

Revisiting Asciminib Resistance: From Binding Affinity to Allosteric Disruption in *BCR::ABL1* Leukemias

Asciminib Direncine Yeniden Bakış: *BCR::ABL1* Lösemilerde Bağlanma Afinitesinden Allosterik Bozulmaya

Sayitoğlu M. et al.: Allosteric Disruption and Asciminib Resistance

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Abstract

Tyrosine kinase inhibitors (TKIs) have reshaped the treatment of *BCR::ABL1* positive leukemias. The first-in-class STAMP (Specifically Targeting the ABL Myristoyl Pocket) inhibitor, asciminib introduces a distinct therapeutic paradigm by targeting the myristoyl pocket and avoids direct competition with ATP. However, resistance to asciminib has been reported in 4-10% of chronic myeloid leukemia (CML) cases. Asciminib resistance is predominantly driven by impaired allosteric regulations: 1) Expanded spectrum of kinase domain mutations "pre-activate" the kinase and confer resistance despite retained drug affinity; 2) Intrinsic primary resistance, from b2(e13)a3 and b3(e14)a3 transcript variants; and 3) Isoform-specific (p190 vs. p210) resistance, with common P-loop mutations exhibit greater resistance in p190 than in p210. This paradigm shift from an "affinity" problem to an "allostery" problem necessitates a corresponding evolution in clinical and laboratory practice. This includes expanding mutational screening, incorporating pre-treatment screening of the *BCR::ABL1* isoforms, and transcript types to appropriately guide therapeutic selection.

Keywords: Asciminib, *BCR::ABL1*, Chronic Myeloid Leukemia (CML), Allosteric Disruption, Drug Resistance, STAMP Inhibitor

Öz

Tirozin kinaz inhibitörleri (TKI'lar), *BCR::ABL1* pozitif lösemilerin tedavisini kökten değiştirmiştir. Sınıfının ilk örneği olan STAMP (ABL Miristoil Cebini Spesifik Olarak Hedefleyen) inhibitörü asciminib, miristoil cebini hedefleyerek farklı bir tedavi paradigması getirerek, ATP ile doğrudan rekabeti önler. Bununla birlikte, kronik miyeloid lösemi (KML) vakalarının %4-10'unda asciminibe direnç bildirilmiştir. Asciminib direnci, ağırlıklı olarak bozulmuş allosterik düzenlemelerden kaynaklanmaktadır: 1) genişlemiş spektrumda kinaz domain mutasyonları, kinazı "önceden aktive eder" ve ilaç afinitesinin korunmasına rağmen dirence neden olur; 2) b2(e13)a3 ve b3(e14)a3 transkript varyantlarından kaynaklanan birincil direnç; ve 3) Yaygın P-loop mutasyonlarına sahip izoform spesifik (p190 ve p210) direnç, p190'da p210'a kıyasla daha yüksek direnç gösterir. "Afinite" sorunundan "allosteri" sorununa doğru yaşanan bu paradigma değişimi, klinik ve laboratuvar uygulamalarında da paralel güncellemeleri gerektirmektedir. Bu güncellemeler, mutasyon taramasının genişletilmesini, *BCR::ABL1* izoformlarının ve transkript tiplerinin tedavi öncesi taramasının dahil edilmesini ve tedavi seçiminin uygun şekilde yönlendirilmesini içermektedir.

Anahtar Kelimeler: Asciminib, *BCR::ABL1*, Kronik Miyeloid Lösemi (KML), Allosterik Bozulma, İlaç Direnci, STAMP inhibitor

1. Introduction: A New Class of Inhibitor, A New Class of Resistance

The *BCR::ABL1* fusion causes constitutively active tyrosine kinase activity which is the hallmark of chronic myeloid leukemia (CML). Tyrosine kinase inhibitors (TKIs) have transformed CML from a fatal disease into a manageable chronic condition [1,2,3]. The long-term success of the TKIs, however, has been constrained by "on-target" resistance, toxicity and intolerance [4,5].

Asciminib (ABL001) is a first-in-class STAMP (Specifically Targeting the ABL Myristoyl Pocket) inhibitor, unlike currently approved ATP-competitive TKIs, targets an allosteric site—the myristoyl pocket—located in the C-lobe of the KD. (Figure 1) [6,7]. In physiological conditions, ABL1 kinase undergoes autoinhibition through N-terminal myristoylation that facilitates intramolecular binding to this pocket and stabilizes the inactive conformation [6,8,9]. In the *BCR::ABL1* fusion protein, N-terminal region motif is replaced by BCR sequences, resulting in loss of myristoyl-mediated autoinhibition and constitutive kinase activation. Asciminib mimics the missing myristoyl group and induces a conformational change that stabilizes the kinase in an inactive state [6,10]. Allosteric inhibition of ABL1 prevents the transition to the active state without competing with ATP, therefore asciminib demonstrates significant efficacy against pathogenic variants of *ABL1* KD, including T315I [11,12,13,14,15,16]. However clinical resistance to asciminib has been reported [17]. We propose that resistance cannot always be explained by impaired drug binding alone and “*allosteric disruption*” provides a more comprehensive framework for understanding asciminib resistance.

