The novel BCR::ABL1 allosteric inhibitor TERN-701 (HS-10382) is potent against mutations resistant to active site tyrosine kinase inhibitors (TKIs) and acts synergistically with TKIs in BCR::ABL1+ cancer cell lines

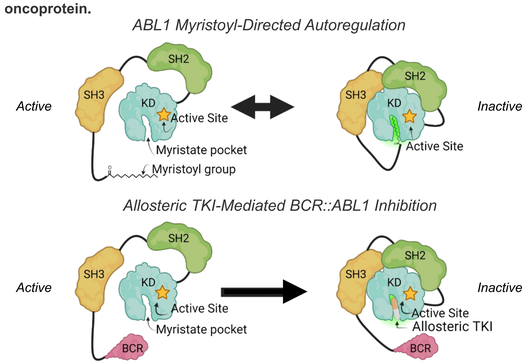
Poster #: CML-593

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1 BACKGROUND

- Chronic Myeloid Leukemia (CML) is a myeloproliferative disorder characterized by a reciprocal translocation between chromosomes 9 and 22, leading to the loss of myristoyl-directed autoregulation and constitutive activation of the BCR::ABL1 oncoprotein.[1,2]
- TERN-701 is a novel allosteric inhibitor of BCR::ABL1, optimized for selectivity and pharmacokinetic parameters, that binds the myristate pocket.
- TERN-701 retains activity against the BCR::ABL1^{T315I} resistance mutation which confers resistance to all approved active site inhibitors except for ponatinib.[3]

Figure 1. Schematic representation of allosteric inhibition of the BCR::ABL1 oncoprotein.



2 METHODS

- Studies were conducted to characterize the potency, selectivity, and potential of TERN-701 to act synergistically with active site TKIs.
- The ABL1 kinase domain (amino acid residues 64-515) was expressed and purified via affinity chromatography from SH9 cells. Activity was validated using a microfluidic mobility shift assay.
- Substrate phosphorylation assays were used to assess the selectivity of TERN-701 against the human kinome. The ability of TERN-701 to inhibit the proliferation of CML cell lines was assessed using CellTiter-Glo®. Synergy between TERN-701 and active site TKIs were assessed using both fixed molar combinations and expanded combination matrices, with cell viability measured using CellTiter-Glo® and interactions quantified using the Bliss, HSA, and Loewe models.

REFERENCES

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[3] Alves R, Goncalves AC, Rutella S, Almeida AM, De Las Rivas J, Trougakos IP, et al. Resistance to tyrosine kinase inhibitors in chronic myeloid leukemia-from molecular mechanisms to clinical relevance. Cancers (Basel). 2021;13(19).
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3 RESULTS

TERN-701 inhibited native BCR::ABL1 in *in vitro* biochemical assays with an IC_{50} = 0.4 nM, and inhibited cell proliferation in native and mutant cell lines with IC_{50}s of 0.6 – 15.6 nM (figure 2A-D), depending on genetic background and BCR::ABL1 mutation status. TERN-701 retained activity against the clinically relevant T315I mutation. With respect to selectivity, in an *in vitro* kinase panel, TERN-701 did not inhibit any kinase by >50% at 1 µM, including full-length ABL1 (figure 2E).[4] TERN-701 was highly potent and selective against only BCR::ABL1+ cell lines in cancer cell line panel, and was more selective than asciminib (figure 3). *In vitro* combination studies revealed that TERN-701 works synergistically with multiple TKIs in the K562 cell line, while additivity and weak synergy was observed in the Ku812 cell line (figures 4 & 5 and tables 1 & 2). As assessed using the BLISS model, synergy was strongest at and below the IC_{50} of each single compound.

Figure 2.

