haematologica
Journal of the Ferrata Storti Foundation

Primary asciminib resistance in a chronic myeloid leukemia patient with the atypical *BCR::ABL1* e13a3 transcript: a case study

by Birte Kristin Jung, Sandra Weissmann, Caroline Schock, Manja Meggendorfer, Torsten Haferlach and Frank Dicker

Received: July 23, 2025.
Accepted: October 7, 2025.

Citation: Birte Kristin Jung, Sandra Weissmann, Caroline Schock, Manja Meggendorfer, Torsten Haferlach and Frank Dicker. Primary asciminib resistance in a chronic myeloid leukemia patient with the atypical BCR::ABL1 e13a3 transcript: a case study.
Haematologica. 2025 Oct 16. doi: 10.3324/haematol.2025.288776 [Epub ahead of print]

Primary asciminib resistance in a chronic myeloid leukemia patient with the atypical *BCR::ABL1* e13a3 transcript: a case study.

Running title (max 50 characters): Primary asciminib resistance in CML

Birte Kristin Jung [1], Sandra Weißmann[1], Caroline Schock[2], Manja Meggendorfer[1], Torsten Haferlach[1], Frank Dicker[1]

Affiliations:

[1]MLL Munich Leukemia Laboratory, Munich, Germany

[2]Outpatient Therapy Center for Hematology and Oncology Offenburg, Offenburg, Germany

Corresponding Author:

Frank Dicker
MLL Munich Leukemia Laboratory
Max-Lebsche-Platz 31, 81377 München, Germany
T: +49-89-990-17-350
Email: frank.dicker@mll.com

Contributions
MM and FD conceived the project. CS provided patient data. BKJ performed data analysis. BKJ and FD wrote the manuscript. All authors reviewed the manuscript.

Data-sharing statement
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Word count: 1500

Competing interests

TH declares part ownership of Munich Leukemia Laboratory (MLL). BKJ, SW, MM and FD are employed by the MLL Munich Leukemia Laboratory. CK declares part ownership of Outpatient Therapy Center for Hematology and Oncology Offenburg.

Acknowledgements

The authors thank all MLL Munich Leukemia Laboratory coworkers for their dedicated work and all physicians who provided samples, cared for patients, and collected data.

Treatment with tyrosine kinase inhibitors (TKIs) has significantly improved the survival of patients with chronic-phase (CP) chronic myeloid leukemia (CML), with the majority now attaining a normal life expectancy. Nevertheless, approximately 20-30% of patients still experience therapy failure under conventional TKIs, often caused by the emergence of resistance mutations at the inhibitor's binding site.[1,2] The newly approved *BCR::ABL1* inhibitor asciminib provides new therapeutic perspectives for CML patients, who have experienced treatment failure due to side effects or insensitivity towards conventional TKIs. Asciminib functions as an allosteric *BCR::ABL1* inhibitor by binding to the myristate pocket of the *ABL1* kinase domain -a mechanism that differs from established ATP-competitive TKIs.[3] Asciminib's unique target region makes it especially suitable for patients with resistance mutations within the ATP-binding site, including the T315I mutation. As well as being used in heavily pretreated patients, asciminib has recently also received FDA approval for treating newly diagnosed CP-CML patients.[4]

This novel approach is particularly relevant due to the critical regulatory role of the ABL1 kinase. The *ABL1* gene encodes for a tyrosine kinase, which plays a pivotal role in cell growth regulation, differentiation, and preserving genomic integrity. In healthy individuals this critical protein is equipped with a built-in regulatory mechanism: The myristoylated *ABL1* N-terminus binds to its myristate pocket, which leads to the proximity of the SH2 and the SH3 domains. The newly formed SH2 -SH3 clamp then attaches to the kinase domain, where it suppresses *ABL1* activity by allosterically blocking the active center of the kinase domain.[5,6] In individuals with CML, the fusion of *BCR* to *ABL1* cuts off the myristoylated N-terminus, which results in a loss of the autoinhibitory cascade and an overly active kinase. In contrast to the standard ATP-competitive TKIs, asciminib does not bind the kinase domain's ATP site, but rather its myristate pocket.[7] In this manner, it can trigger the formation of the SH2-SH3-complex and effectively restore kinase autoinhibition in *BCR::ABL1* -positive cells. Hence, the integrity of the SH2 and SH3 domains is strictly required for the asciminib-mediated suppression of kinase activity.

