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Structure and Dynamics of the ABL1 Tyrosine Kinase and Its Important Role in Chronic Myeloid Leukemia

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ABSTRACT

Abelson (ABL1) tyrosine kinase is an essential component of non-receptor tyrosine kinases and is associated with numerous cellular processes, including differentiation and proliferation. The structural features of ABL1 include a distinct N-terminal cap region, a C-terminal tail, a bilobed kinase, SH2, and SH3 domains. These domains enable its engagement in several signaling cascades and dynamic control. The pathophysiology of chronic myeloid leukemia (CML) is mainly driven by the BCR-ABL1 oncoprotein, arising from dysregulation of ABL1 kinase, namely through its fusion to the breakpoint cluster region (BCR) gene. ABL1 is a crucial target in the treatment of CML as the BCR-ABL1 fusion causes uncontrolled cellular proliferation and resistance to apoptosis. Tyrosine kinase inhibitors (TKIs) targeting the ABL1 tyrosine kinase are playing a critical role in the treatment of CML through the inhibition of persistently activated signaling pathways mediated by the BCR-ABL1 fusion protein. The article examines the structural characteristics of ABL1, how they relate to CML, and the interactions between ABL1 and the current FDA-approved TKIs, emphasizing the kinase's critical function in carcinogenesis and its possible target status for tyrosine kinase inhibitors.

1 | Introduction

The Abelson (ABL) family belongs to the non-receptor tyrosine kinases and consists of two members: c-ABL (ABL1) [1] and Arg (Abl-related gene, ABL2) [2]. These kinases are integral to the regulation of various cellular processes, including cellular stress responses [3], proliferation, differentiation [4], survival, and cytoskeletal reorganization [5]. Structurally, ABL2 shares more than 90% sequence homology with ABL1 across several domains [3], indicating a high degree of conservation and suggesting functional similarities between these kinases [6]. Both of them, like the majority of the non-receptor tyrosine kinases [7], share Src homology-2 (SH2) domain and Src homology-3 (SH3) domain [8]; a proline-rich motif (PxxP) and long C-terminal tail [2]. Beyond these shared structures with ABL2, ABL1 distinguishes itself with additional unique structural features including a bilobed kinase domain (KD), which is also named as SH1 domain, N-terminal cap region, DNA binding domain, G-actin binding domain, nuclear localization, and nuclear export signals. These specialized domains are

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crucial for the nuclear and cytoplasmic functions of ABL1 [7], enabling its involvement in diverse signaling pathways that govern cellular homeostasis and response to external stimuli (Figure 1A) [2, 9].

The ABL1 KD exhibits a bilobal structure characterized by a myristoylated N-lobe, which includes a glycine-rich phosphate-binding loop (P-loop). This loop facilitates the catalytic activity of the kinase and is essential for ATP binding. The N-lobe also contains the αC helix, a critical structural element that can adopt both active and inactive conformations [10], thus contributes to the regulating activity of the kinase [11]. The C-lobe, on the other hand, functions primarily as the substrate-binding site. Positioned between these lobes is the active site of the KD [12], encompassing the activation loop (A-loop) and the ATP-binding pocket, both crucial for catalytic function [13]. SH1 possesses kinase activity and mediates the kinase autophosphorylation upon ATP binding. Tyr412, an essential autophosphorylation site in ABL1 [14], is located within the a-loop and is crucial for switching the protein between its active and inactive conformations [2]. ABL1 activity is tightly regulated by autoinhibition, which is mainly mediated by the SH2 domain and SH3 domain [2, 15]. These domains interact to form an SH2–SH3 clamp structure that maintains the kinase in an inactive conformation [1, 15]. The N-terminal cap region of ABL1 is present in two isoforms: 1a, lacking a myristoyl group, and 1b, modified by covalently attaching a C14 myristoyl group. The presence of the myristoyl group in the 1b isoform has a dual regulatory role [16], influencing both kinase activity and membrane localization [6]. The myristoyl group stabilizes [16] the inactive state of protein through binding the allosteric hydrophobic pocket of the KD [1, 17]. The C-terminal tail of ABL1, encoded by the terminal exon of the ABL1 gene, is multifunctional, containing domains that facilitate DNA binding as well as interactions with both G-actin and F-actin. This region is crucial for the nuclear-cytoplasmic shuttling of ABL1 [18, 19], thereby regulating its participation in cytoskeletal reorganization and nuclear processes [20]. In normal cell conditions, ABL1 is both present in the nucleus and the cytoplasm [21, 22]. Depending on its subcellular location, the ABL1 influences different downstream signaling pathways [22] and different physiological functions [23]. Under normal

