To the editor:

Uncommon BCR-ABL kinase domain mutations in kinase inhibitor–resistant chronic myelogenous leukemia and Ph+ acute lymphoblastic leukemia show high rates of regression, suggesting weak selective effects

In chronic myelogenous leukemia (CML) and Philadelphia chromosome–positive (Ph^+) acute lymphoblastic leukemia (ALL), extensive data are available on the development of the 15 to 20 most common BCR-ABL1 kinase domain (KD) mutations arising after imatinib treatment and their differential response to other tyrosine kinase inhibitors (TKIs). This letter reports on less common KD mutations and suggests reasons for their rarity.

Over the past 5 years, we performed KD mutational analysis covering codons 221 to 500 of the ABL kinase on RNA extracted from blood or bone marrow leukocytes in patients with persistent/recurrent Ph^+ ALL (n = 113) or TKI-resistant CML (n = 870), per published criteria.[1,2] Mutation screening was performed after nested polymerase chain reaction with a sensitivity of 10%-20% mutation-bearing cells.[3] Single nucleotide polymorphisms (noncoding changes observed involving codons 240, 249, 300, 320, 348, 354, and 499 as well as K247R[4]) were excluded, as were mutations occurring at common sites (codons 244, 250, 252, 253, 255, 276, 299, 311, 315, 317, 351, 355, 359, 387, 396, 453, and 459) whose features have been previously described.[3,5-7] We noted 35 different uncommon point mutations arising at 30 codons in 41 patients (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). All of these mutations were unequivocally present by bidirectional sequencing and confirmed to be associated with TKI treatment by demonstration of their absence in previous and/or subsequent samples from the same patient, except for N331S, which was fully predominant on interferon therapy before imatinib. PCR artifact was excluded in those mutations that were not predominant over the unmutated sequence by repeat PCR from cDNA.

For CML, the median time to development of these uncommon KD mutations (41 months of TKI treatment) was similar to that for more common mutations. 4 mutations (L298V, L364I, E450G, and F486S) were present at high levels with another mutation (T315I in 3 and V299L in 1) in 1 case each, consistent with tandem occurrence in the same cells, with 3 occurring after sequential imatinib and dasatinib therapy.[8] Most of the remaining mutations in CML arose in patients with variable imatinib resistance who often had interruptions and dose variations in imatinib due to toxicity or patient noncompliance (Table 1). Mutations noted at time of relapse of ALL/CML-blast phase were associated with prior or concurrent use of imatinib or dasatinib as maintenance therapy.

Overall, rarely observed BCR-ABL1 KD mutations were detected in 3 contexts: (1) in suboptimally treated patients with intermittent TKI therapy; (2) soon after switch from imatinib to another TKI (often regressing with continued therapy with the same TKI); or (3) in lymphoid blasts after maintenance TKI therapy. In repeat testing for partial or resistant disease after 6 to 12 months, all but 5 CML-associated mutations (L273M, E279K, L364I, N331S, and S348L) regressed with imatinib dose escalation or after switch to a new TKI. These results suggest that the range of BCR-ABL1 KD mutations is broad (similar to what is observed with in vitro mutagenesis),[9] but that the common mutations[10] out-compete the others and usually predominate at the time of overt TKI resistance when testing is commonly performed.

Table 1. Clinical features of detection of rare BCR-ABL kinase domain mutations and their response to therapy

Clinical feature	Mutations
Mutations arising in CML chronic or accelerated phase	
Arising with intermittent imatinib treatment	M237V, Y257C, L273M, E279K, E292K, E292V, I293V, S348L, L364I, V422I, W423R, Y435C, E450K, F486S
Arising after nilotinib (no prior imatinib)*	K378R
Arising after dasatinib (no prior imatinib)*	R473Q
Mutation regressed followed imatinib dose escalation (or major molecular response)	V260A, E292V, I293V, G303E, W423R, Y435C
Mutation regressed after switch of TKI (or major molecular response)	E292K, E292Q, E292V, L298V, V304A, L364I, M388L, L411P, I418T, V422I, A433T, L452P, F486S, T495R, M496I
No regression after switch to a new TKI (see supplemental Table 1)	N311S (bosutnib + nilotinib), S348L (dasatinib)
Mutations arising in CML blast phase or relapsed Ph+ ALL	
At relapse of Ph^+ ALL	V338G, E450G (along with T315I)
At CML blast transformation	E279K (myeloid blasts), A337P (T-cell blasts), L364I (along with T315I, B-cell blasts), V379I

*Both mutations regressed in retesting at 6 months after continued nilotinib or dasatinib therapy.

