Abstract #1979

# Preclinical characterization of NVL-330, a selective and brain penetrant HER2 tyrosine kinase inhibitor with broad activity on HER2 oncogenic alterations

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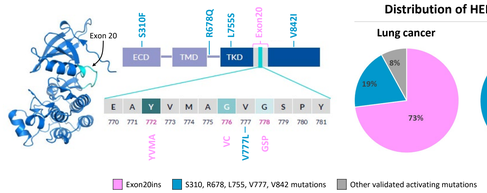
## ADDRESSING A MEDICAL NEED

### HER2 Oncogenic Mutations

- HER2 (ERBB2) can drive tumorigenesis via mutation, amplification, and/or overexpression.[1]
- HER2 exon 20 insertion mutations (exon20ins) induce constitutive kinase activity and are the most frequently occurring HER2 mutations in lung cancer. Examples include Y772_A775dupYVMA (HER2$^{YVMA}$), G776del_insVC (HER2$^{VC}$), and P780_Y781insGSP (HER2$^{GSP}$).[1-3]
- In all solid tumors, point mutations at five hotspots (S310, R678, L755, V777, and V842) account for ~65% of HER2 mutations.[2]







▲ Figure 1 (**Left**) Schematic illustration showing locations of major oncogenic mutations on HER2 protein (PDB: 7JXH). (**Right**) Pie charts showing the distribution of HER2 mutations in NSCLC and all solid tumors.[2]

### HER2-mutant NSCLC Landscape

- Antibody drug conjugate trastuzumab deruxtecan (T-DXd; Enhertu®) has received accelerated approval for previously treated HER2-mutant non-small cell lung cancer (NSCLC), with intracranial activity reported.[4-5]
- Selective HER2 tyrosine kinase inhibitor (TKI) zongertinib has demonstrated preliminary clinical activity in HER2-mutant NSCLC.[6] Its intracranial activity has not been reported.
- Additional HER2-selective or EGFR/HER2-targeting TKIs are under clinical development.

### NVL-330 Preclinical Profile[7]

1. **Activity against oncogenic HER2 mutants and wild-type HER2**
   - 2 – 4% of NSCLC patients have HER2 oncogenic mutations. [8-9]
   - 1 – 5% of NSCLC patients have HER2 amplifications.[8-9]
2. **Sparing wild-type EGFR, to mitigate adverse events and dose-limiting toxicities associated with wild-type EGFR inhibition**
   Inhibition of the closely related ERBB family member wild-type EGFR is associated with adverse events including skin rash and diarrhea.
3. **Activity in the central nervous system (CNS)**
   Almost 50% of HER2-mutant metastatic NSCLC patients develop brain metastases.[10]

**Abbreviations:** amp, amplification; BID, twice per day; CNS, central nervous system; ECD, extra-cellular domain; Exon20ins, exon 20 insertion mutations; HP-β-CD, 2-Hydroxypropyl-β-cyclodextrin; IV, intravenously; Kp, brain-to-plasma partitioning coefficient; Kp,uu, unbound brain-to-plasma partitioning coefficient; MPAS, MAPK pathway activity score; NIH, National Institute of Health; NSCLC, non-small cell lung cancer; PBS, phosphate buffered saline; PDX, patient derived xenograft; phospho-EGFR, phosphorylated EGFR; phospho-HER2, phosphorylated HER2; PO, orally; Q3W, once every three weeks; SEM, standard error of the mean; T-DXd, trastuzumab deruxtecan; T-DXd-R, T-DXd resistant; TKD, tyrosine kinase domain; TKI, tyrosine kinase inhibitor; TMD, transmembrane domain; WT, wild-type.

**References**

[1]Robichaux *et al., Cancer Cell* 2019
[2]Ishiyama *et al., Cancer Research* 2022
[3]Perera *et al., PNAS* 2009
[4]FDA Prescribing Information (Reference ID: 5029058)
[5]Li *et al., ESMO Conference* 2023
[6]Yamamoto *et al., IASLC World Conference of Lung* 2023
[7]Andrews *et al., EORTC-NCI-AACR Conference 2022*
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[9]Liu *et al., Clinical Cancer Research* 2018
[10]Offin *et al., Cancer* 2019
[11]Shaw *et al., NEJM* 2020
[12]Wagle *et al., NPJ Precision Oncology* 2018
[13]Wang *et al., SITC Conference* 2023

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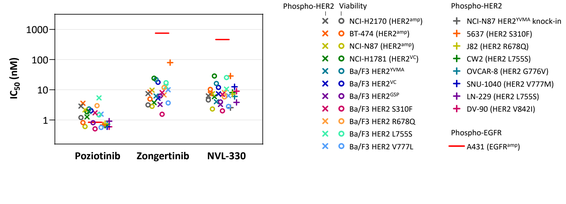
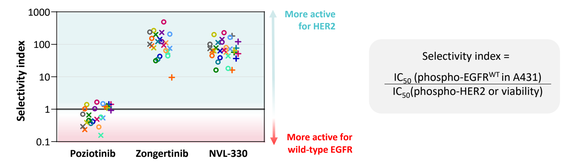
**Disclaimer:** Head-to-head clinical studies comparing NVL-330 with currently approved or investigational therapies have not been conducted.

