ErbB3 Inhibitory Surrobodies™ Inhibit Tumor Cell Proliferation *In Vitro* and *In Vivo*

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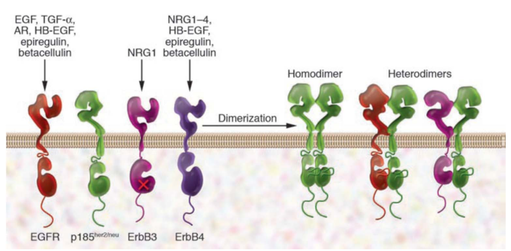
ABSTRACT

ErbB3 is an important regulator of tumorigenesis and is implicated in the development of resistance to several currently used oncology drugs. We have developed a unique, high affinity, antigen-binding structure composed of an immunoglobulin heavy chain and an invariant surrogate light chain called a Surrobody™. We have identified ErbB3 inhibitors through screening of our proprietary Surrobody libraries. Two of these inhibitors appear to work by a previously unrecognized mechanism of action. As a consequence, they not only inhibited cell proliferation and intracellular signaling driven by stimulation with the ErbB3 ligand neuregulin (NRG), but also inhibited signaling and proliferation that was driven by overexpression of ErbB2. In addition, the Surrrobodies inhibited tumor growth *in vivo* in both ErbB2-overexpressing and non-overexpressing cells. In ErbB2 overexpressing cells, both of the anti-ErbB3 Surrobodies significantly augmented the activities of agents that inhibit cell proliferation by different mechanisms. Moreover, although NRG diminished the efficacy of these agents, when they were combined with anti-ErbB3 Surrobodies the effect of NRG was abrogated. In this capacity, the anti-ErbB3 Surrobodies were more effective than an ErbB2/ErbB3 dimerization inhibitory antibody. Despite the fact that these Surrobodies appear to engage ErbB3 differently than previously described anti-ErB3 antibodies currently undergoing clinical testing, they retain all of the beneficial characteristics of this class of agents, including the ability to augment drugs that inhibit EGFR. These anti-ErbB3 agents therefore show substantial promise and greater therapeutic potential than previously described anti-ErbB3 antibodies for development as single agents, in combination with other ErbB directed antibodies or small molecules, or as a component of a bispecific Surrobody.

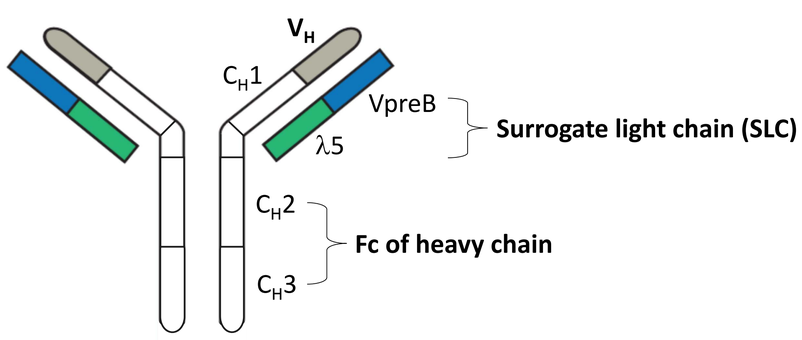
ErbB3
An obligate partner in ErbB2-mediated transformation



Zhang H. et al, J Clin Invest 2007

ErbB3 expression/phosphorylation has negative prognostic value in a number of cancers. Resistance to therapies targeting EGFR or ErbB2 is frequently associated with increased ErbB3 activity and AKT activity.

Schematic of bivalent Surrobody structure



Anti-ErbB3 Surrobody (SgG)

Surrobodies SL-175 and SL-176 were identified from a proprietary fully human library against an ErbB3-Fc fusion protein similar to previously described (1).

- Bind ErbB3 selectively over EGFR and ErbB2
- Amenable to all chromatography methods for antibody purification
- 89-154 mg/L pure protein yield from transient expression in HEK293 cells
- Stable in buffer at room temperature for >1 year
- Size exclusion chromatography indicates protein aggregates at <3%

Target binding *EC50 (pM)*	ErbB3-Fc (ELISA)		Cell surface receptor (FACS)
	human	murine	BxPC-3 cells (human pancreatic)
SL-175	41	124	138
SL-176	49	95	40

Reference

1. Xu L, Yee H, Chan C, Kashyap AK, Horowitz L, Horowitz M, et al. Combinatorial Surrobody libraries. Proc Natl Acad Sci (2008) **105**:10756-61

