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Different lung cancer-associated *ERBB2* variants exhibit distinct therapeutic vulnerabilities.

Background:
Activating mutations in *ERBB2* occur in 1-4% of all non-small cell lung cancers (NSCLCs), and most mutations are exon 20 insertions encoding part of the ERBB2 tyrosine kinase domain (TKD). NSCLCs with *ERBB2* mutations in exons that encode domains outside of the TKD are understudied, including those in the extracellular (ECD) and transmembrane (TMD) domains. Early clinical data suggest that ECD- or TMD-mutant tumors might be less sensitive to zongertinib, however, novel models to study the molecular properties and therapeutic vulnerabilities of tumors harboring these mutations are urgently needed.

Methods:
Patients with NSCLC with *ERBB2*-activating mutations with genomic tumor profiling data from the AACR Project GENIE database were included in this study. The cohort was divided into cases with TKD, ECD, and TMD *ERBB2* mutations, and mutational data across the groups were compared. We also stably expressed different *ERBB2* variants (*ERBB2*^{WT} = wild type, *ERBB2*^{S310F} = ECD, *ERBB2*^{V659E} = TMD, and *ERBB2*^{YVMA} = TKD) in immortalized human tracheobronchial epithelial (AALE) cells to determine their consequences for oncogenic transformation in 3D growth assays, ERBB2 signaling, receptor trafficking by immunofluorescence and sensitivity to trastuzumab deruxtecan (T-DXd) and the novel ERBB2-tyrosine kinase inhibitors (TKIs) zongertinib and sevabertinib.

Results:
Of 487 cases of *ERBB2*-mutant NSCLC from the GENIE database, most had TKD mutations (75%), followed by ECD (18%) and TMD (7%) mutations. ECD or TMD-mutant tumors had a significantly higher tumor mutation burden compared to TKD-mutant tumors (ECD vs TMD vs TKD: 11.1 vs 8.4 vs 5.2 mut/Mb, $p<0.0001$). Interestingly, in comparison to TKD-mutant tumors, ECD-mutant tumors had high rates of co-mutations in *EGFR* (32%, $p<0.001$) and *KRAS* (17%, $p<0.001$), and TMD-mutant tumors in *KRAS* (18%, $p<0.001$) and *PIK3CA* (24%, $p<0.001$). In the engineered lung lines, all three *ERBB2* mutation variants resulted in increased phosphorylation levels of ERBB2 as well as activation of MAPK and PI3K downstream signaling. However, compared to *ERBB2*^{YVMA} and *ERBB2*^{V659E} -transfected cells, *ERBB2*^{S310F}-expressing cells had a significantly lower potential ($p<0.001$) to induce oncogenic transformation, and exhibited reduced sensitivity to the ERBB2-selective TKI zongertinib (IC_{50}: *ERBB2*^{S310F} = 359nM, *ERBB2*^{V659E} = 12nM, *ERBB2*^{YVMA} = 18nM) in 3D growth assays. In contrast, all mutated variants were highly sensitive to the dual EGFR-ERBB2 TKI sevabertinib (IC_{50}: *ERBB2*^{S310F} = 5nM, *ERBB2*^{V659E} = 14nM, *ERBB2*^{YVMA} = 9nM). Strikingly, whereas the *ERBB2*^{WT} and *ERBB2*^{S310F} mutant receptors predominantly localize to the plasma membrane, *ERBB2*^{YVMA} and *ERBB2*^{V659E} mutant receptors localize intracellularly in all cell types tested. Potentially related to this localization pattern, *ERBB2*^{WT} and *ERBB2*^{S310F}-expressing AALE cells were quite sensitive (IC_{50}: <50ng/mL) to T-DXd cytotoxicity, whereas cells expressing the *ERBB2*^{YVMA} and *ERBB2*^{V659E} variants exhibited reduced sensitivity (IC_{50}: 2,400ng/mL), or resistance (IC_{50}: >10,000ng/mL), respectively, to the antibody-drug conjugate.

Conclusion:
Our study reveals that the specific *ERBB2* mutation subtype is functionally relevant for determining the sensitivity profile to T-DXd and the novel ERBB2 TKIs zongertinib and sevabertinib. These findings have implications for understanding the biology of *ERBB2*-mutant NSCLC and for optimizing treatment strategies for patients with this disease.