

ELVN-002; a potent, selective HER2 inhibitor with a differentiated binding mode conferring the potential for enhanced efficacy in combination with HER2-targeting antibody-drug conjugates

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INTRODUCTION

ELVN-002 is designed to achieve an improved therapeutic index compared to current approved and investigational tyrosine kinase inhibitors (TKIs) in HER2-amplified/overexpressed tumors

Current HER2 TKI Landscape

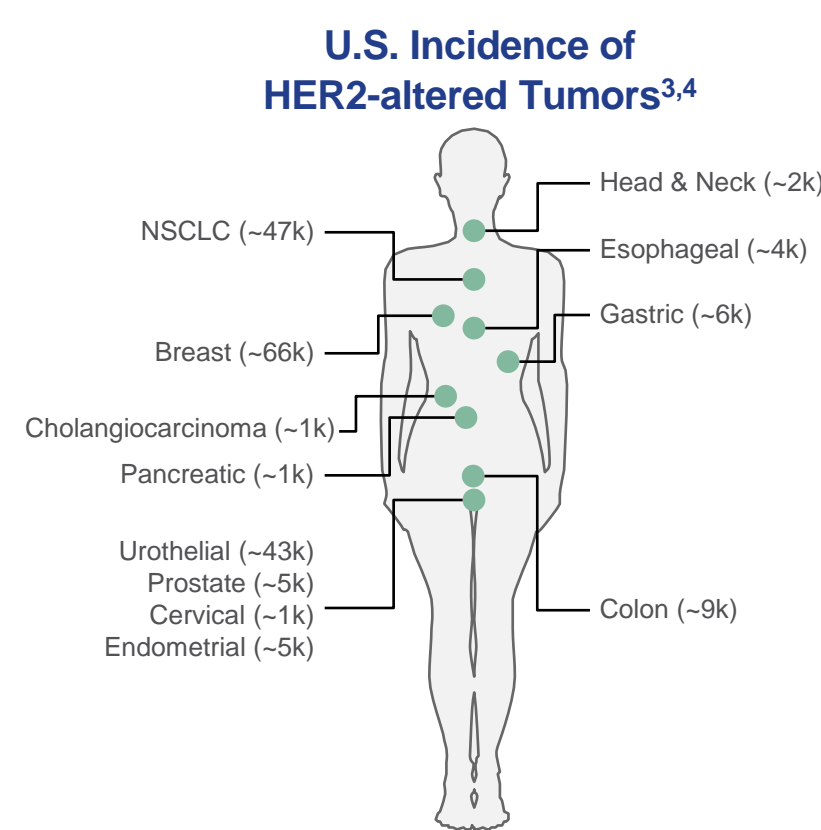
- Tucatinib (Tukysa®) is the only approved HER2-selective TKI in 2L+ HER2+ metastatic breast cancer (MBC) in combination with trastuzumab + chemotherapy, or the HER2-targeting antibody-drug conjugate (ADC) ado-trastuzumab emtansine [Kadcyla®]¹
- Tucatinib only achieves IC₉₀ coverage all day in only ~40% of patients and is also limited by liver toxicity²
- Zongertinib is an investigational HER2 selective inhibitor currently in clinical trials

ELVN-002

- Designed to achieve a wide therapeutic window in HER2-altered and mutant tumors, including in combination
- Highly selective, CNS-penetrant, irreversible inhibitor
- Best-in-class wild-type HER2 potency and ability to maximize target coverage in patients
- Minimal risk of CYP-mediated drug-drug interaction may allow for easier/safer combination with chemotherapy and ADCs
- Drives enhanced HER2 internalization and subsequent degradation, compared to tucatinib and zongertinib, which may lead to an advantage in combining with ADCs

Unmet Need in HER2-Altered Patient Populations

- Overexpression, amplification, or mutation of human HER2 is observed in up to 30% of solid tumors³
- Trastuzumab deruxtecan (T-DXd) is a HER2-targeting ADC recently approved for HER2 altered cancer types⁴
- However, there remains a substantial unmet need for patients who either progress on T-DXd or discontinue treatment due to adverse events