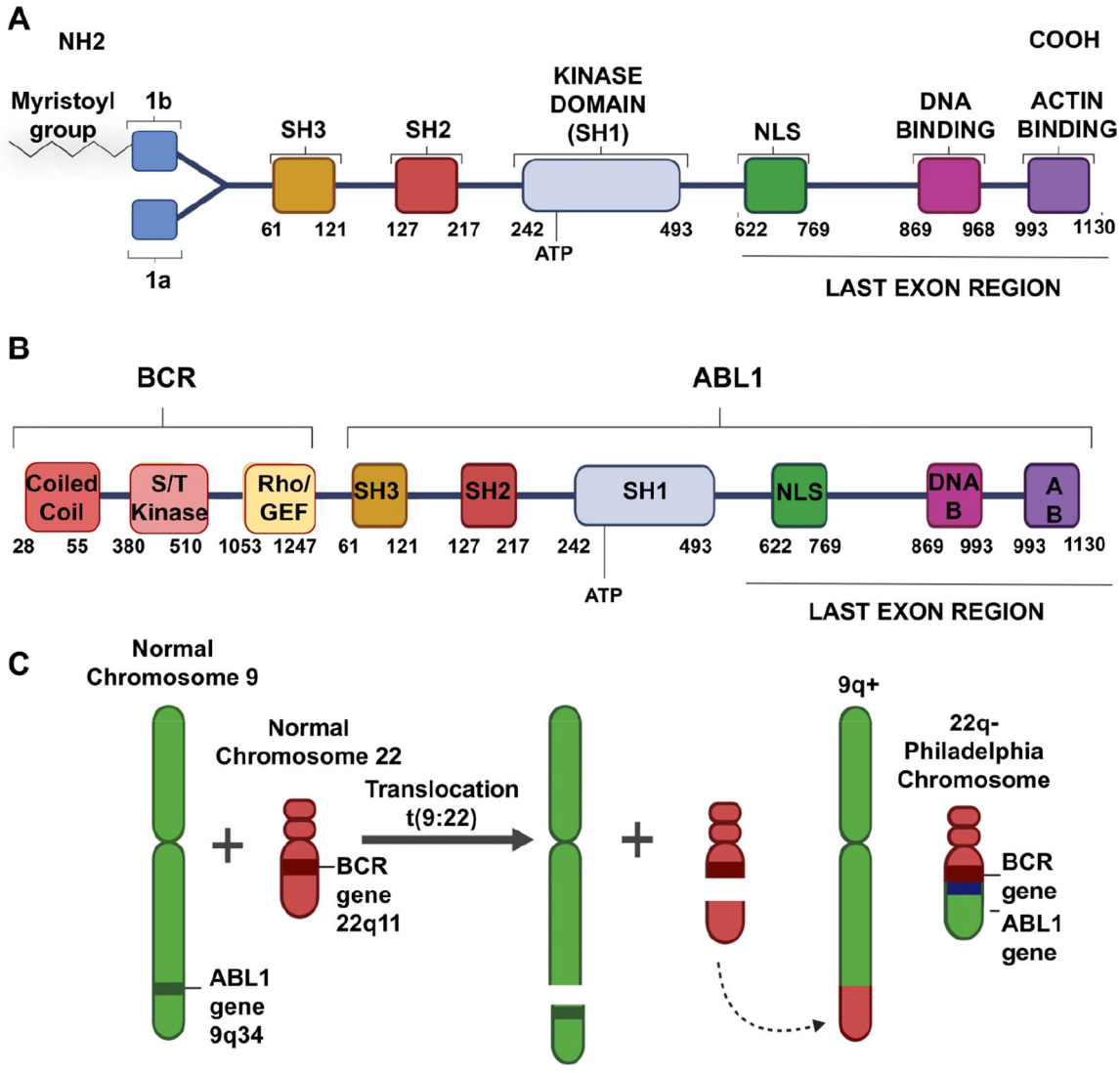


FIGURE 1 | (A) Structural organization of ABL1 1a and 1b isoforms. (B) Structural organization of BCR-ABL1 fusion protein. (C) The schematic representation of BCR-ABL translocation. Created via BioRender.

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cellular conditions, ABL1 remains in an inactive state [24], tightly regulated to prevent aberrant activity [5, 21]. Activation of ABL1 typically occurs through autophosphorylation or interaction with other proteins in responding to specific cellular stimuli [5, 24], like DNA damage [19]. Dysregulated ABL1 kinase activity has been implicated in the pathogenesis of several diseases, including chronic myeloid leukemia (CML) [5], diabetes, neurological disorders, and Niemann-Pick type C disease [23].

CML is a hematologic malignancy classified as a myeloproliferative disorder [25] and is hallmarked by the uncontrolled proliferation of myeloid granulocytic cells in the bone marrow [26]. According to the criteria of the European Leukemia Network (ELN) and MD Anderson Cancer Center (MDACC) [13], CML has three phases [27]: chronic (KP), accelerated phase (AP), and blast crisis (BC) [28]. Increasing percentages of blasts and cytogenetic accumulations characterize each phase. According to the World Health Organization (WHO) criteria, the presence of 10%–19% blasts in peripheral blood or bone marrow defines AP, while ≥ 20% blasts indicate BC [29, 30]. Although most patients are diagnosed with CP, some cases may progress directly to BC [29, 30]. Clinical symptoms are often nonspecific, with fatigue, fever, and weight loss being common due to anemia and splenomegaly [27, 30]. In patients progressing to BC, more severe symptoms such as bone pain and bleeding may occur [27, 30]. However, approximately half of CP patients are asymptomatic and are diagnosed incidentally through routine blood tests [27, 30].

The CML is linked to abnormal ABL1 activity of BCR-ABL1 fusion protein (Figure 1B) that arises from the reciprocal translocation between ABL1 gene on chromosome 9 with the breakpoint cluster region (BCR) gene on chromosome 22 t (9;22)(q34;q11) in bone marrow cells in 95% of CML cases (Figure 1C). The BCR-ABL1 oncoprotein possesses constitutive tyrosine kinase activity [31, 32], driving oncogenic signaling pathways promoting uncontrolled cell proliferation and resistance to apoptosis [9, 33]. BCR-ABL1 oncogene has three isoforms, p190, p230, and p210 [11, 13]. The p190 isoform, which has a shorter BCR segment compared with p210 [13], is predominantly observed in Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL) and is related to a worse prognosis [34], resulting in a more aggressive disease course [35]. The p230 isoform, which has a longer BCR segment compared with p210 and p190 [13], is associated with a rarer and a more benign course called neutrophilic CML [36, 37]. Having a longer BCR fragment may modulate kinase activity, thereby making the disease less aggressive. The p210 isoform is centrally involved to the pathogenesis of CML [13, 38] and is the predominant driver of the disease in most patients [28, 35]. The loss of regulatory sequences and autoinhibition in the p210 isoform leads to constitutive autophosphorylation of the ABL1 KD. This deregulation triggers aberrant downstream signaling pathways, including the JAK2/STAT pathway, the RAS/RAF/MEK/ERK pathway [39], and the PI3K/AKT/mTOR pathway [40], all of which contribute to leukemogenesis by promoting cellular proliferation and inhibiting apoptosis [41]. Given that CML is primarily driven by the p210 isoform, which enhances ABL1 kinase activity, ABL1 has become a critical therapeutic target. Tyrosine kinase inhibitors (TKIs) have been developed to specifically inhibit the constitutive kinase activity of the BCR-ABL1 oncoprotein [13], offering a targeted approach to managing CML and improving patient outcomes [28, 42].

Over the past two decades, clinical and translational biomedical research has led to significant advancements in the treatment of CML [43, 44]. Globally, CML is an uncommon hematological cancer with yearly incidences that vary by nation and range from 0.4 to 1.75 cases per 100,000 [43, 44]. Modern treatments have led to a large increase in patient life expectancy and a steady rise in the prevalence of CML [43, 44]. According to current estimates, there are 10–12 cases of CML for every 100,000 people worldwide [45–47]. As people age, the disease's incidence rises and peaks, particularly in the 65–74 age range [46]. These statistics are crucial for comprehending the epidemiological features and worldwide burden of CML [46]. The life expectancy of patients with CML is now nearly equivalent to that of healthy people because of recent advancements in treatment [48]. As a result, the death rate from CML has dramatically dropped [48]. For instance, the mortality rate with CML has dropped to 1%– 2% from roughly 10%–20% in the early 2000s [48]. The use of targeted medicines, particularly TKIs, is credited with this progress [48].