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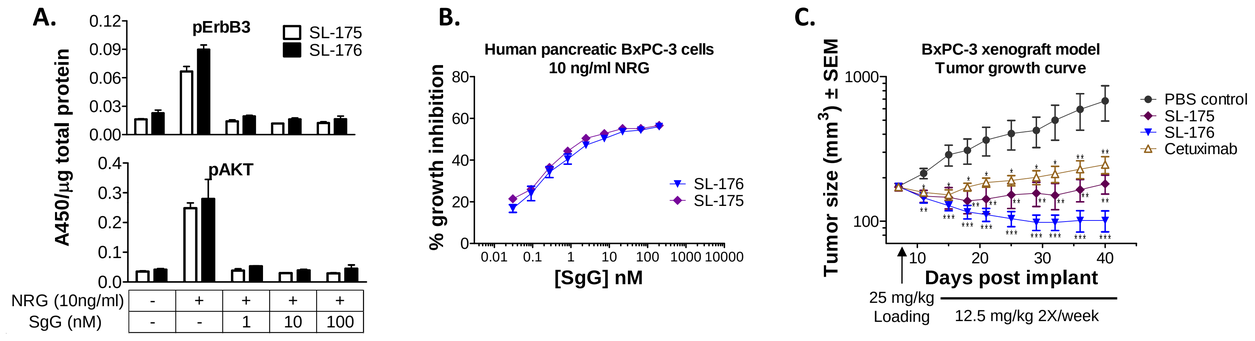
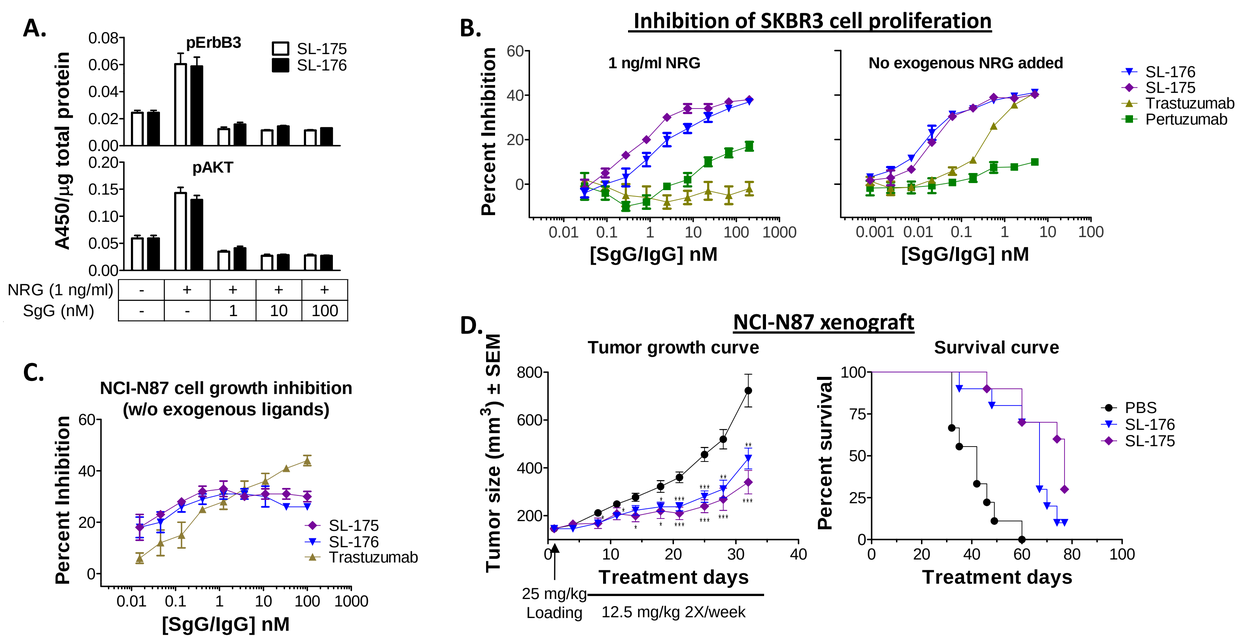
Figure 1: Inhibition of ErbB3 signaling and cancer cell proliferation *in vitro* and *in vivo*



