Poster Number: 4019

Preclinical Activity of ELVN-002: a Potent, Selective, Irreversible and CNS Penetrant HER2 and pan-HER2 Mutant Small-Molecule Inhibitor for the Treatment of HER2-Driven Malignancies

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INTRODUCTION

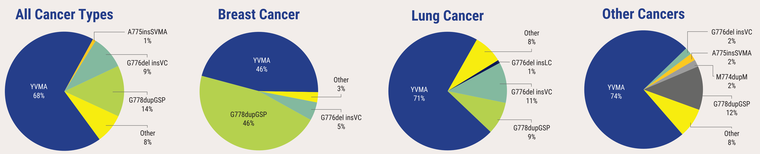
Current HER2 TKI Landscape

- The high degree of structural homology between EGFR and HER2 makes it challenging to design HER2-selective inhibitors
- Most approved and investigational agents are dual EGFR/HER2 inhibitors that are dose-limited by EGFR-driven toxicity
- Tucatinib is the only approved HER2-selective tyrosine kinase inhibitor (TKI), but lacks potency against key mutants, including HER2 YVMA, the most common exon 20 insertion mutation (E20IM) in non–small cell lung cancer (NSCLC), and L755S/P, the most common HER2 mutation in breast cancer (22%)
- Current HER2 TKIs may not achieve sufficient central nervous system (CNS)–free drug levels to fully address brain metastases, leading to disease progression in patients with lung and breast cancer

ELVN-002

- Designed to irreversibly inhibit HER2 and multiple key HER2 mutations, including HER2 YVMA and L755, and
- Selectively inhibit HER2 while sparing EGFR to prevent EGFR-related toxicities, with the potential for improved efficacy in NSCLC and other cancers including for patients with brain metastases

HER2 Exon 20 Insertion Mutation Prevalence[1]



- Exon 20 insertion mutations occur across a spectrum of cancer types with specific mutations that are not sensitive to tucatinib
- The most common HER2 exon 20 insertion mutation is a duplication or insertion of the amino acids YVMA

Table 1: ELVN-002 Potently Inhibits HER2 and HER2 Mutants While Sparing EGFR

	Poziotinib IC_{50} [nM]	Pyrotinib IC_{50} [nM]	Tucatinib IC_{50} [nM]	ELVN-002 IC_{50} [nM]
BT474 HER2^{WT} pHER2 IC_{50}	3.5	13	12	13
Beas2b HER2^{S310F} pHER2 IC_{50}	1.9	2	16	2.8
Beas2b HER2^{L755S} pHER2 IC_{50}	**4**	**3.5**	**99**	**4.7**
Beas2b HER2^{YVMA} pHER2 IC_{50}	**2.1**	**5**	**127**	**4.2**
Beas2b HER2^{YVMA} pHER2 IC_{50} in 100% human serum (fold shift)	69 (33x)	324 (65x)	>1000 (~10x)	33 (8x)
BT474 (HER2^{wt}) cytotox IC_{50}	0.9	2.3	22	3.9
NCI-N87 (HER2^{wt}) cytotox IC_{50}	0.4	2.6	44	3.3
Ba/F3 HER2^{YVMA} cytotox IC_{50}	1.5	3.2	119	5.1
H2073 (EGFR^{wt}) pEGFR IC_{50}	1.4	6.4	>10000	2160
A431 (EGFR^{wt}) pEGFR IC_{50}	1.3	10	>10000	2290
A431 (EGFR^{wt}) cytotox IC_{50}	0.6	75	>10000	*3530*
Human hepatocyte stability, extraction ratio	68	74	76	22
GSH in human liver cytosol (% remaining @ 1h)	80%	34%	-	70%
Kinetic solubility, pH 7.4 (uM)	5.6	< 0.1	9.3	260

In contrast to tucatinib, potent pharmacodynamic activity for HER2 YVMA (71% of E20IM NSCLC) & HER2 L755 (22% HER^{wt} BRC)

In contrast to dual inhibitors, our candidates spare EGFR

ELVN-002 has exceptional drug-like properties and PK profile for a covalent TKI

Table 1: Beas2b cells derived from normal human bronchial epithelium were engineered to express HER2 YVMA, HER2 S310F, or HER2 L755S. pHER2 signal was measured in Beas2b HER2 YVMA cells in the presence of 100% human serum to model the attenuating effect of human plasma protein binding on compound potency in order to provide a more clinically relevant context. pEGFR and pHER2 IC_{50} values were determined by AlphaLISA®, colorimetric ELISA, or in cell western. Cytotoxicity IC_{50} values were determined via Cell Titer Glo® after compound treatment for 3-5 days. All IC_{50} values are [nM] and represent average values from multiple experiments. Hepatocyte stability, GSH (glutathione) reactivity, and kinetic solubility assays represent a subset of our ADME (absorption, distribution, metabolism, and excretion) screening assays.

