ELVN-001, a Next-Generation, ATP-Competitive ABL1 Tyrosine Kinase Inhibitor for the Treatment of Chronic Myeloid Leukemia

Stefan D. Gross[1], Christopher A. Eide[2], Michael R. Burkard[1], Anna Reister Schultz[2], Monette A. Aujay[1], Samuel Kintz[1], Brian J. Druker[2], and Joseph P. Lyssikatos[1]

[1]Enliven Therapeutics, Boulder, Colorado, USA
[2]Division of Hematology and Medical Oncology, Knight Cancer Institute, Oregon Health & Science University, Portland, Oregon, USA

INTRODUCTION

Chronic Myeloid Leukemia (CML):
- CML is a myeloproliferative disease that manifests as uncontrolled granulocyte proliferation with a relatively normal differentiation[1]
- More than 99% of patients with CML harbor a reciprocal translocation between chromosomes 9 and 22 within the breakpoint cluster region (BCR) and the Abelson tyrosine kinase (ABL1) genes
- The resultant BCR::ABL1 oncogene encodes a fusion protein, BCR::ABL1, with constitutive tyrosine kinase activity that leads to aberrant activation of downstream signaling pathways, driving abnormal differentiation, growth, and survival of leukemic cells

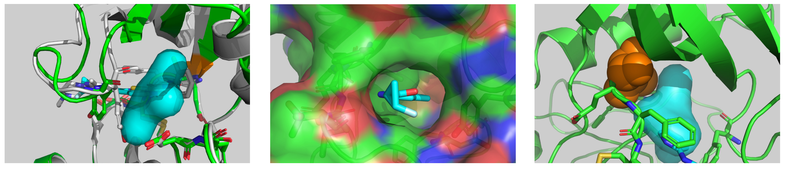
Current state of the disease:
- The development of tyrosine kinase inhibitors (TKIs) targeting the BCR::ABL1 kinase has improved the outcome for patients with CML
- Specifically, 6 TKIs have been approved to treat this disease: imatinib, nilotinib, dasatinib, bosutinib, ponatinib, and asciminib
- Except for asciminib, which allosterically inhibits BCR::ABL1 via interaction with its myristoyl pocket, these TKIs all target the ATP-binding site of the ABL1 kinase domain of the fusion protein
- As a result of these therapies, life expectancy for newly diagnosed patients with chronic-phase (CP) CML now approaches the age-matched general population[2]

Challenges associated with current TKI therapies:
- Approximately 20% of patients move to a different TKI within the first year, and ~40% of patients switch in the first 5 years due to loss of clinical benefit or intolerance[3]
- Therapeutic benefit and quality of life are impacted by treatment-related adverse events (TRAEs), due in part to off-target inhibition of other tyrosine kinases, such as c-KIT, FLT-3, PDGFR, VEGFR2, and c-SRC[4]
- Loss of disease control is often associated with point mutations in the BCR::ABL1 kinase, which impair TKI binding[5]
- One of the most frequently occurring alterations is the T315I mutation, which occurs in approximately 20% of patients with resistant CML[6]
- Only ponatinib and asciminib have been shown to effectively address the T315I mutation clinically, but only at higher doses that could contribute to additional TRAEs

ELVN-001 selectivity has the potential to minimize TRAEs and therefore enable greater target engagement and efficacy
- ELVN-001 is a potent Type 1 inhibitor of BCR::ABL1 that can also address the T315I mutation in vitro and in vivo at concentrations anticipated to be clinically achievable, while sparing key anti-target kinases such as c-KIT, FLT-3, PDGFR, VEGFR2, and c-SRC

Figure 1: Unique Binding Mode Confers Selectivity for Activated BCR::ABL1 (and T315I)

Unique P-Loop "Folded-In" Conformation | Narrow "Selectivity Tunnel" | T315I Active-Site Bypass



Overlay with dasatinib (grey) | T315I crystal structure, surface view | T315I shown as spheres (orange)