2 | Enlightened Structures of ABL1

The ABL1 gene encodes ABL1 tyrosine kinase protein, which is associated with various cellular processes and consists of multiple domains that play complex and diverse roles [11, 20]. It is essential to comprehend the structural features of ABL1, specifically the KD, SH2 and SH3 domains [13], the N-terminal cap region (Figure 2), and its function in the BCR-ABL1 fusion protein [11], To comprehend how mutations and changes can cause illness [27], especially CML [49]. The complex interplay between these domains determines the activity of ABL1 [13], and changes in its structure, such as those seen in the

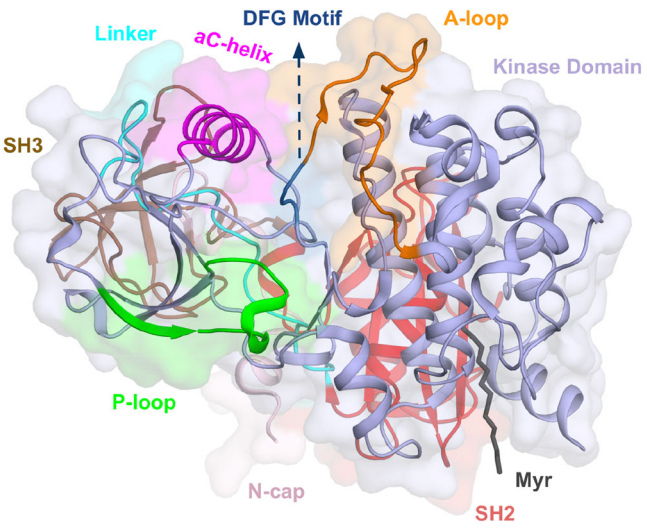


FIGURE 2 | Crystal structure of ABL1 (1b) core in the inactive conformation with N-cap presented in pink, SH3 in brown, SH2 in red, linker in cyan, kinase domain in light purple, P-loop in green, αC-helix in magenta, DFG-motif in blue, A-loop in orange, and myristoylation group in gray ($ABL1^{DOMAINS}$, PDB: 2FO0).

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BCR-ABL1 fusion protein [9], may lead to constitutive activation of ABL1, driving oncogenesis [33].

2.1 | ABL1 KD

The ABL1 kinase exhibits a bilobal architecture characteristic of many kinases [11], this structure consists of a smaller N-terminal lobe and a larger C-terminal lobe [50], with an activation loop (A-loop) situated between these lobes [51]. The relative orientation and interaction between the N-terminal and C-terminal lobes dictate the conformational state of the kinase [11], allowing it to switch between active and inactive forms [51].

N-terminal lobe consists of a five-stranded antiparallel β-sheet and a single α-helix called αC helix. The β1- and β2-strands within the N-terminal lobe are connected by a glycine-rich loop (GRL), also referred to as the P-loop [52]. This P-loop plays a critical role in binding phosphate groups, facilitating the interaction of the kinase with ATP. The protein's transition between active and inactive states is modulated by the orientation of the αC helix. The salt bridge between the positively charged Lys271 residue in the β3-strand coordinates and the negatively charged glutamate residue in the middle of the αC helix (Glu286) [51] coordinates the phosphorylation of the kinase turns ABL1 kinase in catalytically active form [53, 54]. In the lack of this salt bridge, the ABL1 is found in the inactive state [7]. This mechanism of activation is conserved in the structure of virtually all protein kinases [11, 20]. The activation loop (A-loop) within the ABL1 kinase is a pivotal regulatory element controlling ATP binding site accessibility. Within this loop lies the highly conserved aspartate-phenylalanine-glycine (DFG) motif, encompassing residues 381–383 [55]. The orientation of the aspartate (D) and phenylalanine (F) in the DFG motif is critical in determining the kinase's conformational state [56–58]. In the active state, the A-loop adopts an open conformation, termed the DFG-in conformation. In this conformation, the aspartate residue faces the ATP-binding site, facilitating the binding of Mg^{2+} [59], which is crucial for catalytic activity [60]. The phenylalanine residue, meanwhile, is sequestered within a hydrophobic pocket, stabilizing the active state. Conversely, in the inactive state, the A-loop adopts a closed conformation [59], known as the DFG-out conformation [61]. In this state, the aspartate and phenylalanine residues are reoriented, which disrupts the coordination necessary for Mg^{2+} binding and prevents ATP from accessing its binding site. This conformational shift effectively locks the kinase in its inactive state [11, 20], preventing unwanted or premature activation of the kinase's catalytic functions [13].

Upon activation, ABL1 kinase undergoes conformational rearrangements especially in key elements of the N-terminal lobe and a-loop. In the active state, the p-loop is found in an elongated structure that allows ATP binding while αC-helix and a-loop are found in open/in conformation that facilitates substrate binding (Figure 3A). Whereas, in the inactive state, P-loop is found in a more concentrated and compact structure while αC-helix and A-loop are found in closed/out conformation that blocks substrate binding (Figure 3B) [62].

In addition to the active and inactive conformations of ABL1, a third intermediate conformation of ABL1 is discovered in which the A-loop is present in the compact form, blocking the binding of substrate while the DFG motif is found in the DFG-in conformation (Figure 3C). Due to the similarity of this intermediate conformation of ABL1 with inactive Src KD of c-Src, it is called "Src-like inactive conformation." In the Src-like inactive conformation of ABL1, αC-helix protrudes the active site and creates an extended ATP-binding pocket [13] mediating the flipping of the DFG motif to DFG-in conformation [1].

The dynamic regulation of ABL1 kinase through the structural interplay between its N-terminal and C-terminal lobes, as with the conformational flexibility of the A-loop and DFG motif [1], highlights the sophisticated control mechanisms that govern its activity [13, 62]. Understanding these molecular details is critical for developing of targeted therapies, especially in disease CML where dysregulated ABL1 activity plays a central role in pathogenesis.

2.2 | SH2 & SH3 Domains

ABL1's SH2 and SH3 domains act as a clamp [11, 13], and maintain the inactive state of the KD [63, 64]. In the autoinhibition of ABL1, the SH2 domain binds to the distal side of the C-terminal lobe of the kinase with a dense hydrophobic interaction network [11, 13], and this binding restricts the movement of the C-lobe [49]. The SH3 domain prevents the activation of ABL1 by connecting with the N-terminal region of the kinase [11, 13, 49]. SH3 recognizes proline-rich regions and forms an interaction between the SH2 domain and the KD [11], the SH2-kinase binding region (Linker region) [13], which is important for protein conformations [49, 63]. The connector region, which is the region that connects the SH2 and SH3 domains [13, 63], is an important element that regulates the flexibility and stability of the protein [11, 65]. This allows the domains to remain stable on the KD, ensuring [11, 63] that the ABL1 KD is in its inactive state [13, 65]. For ABL1 to function in its active form [13, 66], clamp acting SH2-3 domains must be released [65]. The SH2 domain attached to the C-terminal lobe of the kinase binds to phosphorylated tyrosine residues of ABL1 substrates [13, 64], dissociating from the lobe, and localizes to the N-lobe [11, 49]. Repositioning of ABL1 results in a new conformation, reducing autoinhibition. Phosphorylation of the Tyr-245 residue in the SH2-kinase binding site [11] may prevent binding of the SH3 domain [49], thereby activating ABL1 [64]. The inflexible SH2-SH3 clamp is taken off of ABL1 in both situations [13], enabling substrate phosphorylation through SH2- or SH3-processing [64, 65].

