Baku State University Journal of Life Sciences & Biology ***2024****, v.1 (1), p. 29-37*

journal homepage: http://bsuj.bsu.edu.az/en

BCR-ABL DEPENDENT AND INDEPENDENT MECHANISMS IN TYROSINE KINASE INHIBITORS TREATMENT OUTCOME

Nigar Karimova[a]*, Rovshan Khalilov[b], Aziz Eftekhari[c], Bayram Bayramov[a]

[a]Institute of Genetic Resources, Baku, Azerbaijan
[b]Baku State University, Baku, Azerbaijan
[c]Ege University, Izmir, Turkiye

Received 05 october 2023; accepted 14 november 2023

Abstract

Introducing tyrosine kinase inhibitors (TKIs) has had a transformative impact on treating chronic myeloid leukemia (CML), leading to a remarkable survival rate exceeding 80%. The primary focus of CML treatment has centered around enhancing the effectiveness and specificity of TKIs to inhibit the activation of the BCR-ABL1 kinase and addressing resistance arising from mutations in the BCR-ABL1 oncogene. However, despite successful BCR-ABL1 inhibition, a significant number of patients develop resistance to TKIs, necessitating the exploration of novel therapeutic approaches.

This review is focused on a detailed examination of the latest reports on both BCR-ABL1-dependent and BCR-ABL1-independent mechanisms of resistance to TKI. The investigation of these crucial pathways could lead to the development of promising therapeutic approaches. By employing such combination treatments, residual leukemic cells can be effectively targeted, leading to an increased response rate among CML patients.

Keywords: CML; BCR-ABL; TKI; resistance.

1. Introduction

Chronic myeloid leukemia (CML) is a malignant condition characterized by the clonal expansion of white blood cells originating from the myeloid lineage in the bone marrow. CML develops as a result of a balanced reciprocal translocation, known as the t(9;22)(q34;q11), occurring between chromosomes 9 and 22, leading to the formation of the Philadelphia chromosome [1]. This translocation event results in the fusion of the Breakpoint Cluster Region (BCR) gene with the Abelson proto-oncogene 1 (ABL1) gene, generating the BCR-ABL1 fusion gene. The given chimeric protein, BCR-ABL1, is a highly active tyrosine kinase signaling protein that promotes uncontrolled cell proliferation and inhibits apoptosis, leading to leukemia [2].

* Corresponding author. Tel.: +90 535 721 23 32
E-mail address: nigar.karimova.23@gmail.com

Based on the specific breakpoint location within the BCR gene, various isoforms of the BCR-ABL1 protein are generated. The variant found in over 90% of patients with CML is e13a2/e14a2 (b2a2/b3a2) alternative transcripts, which arise from the fusion of BCR exon 13 or 14 with ABL1 exon 2, resulting in a 210 kDa protein variant. In contrast, the e1a2 transcript codes for a 190 kDa protein, which is less prevalent in CML but frequently observed in cases of acute lymphoblastic leukemia, occurring in approximately 70% of such cases [3].

The fusion gene generates the BCR-ABL1 protein, responsible for uncontrolled cell proliferation, disruption of stromal adhesion, and inhibition of apoptosis by activating downstream signaling pathways, including Janus kinase-signal transducer and activator of transcription (Jak-Stat) and Myc. Within the bone marrow, specific niches play a vital role in generating and progressing chronic myeloid leukemia (CML) [4]. ABL1 component of the BCR-ABL protein includes an SRC-homology-2 (SH2) domain, an SH3 domain, and a kinase domain. Normally, in the absence of BCR fusion, ABL1's myristoylated N-terminal region induces self-inactivation of its kinase activity. However, during the fusion process of BCR-ABL, this myristoylated N-terminal region is eliminated [7].

The BCR-ABL kinase domain contains crucial motifs essential for its function, including the phosphate binding loop (P-loop), the contact site (ATP/IM binding site), the catalytic domain, and the activation loop (A-loop). BCR-ABL1 exhibits activity when ATP binds to the active site within the ABL1 kinase domain and transfers its phosphate group to ABL1 substrates. Nonetheless, tyrosine kinase inhibitors (TKIs) compete with ATP for binding to the active site, thereby hindering BCR-ABL1 activation and preventing the occurrence of leukemia [6]. Since its initial approval by the FDA as a tyrosine kinase inhibitor (TKI) in 2001, Imatinib has revolutionized the course of leukemia therapy, leading to notable long-term overall survival rates. Furthermore, the drug has been joined by four additional TKIs, namely dasatinib, nilotinib, bosutinib, and ponatinib, which have received approval and are now crucial in managing patients with CML. However, approximately 25% of patients encounter TKI resistance at some stage during therapy [3].

