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Kinase Domain Point Mutations in Ph+ Acute Lymphoblastic Leukemia (ALL) Emerge Following Therapy with BCR-ABL Kinase Inhibitors

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Abstract

Background—BCR-ABL kinase domain (KD) mutations are detected in approximately 45% of imatinib-resistant CML. Patterns of KD mutations in Philadelphia chromosome (Ph)+ acute lymphoblastic leukemia (ALL) are less well-studied.

Methods—We assessed KD mutations in relapsed Ph+ ALL following treatments that included one or more kinase inhibitors (n = 24) or no prior KI therapy (n = 12).

Results—ABL KD mutations were detected by direct sequencing in 15 of 17 (88%) relapsed Ph + ALL with prior imatinib (n = 16) or dasatinib (n = 1) treatment, and in 6 of 7 (86%) resistant/ relapsed tumors treated with 2 or more KIs, compared with 0 of 12 relapsed Ph+ ALL never treated with KI. A restricted set of mutations was seen, mostly Y253H and T315I, detected on average 13 months following KI initiation, and mutations were not detected in the initial tumor samples prior to KI therapy in 12 patients assessed. Using a more sensitive pyrosequencing method, we did not detect mutations at codons 315 and 253 in the diagnostic samples from these 12 patients or in 30 Ph+ ALL patients who never relapsed.

Conclusions—ABL KD mutations, especially at codons 315 and 253, emerge upon relapse in the vast majority of patients with Ph+ ALL receiving maintenance KI therapy. Ongoing KI exposure may thus alter the patterns of relapse and favor outgrowth of clones with KI-resistant mutations.

Keywords

kinase inhibitor; therapy resistance; biological selection; lymphoid leukemia; chronic myeloid leukemia

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Introduction

BCR-ABL kinase domain (KD) point mutations are detected in the dominant tumor clone(s) in approximately 45% of CML at the time of disease resistance, developing after an average of 20-35 months of imatinib (Gleevec®) therapy.1-3 Second and third generation kinase inhibitors (KIs), including dasatinib,4 nilotinib5, bosutinib6, INNO-4066, and MK-04577 are now used for imatinib-resistant CML and are each associated with a somewhat different spectrum of KD mutations likely related to differing sensitivity of mutant clones to these agents.8, 9

The algorithms for monitoring following imatinib resistance in CML are becoming well-established.10, 11 The clinical scenario in which BCR-ABL KD mutations are detected in Philadelphia chromosome (Ph)+ acute lymphoblastic leukemia (ALL) is less clear but has mostly been associated with relapsed disease following treatment with chemotherapy followed by maintenance therapy with tyrosine kinase inhibitors.12 Since current therapy for Ph+ ALL includes chemotherapy combined with imatinib therapy,13-16 the timing of emergence and overall pattern of BCR-ABL KD mutations is highly relevant. Furthermore, the differences between the biology of lymphoid blast crisis versus de novo Ph+ ALL remains unclear.17 To determine the pattern of KD mutation in Ph+ ALL, we assessed the patterns of BCR-ABL KD mutations at diagnosis, upon relapse and following salvage therapy with second KIs.

Materials and Methods

Patient population

Patients with Ph+ ALL seen at our institution over the last 5 years that had adequate available RNA from an involved sample (>10% blasts) were analyzed. All but two patients were adults over 19 years old. Diagnostic criteria were those of the World Health Organization International Histological Classification of Tumours.18 All but two ALL cases were of B-cell lineage as determined by an extensive flow cytometric antigen panel; the remaining two cases were myeloid/B-cell biphenotypic lineage. Cases presenting as lymphoid blast crisis of CML were excluded. Therapy for Ph+ ALL, in most cases, was hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone (CVAD) chemotherapy alternating with high-dose ara-C and methotrexate along with imatinib at 400 to 800 mg/day, with imatinib with or without vincristine or POMP as maintenance (individual treatments are summarized in Table I).13 Patients who presented outside of our institution were initially treated with a variety of intensive multiagent chemotherapy regimens with 400-800 mg/day of imatinib. Twelve relapsed Ph+ ALL who had received Hyper-CVAD (+/- rituximab) but no imatinib therapy were also studied for comparison. Therapy for relapsed disease was variable and included dasatinib (with or without additional chemotherapy) in 9,4, 5 others TKIs in 4, and MK-0457 in 3.7 Eight patients underwent stem cell transplant in 1^{st} or 2^{nd} remission.19

For monitoring of treatment response, BCR-ABL PCR studies were done every 1 to 3 months on peripheral blood during the induction phase. Standard cytogenetic analysis of Giemsa (G)-banded metaphases was done on bone marrow aspirates, and fluorescence *in*

situ hybridization using BCR-ABL fusion probes was done along with the PCR studies on blood every 3-6 months during the maintenance phase and during remission and at relapse.

