

Olga Burenkova, Aaron C. Fulgham, Ashish Kalra, Matt D. Onsum, Bryan E. Linggi, Birgit Schoeberl, Emily Pace, Kip A. West, Magdalena O. Leszczyniecka, Violette Paragas, Lin Nie, Lihui Xu, Viara Grantcharova, Dongmei Xiao, Howard Latimer, Sharon Moulis, Stavros G. Kopsiaftis, Washington Alves, Jose Varghese, Matt Wallace, Raghida Bukhalid, Gabriela Garcia, William Kubasek, William Slichenmyer, Michael Feldhaus, Ulrik B. Nielsen
Merrimack Pharmaceuticals, Inc., 700 One Kendall Sq, Cambridge, MA 02139

Abstract

Human monoclonal IgG2 antibodies against ErbB3 were developed and tested in multiple assays for their ability to modulate ErbB3 activity and one candidate, MM-121, was chosen to move forward into pre-clinical testing. MM-121 demonstrated potent inhibition of 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 and prostate cancer. Pharmacodynamic analysis of samples from these studies showed that regular MM-121 administration resulted in inhibition of ErbB3 activity which correlated with efficacy.

Cumulatively, these data supported that MM-121 could have significant clinical benefit for the treatment of cancer and warranted its progression into phase 1 clinical trials.

In this study we investigated the potential clinical utility of MM-121 in combination with chemo or targeted therapies in a variety of xenograft models. We have also treated several cancer cell lines *in vitro* with chemotherapeutic agents and MM-121 to evaluate its combined effect on cell viability.

The data support the continued development of MM-121 in combination with other therapeutic agents.

Mathematical model of the ErbB pathway

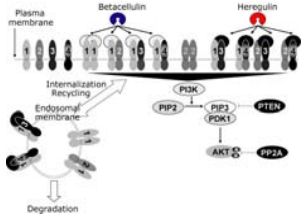


Figure 1. Schematic of the ErbB pathway. The computational model of ErbB signaling is based on literature supported interactions of the four ErbB receptors. Ligand binding, receptor homo- and heterodimerization, receptor internalization, recycling and degradation are included in the model.

MM-121 blocks heregulin induced signaling in multiple cell lines

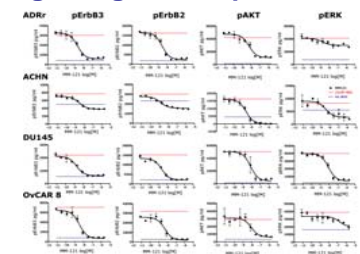


Figure 2. MM-121 inhibition of HRG (25nM)-induced ERBB3, ERBB2, AKT, and ERK phosphorylation was measured by ELISA

In vivo anti-tumor activity of MM-121 as a single agent

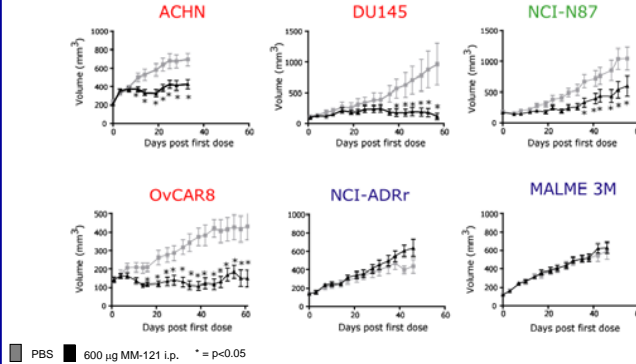


Figure 3. MM-121 Efficacy in xenograft models of human cancer
The efficacy of MM-121 in the inhibition of tumor growth was assessed in multiple xenograft models. Tumors were established in nude mice and animals were dosed intra-peritoneally q3d with 600ug of MM-121. Tumor size (Length x Width) was measured twice a week and measurements were used to calculate tumor volume (p/6 (L x W2)). Red titles indicate strong response, blue indicates non-response, and green indicates partial response.

