

Computational Modeling and Simulation Guide the Development of MM-111, a Bispecific Antibody Targeting ErbB3 in ErbB2-Overexpressing Tumors

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Abstract

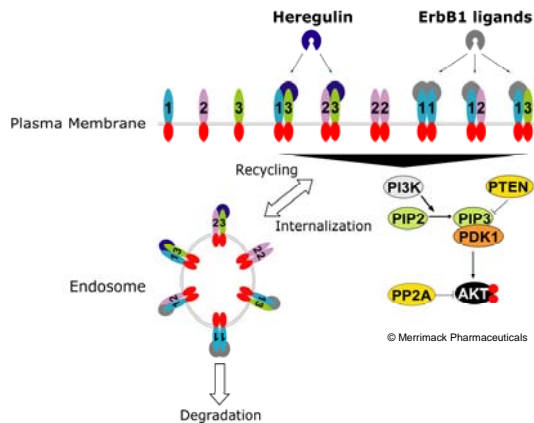
While the importance of ErbB2 in cancer has long been known, the equally important oncogenic role of its preferred dimerization partner, the kinase-dead ErbB3, has only recently been appreciated. To develop next-generation therapeutics against the ErbB2/3 oncogenic unit, we have employed our Network Biology approach coupling quantitative biology with a predictive mechanistic model of ErbB receptor and downstream signaling networks.

The model describes the dynamics of interaction between ErbB1, ErbB2, and ErbB3 in response to heregulin (HRG) binding. Receptor dimerization, phosphorylation, internalization, and degradation along with downstream signaling of the PI3K-AKT cascade are included. The dynamical behavior of the model was constrained using parameter estimation based on high-density experimental signaling data from ErbB2-overexpressing BT474 cancer cells.

Simulating the inhibition of HRG-induced signaling under several conditions indicated that a bispecific antibody directed against ErbB2 and ErbB3 is a novel, highly effective strategy for antagonizing the ErbB2/3 oncogenic unit in the presence of high levels of ErbB2. To take advantage of this insight, we have developed MM-111, which inhibits ligand-induced phosphorylation of ErbB3 by exploiting the overexpression of its dimerization partner, ErbB2, for specific targeting to cancer cells that display both receptors.

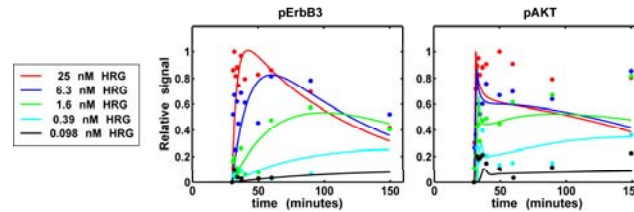
We developed an *in silico* representation of MM-111 and used it to select optimal binding affinities for the ErbB2 and ErbB3 arms. Further computational analysis of the virtual drug predicted that avid, bivalent binding of MM-111 is required for molecular potency, a result which was subsequently confirmed by testing monospecific knockout variants of MM-111. Additionally, by introducing this virtual inhibitor into our ErbB computational model, we have quantitatively predicted the dependence of MM-111 *in vitro* and *in vivo* response on a wide range of ErbB1, ErbB2, and ErbB3 expression levels. Importantly, these results hold across multiple tumor types, suggesting that our computational tools can be used to identify a specific molecular profile that characterizes tumors driven by the ErbB2/3 oncogenic unit for which MM-111 will be efficacious.

Building a computational model of the ErbB pathway



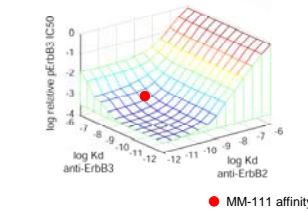
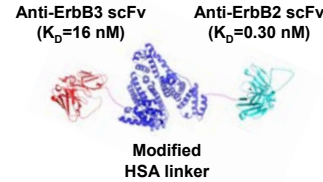
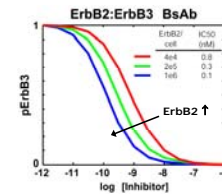
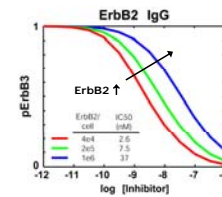
An ordinary differential equation model of heregulin (HRG) signaling was created, incorporating ErbB1, ErbB2, and ErbB3 as well as the PI3-kinase cascade. Receptor homo-/hetero-dimerization and trafficking are also included.

