

ORIGINAL ARTICLE

Mutations of the BCR-ABL-kinase domain occur in a minority of patients with stable complete cytogenetic response to imatinib

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Residual leukemia is demonstrable by reverse transcriptase-polymerase chain reaction in most patients with chronic myeloid leukemia who obtain a complete cytogenetic response (CCR) to imatinib. In patients who relapse during imatinib therapy, a high rate of mutations in the kinase domain of BCR-ABL have been identified, but the mechanisms underlying disease persistence in patients with a CCR are poorly characterized. To test whether kinase domain mutations are a common mechanism of disease persistence, we studied patients in stable CCR. Mutations were demonstrated in eight of 42 (19%) patients with successful amplification and sequencing of BCR-ABL. Mutation types were those commonly associated with acquired drug resistance. Four patients with mutations had a concomitant rise of BCR-ABL transcript levels, two of whom subsequently relapsed; the remaining four did not have an increase in transcript levels and follow-up samples, when amplifiable, were wild type. BCR-ABL-kinase domain mutations in patients with a stable CCR are infrequent, and their detection does not consistently predict relapse. Alternative mechanisms must be responsible for disease persistence in the majority of patients.

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Introduction

More than 80% of newly diagnosed patients with chronic-phase chronic myeloid leukemia achieve a complete cytogenetic response (CCR) (Druker *et al.* *J Clin Oncol* 2006, **10**:18S, Abstract). Although these responses are generally durable, residual disease usually remains detectable by reverse transcriptase-polymerase chain reaction (RT-PCR) for BCR-ABL. Furthermore, recurrence almost invariably occurs after discontinuation of therapy, indicating that the residual BCR-ABL-positive cells have full leukemogenic potential.^{1–3} A recent study found BCR-ABL-kinase domain mutations in CD34+ cells of 5/13 (38%) patients with a CCR,⁴ suggesting that disease persistence may be caused by similar mechanisms as acquired imatinib resistance.^{5,6} However, the relapse rate in this small cohort of patients was high and the majority of patients had

residual leukemia cells detected by FISH, suggesting a potential bias toward patients with a high risk of relapse. We therefore analyzed the incidence of kinase domain mutations in a more representative group of patients with a stable CCR.

Materials and methods

Patient sample collection and processing

This study was approved by the Institutional Review Board of Oregon Health & Science University. Informed consent was obtained from all patients. Bone marrow samples were collected on patients with a stable CCR, defined as having a CCR on consecutive tests with at least 20 analyzable metaphases, at least 3 months apart. Prospectively collected fresh bone marrow samples were processed by isolating mononuclear cells (MNC) by density gradient centrifugation and selecting CD34+ cells using immunomagnetic columns (Miltenyi, Auburn, CA, USA). For samples stored at –80°C verified to meet our definition of stable CCR, MNC were analyzed directly. RNA was extracted using the RNAqueous kit (Ambion, Austin, TX, USA) and reversely transcribed into cDNA using random hexamer primers and Superscript reverse transcriptase (Invitrogen, Carlsbad, CA, USA), followed by quality assessment by one-step RT-PCR for ABL as described.⁷

Mutation screening

Mutation screening was performed in duplicate, using the following parallel procedures for each sample: direct sequencing of BCR-ABL PCR products as described⁸ and denaturing high-performance liquid chromatography (D-HPLC) as described.⁹ Both approaches begin with a PCR reaction spanning both the breakpoint and kinase domain of BCR-ABL. The nested step in the direct sequencing procedure also spans these regions to maximize specificity, whereas the D-HPLC procedure has the modification that four overlapping amplicons covering only the kinase domain of ABL are used (codons 221–292, 277–342, 325–387, 372–434, primers available on request). Sensitivity of D-HPLC has been shown to be twofold greater than conventional sequencing, detecting mutant transcripts that represent at least 15% of the PCR product.⁹ For increased sensitivity, subcloning of PCR products was performed using the TOPO-TA cloning kit (Invitrogen) in samples amplified with the direct sequencing procedure. Sequencing was performed for 10–20 individual clones, with a positive mutation scored when at least two clones were positive for the same mutation. This increased the sensitivity for mutants comprising only 10–20% of the total.

