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Zenocutuzumab, an antibody that can overcome HER3 mediated NRG1 signaling in tumor cells by docking on HER2.

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INTRODUCTION

HER2/HER3 signaling is upregulated in many forms of cancer.

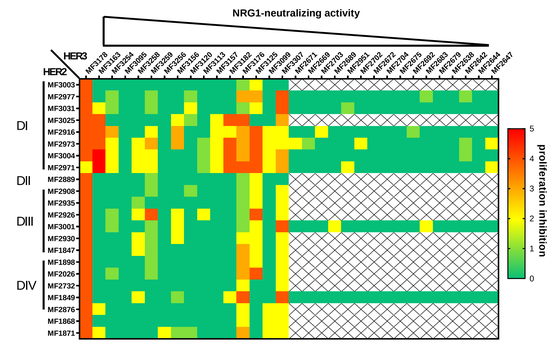
Activation of the HER2/HER3 pathway occurs by the HER3 protein binding either the ligand neuregulin-1 (NRG1; also known as heregulin), or an oncogenic NRG1 fusion protein[1]. Oncogenic NRG1 fusion proteins are expressed as a consequence of a genetic rearrangement of the NRG1 gene and have been identified in a wide variety of tumors[2].

The anti-HER2xHER3 bispecific antibody zenocutuzumab (Zeno) was developed to inhibit NRG1-mediated HER2:HER3 signaling[3].

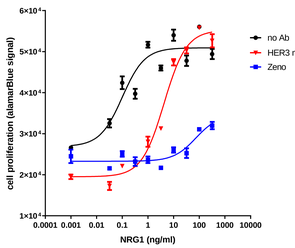
UNBIASED SCREEN FOR HER2×HER3 BICLONICS® IN BXPC-3 CELLS

HER2 and HER3 common light chain Fabs were combined to generate a library of bi-specific antibodies and screened for inhibition of NRG1-dependent growth of the pancreatic adenocarcinoma BxPC3 cells.

The bi-specific antibody with the Fab arms MF3004 (HER2) and MF3178 (HER3) potently inhibited NRG1-dependent growth. Zeno is based on a humanized variant of this bi-specific antibody.



EFFICIENT INHIBITION OF NRG1-DRIVEN CELL PROLIFERATION



N87 gastric carcinoma cells were stimulated with increasing concentrations of NRG1 and treated with Zeno or the parental HER3 mAb. After 3 days the cell density was measured.

Zeno efficiently inhibits NRG1-dependent cell proliferation.

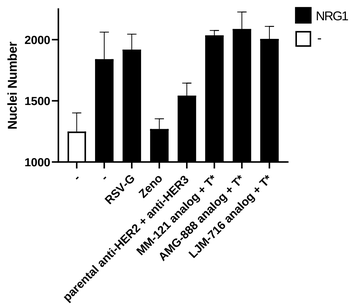
Zeno inhibits NRG1-dependent cell proliferation at higher NRG1 concentration than the bivalent parental HER3 mAb.

ZENO BLOCKS HIGH NRG1-INDUCED PROLIFERATION

SKBR-3 breast cancer cells were stimulated with high NRG1 (EC90) concentrations and treated with the indicated antibodies.

Zeno more potently inhibits NRG1-driven cell proliferation than the combination of HER2- and HER3- antibodies.

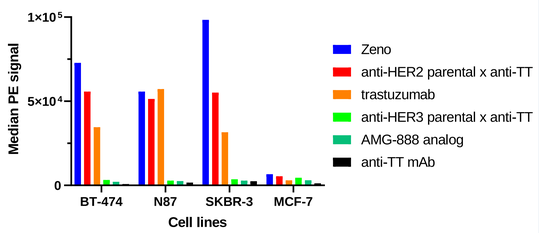
Analogs of MM-121, AMG888, LJM-716 which target HER3 were combined with the HER2 antibody trastuzumab (T*). Zeno is also more effective than the combination of its parental HER2 and HER3 mAbs. RSV-G antibodies were used as negative control.



TARGET CELL BINDING IS LARGELY DETERMINED BY HER2

Antibody binding on cancer cells as measured by the FACS median fluorescence intensity.

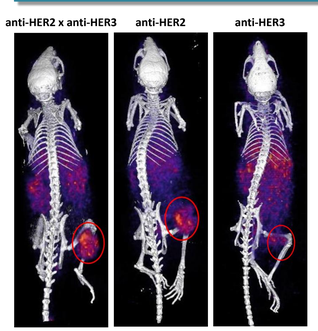
Target cell binding of Zeno compared to bispecific antibodies of the HER2 or HER3 Fab, combined with a negative control tetanus toxoid (TT) Fab. The HER2 mAb Trastuzumab and the HER3 mAb AMG-888 analog were controls.

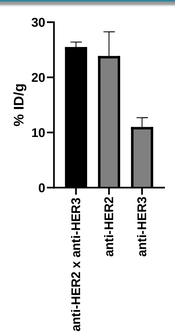


BT-474 (breast, ductal carcinoma), N87 (gastric carcinoma) and SKBR-3 (breast, adenocarcinoma) express high levels (+++) of HER2 while MCF-7 (breast, adenocarcinoma) express low levels (+) of HER2.