OBJECTIVE

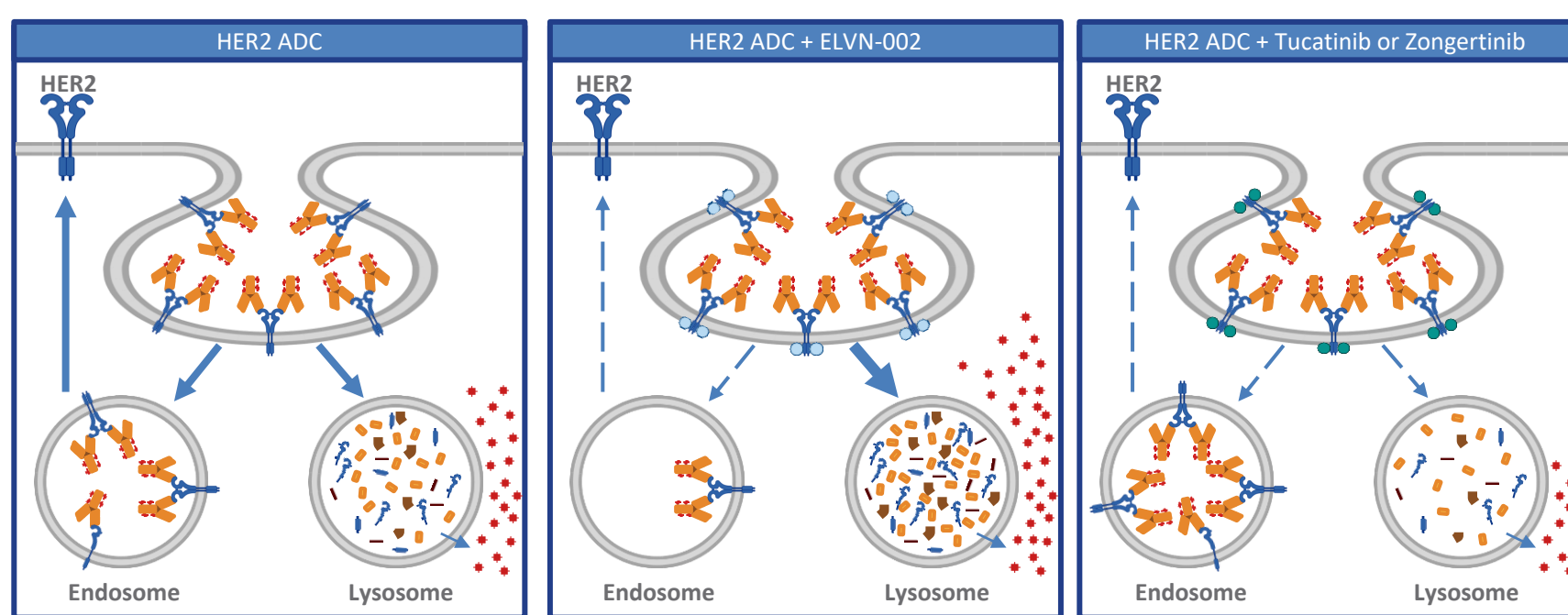
To characterize the impact of ELVN-002 and other clinically-relevant HER2 inhibitors in combination with HER2 targeted antibodies on HER2 cell surface expression and internalization, degradation via ubiquitination, and tumor growth inhibition in xenograft models

ELVN-002 Potentially Represents the Best TKI Combination Partner for ADCs in the HER2+ Space

ADCs are degraded in the lysosome, resulting in intracellular payload release. However, some intact ADC escapes degradation via endocytic recycling

Due to its unique binding mechanism, ELVN-002 drives HER2 internalization and enhances intracellular payload release

In contrast, treatment with either tucatinib or zongertinib results in decreased HER2 internalization



Based on preclinical data, ELVN-002 has the potential to improve the efficacy of HER2 targeted ADCs via enhanced intracellular payload release, while simultaneously inhibiting HER2 signaling

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RESULTS

Receptor Dynamics in HER2-Amplified Cell Lines

Table 1: ELVN-002 potently inhibits HER2^{WT} while sparing EGFR

	Neratinib	Sevabertinib	Zongertinib	Tucatinib	ELVN-002
BT474 HER2 ^{WT} pHER2 IC ₅₀	13	3	23	12	13
BT474 HER2 ^{WT} pHER2 IC ₅₀ in 50% human serum (fold-shift)	37	33 (10)	150 (7)	44 (3)	18 (1)
NCI-N87 HER2 ^{WT} pHER2 IC ₅₀	17	5.7	21	13	13
SKBR3 HER2 ^{WT} pHER2 IC ₅₀	13	4.2	15	15	11
H2073 (EGFR ^{WT}) pEGFR IC ₅₀	3.5	-	2,030	>10,000	2,160
A431 (EGFR ^{WT}) pEGFR IC ₅₀	8	22	1,950	>10,000	2,290