2.3 | N-Terminal Cap Region

According to having N-terminal myristoylation site, two forms of ABL1: 1a and 1b exist [26]. Myristoylation is a critical lipid modification occurring in the N-terminal region of the ABL1 protein [13, 67]. This critical lipid modification acts as an autoinhibitory mechanism by covalently binding to the myristoyl pocket in the C-lobe of the ABL1 KD [26] and altering the organization of the SH3 domain [13, 68–70], therefore keeping the activity of ABL1 under control [68, 69]. The N-terminal

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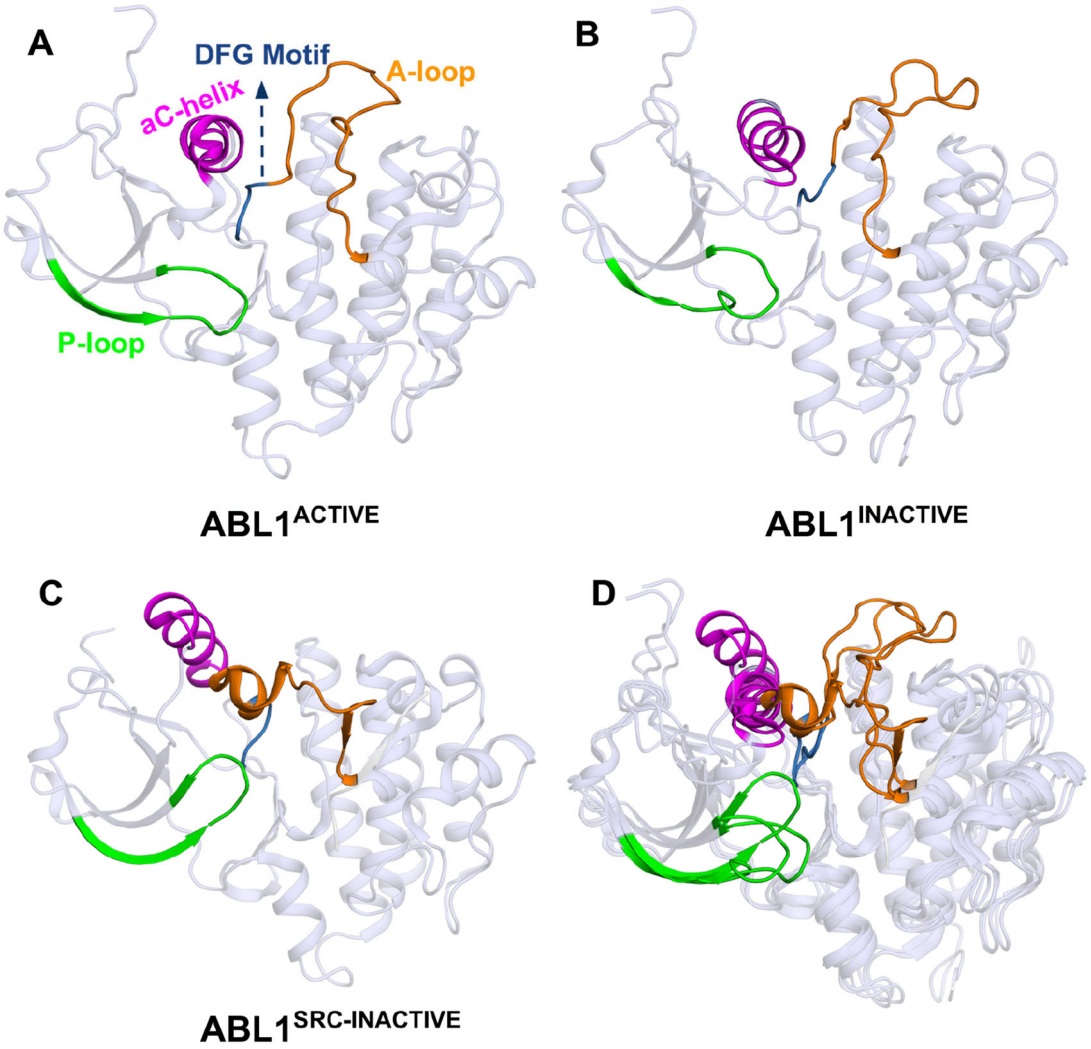


FIGURE 3 | ABL1 kinase domain in different conformations. (A) ABL1 in active (DFG-in) conformation with the P-loop is represented in green, αC-helix in magenta, DFG-motif in blue, A-loop in orange ($ABL1^{ACTIVE}$, PDB: 6XR6). (B) ABL1 in inactive (DFG-out) conformation ($ABL1^{INACTIVE}$, PDB: 6XR7). (C) ABL1 in Src-inactive conformation ($ABL1^{SCR\text{-}INACTIVE}$, PDB: 2G1T). (D) The superposition of the active, inactive, and src-inactive conformations of ABL1 kinase domain.

myristoylation group facilitates the binding of the SH2 domain to the distal side of the kinase's C-terminal lobe via a dense hydrophobic interaction network [13], which induces a 90° bend in the center of the α1 helix located in the C-terminal region [20, 71]. This conformational reorientation accounts for the transition of the kinase to the inactive, auto-inhibited state [13, 26]. The region that facilitates the binding of the myristoylation group to the C-lobe by combining with the SH3 domain is the cap region [13], which ensures tight binding of the SH2 and SH3 domains to ABL1 [68, 69] and also contributes to the formation of the inhibitory structure of the SH2 and SH3 domains [2, 70].

Exons 1 A and 1B help to understand the structural features of the protein by determining which region of the BCR gene the ABL1 gene is inserted into [72, 73]. As distinct exons, 1 A and 1B generate different BCR::ABL1 isoforms [72]. Differences between isoforms may reflect differences in the activities of the BCR::ABL1 fusion protein. For instance, some isoforms may exhibit higher kinase activity [7], while others may affect different signaling pathways and result in differences in the clinical features of diseases [26, 72].