- ELVN-001 is a Type 1 binder (DFG-in) that exhibits a differentiated binding mode in the active form of the ABL1 in which the P-loop adopts a unique "folded-in" conformation
- Because of this unique interaction, ELVN-001 does not suffer from a steric clash with the isoleucine gatekeeper of the T315I mutant form, unlike promiscuous Type 1 inhibitors such as dasatinib that bind in the more common P-loop "extended" form
- ELVN-001 is therefore highly active against this major clinically relevant resistance mutant as well as a subset of less-common resistance mutants

Table 1: ELVN-001 in vitro Profile

	Asciminib	Ponatinib	Nilotinib	ELVN-001
KCL-22 (BCR::ABL^{wt}) cytotox IC_{50} (50% human serum)	7 nM	1 nM	90 nM	19 nM
KCL-22 (BCR::ABL^{T315I}) cytotox IC_{50} (50% human serum)	>1,150 nM	14 nM	> 10,000 nM	131 nM
K-562 (BCR::ABL^{wt}) cytotox IC_{50} (50% human serum)	85 nM	4 nM	228 nM	65 nM
K-562 pCRKL IC_{50} (100% human serum)	NA	36 nM	1,080 nM	112 nM
Human hepatocyte stability, extraction ratio	64[7]	62	62	0
Plasma protein binding (% unbound)	~2	< 1	< 1	40
Cytochromes p450 (CYPs) (% inhibition @ 10 µM)	All < 50%	All < 50%	2C8, 2C9, 3A4, 2C19 > 50%	All < 50%
Human ether-à-go-go-related gene (hERG) IC_{50}	25 µM	2.3 µM	0.13 µM	> 30 µM
Breast cancer receptor proteins (BCRPs) substrate	Yes	Yes	Yes	No

Potential correlation to major molecular response (MMR) in humans

BCRP may play a role in off-target resistance

- ELVN-001 exhibits potent anti-proliferative and biomarker (Tyr207-phosphorylated CRKL) inhibition in native BCR::ABL1 and T315I mutant cell lines in the presence of 50-100% human serum to model the attenuating effects of serum protein binding in vivo
- ELVN-001's low hepatic extraction ratio predicts good human pharmacokinetics (PK) to enable maximum target engagement over the dosing interval
- Low turnover by human hepatocytes and in vitro cytochrome p450 (CYP) isoform inhibition data predict low risk of clinically meaningful drug-drug interactions (DDIs)

Figure 2: ELVN-001 Has a Highly Selective Kinome Profile in vitro and in Cells

ELVN-001 (100 µM ATP)

Kinase	Fold Selectivity
ABL1	1
ABL2/ARG	31
TNIC	41
TNIK	110
LOK/STK10	183
LRRK2	486
FGR	550
ACK1	698
PYK	725
HGK/MAP4K4	973
LCK	>1,000

Large window for ABL2/ARG may result in improved safety[8]

Cellular Phosphorylation IC_{50} (nM)

	c-KIT	FLT-3	PDGFRβ	VEGFR2	c-SRC
ELVN-001	>10,000	>10,000	>10,000	>10,000	>10,000
Ponatinib	30	3.8	89	4.8	630
Nilotinib	200	>10,000	720	2,900	>10,000
Dasatinib	0.6	>1,000	7.1	>1,000	10
Bosutinib	1,000	4,700	7,900	>10,000	16
Imatinib	82	>10,000	230	9600	>10,000

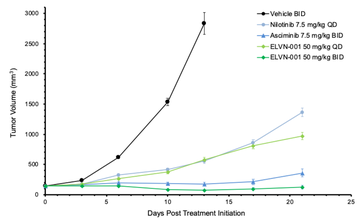
- 372 kinases screened in biochemical assays at 1 µM ELVN-001 (100 µM ATP assay concentration)
- 10 kinases identified as being inhibited by >50% were selected for IC_{50} determination
- Results indicated a >100x window vs all but 2 kinases profiled (ABL2 and TRKC)
- Highly selective vs key TKI-associated kinase anti-targets c-KIT, FLT-3, PDGFRβ, VEGFR2, and c-SRC in cells

