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ELEVENTH ANNUAL MEETING

DISCOVERY OF A SELECTIVE PROTAC DEGRADING UNTAMABLE BCR::ABL1 COMPOUND MUTANTS IN CML AND PH+ALL

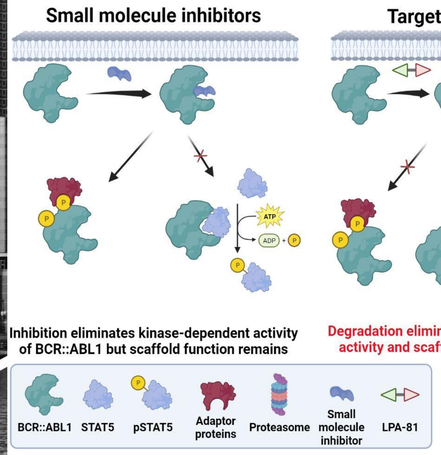
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INTRODUCTION

Chronic myeloid leukemia (CML) is caused by BCR::ABL1, a constitutively active tyrosine kinase generated as a result of the t(9;22)(q34;q11.2) translocation. Tyrosine kinase inhibitors (TKIs) targeting the ATP binding site of BCR::ABL1 (e.g., imatinib) have greatly improved survival in chronic phase CML (CP-CML) but significant clinical challenges remain. Only one-quarter of patients achieve treatment free remission (TFR). More concerning, 15-20% of patients develop TKI resistance and approximately 10% progress to blast phase (BP)-CML, an acute leukemia with poor prognosis. In contrast to ATP site TKIs, the allosteric TKI asciminib (ASC) binds a regulatory site at the bottom of the kinase domain, mimicking the physiological ligand myristate to enforce an inactive kinase conformation. Point mutations in the BCR::ABL1 kinase domain that impair TKI binding are the best characterized mechanism of acquired TKI resistance and have informed the development of successive generations of TKIs. In aggregate, the currently approved ATP site TKIs cover all single BCR::ABL mutants. However, BCR::ABL1 compound mutations (two or more mutations in the same BCR::ABL1 molecule) can confer high level of TKI resistance.



RESULTS

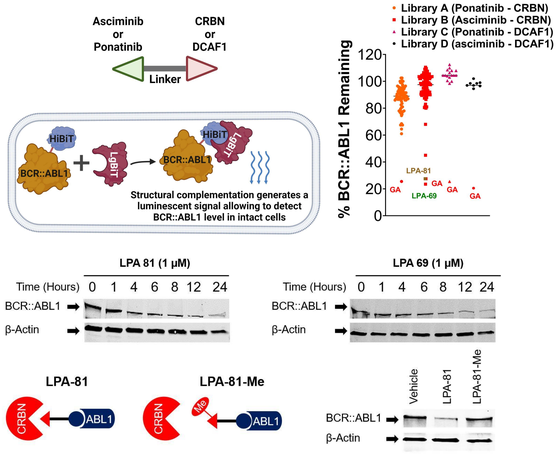


Fig. 1. LPA-81 potently degrades BCR::ABL1 oncoprotein.

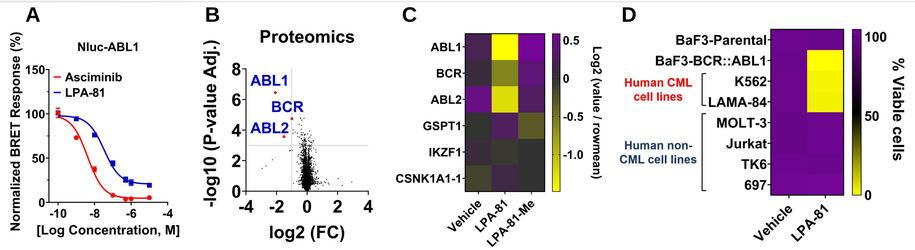


Fig. 2. (A) LPA-81 binds to the myristoyl pocket of ABL1. (B) LPA-81 selectively degrades BCR::ABL1, ABL1, and ABL2. (C) The CRBN neo-substrates were not affected by LPA-81. (D) LPA-81 selectively kills BCR::ABL1 positive cells.

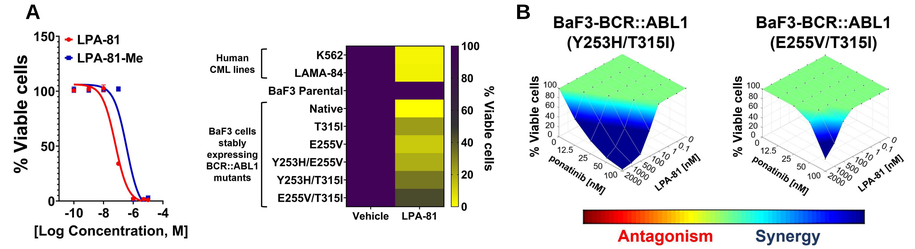


Fig. 3. (A) LPA81 can suppress native BCR::ABL1 and clinically resistant BCR::ABL1 compound mutants. (B) Combining LPA-81 with ponatinib is highly synergistic against BCR::ABL1 compound mutants.

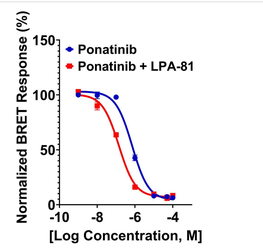


Fig. 4. LPA-81 enhances the binding affinity of ponatinib to the ATP site of BCR::ABL1.

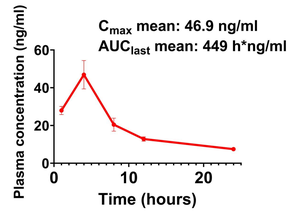


Fig. 5. LPA-81 displays favorable pharmacokinetics in vivo.

CONCLUSIONS

I. LPA-81 selectively degrades BCR::ABL1 oncoprotein that harbor single and/or compound mutations at concentration as low as 0.1 µmol/L (Dmax=98%) within 24hours.

II. LPA-81 enhances the binding affinity of ponatinib to the ATP site of BCR::ABL1 leading to the formation of stable DFG-out conformation of BCR::ABL1.

III. Maximizing the inactive (DFG-out) conformation will enhance BCR::ABL1 degradation via LPA-81 to target both kinase function and scaffold-dependent BCR::ABL1 activities.

IV. LPA-81 is highly stable and exhibited favorable pharmacokinetics (PK) *in-vivo.*

SIGNIFICANCE

I. LPA-81 is capable of selectively degrading native and mutants (single and compound) BCR::ABL1 oncoprotein.

II. LPA-81 is more potent than currently available TKIs against BCR::ABL1 compound mutants *in-vitro*.

III. Developing the first PROTAC-based combinatorial strategy capable of degrading clinically resistant BCR::ABL1 compound mutants.

IV. The stable DFG-out conformation of BCR::ABL1 is required for optimal degradation via LPA-81.

V. Developing novel asciminib-based fluorescent tracer (provisional patent), enabling a high-throughput NanoBRET approach to determine the conformational states of BCR::ABL1 and binding affinity of ligands to the myristoyl pocket of ABL1 (Fig. 2A).

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