A Novel BCR-ABL1 I502T Mutation Associated with Acquired Asciminib Resistance in Chronic Phase CML

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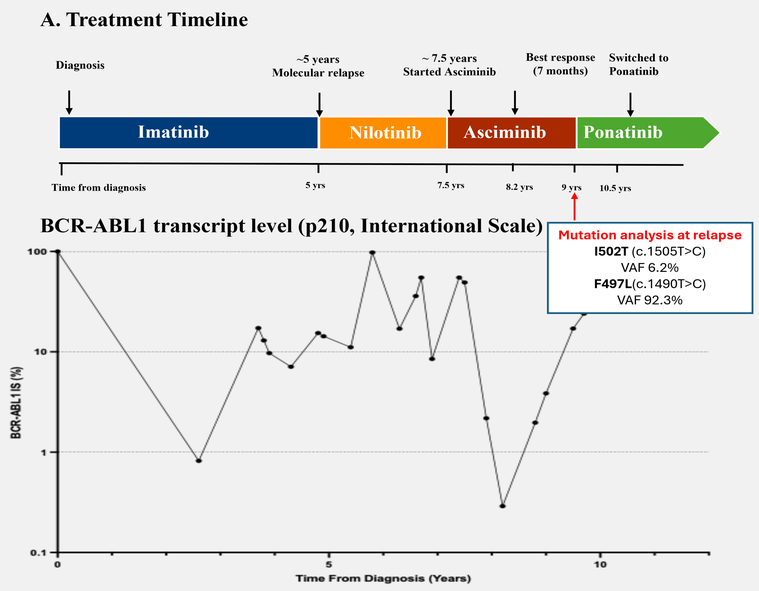
INTRODUCTION

- Asciminib is a first-in-class allosteric BCR-ABL1 tyrosine kinase inhibitor targeting the ABL1 myristoyl pocket and is approved for newly diagnosed and previously treated chronic phase chronic myeloid leukemia (CP-CML).
- Unlike ATP-competitive TKIs, asciminib retains activity against many kinase domain resistance mutations; however, its distinct mechanism selects for resistance-conferring myristoyl pocket mutations such as A337V and I502L.
- Here, we report the identification and functional validation of a novel BCR-ABL1 I502T mutation associated with acquired asciminib resistance in CP-CML.

CASE REPORT

- A 41-year-old woman with CP-CML was initially treated with imatinib for 5 years before transitioning to nilotinib for molecular relapse. She lost response to nilotinib after 2.5 years and was started on asciminib as a third line TKI. BCR-ABL1 kinase domain mutation analysis prior to asciminib initiation was negative.
- She achieved an initial molecular response, with p210 transcripts declining from >55% to a nadir of 0.29% after 7 months on therapy.
- Subsequently, progressive molecular relapse occurred over the following 18 months (p210: 1.97% → 3.86% → 17.05% → 24.08%).
- Mutation analysis at the time of confirmed failure identified a novel variant, I502T (c.1505T>C; VAF 6.2%), located in the myristoyl pocket region of ABL1. A co-occurring F497L variant (VAF 92.3%) was also detected.



METHODS

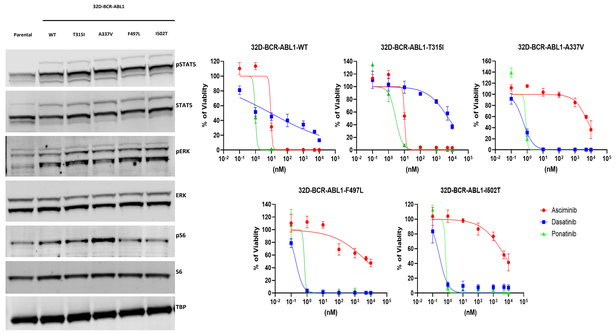


Figure 1. 32D cell line expressing the novel mutation BCR-ABL1-I502T induced resistance to asciminib drug in invitro condition. A) Immunoblot of parental 32D cells and 32D cells expressing BCR-ABL1-WT and mutations (T315I, A337V, F497L and I502T) for basal signaling pathway expression. Blots were probed with STAT5, pSTAT5, ERK, pERK, S6 and pS6. TBP was used as an internal loading control. **B)** Cell viability assay in 32D-BCR-ABL1 WT and mutant cell lines (T315I, A337V, F497L and I502T) treated with asciminib, dasatinib and ponatinib 48 h. The data represents three independent experiments with mean±SD.

