy with a third TKI

At the start of third TKI, 25 patients (52%) were in CP, 10 (21%) in AP, and 13 (27%) in BP. Twenty-four of 34 patients (71%) on dasatinib and 8 of 14 (57%) on nilotinib as third-line TKI had mutations before treatment initiation (Table 2).

The best response to the third TKI in CP was 5 MMR, 3 CCyR, 2 PCyR, 3 mCyR, 6 CHR, and 6 NR. In AP, 1 patient achieved MMR, 1 CCyR, 2 PCyR, 1 mCyR, 4 CHR, and 1 NR. In BP, there were 1 MMR, 2 CCyR, 1 PCyR, 1 mCyR, 2 return to CP (RCP), and 6 NR (Table 3). In the dasatinib group, 7 patients (21%) discontinued treatment because of toxicity despite an acceptable response, including 2 patients who discontinued because of pleural effusion, and 1 each for gastrointestinal bleeding, neutropenia, renal failure, atrial fibrillation, and myalgias. None of the 14 patients on third-line nilotinib was taken off because of intolerance (Table 4).

The median CCyR duration for the 11 patients (2 patients with MMR did not have CCyR) who achieved this response was 16 months (range, 2-49 months); only 3 patients in CP who achieved CCyR (2 also attained MMR) had a response lasting longer than 12 months (28.6+ months, 16.3+ months, and

Table 5. Clinical characteristics of patients still on treatment, nilotinib-dasatinib group

Patient no.	Age, y	Nilotinib				Dasatinib				
		Duration of imatinib, mo	Duration of nilotinib, mo	Best response	Reason off	Bcr-abl mutation before therapy	Dose, mg	Percentage of Ph-before therapy	Best response	Response duration, mo
2	47	43	14	mCyR	Grade II thrombocytopenia	None	140 QD	100	mCyR	34+
3	59	61	18	PCyR	Loss CyR	G250E	50 BID	100	mCyR	30+
7	64	48	5.6	CHR	Loss CHR	H396R	70 BID	100	CCyR	41+
31	66	54	25	MMR	Transformation	A276G	50 BID	55	MMR	30+
32	22	41	11	MMR	Grade II gynecomastia	E459G	50 BID	0	CCyR	26+
33	38	73	8.4	MMR	Transformation	None	100 QD	0	MMR	23+

QD indicates once daily; and BID, twice daily.

18.7+ months). These included 1 patient treated with nilotinib after imatinib and dasatinib failure who had mutations V299L and F486S at the start of nilotinib, and 2 patients treated with dasatinib after imatinib and nilotinib failure (1 with H396R mutation and 1 with E459G mutation at the start of dasatinib). Nine of 34 patients (26%) on dasatinib and 3 of the 14 patients (21%) on nilotinib had disease transformation while on therapy with the third TKI.

After a median follow-up of 16 months (range, 3-34 months), 13 patients continue on therapy (10 CP, 2 AP, 1 BP; 7 on nilotinib, 6 on dasatinib; Tables 5-6). Of the 10 patients in CP, 5 continue with their best response after a median follow-up of 15 months (range, 3-34 months): 2 with sustained CCyR, 1 PCyR, and 2 mCyR. Four other patients have lost their best response: 1 patient lost a CHR, 1 lost a CCyR (now in PCyR), and 2 lost a MMR (1 in CHR, 1 in mCyR); 1 patient has not responded after 10 months. Both AP patients are still on therapy after having lost their best response: 1 lost a CHR and 1 lost a MMR (now in PCyR). The patient in BP is still on therapy and remains in MMR.

With a median follow-up for the total population of 13 months (range, 0.5-41 months) since the start of the third TKI, 25 patients (31%) are still alive, including 15 treated with dasatinib and 10 with nilotinib for a median overall survival of 20 months. The median event-free survival for the total group is 13 months, and the failure-free survival is 5 months. The outcome varies by stage of the disease, with a median failure-free survival of 20 months for patients in CP at the time the third TKI was commenced, compared with only 5 months for patients treated in AP and 3 months for those in BP (Figure 1).

