Efficacy of MM-121 in ER+ and triple negative breast cancer studies

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1) Background

In women, breast cancer is the most common cancer and the fifth most common cause of cancer death. Due to the heterogeneity of breast cancer, the progression free survival can vary widely with stage and type from 98% to 10%. We present preliminary data that MM-121, a first-in-class anti-ErbB3 antibody, is efficacious in a subset of both hormone-dependent (ER+) and triple negative (ER-PR). ErbB-2 breast cancer lines that express the molecule profile consistent with MM-121 monotherapy expression in ER+ and HER2 breast cancer models. Using a combination of computational and experimental approaches, ErbB-2 was identified as a critical target of oncogenic signaling (Fig 1) leading to the development of MM-121, a first-in-class anti-ErbB3 antibody. We have previously demonstrated that MM-121, when used as a single agent, inhibits heregulin-induced signaling events in human breast cancer cell lines. Moreover, MM-121 caused dose-dependent suppression in MDA-MB-175-VII xenograft tumors. Here, we show that MM-121 can inhibit both ErbB-2 and Her2 induced VEGF and inhibition of HRG stimulated MDA-MB-175-VII cell growth greater than either single agent alone.

2) Identification of a MM-121 positive biomarker signature in multiple breast cancer cell lines

3) MM-121 treatment inhibits VEGF secretion in breast cancer cell

A) MM-121 inhibits secretion of pERB3 in BT474 and T47D cells. Western blot of total pERB3 and total ErbB3 (IgG-HER2) confirmed with antibodies against phospho-ERB3 (Tyr1173), ErbB3, phosphoErbB3 (Tyr1238), phospho-Akt (Ser473) and beta-actin (all antibodies were purchased from Cell Signaling Technology). BT474-M3 xenograft tumors. Mice were implanted with estradiol pellets (0.72mg/pellet/60days, Innovative Research of America) and 19x10^6 BT474-3 cells/mouse. 7 days post implantation, mice were randomized based on tumor volume and treated with MM-121 (Q3D) and/or tamoxifen (pellet 5mg/pellet/60days, Innovative Research of America).

4) Treatment with MM-121 inhibits proliferation and induces apoptosis in vitro

A) MDA-MB-175-VII cell viability is inhibited by MM-121. MDA-MB-175-VII (20% FBS) cells (10% FBS) were treated with MM-121 for 48 hrs then lysed and Western blots were probed for Bcl-2 (Santa Cruz) and BAX (Santa Cruz). Established tumors were dosed every 3 days with either PBS or MM-121 at 600 ug/mouse (B, C) or 1500 ug/mouse (A).

5) MM-121 monotherapy is efficacious in vivo

A) MDA-MB-175-VII cell viability is inhibited by MM-121. MDA-MB-175-VII cells (20% FBS) were treated with MM-121 for 6 days and cell viability was assayed by cell titer glo (Promega). B) MM-121 induces cell cycle arrest in MDA-MB-175-VII cells. Cells (2% FBS) were treated with MM-121 for 96 hrs then trypsinized (0.05%) and stained using Propidium Iodide. FlowJo software was used for analysis. C) MM-121 induces apoptosis in MDA-MB-175-VII cells. Cells (20% FBS) were treated with MM-121 for 48 hrs then trypsinized (0.05%) and stained using Propidium Iodide and Annexin V. D) MM-7 cells upregulate Bax in response to MM-121 treatment. MCF-7 cells (10% FBS) were treated with MM-121 for 48 hrs. Images and Western blots were probed for Bcl-2 (Santa Cruz) and BAX (Santa Cruz).

6) MM-121 in combination with paclitaxel inhibits cell growth greater than either single agent alone

A) Tamoxifen treatment reduces pERB3 and total ErbB3 levels in BT474-M3 cells. Western blot of total pERB3 and total ErbB3 (IgG-HER2) confirmed with antibodies against phospho-ERB3 (Tyr1173), ErbB3, phosphoErbB3 (Tyr1238), phospho-Akt (Ser473) and beta-actin (all antibodies were purchased from Cell Signaling Technology). BT474-M3 xenograft tumors. Mice were implanted with estradiol pellets (0.72mg/pellet/60days, Innovative Research of America) and 19x10^6 BT474-M3 cells/mouse. 7 days post implantation, mice were randomized based on tumor volume and treated with MM-121 (Q3D) and/or tamoxifen (pellet 5mg/pellet/60days, Innovative Research of America).

7) MM-121 in combination with paclitaxel inhibits cell growth greater than either single agent alone

A) MDA-MB-175-VII cell viability decreases in vitro in response to MM-121 and paclitaxel combination treatment. MDA-MB-175-VII cells (20% FBS) were treated with MM-121 and/or paclitaxel for 4 days and cell viability was assayed by cell titer glo (Promega). C) MDA-MB-231 xenograft tumors treated with MM-121 in combination with paclitaxel. Mice were randomized based on tumor volume and treated with MDA-MB-231 cells/mouse (low growth factor matrigel, BD Biosciences). Mice were implanted orthotopically with 20x10^6 MDA-MB-231 cells/mouse (low growth factor matrigel, BD Biosciences).