ELVN-002 potently inhibits HER2 amplified cell lines and is less plasma protein bound than other TKIs

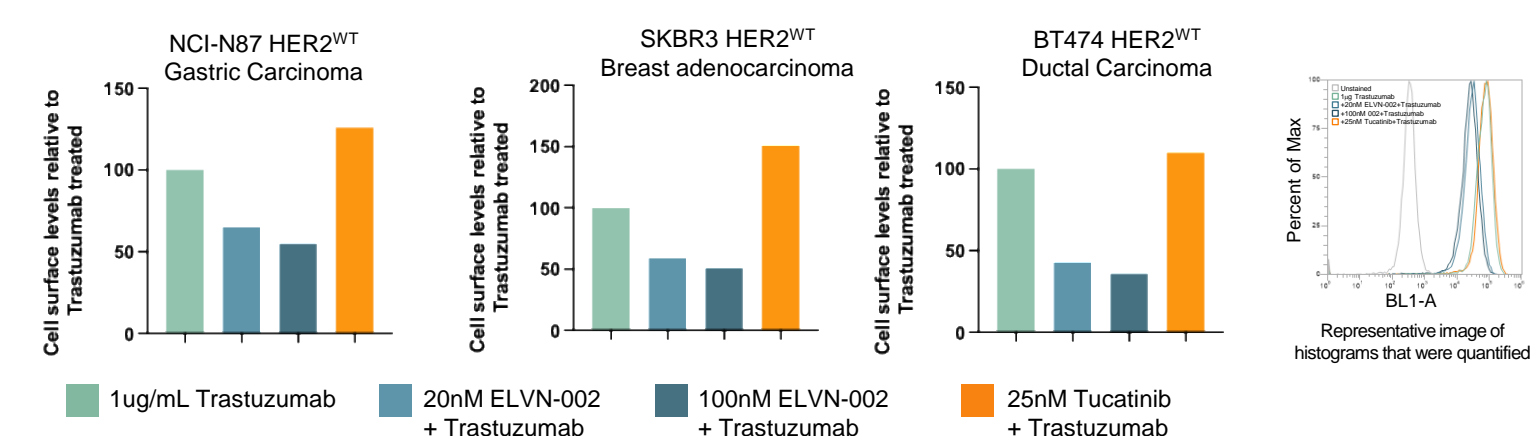
In contrast to dual inhibitors, our candidate spares EGFR

NCI-N87, SKBR3, and BT474 are HER2-amplified cell lines. pHER2 signal was measured in BT474 cells in the presence of 50% human serum to model the attenuating effect of human plasma protein binding on compound pEGFR and pHER2. IC₅₀ values were determined by AlphaSight or cobometric ELISA. All IC₅₀ values are [pM] and represent average values from multiple experiments. We chose target coverage of pHER2 IC₅₀, IC₉₀, and >IC₉₀ for ELVN-002 treatment concentrations. For tucatinib, we targeted IC₉₀, which is comparable to published C₉₀ exposure. For Zongertinib we chose to target IC₅₀ to adequately compare target coverage which is close to relevant C₉₀.

- ELVN-002 is currently being evaluated in combination with HER2-targeted antibodies or ADCs (trastuzumab, T-DXd [Enhertu®], and ado-trastuzumab emtansine [Kadcyla®])
- To understand the impact of combination therapies with HER2 TKIs and HER2-targeted antibodies, we evaluated receptor dynamics in HER2-amplified cell lines (Table 1)
- Other HER2-selective TKIs were also evaluated in combination