The SH3 domain is encoded by *ABL1* exons 2 and 3, which are located N-terminally of the kinase domain within the ABL1 protein. In 98% of *BCR::ABL1* -positive patients, the defining mRNA fusion occurs at *BCR* exon 13 or 14 and *ABL* exon 2, resulting in the e13a2 and e14a2 isoforms.[8, 9] Much rarer transcripts are e1a2, followed by e19a2, accounting for 1.3% and 0.7% of newly diagnosed patients at our laboratory between 2021 and 2024. In these transcripts the SH3, SH2 and kinase domains remain functionally intact. However, *BCR::ABL1* gene fusion can also occur with *ABL1* exon 3, forming the e13a3 and e14a3 variants. Patients with these isoforms lack the entire *ABL1* exon 2 and therefore also parts of the SH3 domain's coding sequence (Figure 1A). Fusions lacking *ABL1* exon 2 accounted for 0.7% of newly diagnosed CML patients in our laboratory between 2021 and 2024.

Recent *in vitro* and protein modelling studies demonstrate that the loss of exon 2 functionally impairs the correct folding of the SH3 domain and consequently *ABL1* autoinhibition. This truncation of the SH3 domain has been demonstrated to confer a high degree of resistance towards the TKI asciminib.[10, 11] In view of asciminib's relatively recent approval and brief history of therapeutic use, however, there is so far only very limited data to verify the described resistance mechanism in clinical use. We here report clinical evidence of the naturally conferred resistance against the novel *BCR::ABL1* inhibitor asciminib in a CML patient with e13a3 transcript type who was treated with asciminib before the original publication of the resistance mechanism.

In October 2019, a 67-year-old male's peripheral blood was referred to our laboratory for evaluation of CML. The patient gave informed consent for the use of laboratory results and clinical data for research purposes according to the Declaration of Helsinki. The study was approved by the laboratory's institutional review board. The patient presented with a white blood cell count of 14.3×10^9/L, a hemoglobin level of 15.2 g/dL and a platelet count of 213×10^9/L. A *BCR::ABL1* -specific multiplex PCR identified a rare e13a3 fusion transcript with a *BCR::ABL1*/*ABL1* ratio of 204.66% upon quantitation with the in-house-developed *BCR::ABL1* p210 quantitative assay.[12] It is important to highlight that the assay is optimized for the quantitation of the major breakpoints e13a2 and e14a2 (Figure 1B). In the typical e13a2/e14a2 fusions, both the *BCR::ABL1* fusion and the *ABL1* reference

amplicons are amplified within exon 2, thus yielding *BCR::ABL1/ABL1* ratios below 100%. In contrast, in a3 fusions lacking exon 2, this quantitation method can yield ratios above 100%, as only *ABL1* transcripts are amplified in the reference gene replicates.

Other MPN-driver mutations in the genes *JAK2*, *CALR* and *MPL* were excluded by sequencing of the respective mutation hot spots. Morphological examinations of the bone marrow smears showed a cell-rich bone marrow with hyperplasia of the granulopoiesis and evidence of all stages of maturation. Erythropoiesis was reduced and the megakaryocytes were regular. Myeloid blasts could not be detected, confirming the diagnosis of CML. Fluorescence *in situ* hybridization on the bone marrow sample confirmed the *BCR::ABL1* fusion in 71/100 interphase nuclei and no additional cytogenetic alterations were found. According to the EUTOS long-term survival score, the patient was at low risk. For all follow-up investigations of the e13a3 transcript, the *BCR::ABL1* p210 quantitative assay was applied.