RNA isolation and quantitative BCR-ABL PCR

White blood cells from bone marrow aspirate or peripheral blood specimens were isolated by centrifugation following red blood cell lysis. Total RNA was extracted using Trizol reagent (Gibco-BRL, Gaithersburg, MD) and RNA quality assessed by agarose gel electrophoresis prior to cDNA synthesis. Real-time quantitative PCR for BCR-ABL was performed in a one-tube reaction with normalization to total ABL levels, and post-PCR sizing of fusion transcript products, as previously described.20, 21

KD mutation detection

To avoid interference from the normal ABL allele, a nested PCR sequencing approach was used with a 1st round amplification of the BCR-ABL transcript followed by two separate PCR reactions that cover exons 221 to 380 and codons 350 to 500 of the ABL kinase domain.22 Standard dideoxy chain-termination DNA sequencing was performed using Big Dye chain terminator reagents on an ABI3700 analyzer and analyzed using Sequence Analysis software V3.3 and the SeqScape software V2 (ABI, Foster City, CA).1 All mutations were confirmed by sequencing of forward and reverse strands, with a sensitivity of 10% mutation-bearing transcripts in the analyzed population. Mutation level for direct sequencing (ds) was reported semiquantitatively in Table I, as only mutant (100%), mostly mutant (75%), mixed (50%), or mostly unmutated (25%).

For mutation analysis of codons 311-317 and codons 250-255, pyrosequencing was performed following first round PCR, as above and different 2nd-round PCR reactions. Second round PCR was performed using one biotin-tagged primer and single-stranded product isolated by avidin-sepharose beads (GE Life Sciences, Piscataway, NJ) and sequenced using a HSQ96 Pyrosequencer (Biotage, Uppsala, Sweden). The sensitivity of the pyrosequencing was 1-5% mutation-bearing transcripts in the total RNA pool. Mutation level for pyrosequencing (py) was reported as % mutated product in Table I, with an established precision of 5% for the assay.

Results

BCR-ABL KD Mutations are detectable at relapse in KI-treated but not KI-naïve ALL

Overall, 63 samples from 36 Ph+ patients with relapsed Ph+ ALL were studied, including 24 with kinase inhibitors (KI) as part of their therapy, and 12 patients who had never received KI therapy prior to relapse as a comparison group. There were 34 adults and 2 children (21M 15F, median 45 years old at diagnosis, range 3-83 years), with median followup of 28 months, range 11-96 months). 30 of 36 (83%) tumors had e1a2 BCR-ABL fusion transcripts associated with the p190 BCR-ABL protein, with the other tumors having b2a2 or b3a2 BCR-ABL fusion transcripts producing the p210 protein

Using a nested PCR-based assay to directly sequence the entire ABL kinase domain (KD) of the BCR-ABL fusion transcript, no KD mutations were noted at relapse in the 12 patients

with Ph+ ALL who had received no prior KI therapy. The frequency and pattern of BCR-ABL KD mutation development among the 24 patients who had received KI therapy prior to relapse is summarized in Table I. BCR-ABL KD mutations were noted by direct sequencing in 15 of 17 (88%) patients with morphological evidence of relapse after only imatinib (n = 16) or dasatinib (n = 1) therapy. The sites of KD mutation were Y253H in 6 (c.1121T>C), T315I in 4 (c.1308C>T), F317L in 2 (c.1315C>A), and E255K (c.1127G>A), P309A (c.1288C>G), E355Q (c.1427G>C), F359V (c.1439T>G), H396R (c.1551A>G), E459K (c.1739G>A) in 1 each, with 3 patients having 2 detectable mutations (Figure 1). All mutations were detected in peripheral blood or bone marrow aspirate sample, although concurrent relapse in the CNS was commonly noted. The median time from diagnosis to mutation detection was 10 months (range 6-24 months), with mutations in codons 253 and 315 most frequently developing in those receiving continuous KI therapy in the preceding months, versus those patients with some breaks in imatinib maintenance therapy. The two patients on imatinib maintenance who relapsed without detectable KD mutations also had intermittent KI therapy.

BCR-ABL KD mutations were also noted in 6 of 7 patients (86%) who presented to our institution with relapsed/persistent Ph+ ALL, after having received two or more KIs. The sites of KD mutation were T315I in 4 (c.1308C>T), Y253H (c.1121T>C), F317L (c.1315C>A) and E355G (c.1428A>G) in 1 for those receiving dasatinib after imatinib, and Y253H (c.1121T>C) in 1 patient receiving nilotinib after imatinib. One patient who had previously received imatinib, nilotinib and dasatinib (in that order) had 3 KD mutations, including T315I, F317L and E355G, all present at mixed levels suggesting 3 distinct clones. The median duration of KI therapy at mutation detection was 10 months, and the median time on second KI therapy was 4 months.