Our results suggest that the anti-HER2 binding arm is the main driver of Zeno avidity.

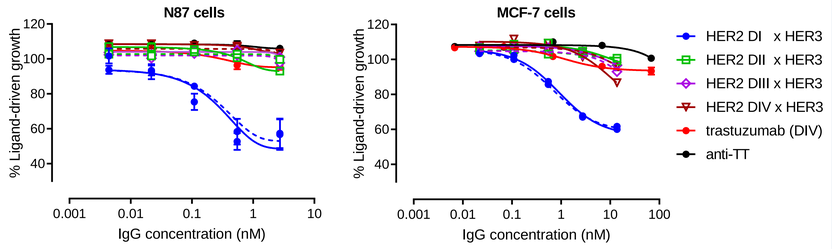
EFFICIENT IN VIVO TUMOR-TARGETING THROUGH HER2 BINDING





HER2, HER3 and HER2xHER3 antibodies with the same Fab arms as Zeno were labelled with 64Cu to evaluate tumor-targeting activity in mice with xenografts of JIMT-1 cells (breast, ductal carcinoma). After 48 hours tumors were imaged and accumulation of radiolabeled antibodies in the tumors was quantified by gamma counts. %ID/g, percentage injected dose per gram tissue.

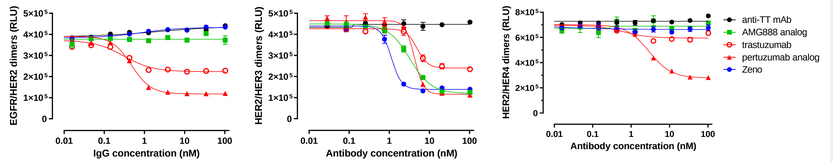
ZENO ACTIVITY IS CORRELATED WITH TARGETING HER2 DOMAIN I



MCF-7 and N87 cells were stimulated with 12.5 nM NRG1.

A titration of bispecific Abs consisting of the Zeno HER3 Fab, combined with different domain-specific anti-HER2 Fabs as indicated (two different HER2 Fabs per group, solid and dashed lines).
Titrations of the Domain IV-binding mAb trastuzumab and a tetanus toxoid mAb (TT) as negative control, were included. Data are represented as means ± SEM.

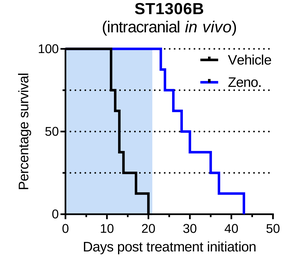
SPECIFIC INHIBITION OF HER2:HER3 DIMERIZATION



Ligand-induced dimerization of ErbB family members (EGFR/HER2; HER2/HER3 and HER2/HER4) was evaluated in β-galactosidase complementation assays.

Zeno specifically inhibited dimerization of HER3 with HER2. Pertuzumab inhibited each HER2-containing dimer pairs (EGFR, HER3 and HER4) and trastuzumab inhibited HER2 dimerization with EGFR and HER3.

INHIBITION OF TUMOR GROWTH IN NRG1-RICH ENVIRONMENT

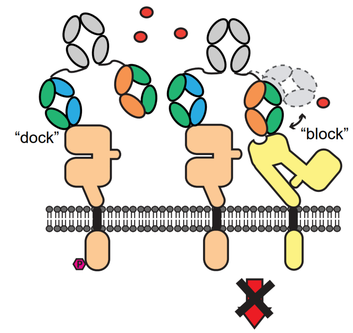


ST1306B patient-derived Xenograft tumor cells were engrafted into the brain of mice by stereotactic injection and tumor growth was monitored by T2-weighed magnetic resonance imaging.

ST1360B have been generated from HER2-amplified breast cancer and express high levels of HER2 and HER3 protein.

Mice treated with Zeno (25mg/kg, twice per week) survived during the 3-week treatment period (blue shaded), while control animals all succumbed.

CONCLUSIONS



Our data shows that Zeno docks to HER2 domain I and effectively blocks HER3 signaling by NRG1.

Efficient *in vivo* tumor targeting is achieved by the docking of Zeno to HER2.

Zeno efficiently inhibits NRG1-driven cell proliferation by blocking HER3 dimerization and signaling.

Zeno efficiently inhibits tumor cell proliferation at high concentrations of NRG1 *in vitro* and *in vivo.*

Zeno offers promise for the treatment of NRG1 fusion cancers. Clinical studies of Zeno in this setting are ongoing.

References

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2) Jonna, Sushma et al. “Detection of NRG1 Gene Fusions in Solid Tumors.” Clinical cancer research : an official journal of the American Association for Cancer Research vol. 25,16 (2019): 4966-4972.
3) Geuijen, Cecile A W et al. “Unbiased Combinatorial Screening Identifies a Bispecific IgG1 that Potently Inhibits HER3 Signaling via HER2-Guided Ligand Blockade.” Cancer cell vol. 33,5 (2018): 922-936.e10.