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Abstract

Lung cancer remains one of the leading causes of death in the United States. Currently available treatments for this disease have shown only modest benefits, and thus, there remains a need for improved therapeutics. Using a combination of computational and experimental biology, we identified ErbB3 as a critical signal transducer in several cancer indications, including lung cancer. Based on this information, we developed a human monoclonal IgG2 antibody (MM-121) against ErbB3 and tested it in multiple assays for its ability to inhibit oncogenic signaling. Previously, we demonstrated that MM-121, used as a single agent, inhibited heregulin-induced signaling events in human cancer cell lines. Additionally, MM-121 caused dose-dependent inhibition of tumor growth in multiple xenograft models of human cancer, including ovarian, renal cell, pancreatic, lung, and prostate cancer. In this study we investigated the efficacy of MM-121 given in combination with either chemotherapy or targeted therapeutics in lung cancer cell lines treated *in vitro* and in lung cancer xenografts. The current data is highly promising as it supports that MM-121 when used as a single therapeutic agent or in combination could have significant clinical benefit for the treatment of lung cancer.

Mathematical model of the ErbB pathway

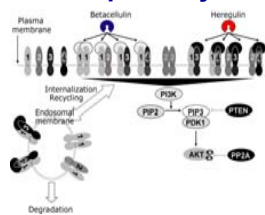


Figure 1. Schematic of the ErbB pathway. The computational model of ErbB signaling is based on literature supported interactions of the four ErbB receptors. Ligand binding, receptor homo- and heterodimerization, receptor internalization, recycling and degradation are included in the model.

MM-121 effects on signaling

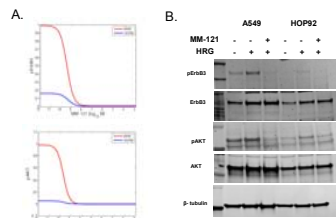


Figure 2. (A) Model simulations predicting the inhibition of pErbB3 and pAkt in lung cancer cells, A549 compared to HOP92 cells treated with MM-121. **(B)** Experimental data (western blot) confirming these predictions. Cells stimulated with heregulin (10nM) for 10 min and treated with MM-121 (250nM).

Activity of MM-121 as a single agent

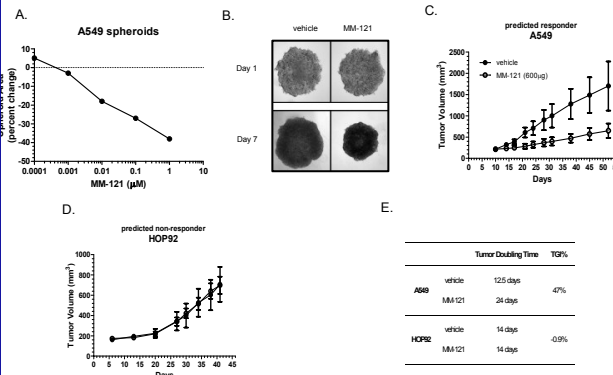


Figure 3. Efficacy of MM-121 tested by spheroid growth assay and xenograft models. **(A)** A549 cells grown as multicellular tumor spheroids (2000 cells/spheroid/well of 96 well plate) and treated with 0.001, 0.01, 0.1, or 1µM MM-121 for 7 days. % change = (Initial Area - Final Area)/Initial Area * 100. **(B)** Spheroids (imaged on Day 1 and Day 7) treated with MM-121 (1µM). Predictions by model simulation tested in lung xenograft models **(C)** A549 and **(D)** HOP92. Tumors were grown s.c. on Nu/Nu mice. Control group received PBS (vehicle) and treatment group (MM-121: 600µg/dose, q3d, i.p.). Tumor volumes were measured twice a week (Tumor volume = 0.52*(LxW²)) **(E)** Tumor doubling time and growth inhibition (%).