Median followup among the cohort of 24 KI-treated patients who relapsed was 29 months (range 11-95 months), with 17 of 24 patients dead of disease at last followup (overall survival, range 12-95 months).

Table II summarizes the cytogenetic findings seen by conventional G-banded karyotype among the 24 KI-treated relapsed tumors. Only 2 cases had t(9;22) as the sole abnormality at diagnosis, with extra copies of the Ph chromosome, as indicated by derivative (der) chromosome 22 or more complex derivative “marker” (mar) chromosomes, seen in 8 cases (all amplifications were confirmed by BCR-ABL fusion FISH study). In cases with complex karyotypes there was usually reemergence of a similar or related clone at time of relapse; however 6 cases at 1st or 2nd relapse had clonal progression or emergence of an apparently distinct (sub)clone from that seen at diagnosis.

Shifts in KD mutation can occur following switch to a different KI

Table I also summarizes the outcome of KD mutations who switched from one KI to another. Complete regression of one mutation and emergence of another was seen in 3 patients (#1, 7, 15), such as the complete shift from E355Q to F317L (c.1315C>A) at 5 months following switch from imatinib to dasatinib. Using quantitative pyrosequencing, we noted that persistence and level of particular mutations sometimes paralleled their predicted sensitivity to particular KIs from in vitro studies,8, 23-25 For example in 3 patients, F317L

and T315I exhibited no regression with dasatinib treatment (#5, 18, 21) but T315I did regress with MK-0457 therapy in 1 of 3 patients (#20) and with stem cell transplant (#18). These data suggest that although mutations can develop rapidly in Ph+ ALL, exposure to a relevant KI is essential for their full emergence and persistence. Several cases that showed shifts to a new KD mutation with introduction of a 2nd TKI had evidence of emergence of a distinct clone (case #7) or clonal progression (cases #1, 15 and 20) by cytogenetic analysis.

BCL-ABL KD mutations are not detectable at diagnosis in Ph+ ALL

In order to assess whether significant levels of BCR-ABL transcripts with KD mutations were present prior to KI use, we studied the diagnostic sample from 12 patients who ultimately developed a mutation at relapse and did not detect any KD mutations. Using a more sensitive quantitative pyrosequencing assay, we also did not any detect mutations in Y253H T315I and F317L in these samples or in the initial diagnostic samples from 25 additional cases of Ph+ ALL who did not relapse (not shown). KD mutations were also rare (2 of 12 patients) in persistent ALL during the first months of treatment, and in those cases mutations were not seen at the relapsed mutational hotspots (Table III).

Discussion

We note that BCR-ABL KD mutation in Ph+ ALL occurs predominantly in the setting of relapse following KI maintenance therapy and involves a restricted subset of mutations that are among the most resistant *in vitro* to imatinib and most of the newer KIs, namely T315I and Y253H.26 Mutations were not observed at relapse in those patients not receiving KI as part of their treatments.

The higher incidence, rapid emergence and restricted group of KD mutations seen here in relapsed Ph+ ALL as compared to KI-resistant CML11, 22, 27 is similar to another series recently reported,28 but our results differ in some respects. By comparing KD mutations present at low levels at the time of diagnosis, Pfeifer et al concluded that acquired therapy resistance in Ph+ ALL is due in part to mutated BCR-ABL clones that exist prior to KI therapy.28 They concluded that this supported the use of KIs as initial therapy to eradicate these pre-existing KD-mutated populations. Given the absence of a substantial population of any given KD-mutated clone detectable prior to mutation growth, our results would favor selection following initiation of KI therapy as the *primary* determinant of mutation emergence. In fact, many of these patients who developed KD mutations at relapse were on continuous KI therapy from the time of diagnosis. The rapidity of KD mutation shifts that we observed in some patients following introduction of a different KI (median 4 months) also supports rapid KD mutation outgrowth in response to KI-driven selective pressure.

The selective pressure reflected in the high rate of KD mutation also provides strong evidence that BCR-ABL expression remains critical to growth of Ph+ ALL at relapse. The more restricted sites of BCR-ABL mutations in Ph+ ALL as compared to KI-resistant CML may be related to need for more active BCR-ABL kinase for growth/transformation of lymphoid cells versus myeloid cells. This hypothesis would also be consistent with the frequent presence in Ph+ ALL of BCR-ABL gene amplification, as evidenced by extra Philadelphia chromosomes and the frequent finding of higher BCR-ABL transcript levels.