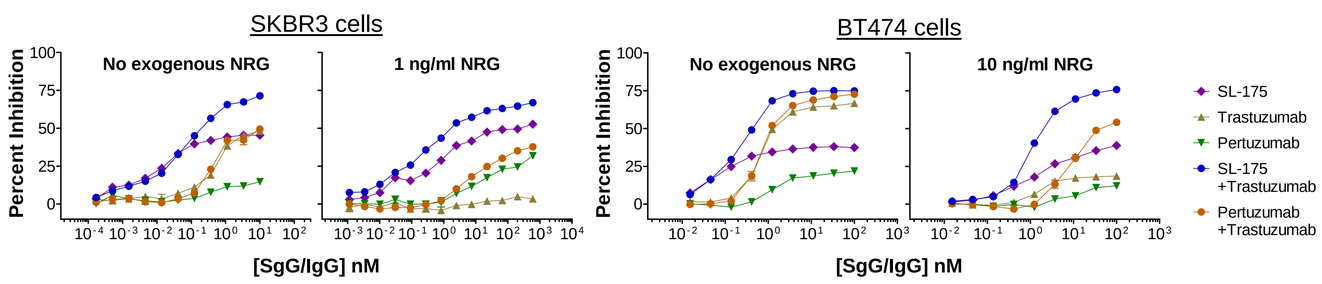
A. Phospho-ErbB3 (panTyr) and phospho-AKT (Ser473) were determined in cell lysates by ELISA in triplicate. **B.** BxPC-3 cells were incubated in serum-free media containing 10 ng/ml NRG in triplicate for 4 days. Relative cell numbers were determined using CellTiterGlo reagents (Promega). SL-175 and SL-176 also inhibited the growth of A431, Colo205, MCF-7, A549, SKBR3, NCI-N87 and BT474 cells. **C.** $5x10^6$ BxPC-3 cells were injected subcutaneously as a matrigel suspension into nude mice. Treatment was carried out as indicated (n=10). Student's T-test was used to assess statistical significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Tumor growth delay for SL-176 and SL-175 was 40.4 ($P < 0.05$) and 19.5 days ($P < 0.05$), respectively.

Figure 2: Inhibition of ErbB2-overexpressing cell proliferation *in vitro* and *in vivo*



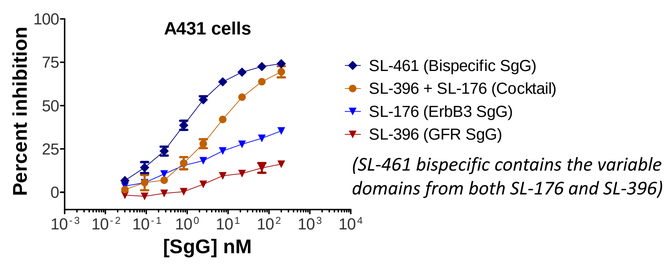
A. Phospho ELISA analysis performed as described in Fig 1. **B,C.** SKBR3 and NCI-N87 cells were incubated in complete media in the absence of NRG for 6 days or for 3 days with 1 ng/ml NRG, as indicated. All experiments were performed in triplicate. **D.** $1x10^7$ NCI-N87 cells were injected subcutaneously as a matrigel suspension into C.B-17 SCID mice. When tumors reached ~$150mm^3$ treatment was carried out as indicated (n=10). Student's T-test was used to assess statistical significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Figure 3: Augmentation of the growth inhibition of ErbB2-overexpressing cells by ErbB2 antibody trastuzumab to a greater extent by ErbB3 Surrobody than ErbB2 antibody pertuzumab



SKBR3 cells were incubated in complete media in the absence or presence of NRG for 6 or 3 days, respectively. BT474 cells were incubated in complete media for 6 days under both conditions. All experiments were performed in triplicate. Similar results were obtained with SL-176.

Figure 4: A bispecific Surrobody targeting two growth factor receptors more potently inhibits tumor cell growth than the combination of parental monospecifics



A431 cells were incubated in serum-free media for 4 days. When tested in combination, the monospecific Surrobodies SL-176 and SL-396 were present at equal concentration as indicated on the graph.

ErbB3 Surrobodies (SL-175 & SL-176)

- Selectively bind and inhibit ErbB3 signaling and cell growth *in vitro* and *in vivo*
- Inhibit ErbB2 overexpressing tumor cell lines *in vitro* and *in vivo*
- Capable of inhibiting ErbB2 overexpressing tumor cell lines in the presence or absence of neuregulin
- ErbB3 Surrobodies augment the activities of ErbB2 antibody trastuzumab to a greater extent than pertuzumab
- A novel bispecific Surrobody targeting ErbB3 and another growth factor receptor demonstrates greater anti-proliferative activity than the combination of the two monospecific Surrobodies
- SL-176 is currently under development and available for partnering