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Quantitative RT-PCR (qPCR) for BCR-ABL was performed as described, and results were expressed as the percent ratio BCR-ABL/glucose-6-phosphate dehydrogenase.¹⁰

Results and discussion

Mutation detection sensitivity

A recent report found a high rate of kinase domain mutations in CD34+ cells isolated from patients with a CCR.⁴ In addition, higher levels of *BCR-ABL* mRNA have been demonstrated in CD34+ cells compared to MNC in patients with CCR.¹¹ These findings suggest that the likelihood of detecting a kinase domain mutation may be higher in CD34+ cells. To determine the optimal cell compartment for screening, we compared *BCR-ABL* amplification and mutation detection in CD34+, CD34- and MNC samples. *BCR-ABL* was amplifiable in 42 of 66 samples (64%). Unselected MNC were analyzed in the initial 18 of these 42 patients, whereas CD34+ cells were selected in the following 24 (median yield 1.32×10^5 (range, 8.4×10^2 to 3×10^6), mean purity of 40%). In samples subjected to CD34+ selection, *BCR-ABL* could be amplified from both CD34+ and CD34- cells in nine patients, in 12 patients only from CD34- cells and in three patients only from CD34+ cells (Table 1). The higher rate of failure in CD34+ cells is likely the result of low cell numbers. Overall, the probability of detecting a mutation was not greater in CD34+ compared to CD34- or unselected MNC samples (1/11 vs 8/39, $P=0.66$, Fisher's exact test). Thus, in our hands enrichment for CD34+ cells does not increase the probability of mutation detection.

At a technical level, two additional factors influence the sensitivity and specificity of mutation detection in the setting of low-level residual disease. Firstly, *BCR-ABL* may or may not be amplifiable. Secondly, the sequencing quality will influence the minimal proportion of mutant allele that is detected. To address some of these issues, we analyzed all specimens by two independent methods. The first approach consisted of direct sequencing of PCR products using two sets of nested primers spanning the *BCR-ABL* breakpoint for amplification and primers spanning the *ABL*-kinase domain for sequencing. The second approach used a primer set spanning the *BCR-ABL* breakpoint for the first round of PCR and four independent primer sets spanning overlapping portions of the *ABL*-kinase domain for the second round, the product of which is subjected to D-HPLC. In 24/66 samples (36%), *BCR-ABL* was undetectable with either method. Twenty-three samples (35%) were informative only by D-HPLC, 12 (18%) with both methods and seven (11%) only by direct sequencing (Figure 1). In the latter, failure was mostly due to incomplete D-HPLC results ($n=5$), when the amplifications failed for one, two or three amplicons. Only two samples amplifiable in the direct sequencing procedure failed D-HPLC outright (Supplementary Table 1). The lower rate of informative

results for the direct sequencing procedure is explicable by the use of two breakpoint-spanning primer pairs rather than a second primer pair spanning only the *ABL*-kinase domain. Although this approach is likely to increase specificity by avoiding second-step amplification of *ABL* from carryover cDNA, it reduces amplification efficacy owing to the longer amplicons. The failure of D-HPLC in samples that were informative by direct sequencing is not readily explicable. Attempts are underway in our laboratory to further optimize conditions for a situation with generally low levels of *BCR-ABL*, such as patients in CCR. In any case, from the standpoint of informativity, D-HPLC was superior to direct sequencing. It is important to note that in our series of patients with low levels of *BCR-ABL* this superiority is the result of a higher rate of successful amplification compared to direct sequencing. When both methods were informative, there was concordance of kinase-domain sequencing results, although results in CD34-positive and -negative cell compartments were occasionally different in this subgroup (Supplementary Table 2). Given that D-HPLC has been shown to detect mutant amplicons as low as 15% of total *BCR-ABL* compared to 30% for direct sequencing,⁹ it is possible that our failure to detect a difference between the two methods is related to the low incidence of mutations. To determine whether the sensitivity of detecting mutations could be improved by subcloning, we sequenced 10–20 individual PCR clones from the samples that were informative by direct sequencing (Table 2). However, only one additional mutation (C305S) was detected, suggesting that subcloning is not generally warranted.