Effects due to differences in the signaling pathways for the p190 versus p230 BCR-ABL protein following KI therapy in lymphoid versus biphenotypic versus myeloid stem cell populations also cannot be excluded. Finally differences in the entry, binding and metabolism of KIs in different leukocyte populations may influence the selection of KD mutated clones in ALL versus CML.

KIs clearly have an important role in therapy of relapsed Ph+ ALL. Dasatinib, in particular, has also been shown to be effective for imatinib-treated resistant/relapsed Ph+ ALL.29, 30 However, the high rate of KD mutation development at relapse suggests that KI-selected mutations can promote disease progression and has implications for the timing and duration of induction and maintenance therapy with KIs in Ph+ ALL.13 Our data suggests that sequential or combination therapy with both imatinib and dasatinib may preferentially select for tumor clones with mutations that mediate resistance to both agents. The most common mutation that develops in this setting, T315I, is cross-resistant to most KIs. Whether other chemotherapeutic agents in maintenance regimens beside KIs plays a role in mediating outgrowth of mutated clones deserves further study. In that regard, strategies to alternate KIs with other therapies during the maintenance phase31 or replacement of KIs with other cytostatic chemotherapies once mutations are detected to allow clonal regression may be strategies worth exploring to minimize biological selection of resistant disease.

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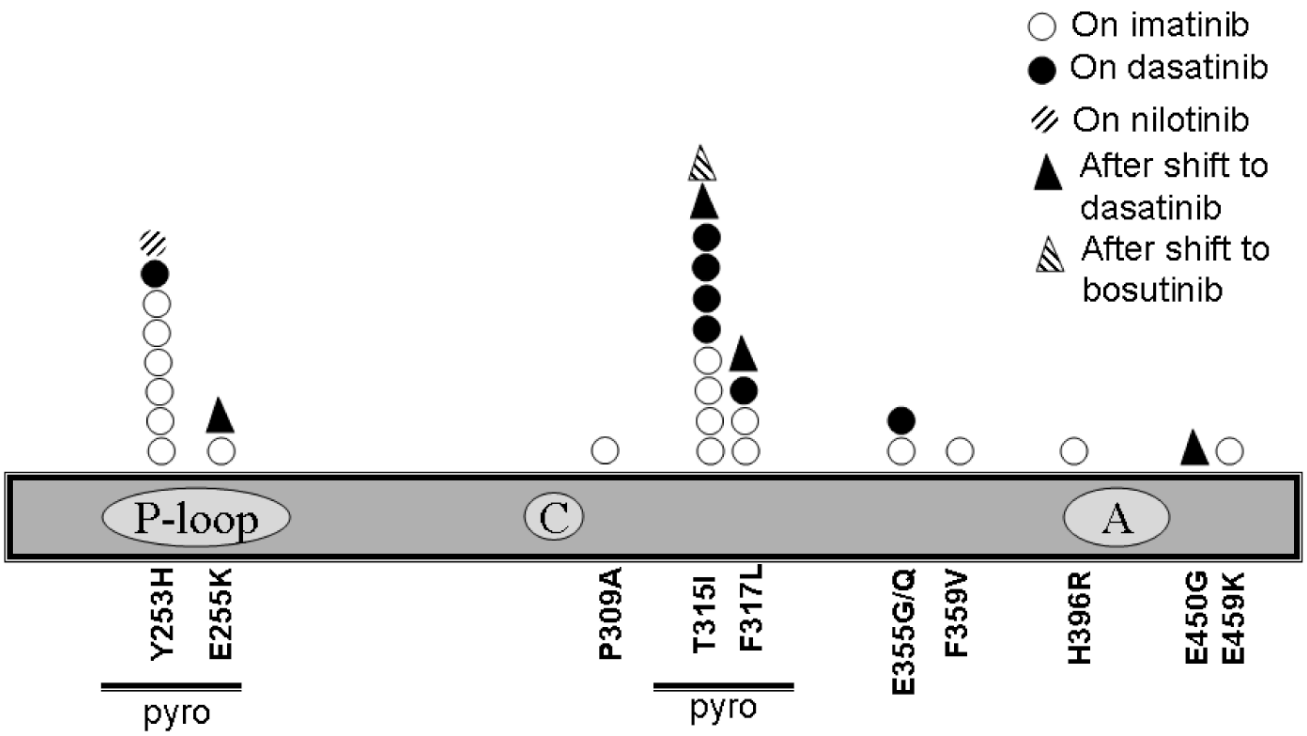