Clone	Asciminib (IC50)	Dasatinib (IC50)	Ponatinib (IC50)
WT	9.1nM	9.1nM	1nM
T315I	10.1nM	5900nM	2.8nM
A337V	6400nM	0.6nM	0.8nM
F497L	5800nM	0.2nM	0.7nM
I502T	5800nM	0.3nM	0.7nM

Table 1. IC50 concentration of asciminib, dasatinib and ponatinib for 32D-BCR-ABL1-WT and mutant cells T315I, A337V, F497L and I502T.

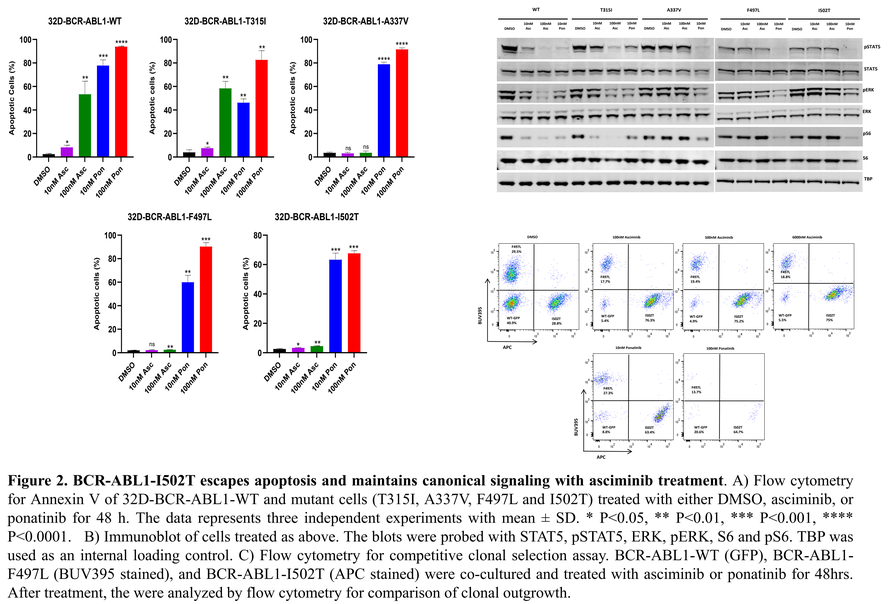


Figure 2. BCR-ABL1-I502T escapes apoptosis and maintains canonical signaling with asciminib treatment. A) Flow cytometry for Annexin V of 32D-BCR-ABL1-WT and mutant cells (T315I, A337V, F497L and I502T) treated with either DMSO, asciminib, or ponatinib for 48 h. The data represents three independent experiments with mean ± SD. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. B) Immunoblot of cells treated as above. The blots were probed with STAT5, pSTAT5, ERK, pERK, S6 and pS6. TBP was used as an internal loading control. C) Flow cytometry for competitive clonal selection assay. BCR-ABL1-WT (GFP), BCR-ABL1-F497L (BUV395 stained), and BCR-ABL1-I502T (APC stained) were co-cultured and treated with asciminib or ponatinib for 48hrs. After treatment, the were analyzed by flow cytometry for comparison of clonal outgrowth.

CONCLUSIONS

I502T is a novel myristoyl pocket mutation that confers high-level asciminib resistance while retaining sensitivity to ATP-competitive TKIs, further expanding the landscape of resistance at this critical drug-binding interface.

CONTACT INFORMATION

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