Annals of Hematology (2025) 104:3893–3898
https://doi.org/10.1007/s00277-025-06385-z

CASE REPORT

Inotuzumab therapy resulted in molecular remission of relapsed/resistant B-cell acute lymphoblastic leukemia with *BCR::ABL1* Lys404Glu mutation

Krzysztof Lewandowski[1] · Maciej Janicki[2] · Marta Barańska[1] · Zuzanna Kanduła[1] · Katarzyna Godziewska[1] · Jolanta Kiernicka-Parulska[1] · Agnieszka Ludwików[2] · Joanna I. Loch[3]

Received: 26 November 2024 / Accepted: 23 April 2025 / Published online: 16 May 2025

Abstract

The Philadelphia positive acute lymphoblastic leukemia (ALL BCR::ABL1 positive) is characterized by the presence of the t(9;22)(q34;q11) resulting in the formation of the BCR::ABL1 fusion, constant high cellular BCR-ABL1 tyrosine kinase activity, abnormal lymphoid cell proliferation and genetic instability of leukemic cells. Recently, great progress has been made due to the incorporation of tyrosine kinase inhibitors (TKI's) into the treatment algorithms. Despite this, a significant number of patients experienced therapy resistance mainly due to disease evolution and/or acquisition of TKI resistant BCR::ABL1 mutation(s). Herein, we report the therapy outcome in a 65 year old woman with high-risk BCR::ABL1 positive ALL treated with the PALG ALL7 (including imatinib, IM) protocol, who obtained complete hematologic remission (CHR) and complete molecular remission (CMR) after treatment with induction,consolidation I and II. Despite the fully matched donor identification, the patient refused to continue with intensive chemotherapy. Therefore, only IM therapy was continued, with periodic drug dose reduction due to hematological toxicity. CHR loss with the absence of BCR::ABL1 KD mutation was noticed 10 months later. The treatment with dasatinib (DAS) 140mg/d and dexamethasone resulted in a second CMR. Due to gastrointestinal toxicity, the DAS dose was reduced to 100mg/d. The second disease relapse was diagnosed 3 months later. BCR::ABL1 KD mutation screening showed the Lys404Glu (K404E) substitution. The 3-week long treatment with ponatinib in the dose of 45mg/d resulted in disease progression. The reinduction therapy with anti-CD22 MoAb (Inotuzumab ozogamycin) led to the 3rd CMR (duration time 9 months). According to our knowledge, it is the first report on the emergence of a BCR::ABL1 Lys404Glu mutation in an ALL patient receiving TKI treatment. Due to the mutation emergence on DAS, and disease progression on subsequent ponatinib treatment, the impact of the mutation acquisition on the 3D structure of ABL1 molecule and the process of TKI's binding to ATP-binding site were additionally studied. The results of the study indicate that the TKI resistance observed in the presented case is probably the result of the impossibility of the formation of stable complexes between ATP-competitive inhibitors and mutant Lys404Glu BCR-ABL1 tyrosine kinase due to conformational molecule changes.

Keywords Acute lymphoblastic leukemia · BCR::ABL1 · APT-competitive tyrosine kinase inhibitors · STAMP inhibitor · BCR::ABL mutations · Molecular dynamic simulation · Inotuzumab

Krzysztof Lewandowski and Maciej Janicki contributed equally to this work.

✉ Krzysztof Lewandowski
lewandowski@ump.edu.pl

✉ Maciej Janicki
mjanicki@amu.edu.pl

[1] Department of Hematology and Bone Marrow Transplantation, University of Medical Sciences, Poznań, Poland

[2] Department of Biotechnology, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

[3] Department of Crystal Chemistry and Crystal Physics, Faculty of Chemistry, Jagiellonian University, Krakow, Poland

Dear Sirs,

ABL1 kinase domain (KD) mutations are present in 5.5%−40% of newly diagnosed acute lymphoblastic leukemia BCR::ABL1 (ALL BCR::ABL1) patients before the treatment initiation and are detectable in the dominant clone in 90% of cases at the time of disease relapse [1, 2].