Discussion

Although imatinib remains the standard treatment for patients with CML, second-generation TKIs have provided additional therapeutic options for patients with CML who fail imatinib. Despite the significant clinical activity demonstrated in clinical trials, the rate of CCyR is only 40% to 50%, leaving a significant number of patients who do not achieve the optimal response; some may eventually lose their response. It has been suggested that patients who do not achieve a MCyR by 12 months from the start of therapy with second-generation TKI or who are still 100% Ph^+ by 3 to 6 months have an increased risk of progression.[20] These data are frequently used to consider a change to alternative therapies. With the availability of 2 of these agents, nilotinib and dasatinib, it has become an increasingly common practice to use one of these agents when patients have failed imatinib and the other second-generation TKI. Early data have suggested that this strategy may be of benefit to some patients. Another study reported a hematologic and cytogenetic response rate of 57% and 30%, respectively, among 23 patients with CML in all phases treated with dasatinib after having failed imatinib and nilotinib (all resistant to nilotinib).[15] Similarly, a recent report indicates that a MCyR was achieved with nilotinib by 32% of patients with CML in CP who had failed prior therapy with imatinib and dasatinib.[16] The results of our present analysis confirm the activity of a third-line TKI (either dasatinib or nilotinib) in patients with CML after failure of both imatinib and nilotinib/dasatinib (second-line therapy). Overall, 33% of patients achieved a MCyR (32% in CP, 40% in AP, and 31% in BP), and 65% of patients in AP or BP achieved a CHR. Furthermore, 7 of 48 (15%) patients achieved an MMR. Of particular interest, however, was the transient duration of cytogenetic response for

Table 6. Clinical characteristics of patients still on treatment, dasatinib-nilotinib group

Patient no.	Age, y	Dasatinib				Nilotinib				
		Duration of imatinib, mo	Duration of nilotinib, mo	Best response	Reason off	Bcr-abl mutation before therapy	Dose, mg	Percentage of Ph-before therapy	Best response	Response duration, mo
2	60	48	17	CCyR	Grade II pleural effusion	Y253H	400 BID	100	MMR	21+
3	46	61	1.4	NR	Transformation	None	400 BID	100	CHR	25+
8	40	64	25	CHR	Grade II pleural effusion	Del 248-275	400 BID	100	PCyR	16+
9	18	26	9.6	mCyR	Grade II thrombocytopenia	None	400 QD	62	CHR	5.7+
11	57	22	11	mCyR	Loss CHR	F317L	400 BID	100	MMR	5.1+
13	46	26.4	34	CCyR	Loss CyR	V299L F486S	400 BID	100	MMR	23+
14	70	42	34	mCyR	Transformation	F317L	200 BID	100	NR	11.2+

QD indicates once daily; and BID, twice daily.

Figure 1. Patient outcome on third-line TKI. (A) Event-free survival. (B) Failure-free survival. (C) Overall survival.

those patients who achieved it. The median CCyR duration was 16 months with only 3 patients (all treated in CP) having a CCyR sustained for more than 12 months.

One reason for the lack of durable cytogenetic remission could be the emergence of new kinase domain mutations as patients are exposed to sequential TKI. Twelve patients had new mutations that emerged after second-line treatment with a TKI; 7 new mutations emerged with nilotinib as second-line therapy (1 patient each developed A276G, Y253F, Y253H, E255V, F311L, F359C, and H396P) and 5 with dasatinib (F317L in 2 instances, Y253H in 1 patient, and 2 patients with 2 coexisting mutations, F359V/F311L, and V299L/F486S). In some instances, the emerging mutation had low in vitro sensitivity to the agent being administered but higher sensitivity to the alternative agent (eg, V299L emerging on dasatinib). In some of these instances, a change of therapy resulted in an adequate response (eg, patient 2 in Table 2). Interestingly, however, some of the mutations have been reported to

have relatively good in vitro sensitivity to the agent being administered when they emerged. Two of the 7 mutations (F311L and H396P) that emerged during nilotinib therapy have a reported cellular proliferation IC_{50} less than 50nM, which suggests adequate in vitro sensitivity. Similarly, 2 of 5 mutations (Y253H and F359V/F311L) emerging on dasatinib as second-line therapy have a cell proliferation IC_{50} less than 3nM suggesting good in vitro sensitivity to dasatinib.[11] For some mutations (eg, A276G and F359C), in vitro sensitivity to second-generation TKI has not been reported.