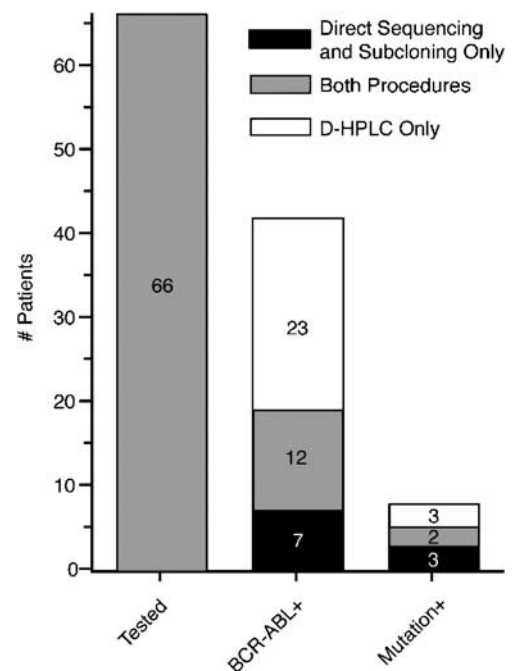


Figure 1 Amplification and mutation detection in patient samples by direct sequencing and D-HPLC procedures. Successful amplification in patients is compared for the direct sequencing procedure vs D-HPLC. Of 66 patients, 24 failed to amplify with either method. Of the 42 patients with detectable *BCR-ABL*, D-HPLC was informative in 35, direct sequencing in 19 and both procedures in 12. Of the eight patients with a mutation, D-HPLC detected the mutation in three, direct sequencing in three (including one mutation detected only by subcloning) and both procedures in two.

Table 1 Comparison of kinase domain mutation detection in CD34 separated samples vs unselected mononuclear cell samples

| Cell compartment | Patients tested | <i>BCR-ABL</i> ⁺ (% tested) | Mutation ⁺ (% amplified) |
|------------------|-----------------|--|-------------------------------------|
| CD34+ | 31 | 11 (35) | 1 (9) |
| CD34- | 31 | 21 (68) | 4 (19) |
| CD34± (combined) | 31 | 24 (77) | 4 (17) |
| MNC | 35 | 18 (51) | 4 (22) |
| Results Combined | 66 | 42 (64) | 8 (19) |

Table 2 Detection of mutations in CCR patients by direct sequencing, subcloning, or D-HPLC

| Procedure | Patients Tested | BCR-ABL ⁺ (% tested) | Mutation ⁺ (% amplified) |
|-------------------------------------|-----------------|---------------------------------|-------------------------------------|
| Nested RT-PCR and Direct Sequencing | 66 | 19 (29) | 4 (21) |
| Nested RT-PCR and Subcloning | 19 ^a | 19 ^a | 5 (26)** |
| Nested RT-PCR and D-HPLC | 66 | 35 (53) | 5 (14)** |

Abbreviations: D-HPLC, denaturing high-performance liquid chromatography; RT-PCR, reverse transcriptase-polymerase chain reaction.

^aSamples subcloned were those that were amplifiable in the direct sequencing procedure.