Figure 1: ELVN-002 Is >100x More Selective for HER2 YVMA Relative to EGFR Than Dual EGFR/HER2 Competitors

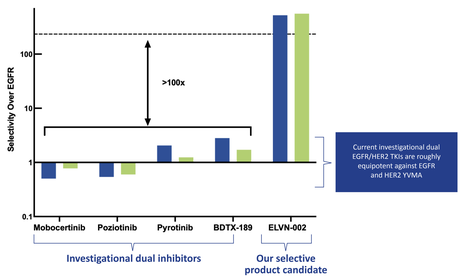


Figure 1: Selectivity over EGFR is defined by the ratio of pEGFR to pHER2 (IC_{50}) measured by pEGFR AlphaLISA® and pHER2 in cell western.

Table 2: ELVN-002 Has Favorable Mutant Coverage Compared to Tucatinib

- ELVN-002 has broad activity across HER2 E20IMs and point mutations commonly found in HER2-mutated cancers
- In contrast, tucatinib has 10 times less activity against the HER2 E20IMs, and over 10 times less activity against HER2 L755S/P mutations, which account for 22% of all HER2 mutations in HER2-mutated BRC
- Tucatinib and ELVN-002 both demonstrate selectivity over EGFR with an IC_{50} of >10,000 nM

	Ba/F3 HER2 Mutation	Proliferation IC_{50} [nM]		Proliferation IC_{50} Fold Over	
		Tucatinib	ELVN-002	Tucatinib	ELVN-002
	Wild-type	29	6	1	1
	P95	33	11	1	2
HER2 Exon 20 Insertion Mutations	A775-G776-ins-C	24	2	1	0.2
	A775-G776-ins-YVMA	225	11	8	2
	A775-G776-ins-YVMS	510	15	18	2
	A775-G776-ins-SVMA	157	6	5	1
	A775-G776-ins-VVMA	294	12	10	2
	A775-G776-ins-MMAY	287	7	10	1
	A775-G776-ins-YVMA-R678Q	642	14	22	2
	G776VC	499	17	17	3
	G776-del-ins-IC	1104	41	38	7
	G776-del-ins-LC	88	13	3	2
	G776-del-ins-VV	1239	34	43	5
	G776-V777-del-ins-CVC	209	13	7	2
	G776-del-ins-AVGC	438	14	15	2
	V777-G778-ins-GC	20	5	1	1
	P780-Y781-ins-GSP	29	3	1	1
Common HER2 Point Mutations	S310F	11	3	0.4	0.5
	S310Y	12	3	0.4	0.5
	R678Q	29	5	1	1
	L755S	418	8	14	1
	L755P	1284	21	44	3
	D769N	7	2	0.3	0.3
	V773M	64	4	2	1
	V777L	11	3	0.4	1
	T798M	3412	194	118	32
	L869R	148	2	5	0.4
	L869R/T798I	2524	43	87	7
	V842I	21	4	1	1
	Ba/F3 parental cell line	>10000	>10000	>10000	>10000
	EGFR	>10000	>10000	>10000	>10000

YVMA: 71% E20IM NSCLC

VC: 11% E20IM NSCLC

22% HER2^{mut} BRC

Table 2: Transfected Ba/F3 cells were treated with compound for 72 hours, followed by readout with CellTiter-Glo® to determine antiproliferation IC_{50} values [nM]. Tucatinib and ELVN-002 were tested in the same study, and IC_{50} values [nM] represent an average of multiple runs.