Figure 1. Summary of location of mutations in the kinase domain of BCR-ABL in relapsed Ph+ ALL

Illustrated is a schematic representation of the kinase domain, including ATP binding pocket (P-loop), catalytic domain (C), and activation loop (A). Mutations which developed on imatinib are shown as open circles, those developing on dasatinib as black circles or triangles, and those on nilotinib and bosutinib as hatched circles. The hatched lines indicate the area covered by the pyrosequencing (pyro) mutation assay. Amino acid designation for the mutations are those of the ABL type 1a protein, with the following approximate KD subdomains: P-loop 248–256, C-helix/catalytic domain 276–290, SH3 contact 294–304, additional imatinib binding pocket 311-318, SH2 contact 331–353, and activation loop 381–402.32

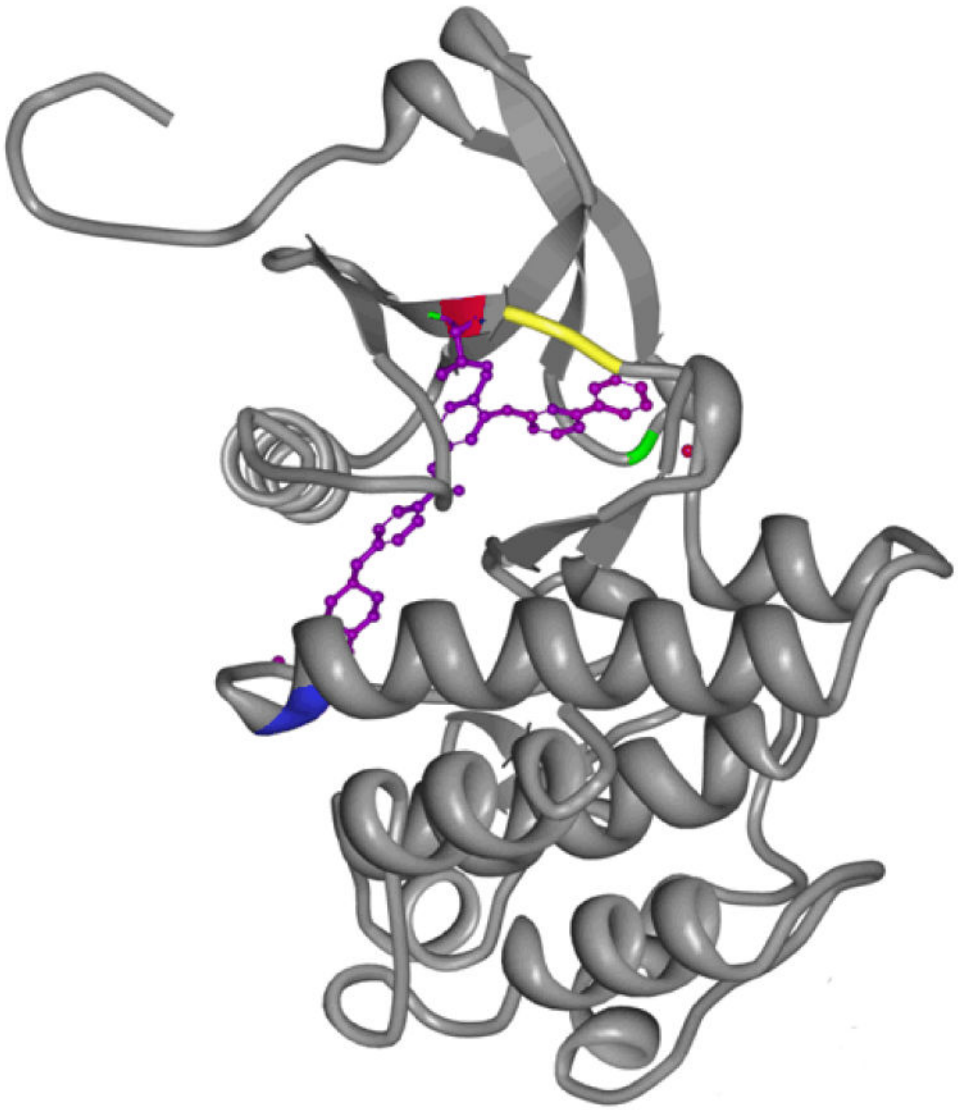

Figure 2. Mapping of BCR-ABL KD mutations seen in relapsed Ph+ ALL

Human Abl kinase domain in complex with imatinib (accession 2HYY,33 accessed from the RCSB Protein Data Bank), was rendered with MBT protein workshop software.34 One ABL chain is shown, with imatinib in its binding pocket highlighted in purple; ABL codon 253 is labeled green, codon 315 is red, codon 317 is yellow, and codon 355 is blue.