TKI resistance can be classified as primary or acquired. Primary resistance refers to the lack of response to treatment, while acquired resistance is characterized by disease progression after an initial positive response to therapy. Notably, acquired resistance develops during the course of treatment, indicating that the tumor has developed mechanisms to evade continuous inhibition of the target. It is essential to mention that the most prevalent mechanisms underlying acquired resistance involve the occurrence of point mutations within the BCR-ABL kinase domain. Disease progression and exposure to multiple TKIs are influential factors affecting the frequency of these mutations [8].

On the other hand, primary resistance mechanisms may involve the overexpression of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2). ABC transporters play a role in intracellular drug accumulation regulation [9]. However, recent biological evidence suggests that curative approaches for TKI-resistant chronic myeloid leukemia (CML) patients should also take into consideration BCR-ABL-independent mechanisms of resistance, with a particular focus on leukemia stem cells (LSCs), immunological aspects and epigenetic alterations. LSCs can persist in CML patients independently of BCR-ABL1 kinase activation. The interaction between LSCs and cells within the hematopoietic niche in the microenvironment may promote the development of resistance [10], (Fig. 1.). Lastly, a relatively new concept highlights the role of molecular minimal residual disease (MRD) during TKI treatment, which may result from LSC persistence. It is possible that MRD positivity could contribute to the development of TKI resistance over time [11].

Thus, in this review, we focus on a detailed description of the mechanisms that have a potential risk of causing TKI resistance and negatively influencing CML subject management.

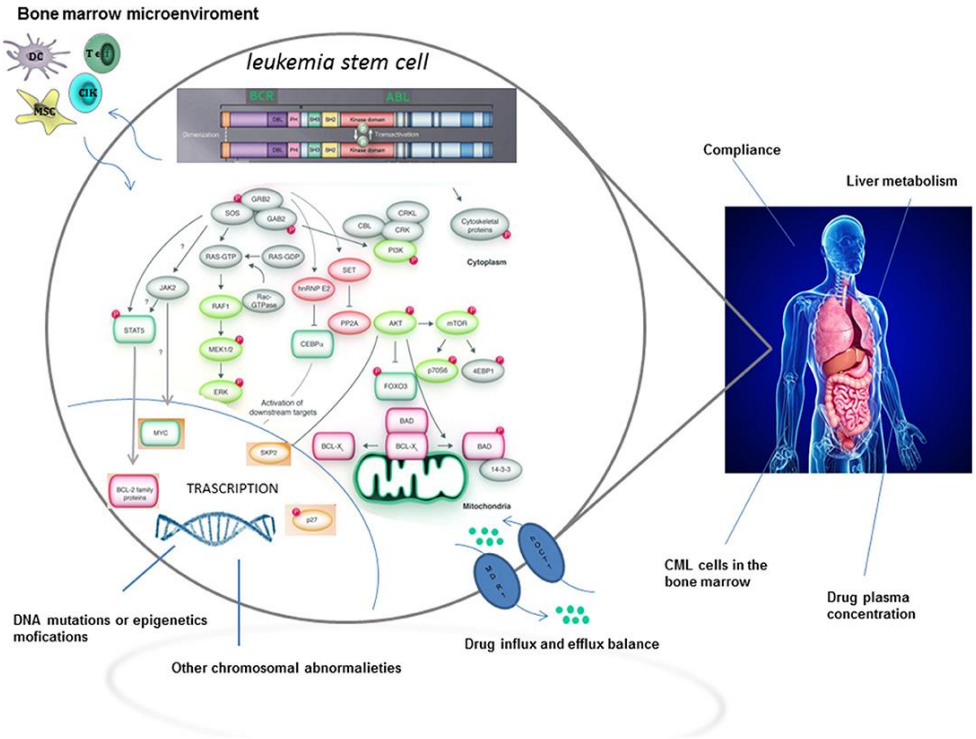

Fig. 1 The mechanisms to cause resistance to TKIs (Loscocco et al., 2019).

BCR-ABL mutations as a major factor in causing the TKI resistance

The most studied mechanism of secondary resistance to ITK therapy is variations in the ABL1 kinase domain. As a result, these alterations are responsible for approximately 40-60% of CML cases with a relapse in hematological condition while on imatinib therapy [12].

Increasing evidence suggests that mutations leading to resistance against kinase inhibitors (KI) primarily occur in four regions of the kinase domain: the gatekeeper region, the A-loop, the G-loop, and the αC-helix. Taking into consideration the specificity of these substructures, they have been extensively investigated for their involvement in the development of drug resistance, particularly secondary mutations that arise as a result of cancer treatment [13].