The patient received first-line treatment with the second -generation TKI dasatinib at a dose of 100 mg/d. This caused a decrease in *BCR::ABL1*/*ABL1* ratio by three orders of magnitude to 0.14% within three months (Figure 2), indicating optimal treatment response. After experiencing a pleural effusion, therapy was switched to 600 mg/d of nilotinib. Six months of nilotinib treatment led to an even further decrease of the *BCR::ABL1* ratio by more than four orders of magnitude from the baseline value, indicating an individual molecular response (IMR) of four.[13] This response level remained constant over time, yet treatment had to be discontinued after 40 months due to the increased risk for cardiovascular adverse events under nilotinib. This 7-week treatment-free period was accompanied by a rapid increase in *BCR*::*ABL1* ratio. Consequently, imatinib 400 mg/d was started, which had to be discontinued due to the onset of diarrhea and edema. Despite the necessity to pause and adjust the patient's treatment several times, satisfactory response rates were achieved under each of the conventional TKIs.

In January 2024 the therapeutic regimen was changed to asciminib in response to the patient's persistent side effects. Within the first three months under asciminib, *BCR::ABL1*/*ABL1* ratio increased by two orders of magnitude, from 0.26% to 3.98% and subsequently reached 10.49%, indicating a loss of IMR. As a consequence of the patient´s non-responsiveness towards asciminib, in April 2024 the *BCR::ABL1* kinase domain was analyzed for resistance mutations by next -generation sequencing of amino acids 184 to 510.[14] No kinase domain mutation was detected, indicating an alternative underlying resistance mechanism. After three months of asciminib treatment, dasatinib was reintroduced at a reduced dose. Under 50 mg/d of dasatinib, treatment response could be successfully restored, leading to an IMR of four.

This case, along with prior *in vitro* evidence and a recent clinical case report, demonstrates that *BCR::ABL1* fusions lacking *ABL1* exon 2 lead to SH3 domain disruption, which significantly compromises the efficacy of asciminib.[10, 11] Although e13a3 and e14a3 are the predominant fusions lacking *ABL1* exon 2, further transcripts with the a3 breakpoint, such as e1a3, e6a3 or e19a3 are possible.

Asciminib is gradually being used in an increasing number of patients. Thus, resistance mechanisms are only now unravelling. Besides point mutations surrounding the myristoyl-binding pocket, mutations occurring in the SH3 domain, such as Y115N, have been found to also mediate asciminib resistance.[3, 15] This further underscores the essential role of the SH3 domain in *ABL1* autoinhibition, as demonstrated in this case study.

Superior efficacy and a favorable safety profile led to FDA approval of asciminib also as a first-line treatment.[3, 4] However, as shown in this case, patients with the e13a3 or e14a3 *BCR::ABL1* fusion should be considered at risk of primary resistance to asciminib. Even more critically, patients with newly diagnosed CML typically exhibit high cancer cell burdens and in some of these cases ineffective treatment with asciminib could favor a fast progression to blast phase.

Despite experiencing side effects, which resulted in several treatment interruptions, the here investigated patient responded well to ATP-competitive TKIs. Further, following asciminib failure, the reintroduction of dasatinib was able to restore the patient's therapeutic response. These findings strongly suggest the usage of ATP-competitive TKIs in patients with e13a3 or e14a3 transcripts and highlight the need for identifying CML patients' *BCR::ABL1* isoforms at diagnosis.

Although the majority of CML patients carry the e14a2 or 13a2 *BCR::ABL1* isoforms, the described asciminib resistance is highly relevant for patients with the a3 transcript type. Our findings are consistent with previous reports of asciminib resistance mediated by the *BCR::ABL1* e13a3 and e14a3 fusion transcripts. The case described here contributes much-needed clinical data that confirms its relevance in a real-world scenario.

References

1. Hughes TP, Shanmuganathan N. Management of TKI-resistant chronic phase CML. Hematology Am Soc Hematol Educ Program. 2022;2022(1):129-137.

2. Hochhaus A, Larson RA, Guilhot F, et al. Long-Term Outcomes of Imatinib Treatment for Chronic Myeloid Leukemia. N Engl J Med. 2017;376(10):917-927.

3. Hughes TP, Mauro MJ, Cortes JE, et al. Asciminib in Chronic Myeloid Leukemia after ABL Kinase Inhibitor Failure. N Engl J Med. 2019;381(24):2315-2326.

4. Hochhaus A, Wang J, Kim DW, et al. Asciminib in Newly Diagnosed Chronic Myeloid Leukemia. N Engl

J Med. 2024;391(10):885-898.

