

Detection of *ABL1* kinase domain mutations in therapy-naïve *BCR-ABL1*-positive acute lymphoblastic leukemia

Mutations in the *ABL1* kinase domain are the main mechanism of resistance to tyrosine kinase inhibitors (TKI) in Philadelphia chromosome-positive (Ph⁺) leukemia. In acute lymphoblastic leukemia (ALL), a very early acquisition of mutations can be observed and reports have described that mutations may exist already before TKI treatment.¹⁻⁴ Initial data came from cloning or allele-specific polymerase chain reaction assays,⁵ which are labor-intensive, do not detect all possible mutations and especially come with the doubt of detecting amplification artefacts. Despite the first description over 18 years ago, *ABL1* mutation screening is not a standard test for ALL patients before initiation of TKI treatment. The current broad availability of highly sensitive next-generation sequencing approaches enables the initial limitations to be overcome. In this study, we used next-generation sequencing to screen 91 *BCR-ABL1*-positive ALL patients for mutations before therapy and identified five (5.5%) with *ABL1* kinase domain mutations at initial diagnosis.

We studied the diagnostic samples of 39 female and 52 male ALL patients diagnosed between April 2007 and November 2016. The median age was 61 years (range, 18-84), and treatment included first- to third-generation TKI (*Online Supplementary Table S1*). Thirty-five patients had the M-bcr (p210) transcript and 56 the m-bcr (p190) transcript. The study was approved by the Internal Review Board and by the Bavarian Ethics Committee, the Bavarian State Medical Association (Bayerische Landesärztekammer) with number 05117. The study adhered to the tenets of the Declaration of Helsinki.

RNA for *BCR-ABL1* detection and mutation analysis was isolated from bone marrow (n=56) or peripheral blood (n=35) with standard protocols and cDNA synthesis was performed using SuperScript™ IV VIL0™ (Thermo Fisher Scientific, Waltham, MA, USA). Six amplicons for sequencing on the MiSeq (Illumina, San Diego, CA, USA) covered amino acids 184-510 of *ABL1* and were generated from pre-amplified *BCR-ABL1*. A 1% detection limit was used for analysis with SeqNext (JSI Medical Systems, Kippenheim, Germany).

Using next-generation sequencing, we found well-characterized *ABL1* kinase domain mutations in five of the 91 (5.5%) diagnostic samples (Table 1, *Online Supplementary Table S1*). Mutations were confirmed in a second independent sequencing run to exclude potential artefacts from random polymerase chain reaction errors. Four patients with a mutation at diagnosis had the m-bcr

transcript (4/56 [7%]) and one was found in a patient with M-bcr (1/35 [3%]). The mutations are present in subclones (<10% of *BCR-ABL1*-positive cells) in patients #2, #4 and #5 (Table 1).

For patient #1, the Q252H (cag>cat) mutation had expanded to 97% of *BCR-ABL1* transcripts at relapse after 5 months of treatment with the GMALL elderly protocol including imatinib, to which the mutation causes resistance (Figure 1). Patients #2 and #3 were both transplanted as part of standard of care at the respective time. Patient #3 (T315I) had achieved complete remission; however, the T315I mutation would be particularly severe because it causes resistance to four TKI (imatinib, nilotinib, dasatinib and bosutinib).⁶ Follow-up data for patients #4 and #5 were not available.

Next, we focused on patients who had a molecular follow-up of at least 6 months (n=35) for longitudinal mutation testing. This excluded patients who died within the first 6 months or those from whom no sample was available for *BCR-ABL1* expression and *ABL1* mutation testing. In 19 of 35 cases, mutation analysis was considered relevant during follow-up due to failure of primary therapy or relapse (defined as a *BCR-ABL1/ABL1* ratio of at least ~1%) (*Online Supplementary Table S1*). Mutation testing was performed between 4 and 71 months after diagnosis (median: 12 months). One or more known resistance mutations were found in 15 of 19 (79%) Ph⁺ ALL cases with relapse or increased *BCR-ABL1/ABL1* expression despite treatment (*Online Supplementary Table S1*). This is in line with the previously published *ABL1* mutation frequency of 70-80% for relapsed Ph⁺ ALL.⁷ Except for the Q252H mutation in patient #1 (Table 1), we did not detect any of the other resistance mutations from the relapse sample at the diagnostic time point with a 1% sensitivity cutoff. For comparison, Soverini *et al.* used a relapse-to-diagnosis backtracking approach to study resistance mutations and found one out of 34 patients with a mutation (Y253H) already at the initial time point.⁸