MM-121 suppresses tumor growth of a KRAS mutant A549 lung cancer cells

As a single agent:

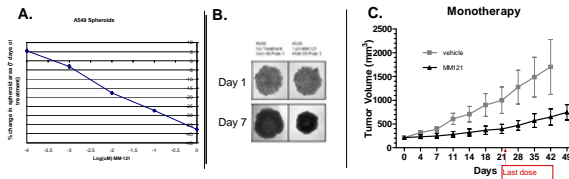


Figure 5. MM-121 inhibits cell growth in-vitro and in-vivo
A. A549 lung cancer cells were grown as multicellular tumor spheroids (2000cells/spheroid/well of 96 well plate) and then treated with 0, .001, 0.01, 0.1, or 1uM MM-121 for seven days ("4" on the graph corresponds to '0' dose). The area was measured under 4x magnification using Metamorph software on day 1 and day 7; % change in spheroid area was calculated ((Initial_Area - Final_Area) / Initial_Area * 100).
B. Photographs of representative spheroids: untreated spheroids grew ~5% in 7 days vs. 35% reduction in size of spheroids treated with 1uM MM-121.
C. Mice bearing A549 subcutaneous xenograft tumors, were treated with 600ug of MM-121 (q3d) resulting in significant tumor growth inhibition that was retained after dosing had stopped

Combination of MM-121 + Erlotinib or Taxol (suboptimal dose) improves efficacy over either agent alone

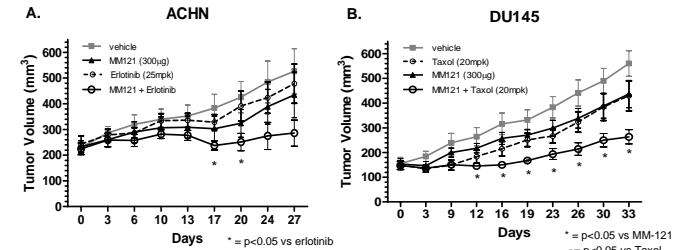


Figure 4. A. The efficacy of MM-121, Erlotinib, and their combination in the inhibition of tumor growth was assessed in ACHN (renal) tumor bearing mice. **B.** The efficacy of MM-121, Taxol and their combination was evaluated in mice bearing DU145 (prostate) xenograft tumors. Tumors were established in nude mice (Charles River Laboratories) and animals were dosed intra-peritoneally (IP) q3d (every three days) with MM-121 (300ug) or Vehicle. Erlotinib (25mg/kg) was administered orally qd (once daily). Taxol (20mg/kg) was given IP on once weekly (q7d) bases. Tumor size (Length x Width) was measured twice a week and measurements were used to calculate tumor volume (p/6 (L x W2)). Inhibition by the combination was greater than either single agent alone resulting in the additive effect.

In combination with other therapeutics:

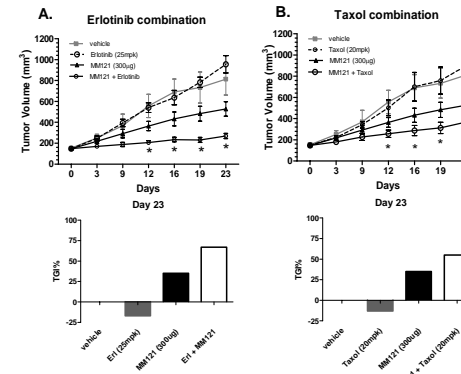


Figure 6. Antitumor activity of MM-121 in combination with either Erlotinib or Taxol against A549 tumor bearing mice.
A. Efficacy of MM-121 (300ug; q3d) and Erlotinib (25mg/kg; qd) combination was significantly better compared to each drug alone. **B.** Significant improvement in efficacy was observed in MM-121 (300ug; q3d) and Taxol (20mg/kg; q7d) combination over either drug as monotherapy.

Summary

- MM-121 inhibits heregulin induced downstream signals such as pErbB3, pErbB2, pAKT, pErk in several cell line *in vitro*
- MM-121, when used as monotherapy, showed anti-tumor activity against multiple tumor types tested *in vivo*
- The combination of MM-121 with either chemotherapy or targeted therapy showed significant anti-tumor response when compared to the single agent treatments
- MM-121 (as a single agent or in combination) effectively inhibits tumor growth of A549 lung cancer cells (containing KRAS mutation) that are otherwise insensitive to either Erlotinib or Taxol.
- These results suggest the potential application of MM-121 in the clinic in combination with other therapeutic agents.