Table 3: ELVN-002 Exhibits Favorable Enzyme Inactivation Kinetics

- The k_{inact}/KI values of ELVN-002 indicate that the compound inactivates HER2 with a 47x higher efficiency than EGFR. In contrast, the enzyme inactivation kinetics of poziotinib indicate that it inactivates EGFR with a 29x higher efficiency than HER2
- For comparison, osimertinib, a selective irreversible EGFR mutant-specific inhibitor (vs wild-type EGFR) that exhibits a favorable efficacy and safety profile in lung cancer patients[2] was also evaluated in these studies. Of note, ELVN-002's HER2/EGFR ratio compares favorably with the EGFR^{L858R}/EGFR and EGFR$^{L858R/T790M}$/EGFR ratios determined for osimertinib of 20x and 50x, respectively

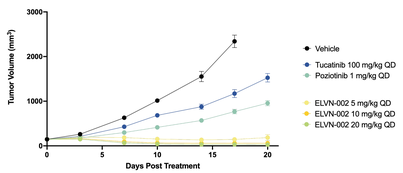
Compound	Kinase	kinact/KI ($M^{-1}s^{-1}$)	kinact (s^{-1})	KI (nM)
ELVN-002	EGFR	1.55E+04	1.05E-02	675.67
	HER2	7.34E+05	1.32E-02	17.92
Poziotinib	EGFR	1.52E+07	9.08E-03	0.60
	HER2	5.21E+05	2.46E-03	4.71

Table 3: Enzyme inactivation kinetics performed at Enzymlogic via COVALfinder®, a TR-FRET binding kinetic assay based on the binding and displacement of an active-site directed fluorescent probe.

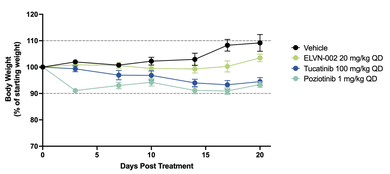
Figure 2: ELVN-002 Demonstrates Robust Anti-Tumor Activity in Beas2b HER2 YVMA Xenograft Model at Well-Tolerated Doses

- ELVN-002 yielded tumor regressions at all doses tested
- Poziotinib's maximum tolerated dose in this model was 1 mg/kg, and this dose yielded an exposure ~8x its human exposure at 16 mg QD; yet only modestly inhibited tumor growth
- Minimal tumor growth inhibition (TGI) vs YVMA observed with tucatinib treatment up to ~14x its human exposure at 300 mg BID
- ELVN-002 treatment was well tolerated, with no significant body weight loss. Poziotinib-treated mice lost significant body weight and required multiple dosing holidays

A: Beas2b HER2 YVMA Xenograft TGI



B: Beas2b HER2 YVMA Xenograft Body Weight Change



Figures 2A and 2B: Beas2b cells derived from normal bronchial epithelium were engineered to express HER2 YVMA. Tucatinib, Poziotinib, and ELVN-002 were dosed daily, PO for 21 days. N=8 NOD-SCID mice per group.

Figure 3: ELVN-002 Demonstrates Robust CNS Anti-Tumor Activity in NCI-N87 HER2^{wt} Intracranial Model at Well-Tolerated Doses

- ELVN-002 yielded sustained tumor regressions in the NCI-N87 intracranial model, and all doses were well tolerated
- Tucatinib treatment of 50 mg/kg and 75 mg/kg BID results in ~4.5x and ~12x, respectively, its human exposure at 300 mg BID
- ELVN-002 exhibited superior CNS anti-tumor activity at up to ~100x lower exposures compared to tucatinib in this model
- ELVN-002 achieved significant free-drug exposure in mouse brain across a plasma concentration range that we estimate will be clinically relevant
- ELVN-002 CSF concentrations compared to free plasma concentrations generally aligned with the reported Kp,uu values

A: NCI-N87 HER2^{wt} Intracranial (CNS) Model

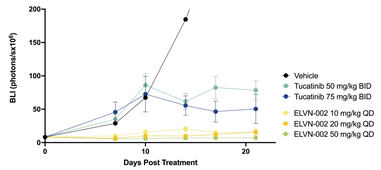


Figure 3A Intracranial efficacy: Mice were injected with luciferase-expressing NCI-N87 cells into the right forebrain, and tumor growth was measured by bioluminescent signal obtained from imaging (IVIS Lumina III). Tucatinib and ELVN-002 were dosed daily, PO for 21 days. N=10 BALB/c nude mice per group.

