



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

604. MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Tgrx-678, a Novel Allosteric Inhibitor of BCR-ABL1, Demonstrates Preclinical Anti-Leukemia Activity, High Oral Bioavailability and Synergism with Ponatinib to Suppress the Highly Resistant Compound MutationsYanxia Shi, PhD¹, Qian Jiang, MD², Linxin Li, DPhil¹, Shuzhen Jiang¹, Tingting Yan¹, Cuifang Zheng¹, Yixin Ai³, Yihan Wang, PhD⁴¹Department of Biological Research, Shenzhen TargetRx, Inc., Shenzhen, China²Peking University People's Hospital, Peking University Institute of Hematology, National Clinical Research Center for Hematologic Disease, Beijing, China³Department of Computational Chemistry, Shenzhen TargetRx, Inc., Shenzhen, China⁴Shenzhen TargetRx, Inc., Shenzhen, China**Introduction**

Clinical resistance to the treatment of BCR-ABL1-positive chronic myeloid leukemia (CML) patients is often conferred by mutation(s) in the ATP-binding site of ABL1 kinase domain. ABL1 is subject to auto-inhibition mediated by myristoylation-triggered conformational change, which can be exploited to overcome resistance, as validated by allosteric inhibitors such as GNF2, GNF5 and asciminib. TGRX-678, a novel allosteric inhibitor designed to target the myristoyl pocket of ABL1, is currently being investigated in a phase I clinical trial (NCT05434312). Herein we report the selectivity, potency, anti-leukemia activity and *in vivo* pharmacokinetic (PK)/pharmacodynamic (PD) characteristics of TGRX-678.

Methods

For *in vitro* study, ABL1b 65-534 fragments were used following the protocol for Z'-LYTE kinase assay kit (Thermo Fisher). For cellular assays, CML cell lines or engineered Ba/F3 cells were incubated with titrating TGRX-678 for 72 hrs, then CellTiter Glo reagent (Promega) was added and the chemiluminescence was measured. For *in vivo* studies, SD rats received TGRX-678 or asciminib and plasma samples were collected for analysis. NOD-SCID mice were inoculated with CML or Ba/F3-BCR-ABL1^{T315I} cells and received oral TGRX-678. Tumor volumes and body weights were recorded for 3 to 5 weeks.

Results

TGRX-678 exhibited minimum off-target inhibition at 1 μ M in a panel of 298 kinases, but inhibited the enzymatic activity of both ABL1 and the T315I mutant with IC₅₀ values of 2.96 nM and 1.15 nM, respectively.

The anti-proliferative activity of TGRX-678 was determined in CML cell lines including K562, KU812, KCL22-S, KCL22-R, LAMA-84 and MEG-01 with IC₅₀ values ranging from 1.1 nM to 6.56 nM. TGRX-678 had no cytotoxicity in 29 cell lines representing other hematological malignancies and solid tumors. Using engineered Ba/F3 cell lines, TGRX-678 was active against the native BCR-ABL1 (IC₅₀ = 4.11 nM) and the clinically prominent mutants including G250E (IC₅₀ = 2.49 nM), Q252H (IC₅₀ = 2.38 nM), Y253H (IC₅₀ = 5.6 nM), E255K (IC₅₀ = 1.01 nM), E255V (IC₅₀ = 2.73 nM) and T315I (IC₅₀ = 66.1 nM). The cellular activity of TGRX-678 was attenuated by mutations in the myristoyl pocket such as A337V, P465S, V468F and I502L or mutations in the SH3-kinase domain interface including P223S and K294E, underpinning its allosteric mode of action.

In SD rats, oral administration of TGRX-678 at 20 mg/kg achieved a maximum plasma concentration (C_{max}) of 2369 ng/mL, which was 1.3-fold higher than that of asciminib. The overall exposure (AUC_{last}) of TGRX-678 (23,147 h·ng/mL) was also 1.7-fold higher than that of asciminib. The oral bioavailability of TGRX-678 was 62%. In xenograft-bearing mice, oral dosing of TGRX-678 at 1 mg/kg QD (for KU812) or 3 mg/kg QD (for K562) was sufficient to completely inhibit tumor growth. In the Ba/F3-BCR-ABL1^{T315I} xenograft mice, TGRX-678 induced dose-dependent tumor regression and reduced tumor volume by 67% at 45 mg/kg QD dose.

We further examined the synergistic effect of TGRX-678 and ponatinib combination. While each agent was inactive to the Y253H/T315I mutant, the combination of TGRX-678 and ponatinib inhibited cell proliferation at suboptimal concentrations. The addition of TGRX-678 dramatically lowered the IC₉₀ for ponatinib from 1489 nM to 60.9 nM, whereas asciminib only reduced the IC₉₀ to 165 nM (Figure A). Similarly, while ponatinib alone had limited effect on cell viability of the E255V/T315I, Q252H/T315I and T315M mutants, the addition of TGRX-678 at a clinically achievable concentration of 720 nM significantly

reduced cell viability to 0-10 % (Figures B-D). In comparison, the addition of asciminib at a much higher concentration of 6000 nM only reduced cell viability to 5-29% (Figures B-D).