Table I

Summary of clinical data in BCR-ABL kinase domain mutational analysis of relapsed Ph+ ALL.

	Clinical setting when KDM detected	Prior KDM status (mo after KI start, method)	KDM mutation at relapse, level/method	Time on KI (mo)	Time from Dx (mo)	KI dose before KDM detected	NC KI	Treatment summary before mutation	Treatment after detection of mutation	Fate of KDM	FU (mo)
KDM emerging at relapse in patients initially diagnosed at our institution											
1	On IM, 1st relapse	nd	253H, 91%/py	15	15	IM 600×14m	N	CVAD>IM/V/Pred	HCVAD/dasat	253H lost 315I and 450G gain	DOD 28.3
2	On IM, 2nd relapse	None (2m/py)	253H, 50%/ds	10	15	IM 400×2m - off tx- IM 800×3m	Y	CVAD/IM Relapse: CVAD/IM	dasat 70	ni	DOD 16.8
3	On IM, 1st relapse	None (0m/py)	253H, 50%/ds P309A, 50%/ds	12	12	IM 600×12m	N	CVAD/IM>IM	none	ni	DOD 30.4
4	On IM, 2nd relapse	None (0m/ds-py)	315I, 20%/py 255K, 70%/py	10	91	IM 600×10m	N	CVAD-R/IDEC>POMP Relapse: V/Dec/IM	bosutinib	ni	DOD 94.6
5	On dasat, 1st relapse in CNS post-bmt	None (0m/py)	315I, 85%/ds-py	12	13	dasat 100×12m	N	CVAD >V/dasat	no KI, salvage chemotherapy	315I persist	AWD 15.5
6	On IM, 1st relapse in CNS post-bmt	nd	317L, 24%/ds-py	16	16	IM 400-300	Y	CVAD, A/RT (CNS) > bmt > IM	none	ni	AWD 12.2
7	On IM, CNS relapse post-bmt	None (3m/ds)	355Q, 50%/ds	6	15	IM 400×3m - off tx-G1400×6m	Y	CVAD>POMP>bmt>IM	dasat 100	317L gain, 355Q lost	AWD 37.5
8	On IM, 1st relapse	None (0m/ds)	359V, 50%/ds	10	10	IM 400>800×10m	N	CVAD/IM	none	ni	DOD 11.8
9	Om IM, 2nd relapse post-bmt	None (2m/ds)	396R, 100%/ds	10	29	IM 400× 5m - off tx- IM 300×10m	Y	I/A>IM/V Relapse: CVAD/IM>bmt	bmt	ni	DOD 39.9
10	On IM, 1st relapse in CNS	None (0m/ds)	459K, 75%/ds	14	14	IM 400>200>600 ×14m	Y	CVAD/R>IM/V/Pred	dasat>bmt	ni	DOD 31.7
Patients referred to our institution for relapsed disease with KDM mutation											
11	On IM, 1st relapse	nd	253H, 100%/ds	13	13	IM 400×12m	?	Larson A-B-C/IM>IM	nilotinib	253H persist	DOD 19.3
12	On IM, 2nd relapse	None (12m/py)	253H, 100%/ds	16	22	IM 400-600 ×16m	Y	CVAD>IM, Relapse: CVAD/IM>IM/V	dasat	ni	DOD 28.6
13	On nilot, 2nd relapse	None (14m/ds-py)	253H, 100%/ds	9	23	IM 400× 1m > off 4m > nilot 800×9m	Y	CVAD>CVAD/IM>bmt Relapse: nilot	none	ni	DOD 39.6
14	On dasat, 3rd relapse	None (2m/py)	253H, 100%/ds	6	24	IM 800×4m > dasat 140×1m	N	V/Pred/Asp>MTX/V/Pred Relapse:V/Pred/Mito>IM/MTX/A>CVAD/IM>dasat	decitabine >bmt	ni	DOD 26
15	On IM, 1st relapse	nd	315I, 50%/ds 253H, 50%/ds	10	10	IM dose/duration unknown	N	CVAD/IM	bosutinib	315I gain 253H lost	DOD 15.1