More than 90 different point mutations involving 50 various amino acids, including the ATP-binding domain (P-loop), catalytic domain, and activation loop (A-loop), have been identified as responsible for clinical imatinib resistance is important to highlight a few especially, G250E, Y253F/H, and E255K/V mutations in the P-loop, T315I mutation in the imatinib binding site, and M351T and F359V/C/I mutations in the catalytic domain (Table 1).

Table 1. Various clinically significant mutations in the BCR-ABL1 kinase domain, and their responsiveness to different tyrosine kinase inhibitors (TKIs) (Poudel et al., 2022).

Mutations in the BCR-ABL kinase domain	Resistance to TKI
T315I, Y253F/H, E255K/V, Q252H, M244V, L248V, G250E, F317L, M351T, M355D, F359V, and H396R/P/A	Imatinib
T315I, E255V/K, V299L, G250E, E255K/V, and F317L/V/I/C	Bosutinib

T315I/A, V299L, and F317L/V/I/C	Dasatinib
T315M/L	Ponatinib
T315I, L248V, Y253H, E255K/V, and F359V/I/C	Nilotinib

Optimal binding of the drug requires structural adjustments in BCR-ABL, which are impeded in P-loop mutants, while the kinase remains stabilized in an active state in A-loop mutants [14]. The T315I mutation, also known as "the gatekeeper" mutation, occurs when threonine is replaced by isoleucine, preventing Imatinib from forming a hydrogen bond with the protein [3]. The presence of a gatekeeper mutation involves the substitution of a small side chain residue with a bulky side chain residue. This change in size hinders the binding of drugs to the protein, leading to potential drug resistance. Recent literature shows that the prognosis of overall survival rates and treatment failure (both TKI and chemotherapy) for T315I carriers is poor. In addition, even though nilotinib and dasatinib, exhibit increased potency and activity against most imatinib-resistant mutations, the T315I alteration remains an exception in their application [3]. Nevertheless, it is crucial to note the progress ponatinib has demonstrated against most kinase domain mutations, including T315I. However, there are complications, such as compound mutations and ponatinib's cytotoxicity [15].

The necessity for an effective and potent drug against mutated BCR-ABL1 protein triggered the development of a new allosteric inhibitor - asciminib. In the latest experiments, asciminib demonstrated significantly favorable clinical results, namely recently, Cortes et al. stated that approximately 50% of CML patients with T315I achieved a major molecular response within a short period [17]. In addition, another large phase I study of asciminib in patients who failed previously showed that 88% of patients induced complete hematological response and 24% of T315I carriers - a major molecular response [16]. Thus, there are ongoing trials to prove and assign asciminib as a front-line therapy (NCT03578367).

Other BCR-ABL-dependent mechanisms of resistance to therapy

A growing interest in TKI resistance mechanisms is stimulated by the increasing number of unresponsive CML patients. Obtained results and ongoing trials identify resistance mechanisms different from the BCR-ABL mutations scheme. For instance, an increase in drug efflux is a pathway that can interfere with the complete inhibition of BCR-ABL1 by TKIs.

Indeed, studies demonstrate that various members of the ABC transporter family have an association with resistance to specific TKIs. Namely, ABCB1 was found to cause resistance to Imatinib and nilotinib, ABCG2 with asciminib and ABCC6 with second-line TKIs dasatinib and nilotinib [18]. Another example is the investigation of the influence of organic cation transporter-1 (OCT-1) on intracellular drug availability. It was shown that OCT-1 might contribute to imatinib resistance; however, it does not affect the outcome of the second and third-generation TKIs. It is worth noting that when a specific transporter associated with resistance is overexpressed, strategies such as using a TKI that is not susceptible to that transporter or adding an inhibitor targeting the transporter can be employed. For instance, Agrawal et al. observed that patients with imatinib-resistant CML, who displayed elevated levels of ABCB1 expression, still exhibited positive responses to second-line treatment with nilotinib. Similarly, there is another study that identified overexpression of ABCG2 as a significant resistance pathway in asciminib-resistant K562 cells and demonstrated that the inhibitor Ko143 (100 nM) could restore the effectiveness of asciminib against those cells [19, 20].

Although common mutations in the BCR-ABL domain are major causes of resistance, recently, there has been more data supporting that a portion of patients gains variations in the myristoyl-binding pocket. Alterations in that region, namely A337V, P465S, V468F, I502L, and C464W, were found to be associated with asciminib resistance, despite the drug's promising results. However, there is the possibility of overcoming this type of resistance with combined therapy of several TKIs [20].