2.4 | BCR-ABL1 Fusion Protein

The BCR-ABL1 fusion protein has some additional regions from the BCR gene in addition to the ABL1 gene [9, 13]. Additional domains from the BCR gene cause the fusion protein to stay continuously active [11, 13], whereas conserved parts of the ABL1 gene, including as the SH2, SH3, and KDs [6], direct signaling events [49]. These additional regions include a coiled-coil region at the N-terminus of BCR that enhances dimerization and oligomerization of BCR-ABL1 [13], resulting in the protein being continuously active [70–74]. The BCR-ABL1 tetramerization is notably mediated by this coiled-female domain [13], suggesting that ABL is activated by proximity-induced trans-phosphorylation and prolonged activation of Y393 residue in the A-loop [75]. BCR-ABL1's oligomeric state causes it to be very active [13], and this high activity increases the virulence of the protein [76]. A major difference between ABL1 and BCR-ABL is the differences in the activation loop and DFG motif locations [11, 20]. ABL1's activation loop is constantly in the open position in BCR-ABL1 [11, 13], however it is closed while the loop is inactive [20], BCR-ABL can bind ATP to

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this open location [13], leading to prolonged tyrosine phosphorylation [20] and faulty activation of intracellular signaling pathways [11, 67] The ABL1 C-terminal region includes a DNA binding domain and actin binding domains, and these domains regulate the cellular localization and nucleus-cytoplasmic transport of ABL1 [20]. Nevertheless, the BCR-ABL1 fusion protein's cellular location and function are greatly impacted by the loss of certain C-terminal regulatory domains [5]. In normal ABL1, the kinase activity is regulated by the SH3 and SH2 domains through autoinhibition, whereas in BCR-ABL1, this autoinhibition mechanism is disrupted by losing the N-terminal cap region and its replacement by BCR sequences. As a result, BCR-ABL1 functions as a continuously active tyrosine kinase [11, 13], leading to uncontrolled cell proliferation [6, 20].

2.5 | BCR-ABL1 TKIs

2.5.1 | FDA-Approved TKIs

In current clinical practice, there are six TKIs approved by the FDA for the treatment of CML, categorized according to their generation and mechanism of action (Table 1). These TKIs are divided into three generations:

- First generation TKI: imatinib (Figure 4);
- Second generation TKIs: dasatinib (Figure 4), nilotinib (Figure 4), bosutinib (Figure 4);
- Third generation TKIs: ponatinib (Figure 4), asciminib (Figure 4).

Imatinib, nilotinib, dasatinib, bosutinib, and ponatinib are ATP-competitive inhibitors binding to the catalytic pocket of the ABL1 KD (Figure 5A) [77, 78]. Specifically, imatinib, nilotinib, and ponatinib bind to the ATP-binding site of BCR-ABL1 [79, 80] when it is in the DFG-out conformation, effectively competing with ATP for binding [81]. In contrast to imatinib and nilotinib, dasatinib binds to the ATP-binding site of BCR-ABL1 [82–84] when it is in the DFG-in conformation [82, 83]. Bosutinib is a more versatile inhibitor that can bind to the catalytic pocket of BCR-ABL1 in both the DFG-in and DFG-out conformations. This ability to bind in multiple conformations enhances their efficacy against a broader range of ABL1 conformations [82], including those found in resistant variants of BCR-ABL1 [84]. Asciminib, a third-generation TKI, employs a unique mechanism distinct from other TKIs (see the Supporting Information). It functions as an allosteric negative element that selectively binds the ABL1 myristoyl pocket instead of the ATP-binding pocket (Figure 5A) [78, 80].

2.6 | First-Generation TKIs

Imatinib is the first FDA-approved TKI used to treat cancer, CML [85]. It binds the ATP-binding pocket of the BCR-ABL1 kinase between the αC helix and A-loop and blocking ABL1 catalytic activity (Figure 5B). Imatinib recognizes the inactive BCR-ABL1 and after binding to the protein [13, 79], it adopts a closed conformation of the A-loop blocking substrate binding

TABLE 1 | FDA-approved BCR-ABL1 TKIs.

FDA-approved BCR-ABL1 TKIs	FDA approval year	Generation	Inhibitory mode of action	BCR-ABL1 tyrosine kinase binding conformation	Structure with BCR-ABL1 (PDB code)
Imatinib	2001	1st Generation	ATP-competitive	Inactive	$ABL1^{IM}$ (2HHY) $ABL1^{IM,\ DAS}$ (7N9G)
Dasatinib	2006	2nd Generation	ATP-competitive	Active	$ABL1^{IM,\ DAS}$ (7N9G)
Nilotinib	2007	2nd Generation	ATP-competitive	Inactive	$ABL1^{NIL,\ ASC}$ (5MO4)
Bosutinib	2012	2nd Generation	ATP-competitive	Both	$ABL1^{BOS}$ (3UE4)
Ponatinib	2012	3rd Generation	ATP-competitive	Inactive	—
Asciminib	2021	3rd Generation	Allosteric Inhibitor	Myristoyl pocket	$ABL1^{NIL,\ ASC}$ (5MO4)

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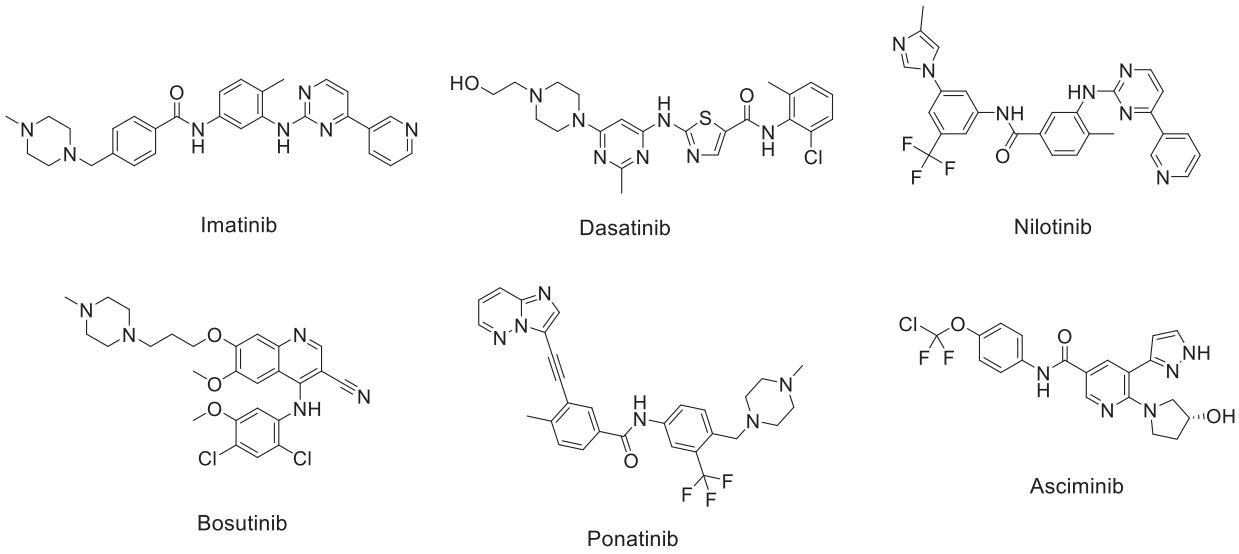


FIGURE 4 | The chemical structures of imatinib, dasatinib, nilotinib, bosutinib, ponatinib and asciminib.