Case

A 65-year-old woman was admitted to the ward with the clinical suspicion of acute leukemia at the end of 2020. An initial CBC showed a hemoglobin level of 6.0 mmol/L, WBC 12.0 G/L (neutrophils 1.77 G/L, lymphocytes 7.55 G/L) and the platelet count of 58.0 G/L. The multicolor flow cytometry (MFC) of the bone marrow (BM) cells performed in November 2020 showed 88% of blastic cells with the following immune characteristics: CD13-CD33 + (dim)CD34 + CD117-HLA-DR + CD38-CD31 + CD3-CD14-CD11b-CD11c-CD64-CD163-CD18-CD56-CD16-CD19 + CD10 + CD20 + CD22 + CD3-CD5-CD4-CD8-CD7-CD2-CD1a-CD66b-CD65-CD15 + CD123-cytCD68-MPO-TdT + cytCD3-cytCD79a + cytIgM-surfacekappa-surfacelambda-//CD45dim, FSC mid, SSC low. Routine cytogenetic evaluation showed karyotype 47 ~ 48,XX,t(9;22)(q34;q11.2)[3], + mar1[2], + mar2[3] [cp4]/46,XX[9]. The molecular evaluation revealed the presence of a *BCR::ABL1* fusion (e1a2, p190). Intrathecal (IT) fluid examination for neoplastic cell presence was negative. Induction therapy including imatinib (IM), according to PALG ALL07 protocol (dexamethasone, vincristine, rituximab) resulted in a complete remission (CR) with negative measurable residual disease (MRD) on multicolor flow cytometry (MFC). The molecular response assessment with qRT-PCR showed BCR::ABL1 copies number of 0.0019%. Therapy response evaluation performed after consolidation therapy (CT I- MTX 1500 mg/m^2, PEG-asparaginase, rituximab, IM) and II (cytarabine, rituximab, IM) confirmed CR, MRD negativity in MCF and qRT-PCR. Meanwhile, a matched unrelated donor was identified. The CTIII (MTX in reduced doses, PEG-asparaginase, rituximab, IM) was given. Unfortunately, the patient refused the continuation of treatment after CT IV (cytarabine, rituximab, IM), including the treatment with allo-HSCT. Therefore, since July 2021, the therapy with IM only in the dose of 200–400 mg daily was continued. Two months later, the BM MFC revealed 89% of ALL cells. *ABL1* KD mutation screening with Sanger sequencing was negative. Normalized copy number (NCN) evaluation showed *BCR::ABL1/GUSB* = 0.150727%. Therapy with dasatinib (DAS) 140 mg/d and dexamethasone 40 mg/d was started in July 2023. The CR with MRD negativity in MFC and qRT-PCR was confirmed one month later. In the end of December 2023, a second disease relapse was diagnosed (bone marrow blast cells—89%). *ABL1* KD mutation screening showed sequence variant NM_005157.6(ABL1):c.1210A > G (Lys404Glu). The ponatinib (PON) treatment in the dose of 45 mg/d was initiated in January 2024. Three weeks later, the BM biopsy showed 85% of blasts positive for CD19 + CD20 + CD22 + CD10 + CD34 + HLA-DR + . The 6 cycles of inotuzumab (INO, anti-CD22 monoclonal antibody conjugated to the cytotoxic antibiotic calicheamicin) were given in the dose 0.8 mg/m^2, 0.5 mg/m2 and 0.5 mg/m^2 on day 1, 8 and 15 of the cycle, respectively. INO therapy was complicated by manageable CTAE grade IV neutropenia and moderate peripheral neuropathy. Nine months latter, CR, MRD(-) and complete molecular remission (CMR) was confirmed.

The introduction of ATP-competitive TKI—IM to the induction chemotherapy of ALL *BCR::ABL1* significantly improved the treatment results. Despite this, 32%−41.3% of patients treated with chemotherapy/TKI/allo-HSCT experienced disease relapse/therapy resistance.

The presence of *ABL1* KD mutations was confirmed in 60–70% of patients with resistant ALL *BCR::ABL1* [3]. The mechanism of mutational resistance differs depending on the localization of the mutation and the type of tyrosine kinase inhibitor [(ATP-competitive or allosteric, specifically targeting the ABL myristoyl pocket (STAMP) of BCR-ABL1 tyrosine kinase (myristic acid mimetic)] used [4]. The ATP competitive inhibitors like DAS or BOS exert inhibitory activity in the DFG-in/DFG-out conformation of BCR-ABL1 tyrosine kinase [5]. Conversely, IM, nilotinib (NILO) and PON reveal inhibitory activity only in the DFG-out conformation of the enzyme [6]. The ATP competitive TKIs resistance is the result of the compromised inhibitor binding to BCR-ABL1 tyrosine kinase through the steric clash, elimination of direct contact residues due to amino acid substitutions, and/or amino acid changes favoring an active conformation of the ABL1 kinase domain. The critical amino acid changes which are responsible for the IM resistance, including those localized in or near