RESULTS

Figure 3: ELVN-001 Anti-Tumor Activity in K562 (Native BCR::ABL) Xenograft

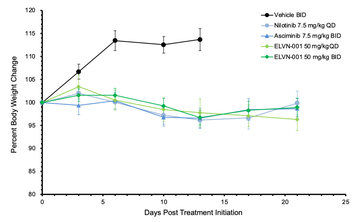
A

Anti-Tumor Efficacy



B

Body Weight

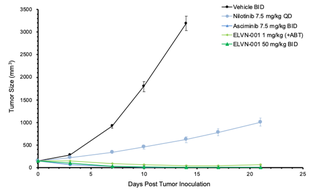


- ELVN-001 exhibited marked anti-tumor activity at both 50 mg/kg QD and BID in a BCR::ABL1 WT K562 subcutaneous NOD-SCID mouse tumor xenograft study (Panel A). Both of these doses were well tolerated based upon body weight loss, which was monitored throughout the course of this study (Panel B)
- At 7.5 mg/kg BID to model its human clinical exposure at a 40 mg BID dose, asciminib elicited significant anti-tumor activity in this model
- At 7.5 mg/kg QD to model its human clinical exposure at a 400 mg BID dose, nilotinib produced significant tumor growth inhibition but no regressions

Figure 4: ELVN-001 Anti-Tumor Activity in an Isogenic Model of T315I CML

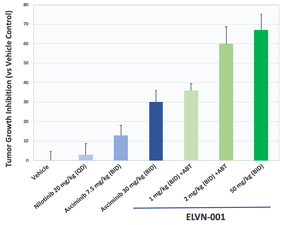
A

WT BCR::ABL1



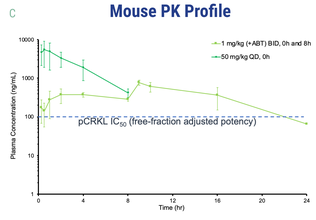
B

T315I BCR::ABL1



C

Mouse PK Profile



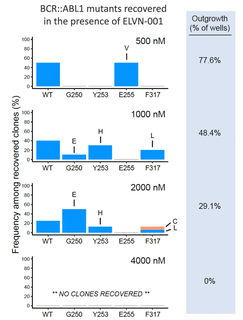
- These in vivo studies employed the KCL-22 WT (Panel A) and T315I mutant (Panel B) BCR::ABL1 isogenic CML cell lines grown as subcutaneous tumor xenografts in the Balb/c nude mouse strain
- ELVN-001 was dosed by employing one of the following three regimens: (1) 50 mg/kg BID alone, (2) 1 mg/kg BID, or (3) 2 mg/kg BID with 100 mg/kg of CYP inhibitor 1-aminobenzotriazole (ABT) administered 1h before first daily dose to better approximate ELVN-001's predicted human PK profile
- In the BCR::ABL1 WT model, nilotinib and asciminib were dosed at 7.5 mg/kg either QD or BID, respectively, to achieve exposures in mice similar to the reported human exposures of these agents at their approved human doses (400 mg BID for nilotinib and 40 mg BID for asciminib)
- In the T315I model, asciminib was also dosed at 30 mg/kg BID to achieve clinically relevant exposures associated with the 200 mg BID dose of this agent employed to treat T315I patients, and nilotinib was dosed at 20 mg/kg QD—a dose that yields exposures in mice well in excess of clinically relevant exposures
- In the BCR::ABL1 WT KCL-22 model, both ELVN-001 and asciminib produced marked tumor regressions at all doses tested, whereas nilotinib yielded tumor growth inhibition (Panel A)
- In the BCR::ABL1 T315I model, ELVN-001 elicited superior tumor growth inhibition (TGI) ranging from 36-67%, compared to both doses of asciminib (13 and 30% TGI) or high-dose nilotinib (3% TGI) (Panel B)
- The exposures of ELVN-001 at 1 mg/kg (+ABT) vs 50 mg/kg (-ABT) reveal that despite markedly lower C_{max} and AUC_{0-24} values for the former vs the latter, the time over the pCRKL free-fraction adjusted (FFA) IC_{50} is a better predictor of activity (Panel C). Importantly, this treatment dose afforded free drug exposures (AUC) greater than five times lower than the exposure measured for ELVN-001 at its non-adverse event level (NOAEL) dose of 5 mg/kg QD in non-human primates, indicating a large safety margin