The model describes HRG signaling dynamics in ErbB2-overexpressing cancer cells



Phospho-ErbB1-3 and phospho-AKT were measured by ELISA in serum-starved BT474 cells. The computational model was fit to the data using a genetic algorithm.

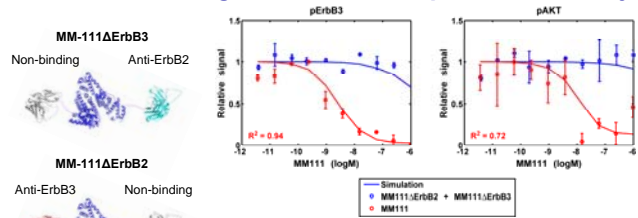
Computational guidance for MM-111 design



Simulations revealed that an ErbB2/3 bispecific antibody (bottom) is superior to either an ErbB2 (top) or ErbB3 (not shown) IgG for inhibiting signaling from the ErbB2/3 heterodimer. All simulations assumed a monovalent $k_{on} = 10^5$ M⁻¹s⁻¹ and $k_{off} = 0.001$ s⁻¹.

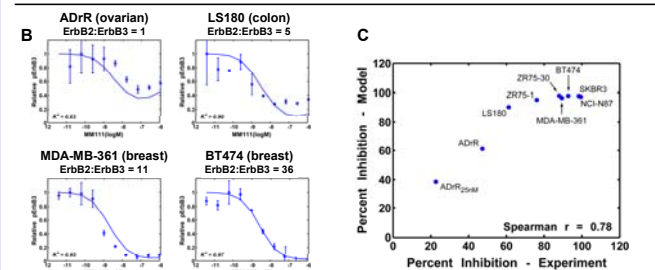
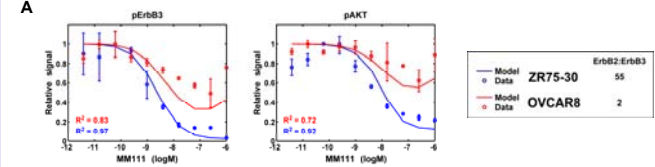
The affinity of the ErbB2 arm is the strongest determinant of MM-111 activity, whereas a high-affinity ErbB3 arm is not required. This design facilitates specific targeting of MM-111 to tumor cells expressing both receptors.

Bivalent binding of MM-111 is required for activity



Simulations predicted that monospecific variants of MM-111 lack efficacy. Experiments confirmed that both arms of MM-111 are required for inhibition of signaling in BT474 cells.

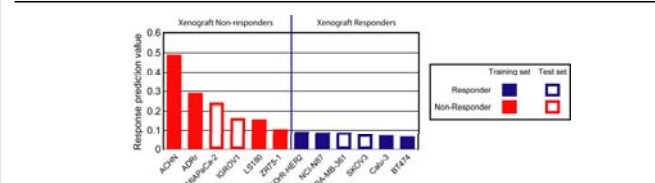
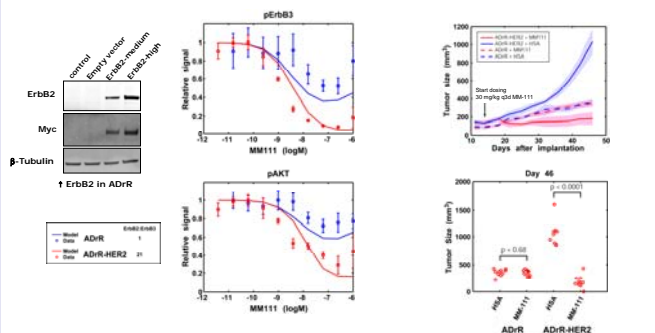
Computational prediction of in vitro response to MM-111 in multiple cell lines



After incubation with MM-111 for 24 hours, cells were stimulated with 5 nM HRG for 10 minutes before lysis. The binding constants of the arms of MM-111 were incorporated into an *in silico* representation of the inhibitor. (A) ErbB2:ErbB3 expression ratio modulates MM-111 inhibition potency. (B-C) The computational model successfully predicts the *in vitro* response of multiple cell lines of varying tumor types and ErbB1, ErbB2, and ErbB3 levels.

Prediction of MM-111 efficacy in vivo

In silico computation → predict → In vivo response



Using our model, we have successfully predicted the xenograft response of multiple cell lines to MM-111.