MM-121 Pharmacokinetic Analysis

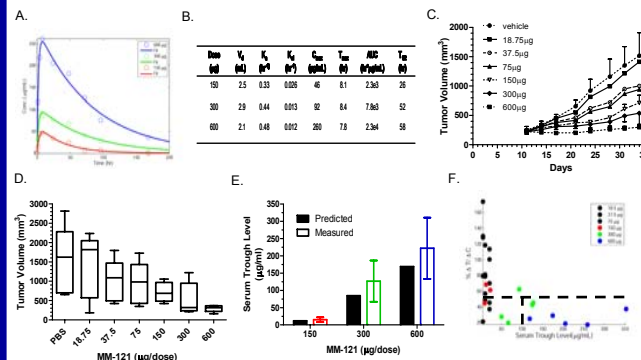


Figure 4. Pharmacokinetic profile of MM-121 and relation to tumor response. A549 xenografts grown s.c. on Nu/Nu mice. **(A)** MM-121 serum levels (color dots) following 1, 4, 8, 24, 48, 72, 96, 168h after one dose (n=4 mice/time point). **(B)** PK parameters at three different doses of MM-121 **(C)** Dose escalation study in MM-121 treated (µg/dose, q3d, i.p.) A549 tumors. **(D)** Tumor volumes at end of study (Day 34). **(E)** Simulation predicted serum levels compared to experimentally measured values **(F)** Tumor growth is inhibited (%ΔT/ΔC) as a function of MM-121 serum levels [experimental data (color dots); simulation data for lower MM-121 doses (black dots)].

MM-121 inhibits pErbB3 and tErbB3 in tumors

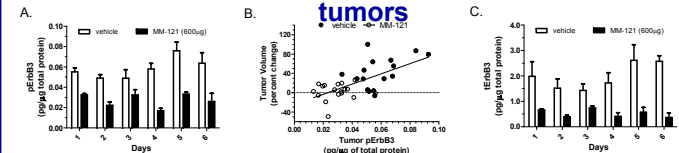


Figure 5. Total and phosphorylated ErbB3 levels in MM-121 treated A549 tumor xenografts. A549 xenografts were grown s.c. in Nu/Nu mice. MM-121 (600µg/dose, q3d, i.p.) was administered on Day 0 and 3. Tumors were harvested at different time points (n=3 mice/time point). **(A)** pErbB3 levels, **(B)** pErbB3 levels related to tumor response [vehicle (○), MM-121 (●)], **(C)** tErbB3 levels.

Combination therapy with MM-121

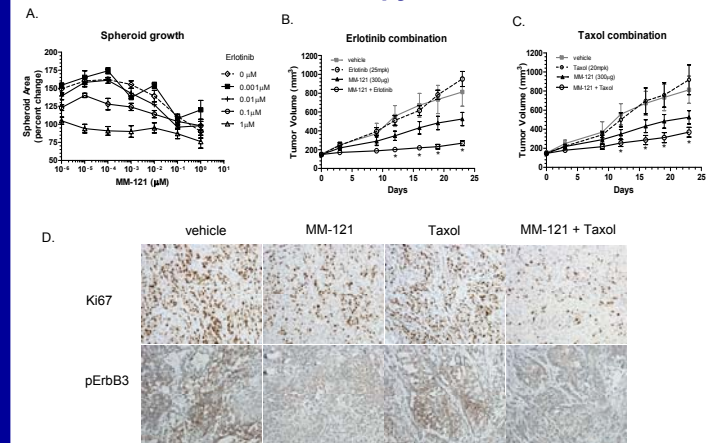


Figure 6. Efficacy of MM-121 in combination with anti-cancer agents. **(A)** Activity of MM-121 in combination with Erlotinib (0.001, 0.01, 0.1, 1µM) in A549 spheroids. Antitumor activity of MM-121 (600µg/dose, q3d, i.p.) in combination with **(B)** Erlotinib (25mg/kg, daily, p.o.) and **(C)** Taxol (20mg/kg, q7d, i.p.) against A549 xenografts; combination therapy shows significantly (*P<0.05) higher tumor growth inhibition. **(D)** Immunohistochemical staining of A549 tumors shows highest inhibition of cell proliferation (Ki67 staining) and ErbB3 phosphorylation in the combination group.

Summary

- Inhibition of ErbB3 and Akt signaling by MM-121 predicted in the computational model was confirmed experimentally by both *in vitro* and *in vivo* analysis.
- MM-121 monotherapy is effectively in inhibiting the growth of lung cancer cells *in vitro* (spheroid) and *in vivo* (xenograft).
- MM-121 serum levels achieved in animals correlated well with tumor response and down regulation of signaling pathways.
- The effectiveness of MM-121 in combination with other clinically approved agents supports its use as a treatment modality for lung cancer patients.