Conclusion

Our findings support that TGRX-678, a novel allosteric inhibitor of BCR-ABL1, is potent against the CML cells and cell lines harboring clinical ATP-site mutations with minimum non-specific cytotoxicity. TGRX-678 exhibits a favorable PK profile and anti-tumor activity *in vivo*. Furthermore, TGRX-678 and ponatinib synergize to overcome the clinically challenging resistance conferred by T315M or T315I-inclusive compound mutations. This data warrant further clinical investigation of TGRX-678 for the treatment of resistant or refractory CML patients.

Disclosures Shi: Shenzhen TargetRx, Inc.: Current Employment, Current holder of stock options in a privately-held company. **Li:** Shenzhen TargetRx, Inc.: Current Employment, Current holder of stock options in a privately-held company. **Jiang:** Shenzhen TargetRx, Inc.: Current Employment, Current holder of stock options in a privately-held company. **Yan:** Shenzhen TargetRx, Inc.: Current Employment, Current holder of stock options in a privately-held company. **Zheng:** Shenzhen TargetRx, Inc.: Current Employment, Current holder of stock options in a privately-held company. **Ai:** Shenzhen TargetRx, Inc.: Current Employment, Current holder of stock options in a privately-held company. **Wang:** Shenzhen TargetRx, Inc.: Current Employment, Current equity holder in private company, Other: CEO of the company.

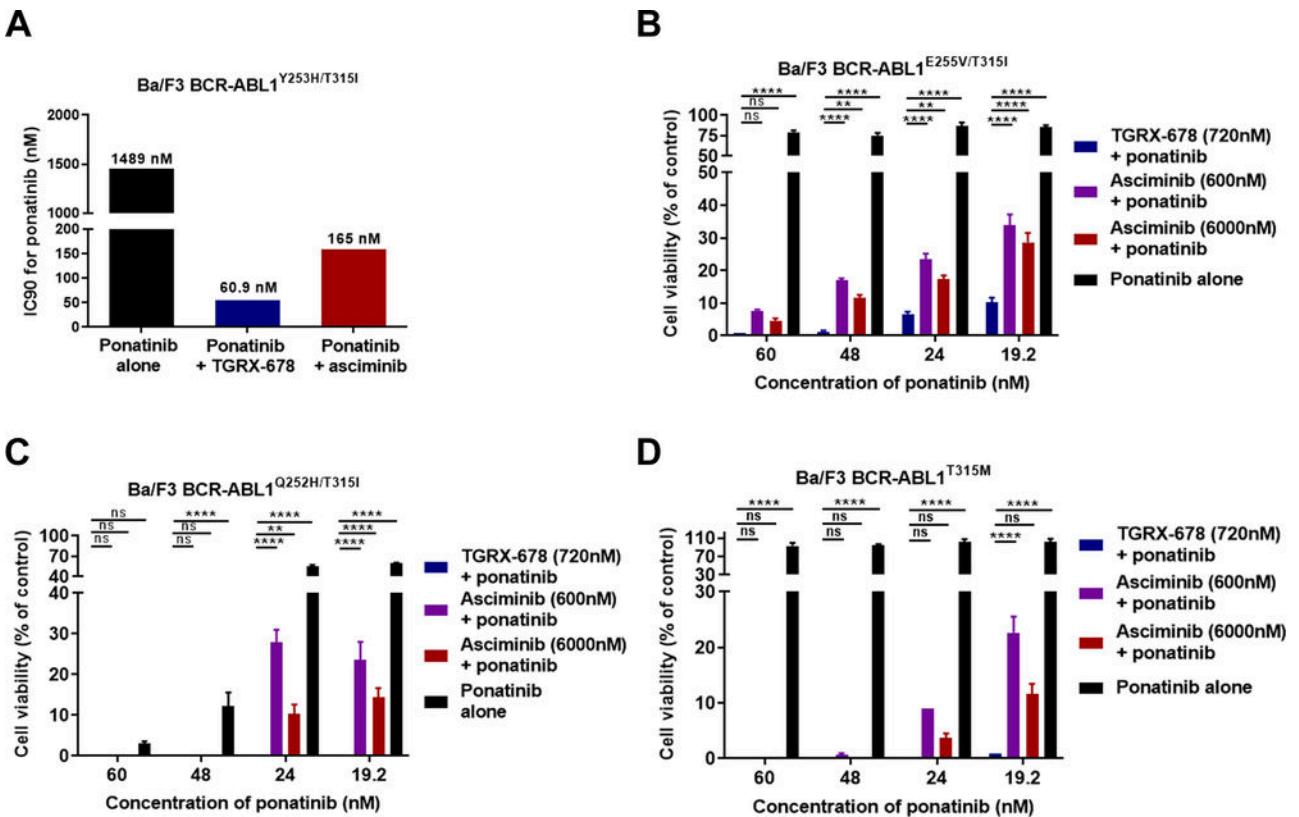


Figure 1

<https://doi.org/10.1182/blood-2023-181391>

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