***P* = 0.29, Fisher's exact test.

Table 3 Results of kinase domain mutation analysis in CCR patients

| Patient No. | Age at study (years) | Time on IM (months) | D-HPLC mutation result | Direct sequencing result | Subcloning result | Time to last follow-up (months) | Current cytogenetic status | Follow-up sample mutation status |
|-------------|----------------------|---------------------|--|--------------------------|------------------------------|---------------------------------|----------------------------|----------------------------------|
| 4 | 80 | 31 | WT (325–387) ^{MNC} | T315I ^{MNC} | T315I (15/15) ^{MNC} | 12 | CCR | WT |
| 6 | 51 | 39 | WT (277–434) ^{MNC} | Y253H ^{MNC} | Y253H (16/16) ^{MNC} | 22 | CCR | WT |
| 7 | 39 | 43 | T315I ^{MNC} | No Amp | NA | 29 | CCR | No Amp |
| 9 | 69 | 32 | F359V ^{MNC} | No Amp | NA | 5 | 100% Ph+ | NA |
| 20 | 27 | 28 | G250E ^{34–} | G250E ^{34–} | G250E(17/17) ^{34–} | 20 | CCR | G250E |
| 34 | 52 | 18 | WT ^{34–/+} | WT ^{34–} | C305S (3/20) ^{34–} | 17 | CCR | NA |
| 35 | 43 | 43 | Y253F ³⁴⁺ | M244V ^{34–} | M244V (20/20) ^{34–} | 14 | CCR | M244V |
| 36 | 24 | 63 | M244V ^{34–/+} G321E ^{34–} E355G ^{34–} | WT ³⁴⁺ | WT (16/16) ³⁴⁺ | 15 | CCR | WT |

Abbreviations: No Amp, No PCR amplification; NA, No sample available.

MNC: result obtained from mononuclear cell sample; 34+: mutation result obtained in CD34-positive cells. 34–: mutation result obtained in CD34-negative cells; Codons successfully analyzed in the D-HPLC procedure are included in parentheses when amplification failed for part of the four amplicons covering the kinase domain. For subcloning results, the number of individual clones with the indicated result are shown in parentheses; mutations were dismissed if not present in more than one subclone.

Frequency of kinase domain mutations

Sixty-six patients with stable CCR were enrolled and *BCR-ABL* amplification was successful in 42 (63%). In the remaining 24 patients, *BCR-ABL* was undetectable, whereas *ABL* was detected by one-step PCR, consistent with good-quality cDNA. The *BCR-ABL*-positive patients had a median age of 54 (range, 24–85) years, median disease duration of 46 months (range, 8–185) and median time on imatinib of 32 months (range, 8–65) (Supplementary Table 1). At the time of starting imatinib, 36 patients had been in chronic phase and six in accelerated phase. At the time of mutation screening, only three patients were within 12 months of diagnosis. Thus, our cohort was comprised almost entirely of patients with long-standing disease. Altogether, we detected nine different point mutations in the *BCR-ABL*-kinase domain of eight of 42 patients (19%) (Table 3). Seven patients were found to harbor mutations associated with clinical resistance to imatinib, including G250E, T315I, Y253H, Y253F, M244V, F359V and E355G.^{6,12–14} G321E was identified in one patient. This mutation was also found in a CCR patient studied by Chu *et al.*⁴ and was shown to confer intermediate imatinib resistance. One patient harbored a C305S mutation, which has not previously been described in patients or by *in vitro* mutagenesis screens.¹⁵ In contrast to all other mutations, C305S was detected only after subcloning and failed to confer IL-3 independence when transfected into Ba/F3 cells (data not shown), suggesting that it may be kinase inactive and thus irrelevant. In four patients with mutations, additional aliquots of the same bone marrow sample were available for analysis. In three patients the mutation was confirmed and in one *BCR-ABL* amplification failed. In total, mutations were present in eight of 66 (12%) patients with stable CCR and in eight of 42 (19%) of those patients with stable CCR who had amplifiable *BCR-ABL*.