In addition to mentioned mechanisms, it is also vital to mention BCR-ABL1 overexpression caused by Ph chromosome duplication. Although its role is not investigated and confirmed as the role of KD mutations, there

are some suggestions that increased levels of BCR-ABL may elevate kinase activity, which may result in kinase domain mutations leading to TKI therapy resistance [21]. Interestingly, elevated BCR-ABL expression is often observed at the latest stages of chronic myeloid leukemia, where reduced sensitivity to TKIs and the development of resistance are commonly encountered [22].

BCR-ABL independent mechanisms and TKIs treatment outcome

Among mechanisms to cause resistance, BCR-ABL independent pathways also should be considered. Despite TKI's impressive results, the primary cause of resistance is that these drugs cannot eliminate leukemia stem cells (LSCs), which play a crucial role in the development and regeneration of CML. It is important to note that LSCs and hematopoietic stem cells (HSCs) share various molecular components involved in maintaining their stemness, including transcription factors, signal transduction factors, regulators of the cell cycle, metabolism, autophagy, and factors associated with the microenvironment. On the other hand, LSCs and HSCs also possess distinct biological properties that affect their interactions with these factors, which may influence therapy outcomes [5].

The majority of acquired resistance cases arise early in the disease progression as a result of the expansion of clones containing Bcr-Abl mutations that hinder its binding to TKIs. Conversely, it has been observed that patients who achieve a complete cytogenetic response still harbor Bcr-Abl positive clones, including LSCs capable of causing relapse upon discontinuing imatinib treatment [23].

The critical complication in the majority of CML patients is that LSCs are not eliminated. Thus, they act as a reservoir of malignant cells that subsequently may relapse upon therapy absence. Also, due to CML-LSCs intratumoral heterogeneity and low frequency in bone marrow, its identification and characterization are problematic. Thus, the key question is regarding the origin of TKI-resistant CML-LSCs: whether they arise from a pre-existing subset of therapy-resistant CML-LSCs or if they emerge as a population that becomes resistant through the process of therapeutic selection [24]. Nevertheless, recent progress provides insight into these issues. Namely, a recent study of the combination of large-scale single-cell gene-expression analysis with cell surface marker screens showed that CML-LSCs possess an aberrant expression of cell surface molecules such as CD33, CD123, IL1RAP, CD26, and CD25. Therefore, these molecules may be applied as markers to distinguish CML-LSCs from normal HSCs [25].

Unfortunately, the latest investigations showed that a highly dormant subset of CML-LSCs can persist even after prolonged treatment with TKIs. Interestingly, the primary molecular difference of LSCs subset from normal HSCs as it exhibits elevated expression levels of various potential therapeutic targets, including TGF-beta, TNF-alpha, Jak-STAT, CTNNB1, and NFKB1A. These distinct molecular characteristics may offer the opportunity for selective targeting of these highly resistant CML-LSCs [26]. Namely, Neviani et al. identified protein phosphatase 2A (PP2A) as a tumor suppressor capable of reducing the survival and self-renewal capacity of quiescent CML-LSCs but not normal quiescent HSCs [27]. Also, according to a recent study, the PP2A activator FTY720 was found to have no significant impact on normal HSCs but significantly impaired the survival and self-renewal capacity of quiescent CML-LSCs. Surprisingly, the primary determinant of these effects on quiescent CML-LSCs was the PP2A-induced inactivation of JAK2 and β-catenin rather than the inactivation of BCR-ABL1 [5].

It is worth mentioning the association between autophagy and resistance in CML. Being a well-conserved catabolic process, it is responsible for protein degradation and antigen presentation. Treatment with TKIs has been shown to induce autophagy, which contributes to the survival of LSCs and the development of TKI resistance. Consequently, selective inhibition of autophagy could potentially reverse TKI resistance and target CML-LSCs, leading to their elimination [5].

According to Baquero et al., basal autophagy was found to be higher in CML-LSCs compared to normal HSC. Moreover, when Lys05 and PIK-III were administered together, a significant decrease in the number of primary CML-LSCs was observed, and xenografted LSCs were effectively eliminated in combination with TKIs. These findings suggest the potential of combining second-generation autophagy inhibitors with TKIs, offering a potential treatment strategy for CML patients with minimal residual disease (MRD). Targeting LSCs is crucial

since their persistence after TKI treatment has been associated with disease relapse, emphasizing the importance of including them as part of the treatment arsenal for CML [28].