2. Beyond Binding Affinity: Resistance as Allosteric Disruption

Early studies of asciminib resistance focused on mutations in the myristoyl pocket (A337V/T, C464W, P465S, V468F, I502L, M472I) where substitutions disrupt drug binding (Figure 2) [18,19]. These variants represent the canonical mechanism of resistance, analogous to ATP-site mutations that reduce inhibitor affinity. However, experimental studies indicated that multiple resistance-associated variants arise outside the myristoyl pocket yet produce comparable reductions in drug sensitivity [20,21,22]. Representative examples include M244V, F359C/I/V variants which alter the conformation of the N-lobe and disrupt SH2 domain docking, thereby impairing the allosteric communication required for kinase autoinhibition rather than directly affecting the drug-binding site [23,24]. We propose that these variants represent a distinct category of resistance *allosteric disruption*, in which the conformational network required for asciminib-mediated inhibition is compromised [21]. Consequently, asciminib may still bind to the myristoyl pocket, but it can no longer efficiently stabilize the inactive kinase conformation. Asciminib does not bind to the ATP site, but some KD mutations (e.g., G250E, Q252H, F317L/V, E355G) destabilize the inactive conformation [24,25,26]. Allosteric disruption framework also can explain why asciminib-resistant variants substantially overlap with mutations previously associated with ATP-competitive TKIs. Instead of affecting independent binding sites, many mutations shift the equilibrium toward an active kinase state, simultaneously reducing susceptibility to both orthosteric and allosteric inhibitors. Compound mutations further reinforce this concept by combining impaired binding with defective conformational regulation, generating multidrug-resistant clones that present increasing therapeutic challenges [25,27].

3. Intrinsic (Primary) Resistance: When the Allosteric Switch is Broken from the Start

Classical *BCR::ABL1* transcripts, b2(e13)a3 and b3(e14)a3 can be contraindicated for asciminib therapy as a form of primary resistance [28,29,30]. These “a3” splice variants are characterized by the absence of *ABL1* exon 2 that lead to a non-functional SH3 domain and destabilizes the autoinhibited kinase conformation. In these patients, the SH3 “clamp” has been already broken from the start. Asciminib can still bind to the myristoyl pocket, but its allosteric signal leads nowhere [29,31]. These findings extend the concept of allosteric disruption beyond acquired mutations to features of the fusion protein itself.

An additional layer of intrinsic resistance is introduced by *BCR::ABL1* isoform diversity. *In-vitro* studies have demonstrated reduced asciminib sensitivity in p190 compared with p210, particularly in the presence of common P-loop mutations [32,33]. Accordingly, the same variant might have a different clinical meaning; a clinician treating a CML (p210) patient with a Y253H variant might consider asciminib. On the other hand, in a Ph+ ALL (p190) patient with Y253H mutation may predict resistance and therapeutic failure.

Transcript variants and isoform-specific differences support a broader interpretation of asciminib resistance. Rather than representing isolated exceptions, they demonstrate that disruption of the allosteric inhibitory network may precede treatment and determine therapeutic efficacy from the outset.

4. Clinical Implications and Recommendations

Current resistance assessment largely focuses on identifying mutations within the KD, particularly those affecting the myristoyl pocket. While essential, this strategy alone is unlikely to capture the full spectrum of clinically relevant resistance mechanisms. On the other hand, recognizing allosteric disruption as a determinant of asciminib resistance has implications for clinical practice. We propose following framework (Table 1) to categorize asciminib resistance mechanisms for clinical use.

Implication 1: Mutation Testing Should Evolve

Mutation analysis following asciminib failure must be expanded to screen for the N-lobe and allosteric interface mutations (e.g., K222E, P223H, L248V) [21,22,34,35].

Implication 2: Pre-Treatment Screening is Essential

Prior to asciminib treatment, identification of *BCR::ABL1* transcript types and fusion isoforms may provide clinically relevant. In particular, patients carrying a3 transcripts or the p190 isoform may represent increased risk of primary therapeutic failure and should be evaluated prospectively [28,33].