B: Tucatinib vs ELVN-002 Brain Exposure

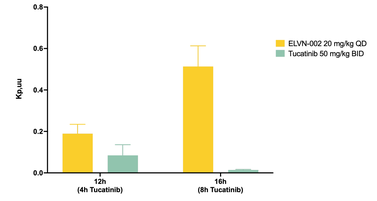


Figure 3B Mouse brain exposures: Steady state Kp,uu for ELVN-002 and tucatinib in non–tumor-bearing BALB/c nude mice (n=3). Measurements were taken after 5 days of dosing at timepoints corresponding to estimated clinically relevant plasma concentrations. Tucatinib was dosed BID and measurements were made at 12h and 16h; however, Kp,uu levels were even lower than at 4h and 8h, respectively. Kp,uu = Free brain concentration (total brain concentration adjusted for brain tissue binding)/ Free plasma concentration (total plasma concentration adjusted for protein binding).

Figure 4: ELVN-002 Demonstrates Robust Anti-Tumor Activity and Additive Activity in Combination With Enhertu at Well-Tolerated Doses

- ELVN-002 yielded deep tumor regressions in the NCI-N87 xenograft model, and all doses were well tolerated
- Low-dose ELVN-002 combined with Enhertu resulted in additive activity and deep tumor regressions in the same model
- In contrast to reversible inhibitors like tucatinib, irreversible inhibitors have been shown mechanistically to drive increased receptor internalization, and there is both preclinical and clinical precedent for additive activity upon combining irreversible TKIs with ADCs in HER-driven settings[4,5]

A: NCI-N87 HER2^{wt} Xenograft TGI: ELVN-002 Mono

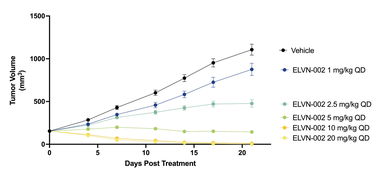


Figure 4A ELVN-002 dose response: ELVN-002 was dosed daily, PO for 21 days. N=8 BALB/c nude mice per group.

B: NCI-N87 HER2^{wt} Xenograft TGI: Enhertu Combo

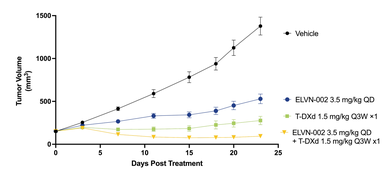


Figure 4B ELVN-002 and Enhertu (T-DXd) combo: ELVN-002 was dosed daily, PO for 21 days. T-DXd was dosed once intravenously. N=8 BALB/c nude mice per group.

SUMMARY AND CONCLUSIONS

ELVN-002 represents a novel therapeutic option for patients with altered HER2

- Inhibition of wild-type and mutant HER2 in vitro while sparing wild-type EGFR
- Potent anti-tumor activity in HER2 YVMA subcutaneous mouse model
- Superior preclinical activity in wild-type HER2 subcutaneous and intracranial models compared to tucatinib
- Improved activity in combination with Enhertu (T-DXd)
- Excellent pharmaceutical properties to support further clinical development of ELVN-002

ELVN-002 is designed to provide a meaningful therapeutic option to patients with brain metastases

ELVN-002 is currently being tested in a Phase 1 clinical trial (NCT05650879) in patients with cancer harboring HER2 alterations

Acknowledgments:

We thank our Pharmaron, Kyinno, and Enzymlogic collaborators for their work on this program

References: 1. Robichaux JP, Elamin YY, Vijayan RSK, et al. Pan-cancer landscape and analysis of ERBB2 mutations identifies poziotinib as a clinically active inhibitor and enhancer of T-DM1 activity. *Cancer Cell*. 2019;36(4):444-457.e7. doi:10.1016/j.ccell.2019.09.001 **2.** Wu YL, Tsuboi M, He J, et al. Osimertinib in resected EGFR-mutated non-small cell lung cancer. *N Engl J Med*. 2020 Oct 29;383(18):1711-1723. **3.** Zhai X, Ward RA, Doig P, Argyrou A. Insight into the therapeutic selectivity of the irreversible EGFR tyrosine kinase inhibitor osimertinib through enzyme kinetic studies. *Biochemistry*. 2020;59(14):1428-1441. **4.** Li BT, Michelini F, Misale S, et al. HER2-mediated internalization of cytotoxic agents in ERBB2 amplified or mutant lung cancers. *Cancer Discov*. 2020;10(5):674-687. doi:10.1158/2159-8290.CD-20-0215 **5.** Abraham J, Montero AJ, Jankowitz RC, et al. Safety and efficacy of T-DM1 plus neratinib in patients with metastatic HER2-positive breast cancer: NSABP Foundation trial FB-10. *J Clin Oncol*. 2019;37(29):2601-2609. doi:10.1200/JCO.19.00858