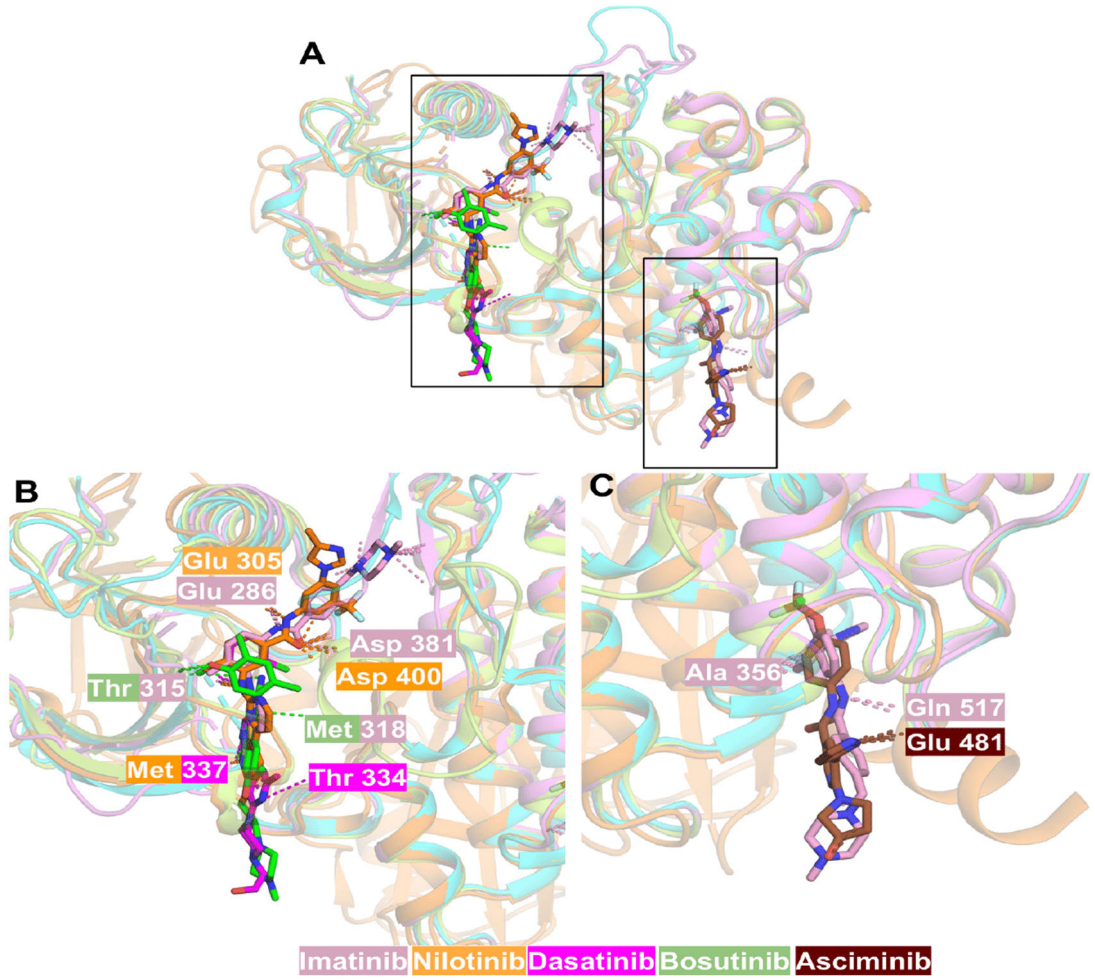


FIGURE 5 | The binding mechanisms of TKIs to BCR-ABL1 as $ABL1^{IM}$ is represented in lime, $ABL1^{IM,\ DAS}$ is represented in pink, $ABL1^{NIL,\ ASC}$ is represented in orange and $ABL1^{BOS}$ is represented in cyan. (A) The superposition of the binding mechanisms of imatinib (pink), nilotinib (orange), dasatinib (magenta), bosutinib (green), and asciminib (brown) to the BCR-ABL1. (B) The interaction between BCR-ABL1 tyrosine kinase and the TKIs imatinib (yellow), nilotinib (orange), dasatinib (magenta), and bosutinib (green). (C) The interaction between BCR-ABL1 tyrosine kinase and the TKIs imatinib (pink) and asciminib (brown).

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while locking the protein in the DFG-out conformation. It also locks the protein in the DFG-out conformation [85, 86] Hydrogen bonding and van der Waals interactions stabilize the imatinib–ABL1 interaction [87, 88]. Beyond inhibition, recent studies in 2021 have shown that imatinib binds to the myristoyl pocket of BCR-ABL1, thereby allosterically activating the KD (Figure 5C) [86].

Although Imatinib is initially effective, Imatinib resistance arises in approximately 15%–20% of CML patients because of BCR-ABL1 mutations that obstruct imatinib binding [87, 88]. Point mutations in the BCR-ABL1 fusion gene are the most common reason of TKI resistance [89]. Within these point mutations, the T315I mutation in the nucleotide binding site of ABL1 (Figure 6A) is the most common mutation [8] and detected in 3%–15% of imatinib-resistant CML patients [90]. The substitution of hydrophilic threonine residue with hydrophobic Isoleucine residue (Figure 6B) prevents the binding of imatinib by interrupting hydrogen bonding between the imatinib and BCR-ABL1. In addition to the disrupted hydrogen bonding [90], the conformational change in the structural cavity of the enzyme centering the mutated residue (Figure 6A) is another reason that blocks the imatinib binding in T315I mutation [91].