Effect of ELVN-002 on Cell Surface Levels of HER2

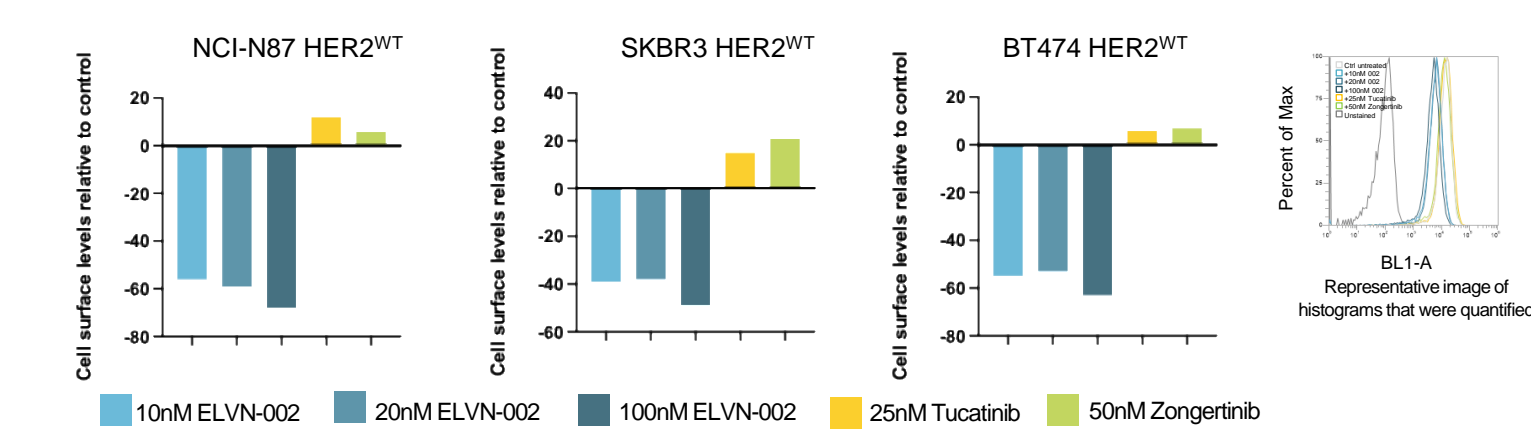
Figure 1: Treatment with ELVN-002 in combination with trastuzumab results in decreased HER2 cell surface levels compared to trastuzumab treatment alone



NCI-N87, SKBR3 and BT474 cells were treated with ELVN-002 or tucatinib +/- trastuzumab for 24 hours, and HER2 cell surface levels were measured using flow cytometry. The above graphs represent the change in HER2 mean fluorescence intensity on the cell surface relative to trastuzumab-treated cells.

- To determine HER2 cell surface levels, flow cytometry was used
- ELVN-002 treatment in combination with trastuzumab resulted in decreased HER2 cell surface levels compared to trastuzumab monotherapy (Figure 1)
- Unlike combination treatment of ELVN-002, treatment of tucatinib in combination with trastuzumab did not change HER2 cell surface levels compared to trastuzumab treatment alone

Figure 2: Treatment with ELVN-002 alone also results in decreased HER2 cell surface levels

