

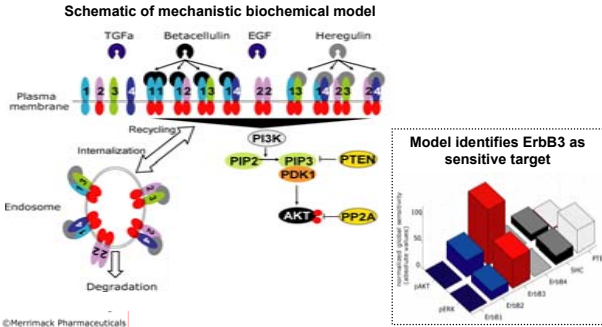
Engineering of a novel bispecific antibody, MM-111, which selectively inhibits ErbB3 in ErbB2 positive tumors and has potent anti-tumor activity

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Abstract

The ErbB family of receptor tyrosine kinases plays a central role in cancer progression due to their potent initiation of the phosphatidylinositol 3-kinase (PI3K) pathway. ErbB3 together with its preferred dimerization partner, ErbB2, initiates a robust signaling cascade in the presence of its ligand, heregulin, and has been identified as a key therapeutic target. We have engineered a bispecific single chain (scFv) antibody fusion molecule, MM-111, which inhibits ligand-induced phosphorylation of ErbB3 with sub-nanomolar potency in ErbB2 over-expressing cancer cells by exploiting the abundant expression of ErbB2 for targeting. We employed computational physicochemical modeling to guide the kinetic optimization of the monovalent binding affinities to the ErbB2 and ErbB3 receptors to increase the potency and specificity of MM-111 for tumor cells. We show that MM-111 inhibits activation of its target ErbB3 and downstream signaling molecules of the PI3K pathway resulting in attenuation of tumor proliferation both *in vitro* and *in vivo*. While the antitumor activity of MM-111 is positively correlated with ErbB2 expression levels, MM-111's potent inhibition of ErbB3 phosphorylation and signaling downstream from this receptor differs markedly from currently available therapies targeting ErbB2 over-expressing tumors and thus provides a novel approach to treatment for these malignancies. In conclusion, our data demonstrate that by combining antibody engineering with computational biology we have developed a novel bispecific antibody with potent anti-tumor properties.

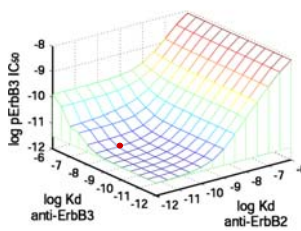
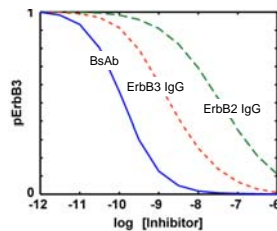
Computational model of the ErbB pathway identifies ErbB3 as sensitive target



Computational model guides MM-111 design

An ErbB2/3 bispecific is the most potent ErbB3 inhibitor in ErbB2 over-expressing cells

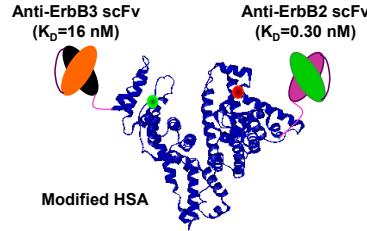
Affinity of ErbB2 arm is more significant than for ErbB3 arm in inhibiting pErbB3 in ErbB2 over-expressing cells



Simulation in cells expressing 1 x 10⁶ ErbB2 receptors/cell

● MM-111 affinity

MM-111 format

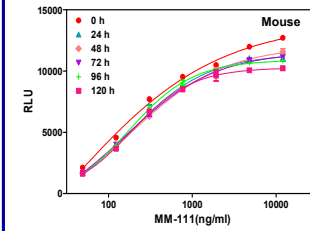


MM-111 features

- High affinity anti-ErbB2 scFv targets MM-111 to ErbB2 over-expressing cells
- Lower affinity anti-ErbB3 scFv inhibits ErbB3 activity
- The ErbB2 and ErbB3 scFv are joined via a modified human serum albumin linker to enhance the serum half-life of MM-111

MM-111 bispecific format is stable in serum

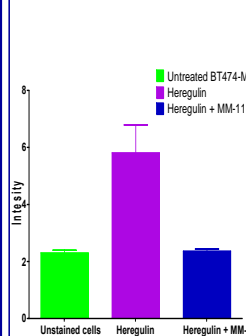
MM-111 retains ability to bind both ErbB2 and ErbB3 following incubation in serum at 37°C at all time points measured



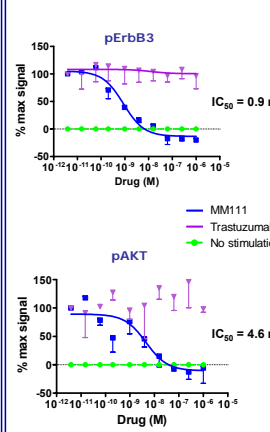
Serum stability of MM-111 was assessed *in vitro* by incubating 100 nM MM-111 in mouse serum at 37°C. MM-111 was removed at the indicated time points and binding to both ErbB2 and ErbB3 was assessed by ELISA with MM-111 captured onto wells coated with recombinant ErbB2 extracellular domain followed by detection with Fc-ErbB3 extracellular domain fusion protein.