While selective pressure of TKI treatment makes the outgrowth of clones with resistance mutations very plausible, the outgrowth in therapy-naïve cells requires a fitness advantage. In previously published biochemical or cell culture studies, the effect of mutations without TKI was evaluated. D276G increased catalytic activity,⁹ and E255K exhibited increased transformation potency.¹⁰ Although T315I showed reduced kinase activity compared to unmutated controls, cell culture assays suggested increased oncogenic potency via different pathways (e.g., phosphorylation).¹¹ In a recent study of pediatric Ph-like ALL (with *EBF1-PDGFRB* rearrangement), Tran and colleagues back-tracked the gatekeeper T681I mutation in *PDGFRB*, which is the analog of the T315I muta-

Table 1. Characteristics of patients with mutations at initial diagnosis.

| Patient | #1 | #2 | #3 | #4 | #5 |
|------------|-------------------------------------|-----------|------------|-----------|-----------|
| Gender | male | female | female | male | male |
| Age | 72 years | 67 years | 52 years | 79 years | 76 years |
| Blasts | 91% | 88% | 12% | 85% | 85% |
| Transcript | p190 | p190 | p190 | p210 | p190 |
| Mutation | Q252H (G>T): 46% Q252H (G>C): 8% | D276G: 3% | T315I: 14% | M244V: 2% | E255K: 1% |
| Treatment | GMALL elderly | alloSCT | alloSCT | NA | NA |
| Outcome | remission followed by relapse | NA | CR | NA | NA |

GMALL: protocol of the German Multicenter Study Group on Adult Acute Lymphoblastic Leukemia; alloSCT: allogeneic stem cell transplantation; CR: complete remission; NA: data not available.

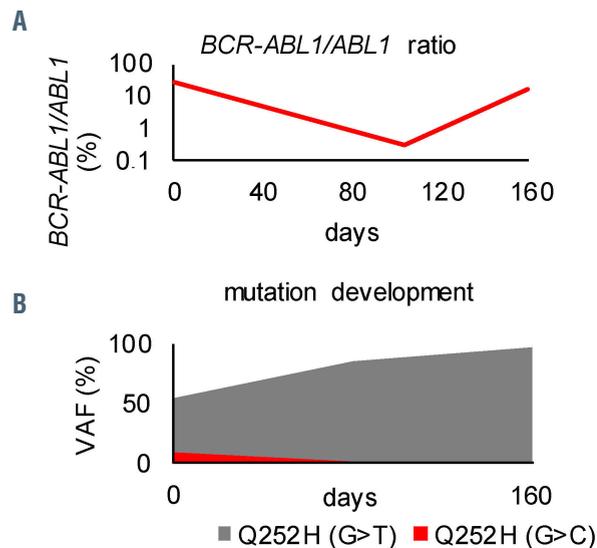


Figure 1. Molecular monitoring of *BCR-ABL1* transcripts and *ABL1* point mutations in a patient with Philadelphia chromosome-positive acute lymphoblastic leukemia. (A) Patient #1 was treated according to the GMALL elderly protocol including imatinib. *BCR-ABL1* expression (*BCR-ABL/ABL1* in %) was measured by quantitative polymerase chain reaction. (B) The variant allele frequency (VAF in %) is given for two follow-up time-points.

tion in *ABL1*. They identified the mutation in three of 23 patients prior to treatment.¹² We speculate that mutations which confer resistance but reduce kinase activity or transformative capacity would not outgrow before TKI treatment and therefore only a subset of resistance mutations can exist in therapy-naïve patients. The high turnover rate of ALL cells should allow a much faster selection of mutated clones with a relative growth advantage if compared to the situation in chronic myeloid leukemia. In an initial dataset of treatment-naïve patients with chronic phase chronic myeloid leukemia, only minor subclones (<1%) were identified, and showed no correlation with endpoints.¹³

In summary, known resistance mutations in the *ABL1* kinase domain were detected in five of 91 (5.5%) therapy-naïve patients with *BCR-ABL1*-positive ALL. For patient #1 (Figure 1) we can show that the mutated clone expands rapidly if treatment with an insensitive TKI is chosen. At present, we see no significant difference in outcome for the five patients with mutations, but the heterogeneity of the cohort and the small number of cases with mutations need to be considered. Further studies will be necessary, especially involving the full spectrum of TKI, which are now increasingly a backbone and guarantor of success of improving outcomes in Ph+ disease. Next-generation sequencing is a direct and sensitive (1%) strategy to identify patients at risk of resistance before any TKI therapy is started. However, 93% (14/15 patients) of mutations found in relapse samples were most likely acquired under the selective pressure of (TKI) treatment. Therefore, testing at initial diagnosis should only be considered in addition to established mutation testing in refractory/relapsed disease.

Constance Baer, Manja Meggendorfer, Claudia Haferlach, Wolfgang Kern and Torsten Haferlach

MLL Munich Leukemia Laboratory, Munich, Germany

Corresponding author:

CONSTANCE BAER - constance.baer@mll.com

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