Follow-up of patients with mutations

Four of eight patients with a mutation showed a concomitant rise of *BCR-ABL* transcript levels of more than twofold within 6 months after sequencing (Figure 2a, no. 9, 20, 35 and 36). This cutoff has been proposed by Branford *et al.*¹³ as predictive for detection of kinase domain mutations. Of these four, two (no. 9 and 36) had subsequent cytogenetic relapse and one (no. 9) died from disease progression. The remaining two patients with a rise in *BCR-ABL* transcript levels maintained a CCR, one (no. 20, G250E) with a dose increase of imatinib from 600 to 800 mg daily. In the other four patients (of the eight with mutations), stable levels of *BCR-ABL* transcript were observed, and follow-up specimens, when amplifiable, were consistently wild type (Figure 2b).

Follow-up of patients without detectable mutations at initial study

Our data suggested that some kinase domain mutations may be detected only transiently. We reasoned that patients with stable CCR may have occasional peaks in *BCR-ABL* mRNA owing to transient amplification of kinase domain mutant clones. We therefore reviewed the qPCR data for the 58 patients without a detectable mutation at initial study in the 12 months before and after the analysis (all patients without mutation detection, including those who failed to amplify for *BCR-ABL* in the initial analysis, but excluding the previously discussed patients with mutations). Patients found in the original sample to have undetectable *BCR-ABL* were included in this analysis because patients with undetectable *BCR-ABL* often fluctuate between detectable and undetectable levels over time.¹⁶ Out of the 39 patients with available data during this period (median number

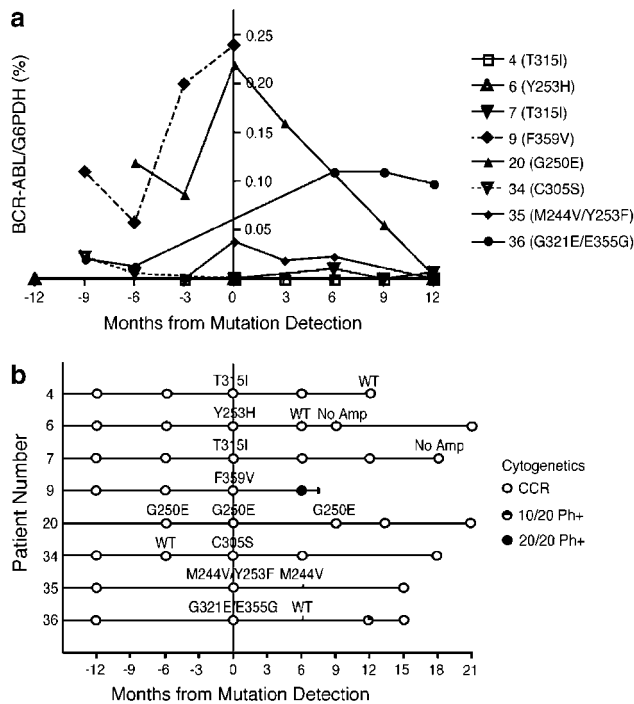


Figure 2 Follow-up data for patients with a kinase domain mutation. (a) Available qPCR data for *BCR-ABL* in patients with a kinase domain mutation was graphed for the period of 12 months before and after the original detection of a mutant. (b) Clinical timelines corresponding to the quantitative RT-PCR data are shown to indicate the cytogenetic and mutation analysis during the study period.

of qPCR tests 5, range, 1–9), 32 had stable transcript levels, two patients had a steady decrease of transcripts from start of treatment and five patients displayed a rise in *BCR-ABL* levels (median 9.7-fold, range, 3.2–25) compared to the previous result (Figure 3a). Sequencing showed wild-type *BCR-ABL* in all five patients at the time of the increase (Figure 3b). Chart review revealed inconsistent compliance with imatinib dosing in three of five patients (no. 2, 21 and 25). For example, patient no. 2 had ceased medication for 2 months before the rise in transcript to a peak of 2.2%, after which dosing was followed consistently and the qPCR level decreased to 0.01%. No reason for the peak could be established in the remaining two cases. Thus, noncompliance is also an important factor that can lead to a rise in *BCR-ABL* transcript levels and should be part of the diagnostic considerations.