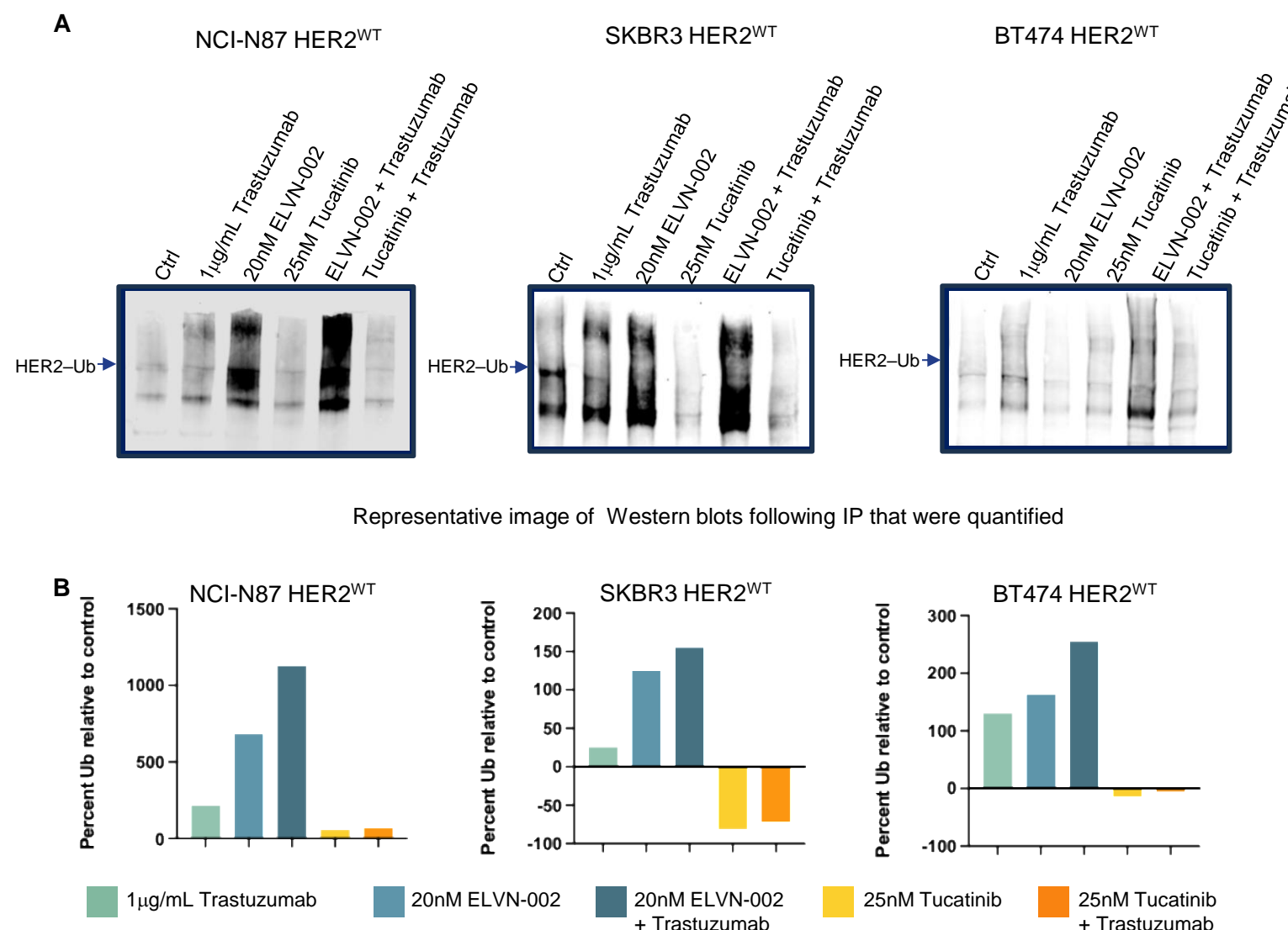


NCI-N87, SKBR3 and BT474 cells were treated with ELVN-002 or other agents for 24 hours, and HER2 cell surface levels were measured using flow cytometry. The above graphs represent the change in HER2 mean fluorescence intensity on the cell surface relative to untreated control cells.

- To determine if receptor dynamics change with monotherapy vs. combination, we again looked at HER2 cell surface levels (Figure 2)
- ELVN-002 treatment alone results in ~2x decrease of HER2 on the cell surface
- In contrast to ELVN-002, treatment with tucatinib or zongertinib does not significantly alter HER2 receptor levels on the cell surface
- These results suggest ELVN-002 alone or in combination with trastuzumab increases HER2 receptor trafficking at the cell surface