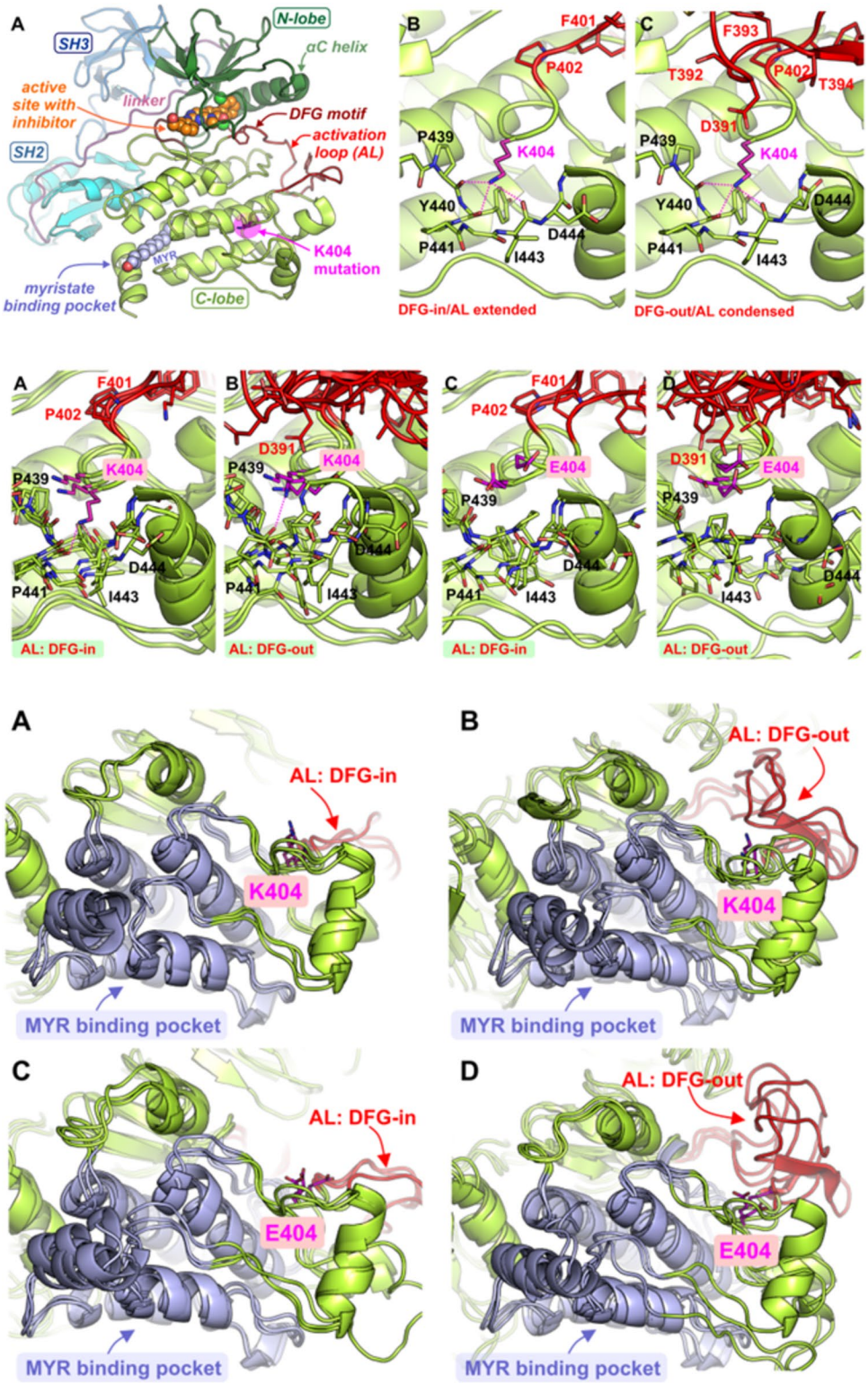
the IM binding site. In the case of DAS, the changes at amino acid positions V299, T315, F317 are critical and responsible for drug resistance. The changes in the amino acid position T315I *ABL1* remain the major clinical challenge in ALL patients, due to the cross-resistance to 1st and 2nd generation TKIs [7].