Conclusions

Our data suggest that the mere detection of a kinase domain mutation in a CCR patient, without a concomitant rise in *BCR-ABL* transcripts, does not predict for relapse, and that in the majority of patients with a stable CCR molecular persistence is not mediated by kinase domain mutations. It is unclear, why in some patients kinase domain mutant clones fail to cause relapse. One possibility is that these transient clones lack self-renewal capacity and are thus unable to maintain long-term hematopoiesis. In regards to the overall prevalence of kinase domain mutations in CCR patients, our results appear to differ from the report of Chu *et al.*,⁴ who found kinase domain mutations in 38%, twofold higher than the rate in our study. Although this difference is not statistically significant (5/13 vs 8/42, $P=0.26$,

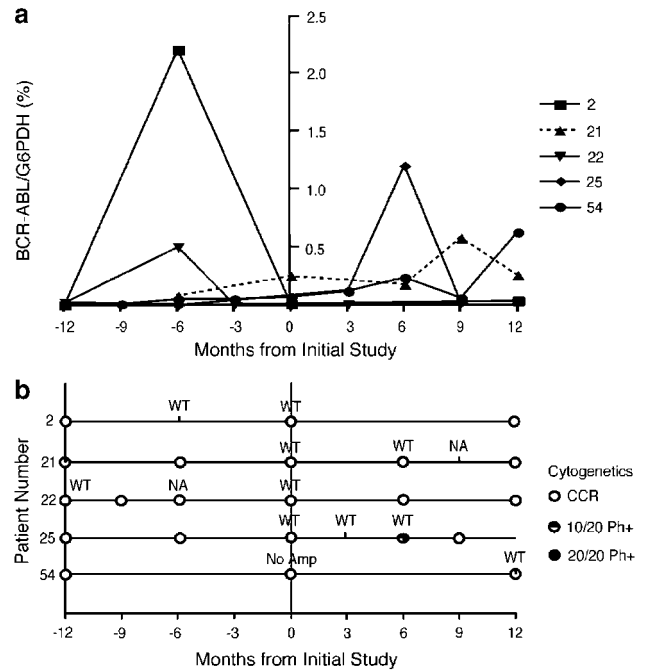


Figure 3 Follow-up analysis for patients without a kinase domain mutation at initial study. (a) Available quantitative RT-PCR data for the five patients with an increase in transcript levels during a period of 12 months before and after initial study, when negative for *BCR-ABL* or no mutations were detected. (b) Clinical timelines matched to the quantitative RT-PCR data are displayed to show the results of kinase domain mutation analysis corresponding to the time points with increased *BCR-ABL* transcript.

Fisher's exact test), this may be due to the relatively small cohort sizes. Chu *et al.*⁴ analyzed CD34+ in all but one patient and multiple clones were routinely sequenced. However, in our study, mutation detection was not more frequent in CD34+ cells and sensitivity was not significantly improved by subcloning, excluding technical differences as the reason for the discrepancy. More likely, the cohort studied by Chu *et al.*⁴ was at higher risk for relapse and kinase domain mutations compared to our patients. This is supported by the fact that the median time on imatinib in our cohort was 32 months compared to 6 months in the study by Chu *et al.*,⁴ suggesting that our cohort was enriched for patients with a stable response to imatinib. In the IRIS trial, a reduction of relapse risk has been demonstrated with CCR duration.¹⁷ Consistent with this, only 5/49 (10%) patients with available cytogenetic follow-up have lost a CCR at a median of 15 months (range, 5–36). Thus, in the majority of CCR patients, mechanisms other than kinase domain mutations must account for disease persistence. These potential mechanisms of disease persistence include insufficient kinase inhibition in leukemic stem cells owing to drug efflux or high levels of *BCR-ABL* expression, or *BCR-ABL* kinase-independent mechanisms such as stem cell quiescence.¹⁸ Elucidating these mechanisms will be crucial for developing strategies that aim at eradication of residual leukemia.