Implication 3: Navigating Therapeutic Sequencing and Combination Therapy

At relapse, comprehensive mutation screening is essential to guide the next treatment choice. Therapeutic combinations, such as dasatinib plus asciminib, may represent the optimal strategy to achieve deep responses and suppress the emergence of resistant clones.

5. Conclusion

The approval of asciminib has opened a highly effective therapeutic front against *BCR::ABL1*-positive leukemias [36,37]. However, this therapeutic advance has also revealed that resistance extends well beyond impaired drug binding. Recent evidence supports a broader conceptual framework in which disruption of the allosteric regulatory network represents a determinant of therapeutic failure.

Viewing resistance through the lens of allosteric disruption has important implications for precision medicine. Future diagnostic strategies should integrate expanded mutation profiling with assessment of *BCR::ABL1* transcript architecture and isoform status, while functional studies should move beyond drug-binding measurements to evaluate conformational regulation. Although prospective validation is still required, this framework provides a unifying explanation for diverse resistance mechanisms and may guide the next generation of biomarker development, therapeutic sequencing, and combination strategies for STAMP inhibitors.

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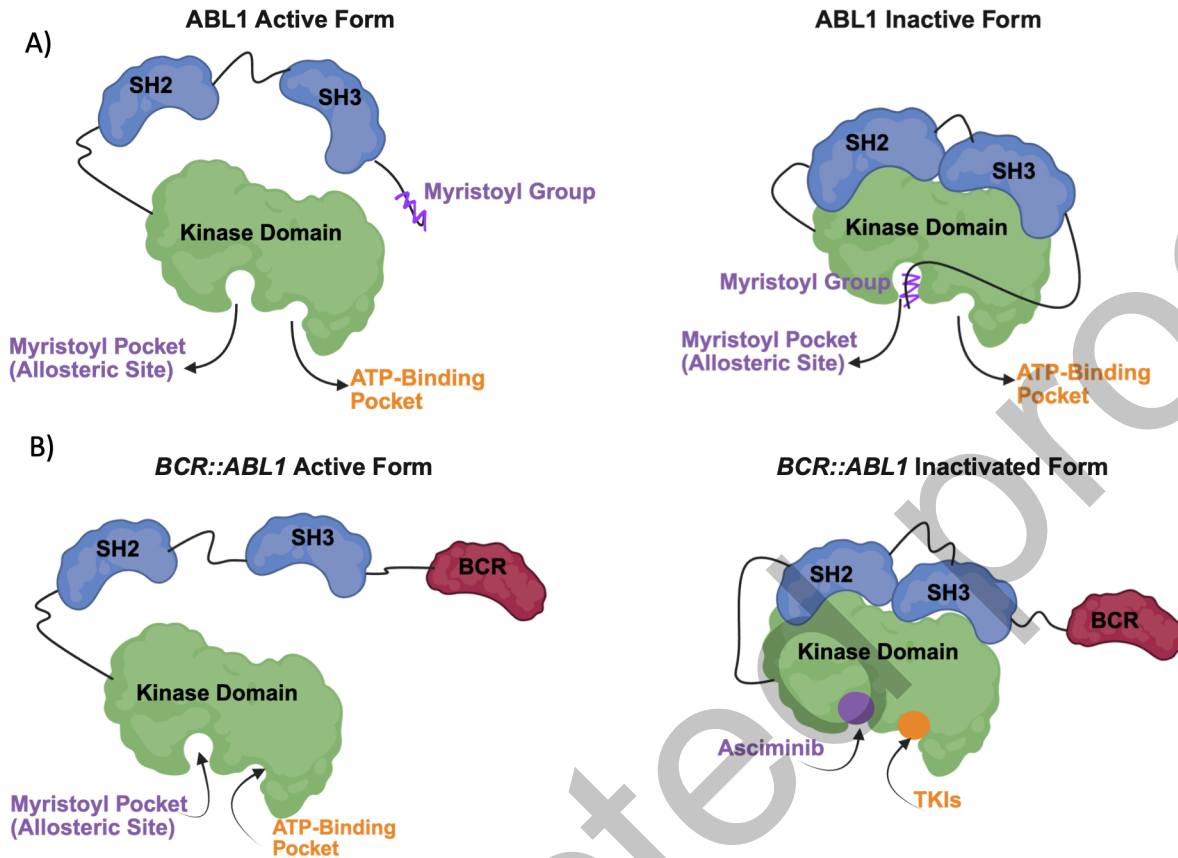


Figure 1. Mechanism of action of ATP-competitive TKIs and Allosteric TKIs. A) Normal ABL1 inactivates itself by clamping of SH2-SH3 domains with myristoylated N-terminus to the myristoyl pocket of the kinase domain. B) BCR::ABL1 is always active until asciminib binds with an allosteric myristoyl pocket or ATP competitive TKIs binds with ATP-pocket turning the kinase into an inactive conformation. Created with BioRender.com.