Immunoprecipitation of Ubiquitinated HER2 to Measure Lysosomal Trafficking

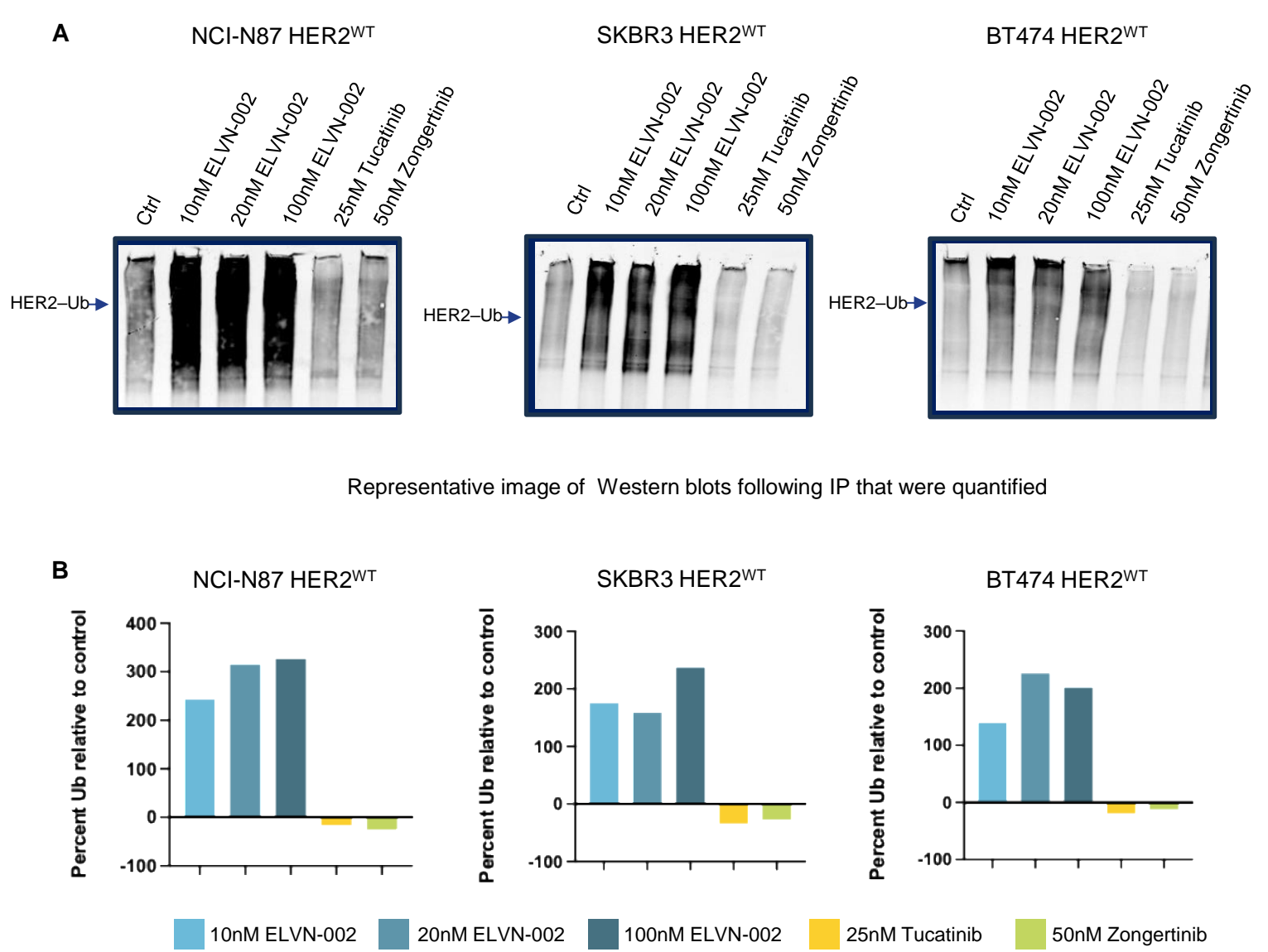
Figure 3: Treatment with ELVN-002 in combination with trastuzumab enhanced HER2 ubiquitination



Top panel: NCI-N87, SKBR3 and BT474 cells were treated with ELVN-002 or other agents +/- trastuzumab and MG132 for 6 hours, then lysed and immunoprecipitated using magnetic beads that are conjugated to an anti-ubiquitin antibody. The beads were incubated with the lysate overnight, then the flow through was collected and the beads were washed (3x). The protein that remained on the beads was collected using non-reducing sample buffer and high heat. The samples were then Western blotted for HER2. Above are representative images of the HER2 Western blots, at least 3 repeat experiments were done. Bottom panel: The graphs represent the quantification of total ubiquitinated HER2 compared to untreated control cells.

- The combination of ELVN-002 and trastuzumab results in additive and increased HER2 ubiquitination, compared to control cells or either treatment group alone
- Unlike treatment with ELVN-002 and trastuzumab, tucatinib alone or in combination results in dampened HER2 ubiquitination

Figure 4: Treatment with ELVN-002 alone also increased levels of ubiquitinated HER2