5. Xie T, Saleh T, Rossi P, Kalodimos CG. Conformational states dynamically populated by a kinase determine its function. Science. 2020; 370(6513):eabc2754.

6. Nagar B, Hantschel O, Young MA, et al. Structural basis for the autoinhibition of c-Abl tyrosine kinase.

Cell. 2003;112(6):859-871.

7. Réa D, Hughes TP. Development of asciminib, a novel allosteric inhibitor of BCR-ABL1. Crit Rev Oncol

Hematol. 2022;171:103580.

8. Salmon M, White HE, Zizkova H, et al. Impact of BCR::ABL1 transcript type on RT-qPCR amplification

performance and molecular response to therapy. Leukemia. 2022;36(7):1879-1886.

9. Molica M, Abruzzese E, Breccia M. Prognostic Significance of Transcript-Type BCR - ABL1 in Chronic Myeloid Leukemia. Mediterr J Hematol Infect Dis. 2020;12(1):e2020062.

10. Leske IB, Hantschel O. The e13a3 (b2a3) and e14a3 (b3a3) BCR::ABL1 isoforms are resistant to asciminib. Leukemia. 2024;38(9):2041-2045.

11. Leyte-Vidal A, DeFilippis R, Outhwaite IR, et al. Absence of ABL1 exon 2-encoded SH3 residues in BCR::ABL1 destabilizes the autoinhibited kinase conformation and confers resistance to asciminib. Leukemia. 2024;38(9):2046-2050.

12. White H, Deprez L, Corbisier P, et al. A certified plasmid reference material for the standardisation of BCR-ABL1 mRNA quantification by real-time quantitative PCR. Leukemia. 2015;29(2):369-376.

13. Schäfer V, White HE, Gerrard G, et al. Assessment of individual molecular response in chronic myeloid leukemia patients with atypical BCR-ABL1 fusion transcripts: recommendations by the EUTOS cooperative network. J Cancer Res Clin Oncol. 2021;147(10):3081-3089.

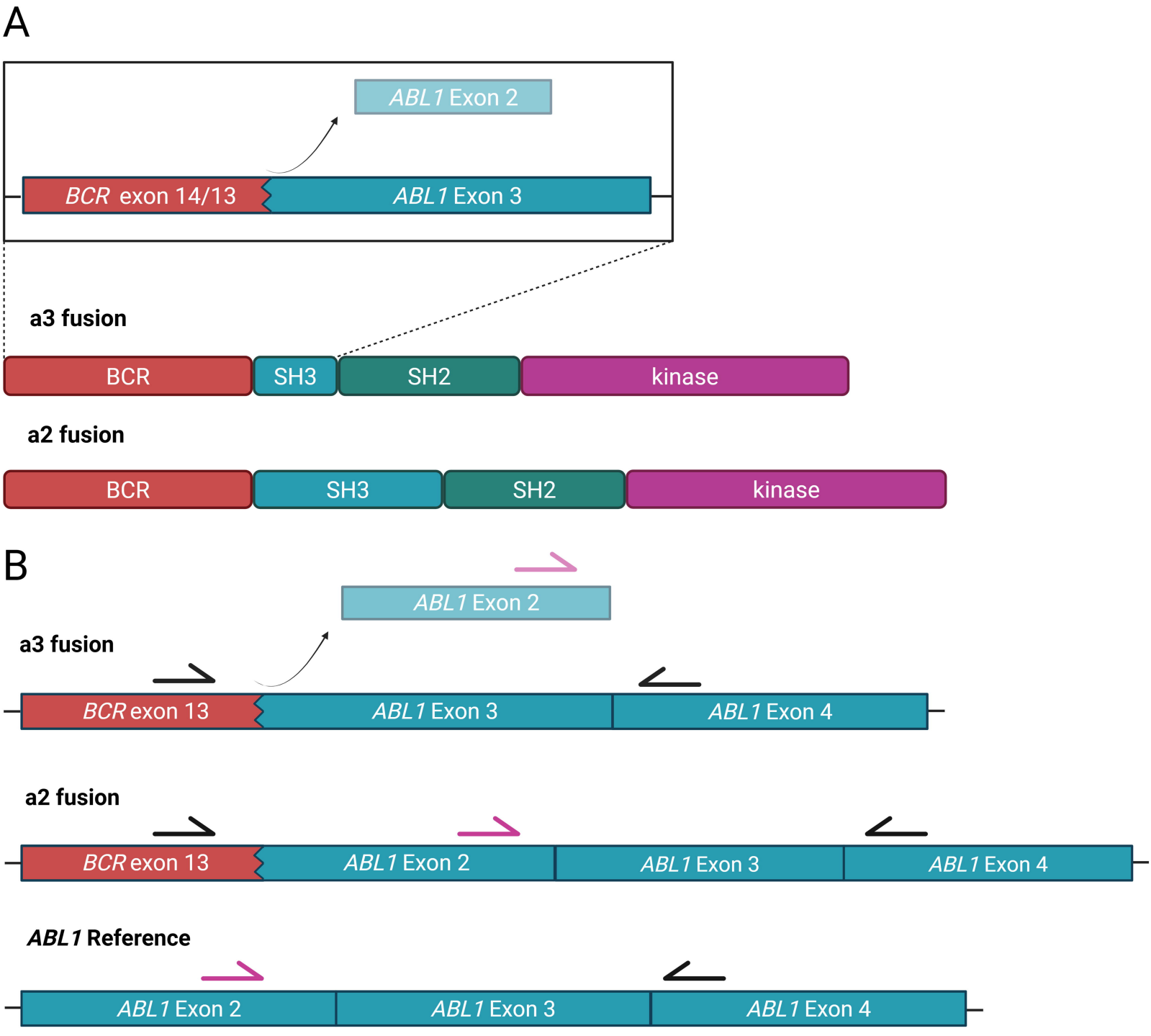
14. Baer C, Meggendorfer M, Haferlach C, Kern W, Haferlach T. Detection of ABL1 kinase domain mutations in therapy-naive BCR-ABL1-positive acute lymphoblastic leukemia. Haematologica. 2022;107(2):562-563.