Table 2: ELVN-001 and Asciminib Have Complementary Mutant Profiles

BCR::ABL1 Mutant Ba/F3 Cell Line	Asciminib Fold-Shift vs WT BCR::ABL1	ELVN-001 Fold-Shift vs WT BCR::ABL1	
WT	1 (4 nM)	1 (35 nM)	
M244V*	1	1	
G250E	0.2	23	P-Loop
Y253F	3	8	P-Loop
Y253H	2	31	P-Loop
E255K	2	37	P-Loop
T315A	2	0.5	ATP Pocket
T315I	2	8	ATP Pocket
F317L	53	28	ATP Pocket
F317V	7	0.7	ATP Pocket
M351T	7	1	SH2 Contact
F359V	14	1	SH2 Contact
H396P	12	0.6	A-Loop
A344P	876	4	Myristoyl Pocket
P465S	818	4	Myristoyl Pocket

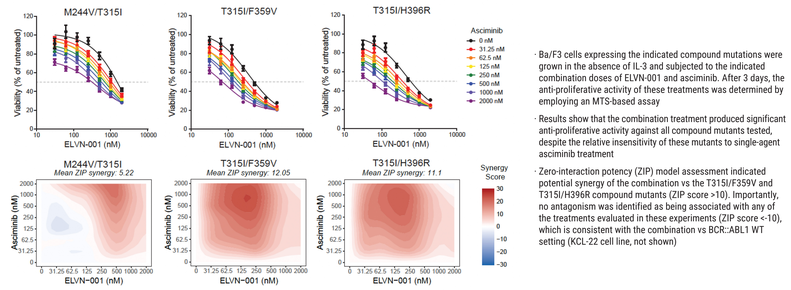
- Both ELVN-001 and asciminib were profiled against the on-target BCR::ABL1 resistance mutants that are most prevalent in the clinic
- Ba/F3 cells expressing the indicated BCR::ABL1 mutations were grown in the absence of IL-3 and subjected to a concentration range of either ELVN-001 or asciminib. After 3 days, the anti-proliferative activity of these agents was determined employing an MTS-based assay
- With the exception of certain P-loop mutations and F317L, ELVN-001 exhibits broad activity against these mutants, consistent with its unique binding mode
- Conversely, asciminib retains activity against the P-loop mutants but exhibits markedly reduced potency vs the SH2 contact and A-loop mutants
- Importantly, ELVN-001 retains potency vs the myristoyl pocket mutations A344P and P465S that exhibit marked insensitivity to asciminib
- In addition, ELVN-001 is active against the M244V P-loop mutation; a clinically relevant asciminib resistance mutation[9]

Figure 5: ELVN-001 Resistance Mutation Screen in BCR::ABL1 WT Ba/F3 Cells



- To screen for the potential spectrum of BCR::ABL1 kinase domain point mutations associated with resistance to ELVN-001, Ba/F3 cells expressing WT BCR::ABL1 (7.5 x 10^5 cells/mL) were treated with N-ethyl-N-nitrosourea (ENU; 50 µg/mL) overnight, washed, resuspended in fresh RPMI 1640 + 10% FBS media, and distributed into 96-well plates at a density of 1 x 10^5 cells/well. Plates were supplemented with graded concentrations of ELVN-001 (500, 1000, 2000, and 4000 nM; 192 wells were surveyed per concentration). Wells were observed for cell growth by visual inspection under an inverted microscope and media color change every two days over the course of four weeks. The contents of wells in which cell outgrowth was observed were expanded in culture medium supplemented with the same concentration as in the initial 96-well plate, then subjected to DNA extraction and PCR amplification and Sanger sequencing of the BCR::ABL1 kinase domain.
- ELVN-001 demonstrated a dose-dependent reduction in overall resistant outgrowth, with no resistant clones recovered at the highest tested dose of 4000 nM. Consistent with cell proliferation panel studies, mutants recovered were confined to select P-loop residues and F317, with no unexpected, novel resistant mutants detected.