STAMP inhibitor Asciminib (ASCI) resistance is usually associated with the emergence of *ABL1* mutants localized at amino acid positions G109D, Y115N, M244V, V289I, A337V/T, E355G, F359V, E462K, G463D/S, P465S, V468F, S501R, I502L [8]. Upfront *ABL1* mutation testing in *BCR::ABL1* ALL is not currently recommended. Conversely, the ABL1 kinase domain mutation testing is obligatory in the case of disease relapse. The decision-making process is especially difficult when an *ABL1* mutation with an unknown drug sensitivity. Therefore, we performed molecular docking of ATP-competitive TKI to native BCR-ABL1 and its K404E mutant (a fragment including KD, SH2 and SH3 domains in closed/autoinhibited form possessing conserved motif DFG at activation loop (AL) pointing inward (DGF-in/AL-extended) or outward (DGF-out/AL-condensed) active site (Fig. 1 upper, middle and bottom panel). Docking revealed that inhibitors were bound to the kinase domain (Table S1) in a mode similar to those observed in crystal structures (Fig. S1). Molecular dynamics (MD) data revealed that all compounds are stable-bound during simulation in native BCR-ABL1 and K404E mutant. Therefore, we decided to check if the mutation affects BCR-ABL1 structure globally. The MD simulations revealed a higher mobility of BCR-ABL1 with activation loop in "DGF-out" conformation in the wild type (WT) and mutated (Lys404Glu) protein in comparison to "DGF-in" form. Moreover, the conformational flexibility of the mutated BCR-ABL1, especially in the region covering residues 382–402 (located close to the K404E mutation), was higher in the mutant than in the native (WT) protein (Fig. S2, S3). These data suggest that the stabilization of the mutated protein in its discrete DGF-out or DGF-in conformation is altered by Lys404Glu mutation, making it impossible to form stable complexes with ATP-competitive inhibitors.

The identified K404E mutation is located in the C-lobe of the KD (Fig. 1), in close vicinity of the myristate binding site (however, outside the ATP-binding pocket) and should not affect the ability to bind myristic acid or its mimetics (Fig. S4). This hypothesis was confirmed by MD data BCR-ABL1 WT and mutant K404E in closed/autoinhibited form, showing no significant conformational changes of myristate binding pocket (Fig. 1). Recently, novel mechanism of ASCI resistance was reported. In this mechanism mutation, M244V permanently stabilized active conformation of BCR-ABL1 by interactions involving αC helix, making the action of ASCI not effective. To check if mutation K404E might have similar effect, we performed MD simulations of KD, SH2 and SH3 domains (WT and K404E variant) in open/activated conformation. Our results revealed that substitution K404E does not affect flexibility of open/activated conformation that is necessary for stabilizing BCR-ABL1 permanently in the active form (Table S2). These observations indicate that ASCI resistance should not occur in the K404E mutant via the newly discovered mechanism described by Leyte-Vidal, et al. in the case of M244V [9].

The question that remains unanswered is how to optimally treat refractory/relapsed (R/R) *BCR::ABL1* ALL patients experiencing pan-ATP competitive TKI resistance. In principle, the therapy in these cases should include the use of drugs with a different mode of action. The data about STAMP (ASCI) monotherapy efficacy in such cases are limited, because the drug is not registered in this indication yet. However, preliminary data confirmed the usefulness of the combination treatment using ASCI and PON or ASCI, PON and INO in R/R *BCR::ABL1* ALL. The use of others targetable drugs, including bi-specific T cell engager (BITE) or chimeric antigen receptor T cells (CAR-Ts) has recently become recommended in R/R *BCR::ABL1* ALL. In an open-label, randomized, phase 3 study 1022 (INO-VATE; NCT01564784), the administration of INO resulted in complete remission/complete remission with incomplete hematologic recovery (CR/CRi) in 73%, and MRD negativity in 81% of patients with CR/Cri. In the phase 1/2 study 1010 (NCT01363297) and open-label, randomized, phase 3 study 1022 (INO-VATE; NCT01564784), the progression-free survival (PFS) was very short (4.4 months and 3.9 months, respectively), despite relatively high response rates [10].

The efficacy of therapy INO plus bosutinib (BOS) was confirmed in R/R disease, but only for patients carrying TKI sensitive *ABL1* mutations [11]. Also, the combination of BITE-blinatumomab (anti-CD19/CD3, BLINA) with BOS, DAS or PON was effective in patients with R/R disease. carrying the TKI sensitive *ABL1* mutation [12]. Another therapeutic option is the administration of 3rd generation TKI olverembatinib-based therapy [13]. Lastly, also the high efficacy of INO and DAS therapy was confirmed in newly-diagnosed ALL *BCR::ABL1* [14].