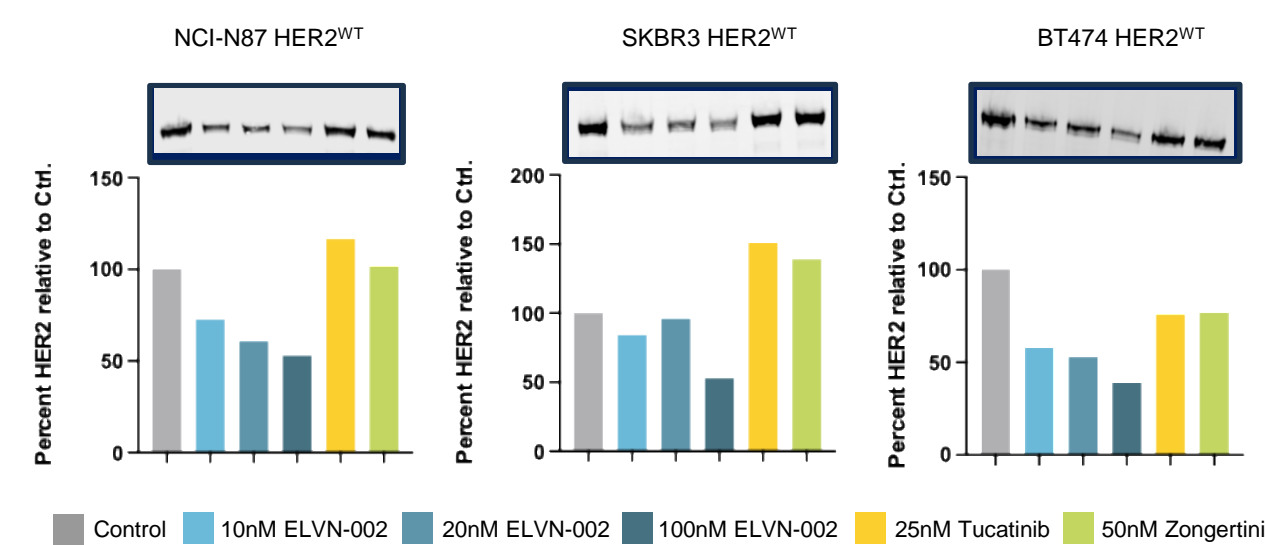


Top panel: NCI-N87, SKBR3 and BT474 cells were treated with ELVN-002 or other agents + 1µM MG132 for 6 hours, then lysed and immunoprecipitated using magnetic beads that are conjugated to an anti-ubiquitin antibody. The beads were incubated with the lysate overnight, then the flow through was collected and the beads were washed (3x). The protein that remained on the beads was collected using non-reducing sample buffer and high heat. The samples were then Western blotted for HER2. Above are images of representative Western blots. Bottom panel: The graphs represent the quantification of total ubiquitinated HER2 compared to untreated control cells. Each experiment was repeated 3 times.

- As reported in the literature, tucatinib alone decreases HER2 ubiquitination⁵
- Zongertinib, while also an irreversible inhibitor, behaves similarly to tucatinib (Figure 4)
- Unlike tucatinib and zongertinib, treatment with ELVN-002 alone increases HER2 ubiquitination (Figure 4)
- The increased HER2 ubiquitination in ELVN-002-treated cells suggests increased lysosomal trafficking and HER2 degradation