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manuscript; M.B.R. reviewed data and the manuscript; H.K. contributed materials and reviewed the manuscript; and J.C. contributed materials, reviewed the data, and wrote the manuscript.

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To the editor:

Long-term survival after nonintensive chemotherapy in some juvenile myelomonocytic leukemia patients with *CBL* mutations, and the possible presence of healthy persons with the mutations

Recently, mutations in the *CBL* gene have been demonstrated in patients with juvenile myelomonocytic leukemia (JMML).[1] Screening for *CBL* mutation using DNA from peripheral blood mononuclear cells (PBMCs) taken after informed consent of the patients' guardians revealed the presence of heterozygous *CBL* mutations in 4 (case nos. 1 and 4, in remission; case nos. 2 and 3, at diagnosis) of 12 JMML patients who possessed wild-type *NRAS*, *KRAS*, and *PTPN11* genes and did not exhibit the clinical features of neurofibromatosis type I (Table 1). This study was approved by the Institutional Review Board of Shinshu University. To elucidate the possibility of the coexistence of cells harboring homozygous mutations, those with heterozygous mutations, and normal clones, we performed genetic analyses using DNA from granulocyte-macrophage colony constituent cells, erythroid colony constituent cells, or nails.[3] The 3 JMML patients (case nos. 1-3) had heterozygous and/or homozygous *CBL* mutations. Taken together with the data obtained by microsatellite analysis for the detection of a loss of heterozygosity and those obtained by multiplex ligation probe amplification analysis for the determination of copy numbers, homozygous *CBL* mutations in 2 patients appeared to result from acquired uniparental disomy (UPD).[4,5] DNA obtained from the nails of case nos. 1 and 2 had heterozygous *CBL* mutations identical to blood samples, suggesting that the patients had germline heterozygous *CBL* mutations. These patients did not exhibit the neurologic abnormalities reported by Niemeyer et al.[6] It is of interest that case nos. 1 and 2 have achieved hematologic improvement after nonintensive chemotherapy and approximately 15- to 28-year survival. Although Muramatsu et al[7] reported no difference in the probability of 2-year overall survival between JMML patients with and those without *CBL* mutations, some JMML patients with the mutations may not always show a poor prognosis.

Because colonies of case no. 1 were not obtained at diagnosis, we cannot exclude the possibility that the patient had lost her homozygous clone when she went into remission. Among 34 JMML patients with *CBL* mutations reported by us and other investigators,[1,7] 31 patients had homozygous mutations at onset, whereas the remaining 3 patients showed heterozygous mutations. Thus, loss of the wild-type allele may play an important role in the disease occurrence of JMML patients with *CBL* mutations.

Case no. 4 had mild hematologic abnormalities at diagnosis, which improved spontaneously. Sequence analyses of genomic DNA and transcribed mRNA from colony constituent cells at 16 years of age revealed a heterozygous *CBL* mutation (1096-1G>T) and the use of novel splice acceptor sites resulting in splice variants. The mutation was present in his lymphocytes, whereas the nails had the wild-type of the gene. None of 100 healthy controls nor the parents of case no. 4 possessed this mutation. Thus, the patient may continue to carry a somatic mutation without disease at present. Although T cells generally do not belong to the leukemic clone in most JMML patients, *RAS* gene mutations were detected in $CD3^+$ cells of 3 JMML patients.[3] It should be elucidated whether the heterozygous 1096-1G>T transversion contributes to spontaneous hematologic remission and a somatic mutation in lymphocytes. The father of case no. 1, who has been healthy and has shown normal hematologic findings, harbored the same heterozygous *CBL* mutation as his daughter in his nails as well as PBMCs, indicating an inherited germline mutation. Persons with heterozygous *CBL* mutations who appear healthy may be present.

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