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References

- 1 Cortes J, O'Brien S, Kantarjian H. Discontinuation of imatinib therapy after achieving a molecular response. *Blood* 2004; **104**: 2204–2205.
- 2 Higashi T, Tsukada J, Kato C, Iwashige A, Mizobe T, Machida S *et al*. Imatinib mesylate-sensitive blast crisis immediately after discontinuation of imatinib mesylate therapy in chronic myelogenous leukemia: report of two cases. *Am J Hematol* 2004; **76**: 275–278.
- 3 Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL *et al*. Dynamics of chronic myeloid leukaemia. *Nature* 2005; **435**: 1267–1270.
- 4 Chu S, Xu H, Shah NP, Snyder DS, Forman SJ, Sawyers CL *et al*. Detection of BCR-ABL kinase mutations in CD34+ cells from chronic myelogenous leukemia patients in complete cytogenetic remission on imatinib mesylate treatment. *Blood* 2005; **105**: 2093–2098.
- 5 Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN *et al*. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* 2001; **293**: 876–880.
- 6 Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J *et al*. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* 2002; **2**: 117–125.
- 7 Diamond J, Goldman JM, Melo JV. BCR-ABL, ABL-BCR, BCR, and ABL genes are all expressed in individual granulocyte-macrophage colony-forming unit colonies derived from blood of patients with chronic myeloid leukemia. *Blood* 1995; **85**: 2171–2175.
- 8 Willis S, Lange T, Demehri S, Otto S, Crossman L, Niederwieser D *et al*. High sensitivity detection of BCR-ABL kinase domain mutations in imatinib-naïve patients: correlation with clonal cytogenetic evolution but not response to therapy. *Blood* 2005; **106**: 2128–2137.
- 9 Deininger MW, McGreevey L, Willis S, Bainbridge TM, Druker BJ, Heinrich MC. Detection of ABL kinase domain mutations with denaturing high-performance liquid chromatography. *Leukemia* 2004; **18**: 864–871.
- 10 Press RD, Love Z, Tronnes AA, Yang R, Tran T, Mongoue-Tchokote S *et al*. BCR-ABL mRNA levels at and after the time of a complete cytogenetic response (CCR) predict the duration of CCR in imatinib-treated patients with CML. *Blood* 2006; **107**: 4250–4256.
- 11 Bhatia R, Holtz M, Niu N, Gray R, Snyder DS, Sawyers CL *et al*. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. *Blood* 2003; **101**: 4701–4707.
- 12 von Bubnoff N, Schneller F, Peschel C, Duyster J. BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. *Lancet* 2002; **359**: 487–491.
- 13 Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J *et al*. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood* 2003; **102**: 276–283.
- 14 Hughes TP, Deininger MW, Hochhaus A, Branford S, Radich JP, Kaeda J *et al*. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors—review and recommendations for 'harmonizing' current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. *Blood* 2006; **108**: 28–37.
- 15 Azam M, Latek RR, Daley GQ. Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR-ABL. *Cell* 2003; **112**: 831–843.
- 16 Lange T, Bumm T, Mueller M, Otto S, Al-Ali HK, Grommisch L *et al*. Durability of molecular remission in chronic myeloid leukemia patients treated with imatinib vs allogeneic stem cell transplantation. *Leukemia* 2005; **19**: 1262–1265.
- 17 O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F *et al*. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* 2003; **348**: 994–1004.
- 18 Copland M, Hamilton A, Elrick LJ, Baird JW, Allan EK, Jordanides N *et al*. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML, but does not eliminate the quiescent fraction. *Blood* 2006; **107**: 4532–4539.

Supplementary Information accompanies the paper on the Leukemia website (<http://www.nature.com/leu>)