## IN VITRO POTENCY AND SELECTIVITY

### NVL-330: a Broadly Active and Selective HER2 TKI

- NVL-330 showed broad activity on HER2 oncogenic alterations, including HER2 exon20ins, activating point mutations, and amplified wild-type HER2.
- NVL-330 and zongertinib had similar HER2 potency and selectivity over wild-type EGFR.
- Poziotinib, a pan-ERBB inhibitor, did not demonstrate selectivity over wild-type EGFR.

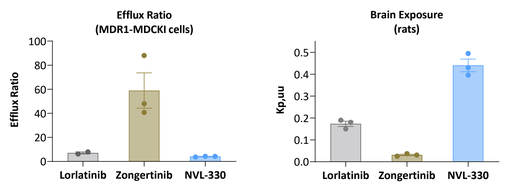



▲ Figure 2 (**Top**) $IC_{50}$ values of NVL-330 in phospho-HER2 or phospho-EGFR AlphaLISA® assays or CellTiter-Glo® viability assays. (**Bottom**) Selectivity indexes over wild-type EGFR as defined by the formula in the graph. AlphaLISA® assays were conducted after a 4-hour treatment. CellTiter-Glo® viability assays were conducted after a 2-day (Ba/F3), 5-day (NCI-H1781), or 3-day (all other cell lines) treatment. The phospho-EGFR assay in A431 cells was conducted without EGF. The phospho-HER2 assay in 5637 cells was conducted with 30 ng/mL EGF. The NCI-N87 HER2$^{YVMA}$ knock-in cell line was generated via CRISPR/Cas9. Geometric means plotted (n = 2 – 51, except n=1 for the following: poziotinib in NCI-H2170 phospho-HER2, zongertinib in BT-474 phospho-HER2, and zongertinib in NCI-H2170 viability assays).

## CNS PENETRANCE

- Low efflux (measured in the MDR1-MDCKI cells) and high brain-to-plasma partitioning (measured by rat Kp,uu) are positive predictors of brain exposure in humans.
- NVL-330 had an efflux ratio and Kp,uu comparable to lorlatinib, a CNS-active TKI benchmark.[11]
- Zongertinib had a high efflux ratio and a low Kp,uu.

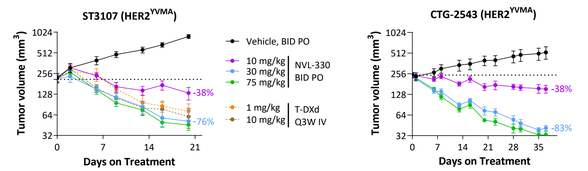



▲ Figure 3 (**Left**) Efflux ratio defined as $P_{app}(B\text{-}A)/P_{app}(A\text{-}B)$ in MDR1-MDCKI cells (NIH; n = 2 – 3). (**Right**) Unbound brain-to-plasma partitioning coefficient (Kp,uu) determined at 1 hour after a single oral dose of 10 mg/kg of lorlatinib, zongertinib or NVL-330 in Wistar Han rats (n = 3), adjusted for fraction unbound in rat plasma and brain homogenate. Average ± SEM plotted.

## IN VIVO ANTITUMOR ACTIVITY

### Activity in HER2 Exon20ins PDX Models

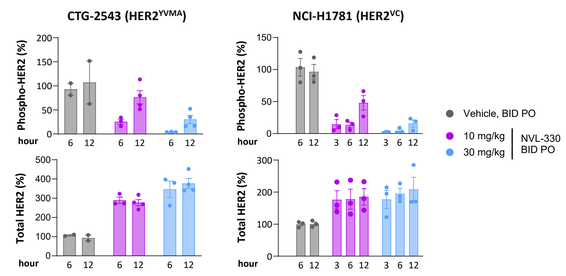
- NVL-330 showed dose-dependent antitumor efficacy in patient derived xenograft (PDX) models harboring HER2$^{YVMA}$, with regression starting at 10 mg/kg BID PO.
- T-DXd achieved tumor regression in the ST3107 model.