2.7 | Second-Generation TKIs

Second-generation TKIs, including dasatinib, nilotinib, and bosutinib, have been developed to address imatinib resistance in CML patients [92]. These agents are effective against most imatinib-resistant mutations, though they remain ineffective against the T315I mutation [88]. Nilotinib binds ABL1 in its inactive form with higher affinity than imatinib as a result of extended hydrogen bonding and van der Waals interactions [13, 93]. Unlike previous TKIs, dasatinib binds to the ATP-pocket of BCR-ABL1 in DFG-in conformation [13, 82]. Dasatinib inhibits BCR-ABL1 kinase activity by forming the hydrophobic pocket, which is more favored in the active form of the protein. Dasatinib extends in the reverse orientation of the imatinib and makes numerous hydrogen bonds and van der Waals interactions with the BCR-ABL1 hinge region [13]. Bosutinib can bind to both DFG-in and DFG-out conformations of the ABL1 [84, 93]. The binding mechanism of bosutinib to the ATP-pocket of BCR-ABL1 is similar to the binding profile of the dasatinib except from the interaction of bosutinib with T315 residue (Figure 4B). Differently from the dasatinib, bosutinib binds T315 residue via multiple van der Waals interactions. However, these interactions are disrupted in T315I mutation and makes bosutinib ineffective against T315I mutation [13].

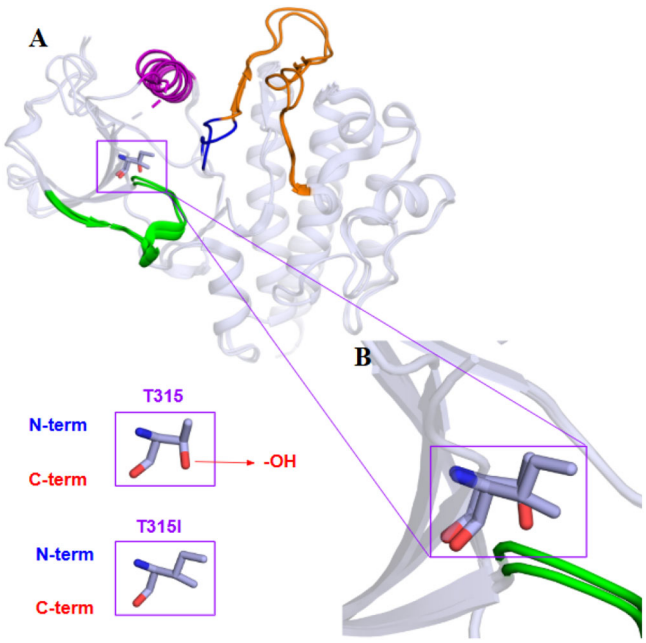


FIGURE 6 | Comparison of the kinase domain structures of WT-ABL1 ($ABL1^{T315}$, PDB: 4WA9) and the T315I mutant ($ABL1^{T315I}$, PDB: 4TWP). (A) Superposition of inactive $ABL1^{T315}$ and active $ABL1^{T315I}$, highlighting key structural elements: the P-loop (green), αC-helix (magenta), DFG-motif (blue), and A-loop (orange). (B) Detailed view of the amino acid structures at the 315th residue in $ABL1^{T315}$ and $ABL1^{T315I}$.

2.8 | Third-Generation TKIs

Third-generation TKIs, the ATP-competitive ponatinib and the allosteric asciminib, have been designed to overcome resistance mechanisms observed with earlier generations [88], including the T315I mutation [87, 94]. As seen in prior generations of TKIs, pan inhibitor ponatinib competes with ATP to bind to the BCR-ABL1 kinase ATP pocket when its in DFG-out, inactive conformation [81]. The efficiency of ponatinib against T315I mutation is based on the van der Waals interactions of ethynyl linker group in the ponatinib with T315I residue rather than hydrogen bonding [13]. Asciminib represents a novel approach as the only FDA-approved allosteric inhibitor for CML. It binds selectively to the ABL1's myristoyl pocket (Figure 4C) instead of the ATP-binding site [5], inducing conformational changes that convert ABL1 into an inactive state and reduce its kinase activity [95–97].

Overall, the development and the use of these TKIs have led to significant advances in the management of CML, though challenges remain, particularly in addressing resistance mutations and optimizing therapeutic efficacy. Still, novel approaches for the treatment of CML are required to overcome these challenges.

2.8.1 | TKIs Currently in Clinical Trials

Beyond the FDA-approved TKIs, several BCR::ABL1 inhibitors currently in clinical trials are showing promise as potential treatment options for CML. Some of the key inhibitors under clinical trials include flumatinib (Figure 7), olverembatinib (Figure 7), vodobatinib (Figure 7), vamotinib (PF-114) (Figure 7), rebastinib (Figure 7), AT9283 (Figure 7), danusertib (Figure 7), and MK-0457 (Figure 7) (Table 2) [98].

2.9 | Flumatinib

Flumatinib is a second-generation TKI derived from imatinib [99, 100]. It was approved by the National Medical Products Administration in 2019 as a first-line treatment for CML. The

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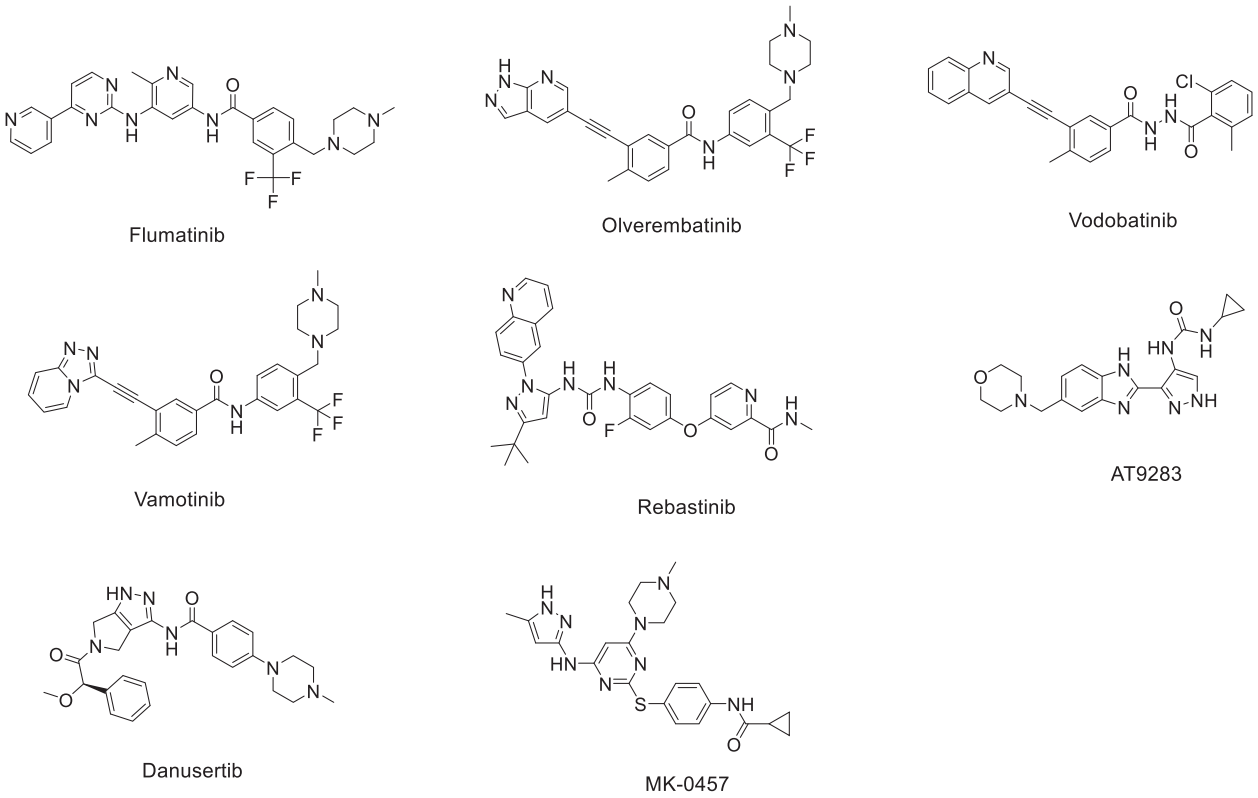