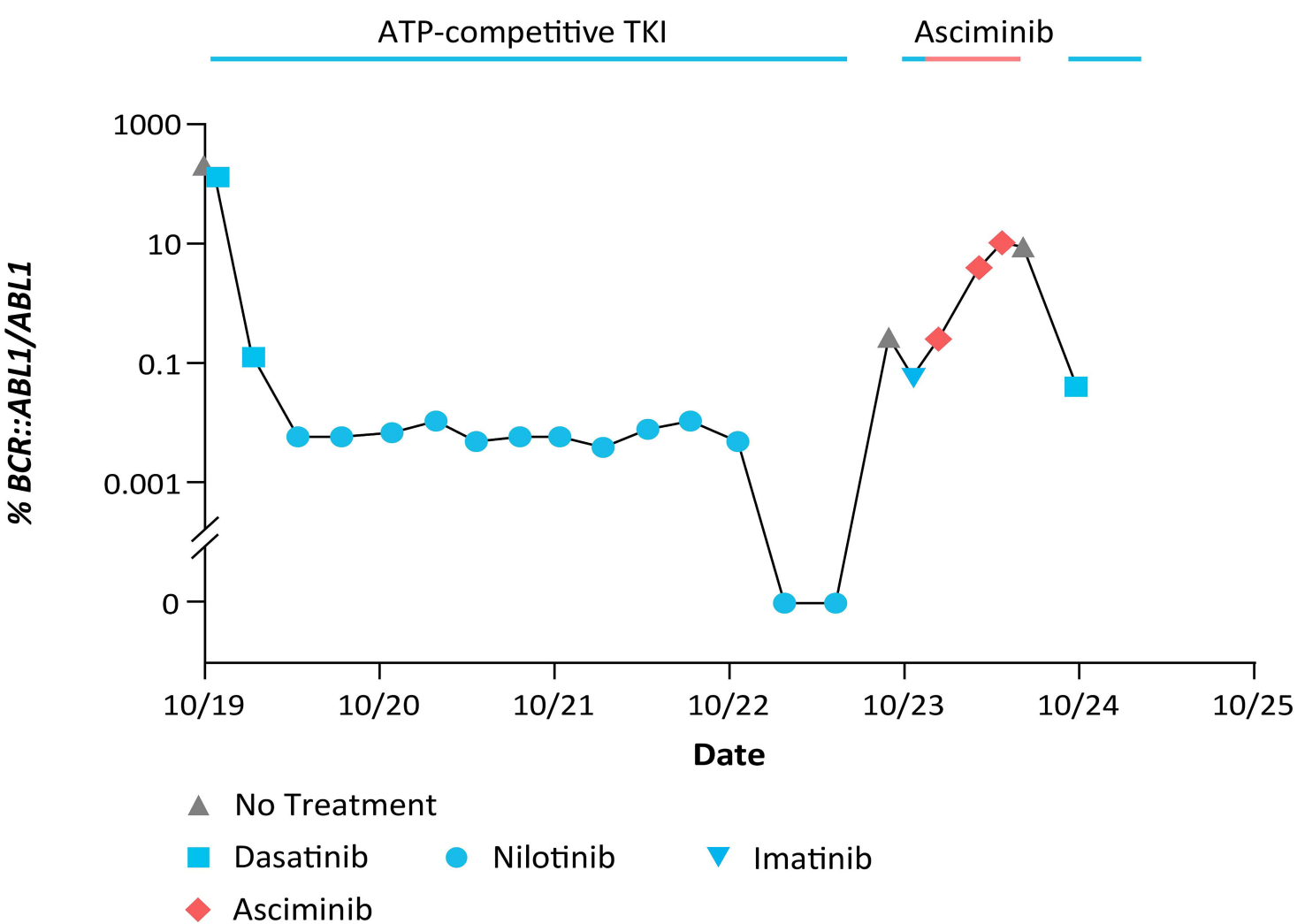
15. Cross NCP, Ernst T, Branford S, et al. European LeukemiaNet laboratory recommendations for the diagnosis and management of chronic myeloid leukemia. Leukemia. 2023;37(11):2150-2167.