	Clinical setting when KDM detected	Prior KDM status (mo after KI start, method)	KDM mutation at relapse, level/method	Time on KI (mo)	Time from Dx (mo)	KI dose before KDM detected	NC KI	Treatment summary before mutation	Treatment after detection of mutation	Fate of KDM	FU (mo)
16	On dasat, 2nd relapse	nd	315I, 100%/ds	5	26	IM 400 > Dasat 140×5m	Y	D/V/Pred/IM> MTX/Etop/D/VP Persist: > dasat 140	MK-0457	315I persist	DOD 42.8
17	On dasat, 3rd relapse	nd	315I, 100%/ds	?	23	IM 400-800 ×12m > dasat 100×5m	N	CVAD Relapse: bmt>IM 400-800	MK-0457	315I persist	DOD 38.1
18	On dasat	nd	315I, 66%/py	6	6	IM 800 ×4m > dasat 70×1m	?	IM>dasat	HCVAD-dasat > bmt	315I lost 255K gain	AWD 14.8
19	On IM, 3rd relapse	None (5m/ds-py)	315I, 100%/ds	12	12	IM 400×5m	Y	AALL-0232>bmt Relapse: AALL-0232> CVAD/IM	dasat and chemotherapy	ni	DOD 18.5
20	On dasat, 3rd relapse	nd	315I, 63%/ds-py 317L, 24%/ds-py 355G, 25%/ds	?	32	IM > dasat dose/duration unknown	?	CVAD/IM Relapse: sarafenib >dasat	MK-0457	315I and 355G loss	AWD 50.8
21	On IM, CNS relapse post-bmt	nd	317L, 75%/ds	24	26	IM 600-400 ×12m	Y	BFCI-ALL>IM-bmt Relapse: CVAD/IM	dasat	317L persist	DOD 57
Patients with relapsed disease with no KDM mutation detected											
22	nr	None (0m/ds)	None	nr	nr	IM 400-600 ×9m	N	CVAD/R/IM	INNO-406	nr	DOD 26.4
23	nr	None (0m/ds)	None	nr	nr	IM 800×3m> off 5m> IM 800×2m	Y	D-V-Asp > bmt>IM	ni	nr	AWD 11.2
24	nr	nd	None	nr	nr	IM 400×24m BMS×1m	Y	HCVAD > IM/V/Pred > HCVAD/dasat	nr	nr	AWD 34m

[1]All cases were e1a2/p190 BCR-ABL transcript type except for case 1 (b2a2/210), case 2 (b3a2/p210) and case 24 (b3a2 & b2a2/p210).

Abbreviations: AWD: alive with disease, bmt: stem cell transplantation, CNS: central nervous system, DOD: died of disease, ds: direct sequencing method (20% sensitivity, semiquantitative), DX: diagnosis, FU: followup, KDM: BCR-ABL kinase domain mutations, KI inhibitor, m: months, NC KI: non-continuous/breaks in KI maintenance therapy, nd: not done, ni: no information, nr: not relevant py: pyrosequencing method (1-5% sensitivity, quantitative +/- 5%). Doses of KI are listed as milligrams/day.
Treatments: ">" indicates sequential treatment, "/" indicates concurrent treatments, A: ara-C, Asp: asparaginase, BFCI-ALL: daunorubicin, vincristine, high-dose methotrexate, L-asparaginase, prednisone, bmt: stem cell transplant, D: daunorubicin, dasat: dasatinib (Sprycel), Dex: dexamethasone, etop: etoposide, HCVAD: hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone alternating with high-dose ara-C and methotrexate, I: idarubicin, HU: hydroxyurea, IM: imatinib mesylate (Gleevec®), Larson A: vincristine, prednisone, l-asparaginase, and doxorubicin, Mito: mitoxantrone, MTX: intrathecal methotrexate, nilot: nilotinib (Tasigna, AMN107), Pred: prednisone, POMP: 6-MP and methotrexate, with or without vincristine and steroids, R: rituximab, V: vincristine.

Karyotype at relapse	Comment
45,XX,-9,t(10;19)(q23;q13.3),t(16;22)(p13.3;q12),del(20)(q11.2q13.3)	Cryptic BCR-ABL, t(10;19) likely constitutional change
46,XY,add(7)(q36),del(9)(q22),t(9;22)(q34;q11.2),del(13)(q14q22), add(21)(p11)	Related complex clones with gain of add7q at relapse
47,XX,-9,t(9;22)(q34;q11.2),+der(22)t(9;22)×2	Several related subclones seen at diagnosis/ relapse
46,XX,t(9;22)(q34;q11.2)	No change at relapse
46,XY,del(6)(q13q23),t(9;22)(q34;q11.2)	Gain of del6q at relapse
46,XY/ BCR-ABL FISH+ 1st relapse: 46,XX,t(9;22)(q34;q11.2) [1 of 20 metaphases] 46,XX,t(9;22)(q34;q11.2),del(20)(q12) [1 of 20 metaphases] 46,XX,t(3;21)(q21;q22),t(9;22)(q34;q11.2) [1 of 20 metaphases] 2nd relapse: 45,XX,-8,t(9;22)(q34;q11.2),t(11;15)(q13;q26), add(12)(q24.3),add(16)(q24)	Subclonal diversity evident that correlates with shifts in KDM upon dasatinib introduction
49,XY,+4,t(9;22)(q34;q11.2),+der(22)t(9;22),+mar	Several related clones at diagnosis, Clonal shift at relapse
45,XX,del(1)(q32q42),-7,t(9;22)(q34;q11.2),-13,-16,+19,+1-3mar 48,XX,der(2)t(1;2)(q11;q37),t(9;22)(q34;q11.2),del(19)(q13.2), +der(22)t(9;22)×2 Karyotype at subsequent relapse/persistence	