Effect of ELVN-002 on HER2 Degradation

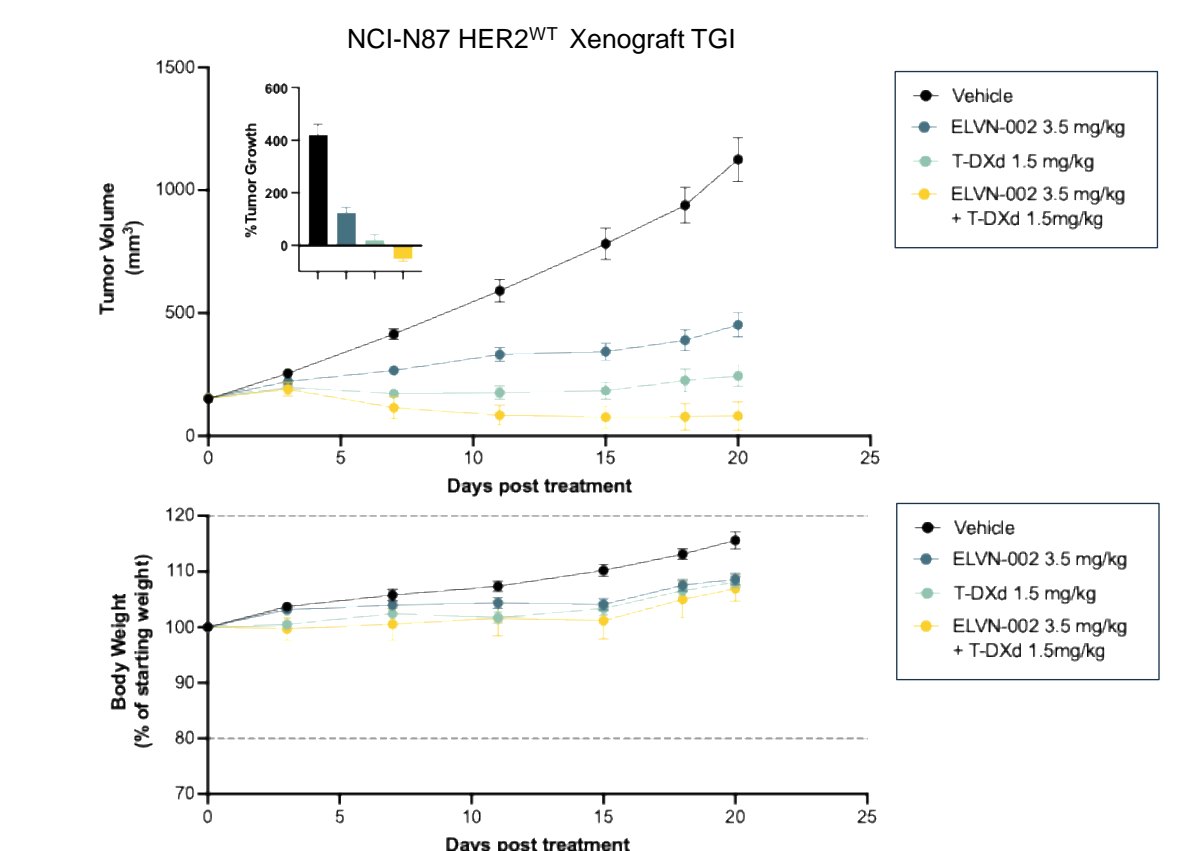
Figure 5: Consistent with increased HER2 ubiquitination and decreased cell surface expression, HER2 is partially degraded in ELVN-002-treated cells



NCI-N87, SKBR3, and BT474 cells were treated with ELVN-002 or other agents for 24 hours. Cells were lysed and Western blotted for total HER2.

- ELVN-002 treatment results in partial degradation of HER2 (Figure 5), with the potential to increase efficacy of HER2 ADCs by increasing cytotoxic payload delivery
- Consistent with dampened HER2 ubiquitination and steady cell surface levels, tucatinib and zongertinib result in little to no effect on HER2 degradation

Figure 6: ELVN-002 in combination with T-DXd Demonstrated Robust Anti-Tumor Activity at Well-Tolerated Doses



Anti-tumor Activity of ELVN-002 in Xenograft Mouse Model

- Low dose ELVN-002 combined with T-DXd resulted in additive activity and deep tumor regressions in the NCI-N87 xenograft model (Figure 6)
- In contrast to other HER2-selective inhibitors, ELVN-002 mechanically drives increased receptor internalization and therefore represents a potential best in class HER2 TKI for combination with ADCs

CONCLUSIONS

- Because of ELVN-002's differentiated profile and mechanism of action, it has the potential to be a superior treatment in HER2-expressing cancers in combination with ADCs, other modalities, and as a single agent
- Treatment with ELVN-002 leads to significantly increased HER2 ubiquitination and internalization compared to other HER2-selective inhibitors
- Reduced cell surface HER2, in conjunction with enhanced HER2 ubiquitination, suggests that ELVN-002 induces greater HER2 receptor flux at the cell surface
- Non-clinical data presented here suggest combination treatment with ELVN-002 and HER2-targeted ADCs has the potential to increase the ADC payload release intracellularly and increase tumor death
- Similar results have been reported for other HER2/EGFR irreversible inhibitors (pyrotinib, neratinib, and pozotinib), but these drugs are limited by toxicity associated with EGFR inhibition⁶
- The reversible HER2 inhibitor, tucatinib, does not increase internalization^{5,7} and data here suggests that zongertinib, although an irreversible inhibitor, does not increase HER2 internalization
- ELVN-002 is being evaluated as monotherapy in HER2+ and mutant solid tumors and in combination with ADCs, trastuzumab, and chemotherapy in HER2+ solid tumors (NCT05650879; NCT06328738)