Figure Legends

Figure 1: Fusion of *BCR* to *ABL1* exon 3 leads to a shortened SH3 domain and altered amplification via p210 LightCycler assay. (A) Schematic of *BCR*::*ABL1* domains and exons with BCR (red), SH3 (blue), SH2 (green) and the kinase domain (purple) in the a3- and a2 transcript types. (B) Schematic of p210 LightCycler assay design with arrows indicating the primer binding sites. The *ABL1* reference forward primer is capable of binding in *ABL1* exon 2 of the reference gene, but also the *BCR*::*ABL1* fusion gene in the case of a2 fusion transcripts. In a3 fusion transcripts double binding is not possible and *BCR*::*ABL1*/*ABL1* ratio calculation is altered, leading to ratios exceeding 100%.

Figure 2: Patient with a13a3 fusion is resistant towards asciminib. Course of *BCR*::*ABL1*/*ABL1* ratio under ATP-competitive TKIs and asciminib over time measured via the *BCR*::*ABL1* p210 LightCycler assay. Each symbol indicates one measurement of *BCR*::*ABL1*/*ABL1* ratio at different treatment stages: Grey triangles indicate no treatment, blue circles indicate treatment with ATP-competitive inhibitor and red squares indicate treatment with asciminib. Bars above the graph indicate in blue period of treatment with ATP-competitive TKI, in red treatment period with asciminib, and no bar indicates a treatment free period.


A
ABL1 Exon 2
BCR exon 14/13
ABL1 Exon 3
a3 fusion
BCR
SH3
SH2
kinase
a2 fusion
BCR
SH3
SH2
kinase
B
ABL1 Exon 2
a3 fusion
BCR exon 13
ABL1 Exon 3
ABL1 Exon 4
a2 fusion
BCR exon 13
ABL1 Exon 2
ABL1 Exon 3
ABL1 Exon 4
ABL1 Reference
ABL1 Exon 2
ABL1 Exon 3
ABL1 Exon 4


ATP-competitive TKI
Asciminib
1000
10
0.1
0.001
0
% BCR::ABL1/ABL1
10/19
10/20
10/21
10/22
10/23
10/24
10/25
Date
No Treatment
Dasatinib
Nilotinib
Imatinib
Asciminib