Figure 6: ELVN-001 and Asciminib vs Select Compound Mutations in BCR::ABL1



- Ba/F3 cells expressing the indicated compound mutations were grown in the absence of IL-3 and subjected to the indicated combination doses of ELVN-001 and asciminib. After 3 days, the anti-proliferative activity of these treatments was determined by employing an MTS-based assay
- Results show that the combination treatment produced significant anti-proliferative activity against all compound mutants tested, despite the relative insensitivity of these mutants to single-agent asciminib treatment
- Zero-interaction potency (ZIP) model assessment indicated potential synergy of the combination vs the T315I/F359V and T315I/H396R compound mutants (ZIP score >10). Importantly, no antagonism was identified as being associated with any of the treatments evaluated in these experiments (ZIP score <-10), which is consistent with the combination vs BCR::ABL1 WT setting (KCL-22 cell line, not shown)

SUMMARY AND CONCLUSIONS

ELVN-001 represents a potential best-in-class therapeutic option for patients with CML
- Type I small-molecule inhibitor of both WT and multiple clinically relevant BCR::ABL1 mutants with exceptional drug-like properties predictive of good human clinical PK with a clean safety profile and minimal risk for DDIs
- Highly active against both the WT and the T315I mutant BCR::ABL1 both in vitro and in vivo
- Profound selectivity vs the broad kinome in biochemical assays
- No detectable cellular activity against key TKI kinase anti-targets c-KIT, FLT-3, PDGFR, VEGFR2, and c-SRC, all of which are known to underlie multiple TRAEs
- Currently undergoing clinical evaluation as a single agent in a Phase I trial (NCT05304377)

ELVN-001 and asciminib potential combination strategy
- Each agent targeting distinct sites in the ABL1 substituent of the BCR::ABL1 oncogene
- Highly complementary BCR::ABL1 mutant profile coverage in Ba/F3 mutant panel
- Demonstrated combination activity against key compound mutants in BCR::ABL1, with trend toward synergy and no evidence of antagonism
- No additional mutations identified in a mutagenesis screen beyond certain P-loop mutants and F317V/C/L, supporting potential for highly effective combination approach with minimal risk for additive or synergistic TRAEs

References: 1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;17:2391-2405. doi:10.1182/blood-2016-03-643544 **2.** Flynn AE, Atallah E. Quality of life and long-term therapy in patients with chronic myeloid leukemia. *Curr Hematol Malig Rep.* 2016;11(2):80-85. doi:10.1007/s11899-016-0306-5 **3.** Henk HJ, Winestone LE, Wilkes JJ, et al. Trend in TKI use, adherence, and switching patterns in patients with CML: before and after the availability of generic imatinib. *J Clin Pathways*. 2020;6(6):35-42, s1. doi:10.25270/ jcp.2020.8.00001 **4.** Pophali PA, Patnaik MM. The role of new tyrosine kinase inhibitors in chronic myeloid leukemia. *Cancer J*. 2016;22(1):40-50. doi:10.1097/PPO.0000000000000165 **5.** Soverini S, Mancini M, Bavaro L, Cavo M, Martinelli G. Chronic myeloid leukemia: the paradigm of targeting oncogenic tyrosine kinase signaling and counteracting resistance for successful cancer therapy. *Mol Cancer.* 2018;17(1):49. doi:10.1186/s12943-018-0780-6 **6.** Mogduno M. New resistance mechanisms for small molecule kinase inhibitors of Abl kinase. *Drug Disc Today.* 2014;11:5-10. doi:10.1016/j.ddtec.2013.12.001 **7.** Schoepfer J, Jahnke W, Berellini G, et al. Discovery of asciminib (ABL001), an allosteric inhibitor of the tyrosine kinase activity of BCR-ABL1. *J Med Chem.* 2018;61(18):8120-8135. doi: 10.1021/acs.jmedchem.8b01040 **8.** Chislock EM, Ring C, Pendergast AM. Abl kinases are required for vascular function, Tie2 expression, and angiopoietin-1-mediated survival. *Proc Natl Acad Sci USA*. 2013;110(30):12432-12437. doi:10.1073/ pnas.1304188110 **9.** Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. *Leukemia*. 2020;34:966-984. doi:10.1038/s41375-020-0776-2

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