	Karyotype at relapse	Comment
	45,XY,del(3)(p13),del(4)(q31.1q35),add(4)(q35),-7, t(9;22)(q34;q11.2),-10,-16,+2mar	Similar complex clones with TKI switch
	46,XY,del(5)(q21q31),t(9;22)(q34;q11.2)	
), der(22)t(9;22)	46,XX,del(1)(p32),t(2;2)(q37;q35),der(5)inv(5)(q11.2q23) dup(5)(q23q31),der(9)del(9)(p21)t(9;22)(q34;q11.2), t(17;22)(q11.2;q13),der(22)t(9;22)	Similar complex clones at relapses
	45,XX,add(3)(q29),-7,der(9)del(9)(p21)t(9;22)(q34;q11.2), der(22)t(9;22)	Related but distinct complex clones at multiple relapses
	39-44,XY,t(2;21)(p13;q22),del(5)(q31q35),add(8)(p23), del(9)(p22p24),t(9;22)(q34;q11.2),der(10)t(1;10)(q21;q26),add(11)(p15), add(12)(p13),-17,-19,del(20)(q11.2)	Related but distinct complex clones at multiple relapses
	45,XY,-7,t(9;22)(q34;q11.2),der(12)t(7;12)(q11.2;p11.2)	Related complex clones with disease persistence
	41-45,X,-X,t(9;22)(q34;q11.2),ins(10)(p15q25q26), der(19)t(1;19)(q23;p13.3)	
	6,XY,i(8)(q10),t(9;22)(q34;q11.2)	Gain of i8q associated with KDM shift with relapse after SCT
11)(p14),-14, del(14)(q21),add(18)(q22),der(22)t(9;22)		
	46,XX,-7,t(9;22)(q34;q11.2),der(14)t(7;14)(q11.2;q22), der(16)t(1;16)(q23;q13),+der(22)t(9;22)	Related but distinct complex clones at multiple relapses
	45-46,XY,inv(3)(q21q26),-7,t(9;22)(q34;q11.2), del(10)(q11),add(11)(p15),del(12)(p11.2),add(16)(q24),-18,+1-2mar	Distinct clones at 1st/2nd relapse
	Karyotype at relapse	

	Karyotype at relapse	Comment
	46,XX,der(9)del(9)(p13)t(9;22)(q34;q11.2), -19,add(20)(q13.3),der(22)t(9;22),+mar	Related complex clones seen at diagnosis/ relapse
	46,XX,t(9;13)(q32;q12),t(9;22)(q34;q11.2)	
	44,XX,der(9)add(9)(p11.2)t(9;22)(q34;q11.2),-13,-13	

ive chromosome, mar: marker (unassigned) chromosomal material, i:

Table III

Detection of BCR-ABL kinase domain mutations by direct sequencing during disease course of Ph+ ALL.

At time of diagnosis	Residual disease during therapy including KI	At relapse with no prior KI treatment	At relapse with one prior KI treatment	At relapse after two or three KIs
0/12[1]	2/12[2]	0/12[3]	15/17[4]	6/7

Direct sequencing refers to full ABL kinase domain Sanger sequencing performed following a nested PCR reaction (See methods).

[1]Tested were the diagnostic or pre-KI-treated samples from 12 patients with Ph+ ALL that eventually relapsed with a KD mutation.

[2]Tested were samples with residual Ph+ ALL in the first 6 months of treatments with chemotherapy and KI. Low levels of S438C (c.1674C>G) and Q252H (c.1120G>T) mutation detected by direct sequencing in 1 sample each.

[3]Tested were 12 relapse samples from patients with Ph+ ALL who received chemotherapy regimens with no KI.

[4]16 pts had been treated with IM only, 1 with dasatinib only prior to KDM detection. 10 of these pts were diagnosed at our institution (summarized in top portion of Table I), with the remainder being referred for relapsed ALL treated elsewhere (summarized below in Table I).

Abbreviations: KI: kinase inhibitor, KDM: ABL kinase domain mutation.