Currently, it is common knowledge that specific dysregulated BCR-ABL signaling pathways play a significant role in TKI resistance. For instance, an essential limitation of Imatinib is that CML-LSCs are not eliminated during imatinib therapy, meaning that they use survival signals different from BCR-ABL to survive and resist during imatinib treatment [5].

A study by Ma et al. demonstrated increased activity in RAF/MEK/ERK signaling pathway responsible for BCR-ABL-independent imatinib resistance through CML-LSCs. Thus, to overcome BCR-ABL independent resistance to Imatinib, it might be necessary to simultaneously inhibit both BCR-ABL and RAF/MEK/ERK signaling. Imatinib and Trametinib, a MEK-inhibitor, were recently combined in a mouse model, showing the successful killing of CML-LSCs and proving the principle of testing this combination in humans [11].

Using exon microarrays, Gerber et al. identified ninety-seven genes that were differentially expressed between CML-LSCs and normal HSCs. These genes play important roles in various cellular processes such as metabolism, proliferation, cell surface interactions, self-renewal, differentiation, and inflammation. Interestingly, certain genes that were found to be overexpressed in CML-LSCs encode cell surface proteins, including IL2Rα (CD25), DPP4 (CD26), PTPRD, CACNA1D, IL1RAP, SLC4A4, and KCNK5. The presence of these proteins on the cell surface makes them potential targets for immune-based strategies [29].

Cancers are considered to be influenced by epigenetic changes in addition to genetic abnormalities, and CML is not an exception. Epigenetic dysregulation plays a significant role in the development of TKI resistance, leading to the escape of leukemic clones and the propagation of the disease. Epigenetic alterations are driven by various systems, including modifications of histones and DNA, as well as the involvement of non-coding RNAs. Enzymes such as histone acetyl- or methyl-transferases, histone deacetylases, and demethylases are responsible for adding or removing specific variations at amino acid residues or CpG islands in DNA.

Recent studies have revealed an association between CML progression, TKI resistance, and CpG islands. The increased methylation of specific genes, such as transcription factor AP-2 alpha (TFAP2A), early B-cell factor 2 (EBP2), and autophagy-related 16-like 1 (ATG16L1), has been observed in patients with blastic phase compared to those in the chronic phase. Furthermore, methylated cases at baseline exhibited a higher frequency of methylation in the ATG16L1 gene among CML patients. It was also found that methylated cases at baseline had a lower probability of achieving a major molecular response (MMR) at 12 or 18 months than unmethylated cases [30]. In CML blast crisis patients, somatic mutations in ASXL1, DNMT3A, RUNX1, and TET2 have been associated with poor response to TKIs and disease. However, the exact causal relationship between these mutations and TKI resistance, disease progression, and relapse is not yet fully understood. EZH2, another epigenetic regulator, has emerged as a factor associated with TKI resistance in CML. It serves as a histone methyl-transferase and is a component of the polycomb repressive complex 2 (PRC2). Studies conducted in a CML mouse model revealed that EZH2 was overexpressed in leukemic stem cells (LSCs), and its dysregulation contributed to resistance against TKIs and offered protection to LSCs. Inactivation of EZH2 using CRISPR/Cas9-mediated gene editing resulted in reduced initiation, maintenance, and survival of LSCs, independent of BCR-ABL1 mutations. Dysregulation of PRC2, which involves the reprogramming of EZH2 and H3K27me3, was also observed in CML stem cells, leading to the evasion of apoptosis and the survival of LSCs [31, 32].

Last but not least, it is important to note the role of the immune system in CML, not only for disease development and progression but also impact on prognosis and therapy response. It is challenging to establish a direct association between the immune system and CML. The major complication is an accumulation of immature myeloid cells known as myeloid-derived suppressor cells (MDSCs), originating from the malignant BCR-ABL1 clone, which leads to suppression of both the innate and adaptive immune systems, thus contributing to the development of CML [33]. However, there is growing evidence of a linkage between the absence of relapse and immunological control of CML in patients who discontinued TKIs. For instance, there are studies to support the relationship between the immune system, checkpoint inhibitors, microenvironment and long-term molecular response. Namely, patients with successful results of TKI therapy and deep molecular response possessed a reduced number of MDSC.