Of particular concern is the emergence of T315I as this mutant is not inhibited by any of the available TKIs. It could be that consecutive treatment with the available TKI may cause selection of this mutation. In our series, 3 patients had T315I at the start of the second TKI, and this remained detectable before the start of the third TKI. As expected, none of these patients achieved an MCyR. Importantly, none of the patients developed the T315I mutation during third-line treatment.

Several reports have suggested that there is little cross-intolerance between imatinib and dasatinib[21] or nilotinib.[22] In our series, 7 of 48 (15%) patients (all on dasatinib) discontinued third-line TKI treatment because of grade 2 or 3 side effects, including myalgia, renal failure, gastrointestinal bleeding, and pleural effusions. Of these 7 patients, 2 also were taken off of nilotinib as second-line therapy because of side effects (one developed a pleural effusion with dasatinib and ECG abnormalities on second-line therapy with nilotinib, whereas another patient had a pleural effusion on dasatinib and had pulmonary edema on second-line therapy with nilotinib). None of these 7 patients had intolerance to imatinib. The relatively high incidence of side effects while on the dasatinib arm as third-line TKI could be because these patients were heavily pretreated before starting dasatinib (median, 5 prior therapies; range, 2-8 prior therapies). Cytogenetic data are available for 6 of these 7 patients as 1 patient discontinued dasatinib because of side effects (myalgias) shortly after the start of therapy. Of these 6 patients, 2 achieved a MCyR with therapy, and it was sustained in 1 patient at the time therapy had to be discontinued. The one patient with a durable MCyR response was given a starting dose of 50 mg twice daily. The other patients received 70 mg twice daily (n = 3), 140 mg once daily (n = 2), and 100 mg once daily (n = 1). It is possible that, in more heavily treated patients, alternative doses and schedules should be investigated to maximize safety and efficacy.

The long-term efficacy of TKI therapy is frequently expressed in terms of event-free survival or duration of MCyR. However, these definitions frequently underestimate other reasons for failure, such as intolerance or lack of cytogenetic response. To paint a more realistic picture of the true benefit of this strategy, we analyzed the failure-free survival where all of these endpoints were considered as failure in addition to the more conventional definition of loss of MCyR or CHR, transformation, or death. When analyzed in this way, the strategy of using a second-generation TKI as third-line therapy results in minimal value for most patients as the time to failure is only 3 to 5 months for patients in advanced stages and 20 months for those in CP. The transient benefit may be valuable to serve as a bridge to SCT where an improved hematologic and cytogenetic status may be of benefit. Indeed, two-thirds of the patients treated in this series in AP or BP achieved a CHR. This strategy cannot be relied on as a long-term solution for these patients. Allogeneic SCT should be considered in all patients who fail 2 TKI who are adequate candidates for this approach. For others, alternative strategies are required.

In conclusion, the use of a second-generation TKI after failure of 2 TKIs may induce responses in some patients, but these are usually not durable except in occasional patients in CP. There remains no clear difference between the different sequences explored in our study (ie, imatinib → dasatinib → nilotinib, vs imatinib → nilotinib → dasatinib). Patients who fail 2 TKIs should be offered allogeneic SCT if they are eligible candidates, and new strategies are needed for all others.

Authorship

Contribution: R.J.G. collected, analyzed, and interpreted the data, performed the research, and wrote the manuscript; H.K., S.O., A.Q.-C., S.F., and Z.E. treated the patients and edited and reviewed the manuscript; and J.C. designed and performed the research, analyzed and interpreted the data, performed the statistical analysis, treated the patients, and helped write the manuscript.

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