A
SH3
N-lobe
αC helix
active site with inhibitor
linker
DFG motif
activation loop (AL)
SH2
K404 mutation
myristate binding pocket
MYR
C-lobe
B
F401
P402
P439
K404
Y440
D444
P441
I443
DFG-in/AL extended
C
F393
T392
P402
T394
D391
P439
K404
Y440
D444
P441
I443
DFG-out/AL condensed
A
F401
P402
K404
P439
D444
P441
I443
AL: DFG-in
B
D391
K404
P439
D444
P441
I443
AL: DFG-out
C
F401
P402
E404
P439
D444
P441
I443
AL: DFG-in
D
D391
E404
P439
D444
I443
AL: DFG-out
A
AL: DFG-in
K404
MYR binding pocket
B
AL: DFG-out
K404
MYR binding pocket
C
AL: DFG-in
E404
MYR binding pocket
D
AL: DFG-out
E404
MYR binding pocket

◂**Fig. 1** Upper panel. Crystal structure of ABL1 kinase and neighborhood of residue 404 in autoinhibited/closed form and DFG motif in different conformations. **A** Crystal structure of the ABL1 protein (PDB: 1opl) with N-lobe (dark green) and C-lobe (light green) of kinase domain and SH2 (cyan) and SH3 (blue) domains; myristoyl residue (in spherical representation) bound in the C-lobe in myristate binding pocket is colored light violet; linker connecting domains SH2 and SH3 is colored pink; model inhibitor (in spherical representation) is colored orange; AL loop is colored red; position of Lys404 is marked with magenta circle. Interactions of Lys404 with is neighboring residues in native ABL1 structure possessing (**B**) AL loop in the extended conformation (DFG-in; PDB: 3ue4) and (**C**) with AL loop in the condensed conformation (DFG-out; PDB: 3cs9). Activation loop is marked in red, while residue 404 in magenta. Middle panel. Molecular dynamics simulation of BCR-ABL1 kinase; in each panel three selected conformations are presented. Neighborhood of (**A**) Lys404 in DFG-in native (WT) form; (**B**) Lys404 in DFG-out native (WT) form; (**C**) Glu404 in DFG-in K404E mutant; (**D**) Glu404 in DFG-out K404E mutant. Rmsf values are shown in Fig. S2. Conformations were obtained from triplicate MD data. Activation loop is marked in red, while residue 404 in magenta. Bottom panel. Molecular dynamics simulation of myristate biding pocket in BCR-ABL1 kinase; in each panel, three selected conformations are presented (the same as in Fig. S2). Conformation of myristate binding pocket (violet) is not significantly by K404E mutation. Panel (**A**) native (WT) BCR-ABL1 in DFG-in and (**B**) DFG-out form. **C** Mutant K404E in DFG-in and (**D**) in DFG-out form. Rmsf values for myristate biding pocket are shown in Fig. S2. Conformations were obtained from triplicate MD data. Activation loop is marked in red, while residue 404 in magenta

In our opinion, the identification of *ABL1* mutation(s), together with a better understanding of its impact on the structure and function of the BCR::ABL1 tyrosine kinase, may change the future strategy of treatment of R/R *BCR::ABL1* ALL. This may include the usage of BITE, type I, type II and/or allosteric TKI in different combinations, depending on the *ABL1* mutation profile.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00277-025-06385-z.

Acknowledgements The computations were carried out in the Poznan Supercomputing and Networking Center (grant pl0074-02).

Author contributions [K.L., M.B., J.L.,] designed the study, [Z.K.] prepared material and carried out molecular genetic testing, [J.K.-P.] performed and interpreted flow cytometry study results. G.K. carried out cytogenetic testing, [M.J., J.L.] performed bioinformatic structural analyses (structure prediction, MD simulation, docking studies, [K.L., M.B.] interpreted clinical data, [K.L., J.M., J.L.] wrote the manuscript, [K.L., J.M., L.J., L.A.] reviewed and edited the manuscript. All authors read and approved the final manuscript. Krzysztof Lewandowski and Maciej Janicki contributed equally to this work.

Funding None.

Data availability No datasets were generated or analysed during the current study.

Declarations

Consent to participate Informed consent was obtained from patient included in the study.

Conflict of interest The authors declare no competing interests.