Furthermore, it was found that a deep molecular response correlates with increased NK-cell and CD8+ T-cell counts in the peripheral blood of CML patients [34]. In addition, the levels of checkpoint receptors such as PD1, CTLA4, and TIM3 were elevated in CD4+ and CD8+ T cells of CML patients compared to healthy volunteers. In other words, enhanced net effector immune responses and decreased PD-1 and immune suppressors may promote sustained deep molecular response in CML [34, 35]. It is worth mentioning that cytokines and chemokines IL-1 alpha, IL-1 beta, IL-6, G-CSF, TNF-, CCL3, and CCL4 are potentially responsible for the reduction of CXCL12 functional expression. However, among these, anti-G-CSF antibody therapy increased CXCL12 expression and CML-LSC numbers in the bone marrow and reciprocally decreased CML LSC numbers in the spleen, while only G-CSF in vitro decreased CXCL12 expression in bone marrow stromal cells [39]. In addition, according to an investigation by Bruck et al., the levels of PD-1, TIM3, and CTLA4 in their CD4+ and CD8+ cells were increased in comparison with controls. At the same time, the given levels were decreased in patients with successful outcomes of TKI therapy, demonstrating a strong association between response to treatment and the immune system [36]. There are many ongoing experiments to assume that immune biomarkers could be applied to predict molecular response in CML patients undergoing TKI treatment.

2. Conclusion

Despite the significant progress in CML treatment through the use of TKIs, there are still challenges to overcome. Disease progression to the latest stages, therapy resistance, and long-term dependence on TKI therapy are major complications of CML management. Current approaches to overcome TKI resistance have primarily focused on enhancing the effectiveness and selectivity of drugs targeting the BCR-ABL1 oncogene, as well as addressing resistance caused by alterations in this oncogene. Nevertheless, BCR-ABL-dependent and BCR-ABL-independent mechanisms contribute to the development of TKI resistance. Thus, there is a high demand for novel therapeutic strategies that specifically target leukemic stem cells (LSCs) to address the challenges faced by a significant portion of CML patients who do not achieve satisfactory outcomes with TKIs or combining TKIs with other agents targeting alternative survival pathways.

Also, unresponsive CML patients who do not exhibit modifications in the kinase domain should undergo screening to identify alternative resistance mechanisms, as it may assist in determining the appropriate combination-treatment approach for these individuals.

Combining new drugs that target the microenvironment, epigenetic factors, or metabolic pathways responsible for the persistence of CML LSCs holds promise in improving the cure rate by addressing BCR-ABL-independent mechanisms of resistance, which are responsible for TKI treatment failure.

References

[1] Vuelta E, García-Tuñón I, Hernández-Carabias P, Méndez L, Sánchez-Martín M. Future Approaches for Treating Chronic Myeloid Leukemia: CRISPR *Therapy. Biology (Basel). 2021,* 10(2), p. 118. https://doi.org/10.3390/biology10020118

[2] Pietarinen PO, Eide CA, Ayuda-Durán P, Potdar S, Kuusanmäki H, Andersson EI, et al. Differentiation status of primary chronic myeloid leukemia cells affects sensitivity to BCR-ABL1 inhibitors. *Oncotarget*. 2017, 8(14), p. 22606-22615. https://doi.org/10.18632/oncotarget.15146

[3] Poudel G, Tolland MG, Hughes TP, Pagani IS. Mechanisms of Resistance and Implications for Treatment Strategies in Chronic Myeloid Leukaemia. *Cancers*. 2022, 14(14). https://doi.org/10.3390/cancers14143300

[4] Apperley JF. Chronic myeloid leukaemia. *Lancet*. 2017, 385, p. 1447-1459. https://doi.org/10.1016/S0140-6736(13)62120-0

[5] Loscocco F, Visani G, Galimberti S, Curti A, Isidori A. BCR-ABL Independent Mechanisms of Resistance in Chronic Myeloid Leukemia. *Frontiers in Oncology*. 2019, 9. https://doi.org/10.3389/fonc.2019.00939

[6] Vinhas R, Lourenco A, Santos S, Lemos M, Ribeiro P, de Sousa AB, Baptista PV, Fernandes AR. A novel BCR-ABL1 mutation in a patient with Philadelphia chromosome-positive B-cell acute lymphoblastic leukemia. *Oncology Targets and Therapy. 2018,* 11, p.8589-8598. https://doi.org/10.2147/OTT.S177019

[7] Hantschel O, Nagar B, Guettler S, Kretzschmar J, Dorey K, Kuriyan J, Superti-Furga G. A myristoyl/phosphotyrosine switch regulates c-Abl. Cell. 2003, 112, p.845-857. https://doi.org/10.1016/S0092-8674(03)00191-0

[8] Alikian M, Gerrard G, Subramanian PG, Mudge K, Foskett P, Khorashad JS, Lim AC, Marin D, Milojkovic D, Reid A, Rezvani K, Goldman J, Apperley J, Foroni L. BCR-ABL1 kinase domain mutations: Methodology and clinical evaluation. *American Journal of Hematology.* 2012, 87(3), p. 298-304. https://doi.org/10.1002/ajh.22272

