Fusions involving the neuregulin 1 gene (NRG1) occur at low frequency in pancreatic, lung, and other cancers. NRG1 fusion oncoproteins bind to HER3, leading to heterodimerization with HER2 and potent activation of downstream signaling mainly via the PI3K-AKT pathway. Zeno cetuximab (Zeno), an ADC-containing bi-specific antibody, uniquely ‘locks’ on HER3, to position the antibody and subsequently ‘locks’ NRG1 from interacting with HER3, effectively preventing HER2/HER3 heterodimerization and downstream signaling. Our goal in this study was to evaluate the efficacy of Zeno in preclinical models of NRG1 fusion-positive cancers.

We tested Zeno in a panel of isogenic and patient-derived cell line and xenograft (PDX) models of lung, breast and pancreatic cancers. Cell lines either expressed an NRG1 fusion endogenously (MDA-MB-157-VII, DOC4-NRG1) or by lentiviral transfer of cDNAs (ATP6i-NRG1 and SLCO4A2-NRG1 in H6C7, pancreatic ductal cell line; CD45-NRG1 and VAMP-NRG1 in immortalized human bronchial epithelial cells; and DOC4-NRG1 in MCF-7 breast cancer cells). PDX models were generated from NSCLC samples harboring CD45-NRG1 (ST3204) or SLCO4A2-NRG1 (LUAD-0061AS) fusions and from a high grade squamous ovarian cancer harboring a CD74-NRG1 fusion (OV-10-0050). Zeno treatment of NRG1 fusions-expressing breast, pancreatic, and lung cancer cell lines resulted in dose-dependent reduction of growth, and abrogated phosphorylation of HER2, HER3, AKT, p70S6 kinase and STAT3 in all cell lines tested. Phosphorylation of HER2, EGFR and MEK/ERK was inhibited, albeit with some variation, in a cell line-specific manner. Growth of isogenic control cell lines without NRG1 fusion was not significantly altered by Zeno treatment. In breast and lung cancer cell lines, Zeno treatment down-regulated cyclin D1 level and induced expression of the negative cell cycle regulator P21 or P27.

Evidence of apoptosis activation (cleaved PARP, expression of BIM and PUMA) was also observed in cells exposed to Zeno. Treatment of mice bearing LUAD-0061AS, ST3204 and OV-10-0050 PDX tumors (2, 8, 25 mg/kg, QW) caused a dose-dependent inhibition of tumor growth, with tumor shrinkage observed at the highest dose which is equivalent to the current human dose (750 µg QW). Finally, we assessed the ability of Zeno to induce antibody-dependent cellular cytotoxicity (ADCC) using a chromium release assay and peripheral blood mononuclear cells. Zeno induced significant cytotoxicity in MDA-MB-157-VII cells while a non-ADCC enhanced, non-specific IgG had no effect.

Here we show that Zeno effectively blocks the growth of NRG1 fusion-positive cell lines and xenograft models of tumors arising from lung, pancreas and other organs. In vitro, Zeno has no significant effect on parental cells and only inhibits viability induced by expression of a NRG1 fusion. In vivo, Zeno demonstrated significant efficacy at clinically relevant dose levels illustrating potent therapeutic activity. These results support the continued development of Zeno to treat patients with this molecularly defined subset of cancers.