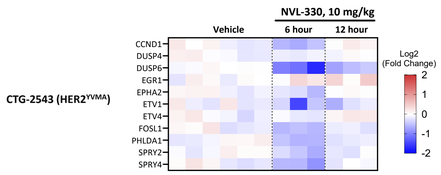



▲ Figure 4 Antitumor activity in subcutaneous ST3107 (**Left**) and CTG-2543 (**Right**) NSCLC PDX models in mice. Vehicle was 20% HP-β-CD pH=3 – 3.5 for NVL-330. T-DXd was diluted in PBS before IV administration. Average tumor volume changes for NVL-330 treated mice (10 and 30 mg/kg) compared to baseline are annotated. All treatments were well tolerated, without significant body weight loss. Average ± SEM plotted (n = 5 mice per group; n = 4 after day 20 for 10 mg/kg NVL-330 group in the CTG-2543 model).

### Pharmacodynamic Activity

- NVL-330 suppressed phospho-HER2 and increased total HER2 in CTG-2543 (HER2$^{YVMA}$) and NCI-H1781 (HER2$^{VC}$) tumors.
- NVL-330 suppressed downstream MAPK pathway activity, as measured by reductions in mRNA levels of DUSP6 and other MAPK pathway activity score (MPAS) genes.[12]

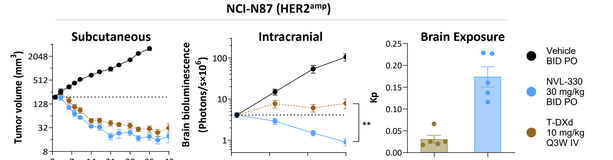



▲ Figure 5 Pharmacodynamic analyses in CTG-2543 and NCI-H1781 tumor samples collected from mice after 3-day treatment with vehicle or NVL-330. (**Top**) MSD analysis of phospho-HER2 Y1248 and total HER2 in tumors collected at multiple time points after the final dose, plotted as percent of average of vehicle. (**Bottom**) Changes in MPAS gene transcript levels in CTG-2543 tumors determined by RNA-seq. Each column represents one mouse. Vehicle treated tumors collected at 6-hour and 12-hour were combined as one group in data analysis.

### Intracranial Activity of NVL-330 and T-DXd

- NVL-330 (30 mg/kg BID) induced subcutaneous and intracranial NCI-N87 tumor regression.
- T-DXd (10 mg/kg Q3W, a clinically relevant dose in mice) induced NCI-N87 tumor stasis intracranially, despite subcutaneous tumor regression.
- NVL-330 demonstrated higher brain penetrance than T-DXd at the intracranial study endpoint.

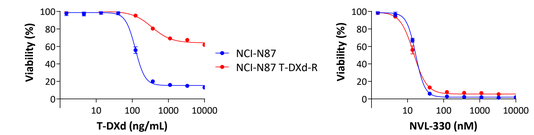



▲ Figure 6 Antitumor activity in NCI-N87-Luc subcutaneous (**Left**, n = 5) and intracranial (**Middle**, n = 10 – 18) tumor models in nude mice. Intracranial tumor burden was assessed by bioluminescence imaging. The treatment started 12 days after intracranial tumor implantation. Statistical analysis was conducted using 2-way ANOVA with the Geisser-Greenhouse correction. **, p < 0.01. (**Right**) Brain-to-plasma partitioning coefficient (Kp) from mice on day 21 at the dosing trough in the intracranial study. N=5. Average ± SEM plotted.

## ACTIVITY POST T-DXD

### NVL-330 Activity Following T-DXd Acquired Resistance

- The T-DXd resistant cell line (NCI-N87 T-DXd-R) was generated via long-term culturing of NCI-N87 cells with T-DXd, which resulted in payload (DX-8951 derivative) resistance.[13]
- NVL-330 was equally effective in NCI-N87 and NCI-N87 T-DXd-R cells in vitro.




▲ Figure 7 Dose response curves of NCI-N87 and NCI-N87 T-DXd-R cells towards T-DXd (**Left**) and NVL-330 (**Right**) determined in a 6-day CellTiter-Glo® viability assay. Average ± SEM plotted (n = 6, from two independent experiments).

## CONCLUSIONS

- NVL-330 had broad preclinical activity on HER2 oncogenic alterations, including HER2 exon20ins, HER2 activating point mutations, and amplified wild-type HER2.
- Preclinical comparison with zongertinib
  - Similar potency and selectivity over wild-type EGFR;
  - NVL-330 demonstrated higher CNS penetrance.
- Preclinical comparison with T-DXd
  - NVL-330 achieved deeper response and higher CNS penetrance in an intracranial tumor model;
  - NVL-330 had activity in cells with acquired resistance to T-DXd.
- Initiation of a Phase 1 clinical trial for NVL-330 is planned in 2024.



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