[9] Dohse M, Scharenberg C, Shukla S, Robey RW, Volkmann T, Deeken JF, et al. Comparison of ATP-binding cassette transporter interactions with the tyrosine kinase inhibitors imatinib, nilotinib, and dasatinib. *Drug Metabolism and Disposition.* 2010, 38, p. 1371-1380. https://doi.org/10.1124/dmd.109.031302

[10] Talati C, Pinilla-Ibarz J. Resistance in chronic myeloid leukemia: Definitions and novel therapeutic agents. *Current Opinion in Hematology.* 2018, 25, p. 154-161. https://doi.org/10.1097/MOH.0000000000000403

[11] Ma L, Shan Y, Bai R, Xue L, Eide CA, Ou J, Zhu LJ, Hutchinson L, Cerny J, Khoury HJ, et al. A therapeutically targetable mechanism of BCR-ABL-independent imatinib resistance in chronic myeloid leukemia. *Science Translational Medicine.* 2014, 6, 252ra121. https://doi.org/10.1126/scitranslmed.3009073

[12] Soverini S, Bassan R, Lion T. Treatment and monitoring of Philadelphia chromosome-positive leukemia patients: Recent advances and remaining challenges. *Journal of Hematology & Oncology.* 2019, 12, p.39. https://doi.org/10.1186/s13045-019-0729-2

[13] Hu R, Xu H, Jia P, Zhao Z. KinaseMD: Kinase mutations and drug response database. *Nucleic Acids Research*. 2021, 49(D1), D552. https://doi.org/10.1093/nar/gkaa945

[14] Yin CC, Cortes J, Galbincea J, Reddy N, Breeden M, Jabbour E, Luthra R, Jones D. (). Rapid clonal shifts in response to kinase inhibitor therapy in chronic myelogenous leukemia are identified by quantitation mutation assays. Cancer Science, 2010, 101(9), 2005-10. https://doi.org/10.1111/j.1349-7006.2010.01627.x.

[15] Schumacher JA, Szankasi P, Bahler DW, Ho AK, Kelley TW, Yang K. A pyrosequencing-based test for detection and relative quantification of the BCR-ABL1 T315I point mutation. *Critical Reviews in Oncology/Hematology*. 2015, 93(3), p.277-292.

[16] Hughes TP, Mauro MJ, Cortes JE, Minami H, Rea D, DeAngelo DJ, Breccia M, Goh YT, Talpaz M, Hochhaus A, et al. Asciminib in Chronic Myeloid Leukemia after ABL Kinase Inhibitor Failure. *New England Journal of Medicine*. 2019, 381, p. 2315-2326. https://doi.org/10.1056/NEJMoa1902328

[17] Cortes JE, Hughes TP, Mauro MJ, Hochhaus A, Rea D, Goh YT, Janssen J, Steegmann JL, Heinrich MC, Talpaz M, et al. Asciminib, a First-in-Class STAMP Inhibitor, Provides Durable Molecular Response in Patients (pts) with Chronic Myeloid Leukemia (CML) Harboring the T315I Mutation: *Primary Efficacy and Safety Results from a Phase 1 Trial. Blood*. 2020, 136, p. 47-50. https://doi.org/10.1182/blood-2020-139677

[18] Eadie LN, Saunders VA, Branford S, White DL, Hughes TP. The new allosteric inhibitor asciminib is susceptible to resistance mediated by ABCB1 and ABCG2 overexpression in vitro. *Oncotarget*. 2018, 9, p. 13423. https://doi.org/10.18632/oncotarget.24393

[19] Agrawal M, Hanfstein B, Erben P, Wolf D, Ernst T, Fabarius A, Saussele S, Purkayastha D, Woodman RC, Hofmann WK, et al. MDR1 expression predicts outcome of Ph+ chronic phase CML patients on second-line nilotinib therapy after imatinib failure. *Leukemia*. 2014, 28, p. 1478-1485. DOI: 10.1038/leu.2014.6.