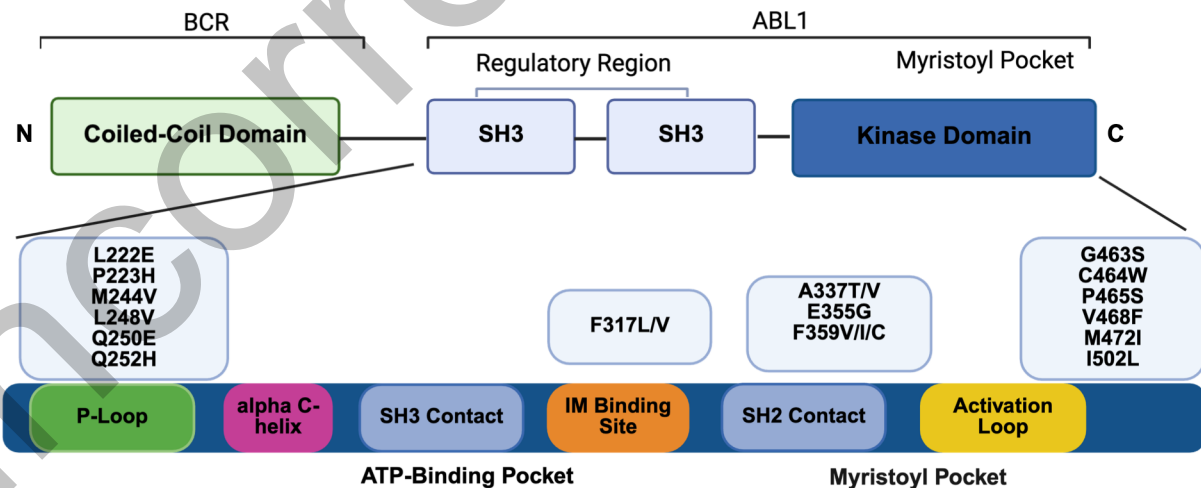


Figure 2. BCR::ABL1 fusion and amino acid substitution in the ABL1 domain identified in asciminib resistance samples. Created with BioRender.com.

Table 1. An Expanded Mechanistic Framework for Asciminib Resistance

Mechanism of Resistance	Location / Variant	Specific Examples	Putative Molecular Mechanism	Clinical Implication / Recommendation
Affinity Loss (Failure of Binding)	Myristoyl pocket (allosteric site)	A337T/V, G463S, C464W, P465S, V468F, M472I, I502L	Steric hindrance or loss of binding interactions in the myristoyl pocket. These directly impair asciminib binding and/or reduce efficacy.	ABL1 mutation testing at relapse. May be overcome by asciminib dose-escalation or switch to ATP-TKI (e.g., ponatinib).
Allosteric Disruption (Failure of Function)	Kinase N-Lobe / Interface	M244V, P223H, L248V, K222E	Retains asciminib binding. Mutation "pre-activates" kinase, likely via α -C helix, breaking the allosteric signal path.	Requires expanded functional assays (not just affinity). High rate of cross-resistance to ATP-TKIs. Consider combination therapy.
	P-Loop / ATP-Site	G250E, Q252H, F317L/V, E355G, F359V/I/C	Dual-effect: "pre-activates" kinase <i>and</i> simultaneously alters ATP-pocket.	"Significant overlap". Must guide choice by specific mutation profile.
	Compound Mutations	Myristoyl pocket variants+ ATP-Site variant	Dual region resistance	Complicates sequencing. Must guide choice by specific mutation profile.
Primary/ Intrinsic Resistance	Transcript Variant (SH3 Defect)	b2(e13)a3, b3(e14)a3	Lacks ABL1 exon 2. Results in a non-functional SH3 domain, making the inhibitory "clamp" impossible to form.	Pre-treatment RT-PCR screening is essential. These variants are potential high risk setting for asciminib resistance.
	Isoform (Context-Dependent)	p190 + P-Loop (e.g., E255V, Y253H)	>100-fold higher IC50 for same mutation vs. p210 background.	Consider pre-treatment isoform testing. p190 (Ph+ ALL) is a relative contraindication, esp. if P-loop mutation is present.
Non-BCR::ABL1 Mechanism	Efflux Pump	ABCG2, ABCB1 Upregulation	Reduced intracellular asciminib concentration.	Test for pump expression at relapse if no mutation is found. May be overcome by switching to a STAMPI not effluxed (e.g., TGRX-678).