References

1. Baer C, Meggendorfer M, Haferlach C, Kern W, Haferlach T (2022) Detection of ABL1 kinase domain mutations in therapy-naïve BCR-ABL1-positive acute lymphoblastic leukemia. Haematologica 107(2):562. Available from: /pmc/articles/PMC8804577/. cited 2024 Oct 3
2. Pfeifer H, Wassmann B, Pavlova A, Wunderle L, Oldenburg J, Binckebanck A et al (2007) Kinase domain mutations of BCR-ABL frequently precede imatinib-based therapy and give rise to relapse in patients with de novo Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). Blood 110(2):727–34. Available from: https://pubmed.ncbi.nlm.nih.gov/17405907/. cited 2024 Oct 3
3. Hochhaus A, Kreil S, Corbin AS, La Rosée P, Müller MC, Lahaye T et al (2002) Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. Leukemia 16(11):2190–6. Available from: https://pubmed.ncbi.nlm.nih.gov/12399961/. cited 2024 Oct 3
4. Roskoski R (2016) Classification of small molecule protein kinase inhibitors based upon the structures of their drug-enzyme complexes. Pharmacol Res 103:26–48. Available from: https://pubmed.ncbi.nlm.nih.gov/26529477/. cited 2024 Oct 3
5. Zhou T, Commodore L, Huang WS, Wang Y, Thomas M, Keats J et al (2011) Structural mechanism of the Pan-BCR-ABL inhibitor ponatinib (AP24534): lessons for overcoming kinase inhibitor resistance. Chem Biol Drug Des 77(1):1–11. Available from: https://pubmed.ncbi.nlm.nih.gov/21118377/. cited 2024 Oct 3
6. Rossari F, Minutolo F, Orciuolo E (2018) Past, present, and future of Bcr-Abl inhibitors: from chemical development to clinical efficacy. J Hematol Oncol 11(1). Available from: https://pubmed.ncbi.nlm.nih.gov/29925402/. cited 2024 Oct 3
7. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F et al (2009) AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell 16(5):401–12. Available from: https://pubmed.ncbi.nlm.nih.gov/19878872/. cited 2022 Feb 1
8. Lewandowski K (2024) Asciminib a new player in treatment of TKI-resistant/intolerant chronic phase chronic myelogenous leukemia. Acta Haematol Pol 55(2):62–72. Available from: https://journals.viamedica.pl/acta_haematologica_polonica/article/view/98474. cited 2024 Dec 30

9. Leyte-Vidal A, Garrido Ruiz D, DeFilippis RA, Leske IB, Rea D, Phan S et al (2024) BCR::ABL1 kinase N-lobe mutants confer moderate to high degrees of resistance to asciminib. Blood 144(6):639–45. Available from: https://pubmed.ncbi.nlm.nih.gov/38643492/. cited 2024 Nov 26
10. Stock W, Martinelli G, Stelljes M, DeAngelo DJ, Gökbuget N, Advani AS et al (2021) Efficacy of inotuzumab ozogamicin in patients with Philadelphia chromosome–positive relapsed/refractory acute lymphoblastic leukemia. Cancer 127(6):905. Available from: /pmc/articles/PMC7983935/. cited 2024 Oct 4
11. Jain N, Cortes JE, Ravandi F, Konopleva M, Alvarado Y, Kadia T et al (2017) Inotuzumab ozogamicin in combination with bosutinib for patients with relapsed or refractory Ph+ aLL or CML in lymphoid blast phase. Blood 130
12. Assi R, Kantarjian H, Short NJ, Daver N, Takahashi K, Garcia-Manero G et al (2017) Safety and efficacy of blinatumomab in combination with a tyrosine kinase inhibitor for the treatment of relapsed philadelphia chromosome-positive leukemia. Clin Lymphoma Myeloma Leuk 17(12):897–901. Available from: https://pubmed.ncbi.nlm.nih.gov/28927784/. cited 2024 Oct 4
13. Liu W, Wang C, Ouyang W, Hao J, Ren J, Peng L et al (2024) Efficacy and safety of olverembatinib in adult BCR::ABL1-positive ALL with T315I mutation or relapsed/refractory disease. Br J Haematol 205(6). Available from: https://pubmed.ncbi.nlm.nih.gov/39363594/. cited 2025 Apr 11
14. Patel AA, Duvall AS, Saygin C, Weiner H, Dworkin E, Noorani A et al (2024) Interim Results of a Phase II Study Investigating Dasatinib and Inotuzumab Ozogamicin-Based Induction for Newly-Diagnosed Philadelphia-Chromosome Positive Acute Lymphoblastic Leukemia (Ph+ ALL). Blood 144(Supplement 1):1432–1432. Available from: https://doi.org/10.1182/blood-2024-193442. cited 2025 Apr 11