[20] Qiang W, Antelope O, Zabriskie MS, Pomicter AD, Vellore NA, Szankasi P, Rea D, Cayuela JM, Kelley TW, Deininger MW, et al. Mechanisms of resistance to the BCR-ABL1 allosteric inhibitor asciminib. *Leukemia*. 2017, 31, p. 2844-2847. doi: 10.1038/leu.2017.264

[21] Patel AB, O'Hare T, Deininger MW. Mechanisms of Resistance to ABL Kinase Inhibition in Chronic Myeloid Leukemia and the Development of Next Generation ABL Kinase Inhibitors. *Hematology/Oncology Clinics of North America*. 2017, 31, p. 589-612. doi: 10.1016/j.hoc.2017.04.007

[22] Braun TP, Eide CA, Druker BJ. Response and resistance to BCR-ABL1-targeted therapies. *Cancer Cell.* 2020, 37, p. 530-542. doi: 10.1016/j.ccell.2020.03.006

[23] Ichim CV. Kinase-Independent Mechanisms of Resistance of Leukemia Stem Cells to Tyrosine Kinase Inhibitors. *Stem Cells Translational Medicine*. 2014, 3(4), p. 405-415. https://doi.org/10.5966/sctm.2012-0159

[24] Giustacchini A, Thongjuea S, Barkas N, Woll PS, Povinelli BJ, Booth CAG, et al. Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia. *Nature Medicine*. 2017, 23, p. 692-702. doi: 10.1038/nm.4336

[25] Warfvinge R, Geironson L, Sommarin NE Micael, Lang S, Karlsson C, Roschupkina T, et al. Single-cell molecular analysis defines therapy response and immunophenotype of stem cell subpopulations in CML. *Blood*. 2017, 129, p.2384-2394. doi: 10.1182/blood-2016-07-728873

[26] Mead AJ. Single cell genomics in chronic myeloid leukemia. *Hemasphere*, 2018, 2, p. 54-55.

[27] Neviani P, Harb JG, Oaks JJ, Santhanam R, Walker CJ, Ellis JJ, et al. PP2A-activating drugs selectively eradicate TKI-resistant chronic myeloid leukemic stem cells. *J Clin Invest.* 2013, 123:4144–57. 10.1172/JCI68951

[28] Baquero P, Dawson A, Mukhopadhyay A, Kuntz EM, Mitchell R, Olivares O, et al. Targeting quiescent leukemic stem cells using second generation autophagy inhibitors. *Leukemia*. (2019), 33:981–94. 10.1038/s41375-018-0252-4

[29] Gerber JM, Gucwa JL, Esopi D, Gurel M, Haffner MC, Vala M, et al. Genome-wide comparison of the transcriptomes of highly enriched normal and chronic myeloid leukemia stem and progenitor cell populations. *Oncotarget*. 2013, 4:715–28. 10.18632/oncotarget.990

[30] Keramatinia A, Ahadi A, Akbari ME, Mohseny M, Mosavi Jarahi A, Bahadori-Monfared A, et al. The role of DNA epigenetics and clinical significance in Chronic Myeloid Leukemia: *A review. Cell Mol Biol.* 2018, 64:58–63. 10.14715/cmb/2018.64.9.9

[31] Xie H, Peng C, Huang J, Li BE, Kim W, Smith EC, Fujiwara Y, Qi J, Cheloni G, Das PP, et al. Chronic Myelogenous Leukemia- Initiating Cells Require Polycomb Group Protein EZH2. *Cancer Discov*. 2016, 6:1237–1247. doi: 10.1158/2159-8290.CD-15-1439. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

[32] Scott MT, Korfi K, Saffrey P, Hopcroft LEM, Kinstrie R, Pellicano F, Guenther C, Gallipoli P, Cruz M, Dunn K, et al. Epigenetic Reprogramming Sensitizes CML Stem Cells to Combined EZH2 and Tyrosine Kinase Inhibition. *Cancer Discov.* 2016, 6:1248. doi: 10.1158/2159-8290.CD-16-0263. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

[33] Hughes A, Yong ASM. Immune effector recovery in chronic myeloid leukemia and treatment-free remission. *Front Immunol.* 2017, 8:1–12. 10.3389

[34] Mukaida N, Tanabe Y, Baba T. Chemokines as a conductor of bone marrow microenvironment in chronic myeloid leukemia. *Int J Mol Sci.* 2017, 18:E1824. 10.3390/ijms18081824

[35] Zhang B, Ho YW, Huang Q, Maeda T, Lin A, Lee SU, et al. Altered microenvironmental regulation of leukemic and normal stem cells in chronic myelogenous leukemia. *Cancer Cell*. 2012, 21:577–92. 10.1016/j.ccr.2012.02.018

[36] Brück O, Blom S, Dufva O, Turkki R, Chheda H, Ribeiro A, et al. Immune cell contexture in the bone marrow tumor microenvironment impacts therapy response in CML. *Leukemia*. 2018, 32, p. 1643-1656. doi: 10.1038/s41375-018-0175-0.