FIGURE 7 | The chemical structures of flumatinib, olverembatinib, vodobatinib, vamotinib, rebastinib, AT9283, danusertib, and MK-0457.

TABLE 2 | Current clinical trials for BCR-ABL1 TKIs.

Inhibitors	ClinicalTrials. gov number	Clinical trial phase
Flumatinib	NCT04677439, NCT04933526, NCT05353205, NCT02204644	IV
Olverembatinib (HQP1351)	NCT05376852, NCT05311943, NCT03883100, NCT03883087, NCT0412668, NCT04260022	II/III
Vodobatinib (K0706)	NCT02629692	I/II
Vamotinib (PF-114)	NCT02885766	I/II
Rebastinib (DCC-2036)	NCT00827138	I
AT9283	NCT01431664, NCT00522990	I/II
Danusertib (PHA-739358)	NCT00335868	II
MK0457	NCT00405054	II

BCR::ABL1 inhibition mechanism of flumatinib is based on the strong hydrophobic interactions between its unique pyridine and trifluoromethyl groups and hydrophobic residues in ABL kinase [100]. Compared with imatinib, flumatinib has greater selectivity and stronger potency against the BCR::ABL1 kinase, resulting in improved effectiveness and fewer side effects [98, 100]. According to a recent phase III research, among patients with newly diagnosed CML-CP, flumatinib is more effective than imatinib and has a lower incidence of adverse effects [100].

2.10 | Olverembatinib

Olverembatinib (HQP1351) is a third-generation TKI targeting several BCR::ABL1 kinase isoforms, including the T315I mutation [98, 101]. As clinical studies suggest, it is effective in patients who developed resistance to previous TKIs. Phase I trials demonstrate the minimal interaction of olverembatinib with other kinases, which reduces the potential side effects and increases its selectivity [101]. These results suggest olverembatinib as an effective treatment option for CML.

2.11 | Vodobatinib

Vodobatinib (K0706) is another promising third-generation TKI that targets both wild-type and mutant BCR::ABL1 isoforms. With the exception of the T315I mutation, vodobatinib has shown notable effectiveness against a variety of BCR::ABL1 mutations in vitro [98]. Clinical studies [102] suggest vodobatinib as a novel treatment option for CML patients who have become resistant to earlier-generation TKIs.

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2.12 | Vamotinib (PF-114)

As a structural analog of ponatinib, PF-114 is a fourth-generation TKI that has increased selectivity as a result of the presence of triazolopyridine moiety and minimized side effects. In vitro studies have shown that PF-114 is highly effective against wild-type BCR::ABL1 and several mutant isoforms, including T315I. Its enhanced selectivity for ABL kinase positioning PF-114 as a promising next-generation therapy for CML patients [103].

2.13 | Rebastinib

Rebastinib (DCC-2036) is a unique "switch control" inhibitor targeting multiple tyrosine kinases, including ABL1 [98, 104, 105]. Rebastinib, in contrast to conventional ATP-competitive inhibitors, binds to the switch control pocket of ABL KD and blocks the conformational change of the protein required for its activation. This unique binding mechanism of rebastinib maintains the kinase in an inactive state [104, 105], even in the presence of T315I mutation [98], and highlights rebastinib as a new treatment option.

2.14 | Aurora Kinase Inhibitors

AT9283 [106], danusertib, and MK-0457 [107] are multi-targeted aurora kinase inhibitors that have the potential to treat CML particularly in patients who have developed resistance to traditional TKIs. Inhibition of Aurora kinases (Aurora A, Aurora B, and Aurora C) has proven effective in treating hematologic malignancies such as CML [106]. AT9283 effectively inhibits key kinases including Aurora A, Aurora B, and ABL1. Its broad kinase inhibition profile suggests it could be beneficial for CML patients who no longer respond to first-line treatments [106]. Similarly, danusertib is a potent pan-aurora kinase inhibitor that targets ABL kinase, including the T315I mutation, by binding to its ATP pocket and preventing the activation of protein [107]. MK-0457, another pan-aurora kinase inhibitor, has demonstrated significant activity against CML cells, including those with the T315I mutation [107, 108]. The potential of these multi-targeted inhibitors to be used in combination therapies opens new possibilities for improving CML treatment [109]. Together, these inhibitors offer promising alternatives for patients with TKI-resistant CML.

3 | Conclusion and Future Aspects

ABL1 has a crucial role in several cellular processes, and its kinase activity is tightly regulated under normal conditions. In CML, aberrant ABL1 kinase activity is the main driving force behind disease pathogenesis, making ABL1 a key therapeutic target. By successfully controlling ABL1's kinase activity, TKIs that target the protein have shown promise for the treatment of CML. But problems with the drug resistance and reduced effectiveness against mutations continue to be major roadblocks for TKI treatments. Therefore, novel approaches that overcome these constraints are imperative to progress the management of CML. Thus, a better understanding of the structural features of ABL1 and its interactions with TKIs may not only illuminate CML pathogenesis but also help to develop novel therapeutic strategies that are more effective and sensitive to resistance mechanisms. In this context, multidisciplinary collaborations may enable to develop new, more durable and efficient drug designs, and the integration of structural biology and clinical research may open new horizons in the treatment of CML.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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