MM-111 inhibits ErbB3 activity *in vitro*

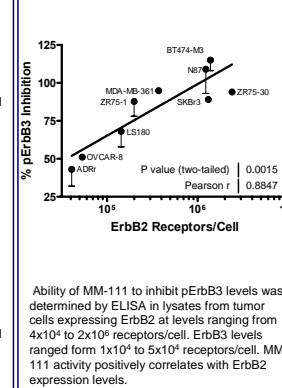
MM-111 blocks heregulin binding to ErbB3 in BT474-M3 cells



MM-111 inhibits pErbB3 and pAKT in BT474-M3 cells



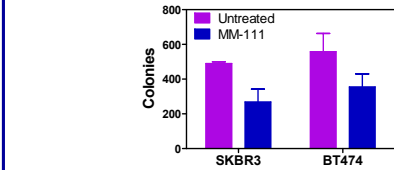
MM-111 activity correlates with ErbB2 levels



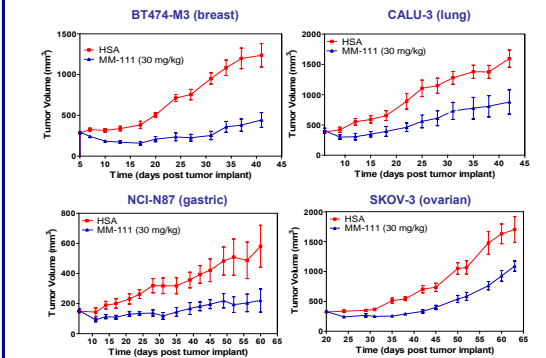
Ability of MM-111 to inhibit pErbB3 levels was determined by ELISA in lysates from tumor cells expressing ErbB2 at levels ranging from 4x10⁴ to 2x10⁶ receptors/cell. ErbB3 levels ranged from 1x10⁴ to 5x10⁴ receptors/cell. MM-111 activity positively correlates with ErbB2 expression levels.

MM-111 inhibits cancer cell growth *in vitro* and *in vivo*

MM-111 treatment inhibits colony formation in BT474 and SKBR3 ErbB2 over-expressing breast tumor cell lines following 14 days of chronic treatment



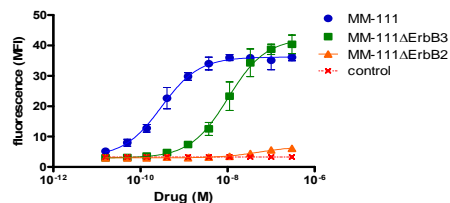
MM-111 treatment delays growth of human tumor xenograft models



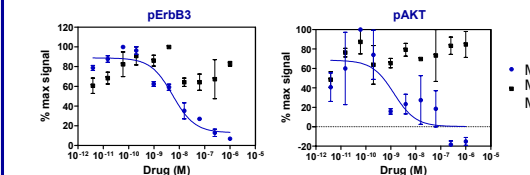
Mice were administered MM-111 every 3 days at the indicated dose. HSA was dosed at 17.5 mg/kg, an equimolar dose to 30 mg/kg MM-111, on the same schedule.

MM-111 bispecific format is optimal for activity

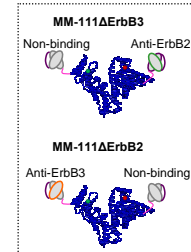
MM-111 shows avidity binding to ErbB2+/ErbB3+ cells compared to monospecific forms



Both arms of MM-111 are necessary for inhibition of signaling in BT474-M3 cells



- MM-111
- MM-111ΔErbB3 + MM-111ΔErbB2



Summary

- ErbB3, the preferred dimerization partner to ErbB2, has been identified as a sensitive target in the ErbB pathway
- Computational models have guided the design of MM-111, a bispecific antibody with high affinity for ErbB2 and potent inhibition of ErbB3 activity in ErbB2 over-expressing cell lines
- The MM-111 format is stable under physiological conditions
- MM-111 has shown potent anti-tumor activity in several *in vivo* models
- MM-111 offers a novel approach for the treatment of tumors that over-express ErbB2