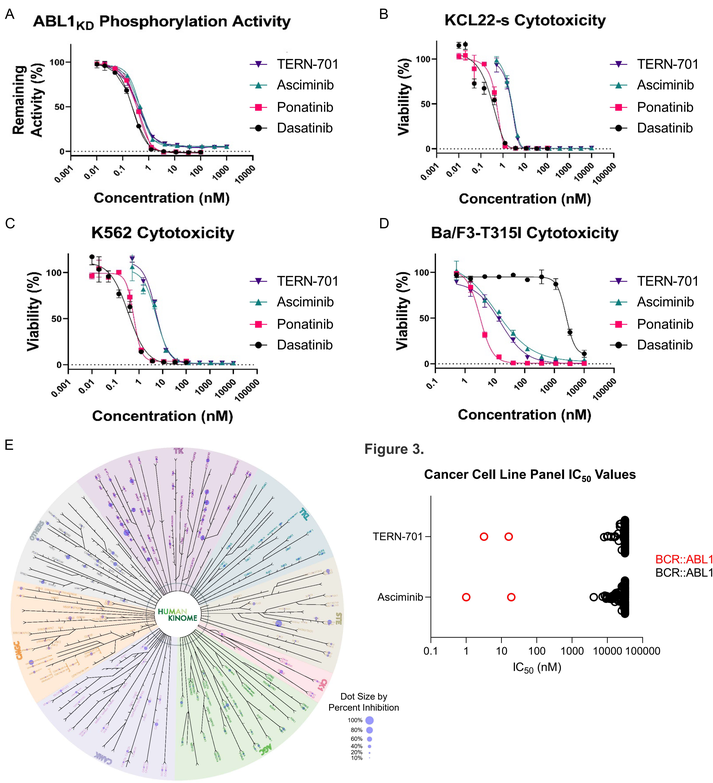
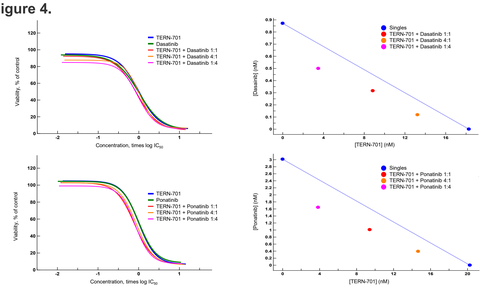


Figure 3.

Figure 4.

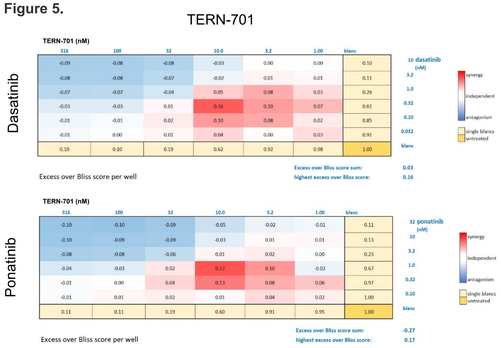


Representative cytotoxicity curves (L) for molar combinations of TERN-701 and dasatinib (top) and ponatinib (bottom). Right panels are representative synergy isobolograms set against the IC_{75} values for single agents and fixed molar combinations with dasatinib (top) or ponatinib (bottom). Points falling below the blue additivity line indicate synergistic interaction. Synergy as quantified by curve shift and isobologram analyses are summarized in table 1.

Table 1

Compound	Combination Partner	Cell Line	Summary of Observations
TERN-701	Imatinib	K562	Synergy at IC_{75} and IC_{90}
		Ku812	Additivity
	Dasatinib	K562	Synergy at all molar combinations
		Ku812	Additivity
	Ponatinib	K562	Synergy at all molar combinations
		Ku812	Additivity
Asciminib	Imatinib	K562	Synergy at IC_{75} and IC_{90}
		Ku812	Additivity
	Dasatinib	K562	Synergy at IC90
		Ku812	Additivity
	Ponatinib	K562	Synergy at IC_{75} and IC_{90}
		Ku812	Additivity

Figure 5.



Representative heat maps of K562 cells treated with TERN-701 and Dasatinib (top) or Ponatinib (bottom). Higher values indicate stronger synergy. Summarized BLISS synergy data is provided in table 2.

Table 2

Compound	Combination Partner	Cell Line	Summary of Observations	Strongest BLISS score
TERN-701	Imatinib	K562	BLISS synergy observed at and below IC_{50} of single compounds	0.12
		Ku812	Weak synergy observed	0.05
	Dasatinib	K562	BLISS synergy observed at and below IC_{50} of single compounds	0.16
		Ku812	Weak synergy observed	0.08
	Ponatinib	K562	BLISS synergy observed at and below IC_{50} of single compounds	0.17
		Ku812	Weak synergy observed	0.07
Asciminib	Imatinib	K562	BLISS synergy observed at and below IC_{50} of single compounds	0.18
		Ku812	Synergy observed	0.13
	Ponatinib	K562	BLISS synergy observed at and below IC_{50} of single compounds	0.17
		Ku812	Weak synergy observed	0.05
	Dasatinib	K562	BLISS synergy observed at and below IC_{50} of single compounds	0.17
		Ku812	Synergy observed	0.15

4 CONCLUSION

- TERN-701 is a potent and selective allosteric inhibitor of BCR::ABL1 in cell-free and cell-based assays, with comparable potency and synergy profiles to that of asciminib while potentially being more selective.
- TERN-701 retains activity against the T315I gatekeeper mutation, which confers resistance to all approved active site TKIs except for ponatinib, which has known safety liabilities.
- A global phase 1 trial assessing safety and initial efficacy of TERN-701 monotherapy is on track to begin by the end of 2023.
- These data support the continued development of TERN-701 for the treatment of CML through monotherapy and potentially combination approaches.
- TERN-701 is being evaluated in a Ph 1 dose escalation/expansion study as HS-10382 in China.