A HER3 antibody that blocks ligand-independent HER2-HER3 dimerization inhibits growth of HER2-dependent tumors and sensitizes to HER2 and PI3K inhibitors

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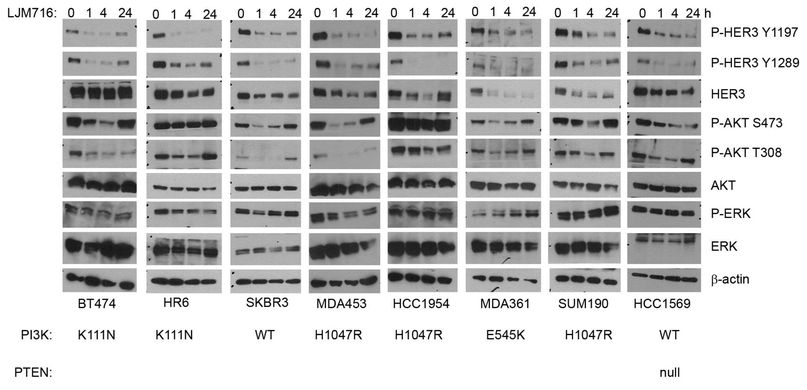
Introduction

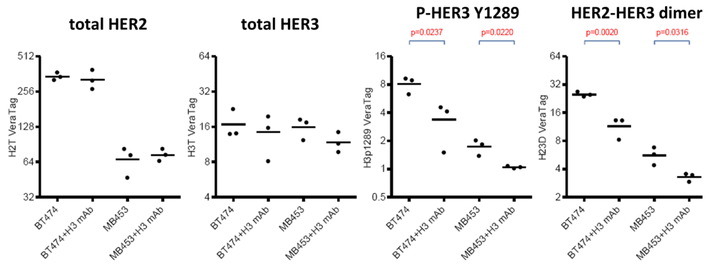
- Inappropriate HER2/HER3 dimerization as a result of HER2 over-expression in cancer results in HER3 mediated activation of the oncogenic PI3K pathway.
- HER2-targeted agents such as trastuzumab, pertuzumab or lapatinib inefficiently inhibit HER2-mediated HER3 activation allowing persistent HER3 signaling that is speculated to limit clinical responses.
- Consequently, the combination of a HER3 targeted agent with HER2 agents may be of clinical benefit.
- Furthermore, HER3 activation has recently been implicated in the relief of a feedback loop induced by PI3K inhibitors.
- This compensatory phosphorylation of HER3 counteracts the pharmacological inhibition of PI3K/Akt and limits the full activity of PI3K/Akt antagonists.
- We hypothesize that complete inhibition of HER3 is required for the full effect of PI3K/Akt inhibitors against HER2+ tumors.

LJM716 inhibits HER3-PI3K signaling

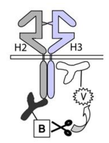


Various cell lines were treated with 10 µg/ml of LJM716 for times as indicated. Whole cell lysates were prepared and separated in a 7% SDS gel followed by immunoblot analysis with indicated antibodies.

HER3 antibody disrupts HER2/HER3 interactions

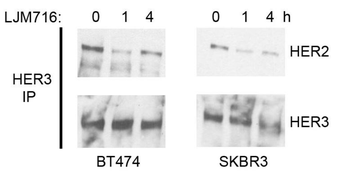


Mice bearing BT474 or MDA453 xenografts were treated with at least two doses of 20mg/kg LJM716 and sacrificed 4 hours after the last dose. The formalin fixed paraffin embedded tumor sections were subjected to VeraTag analysis. A pair of antibodies, one of which is conjugated to biotin and the other a fluorescent molecule (VeraTag) suitable for analysis by capillary electrophoresis, bind to distinct epitopes on HER2 or HER3. The VeraTag molecules are attached to the antibodies via photo-cleavable linkers. Methylene blue, conjugated to streptavidin, binds to the biotin-labeled antibody and is photo-activated by red light. The released singlet oxygen, as a result of methylene blue catalyzed photosensitization, cleaves VeraTag molecules in close proximity to the antibody-biotin-streptavidin complex.



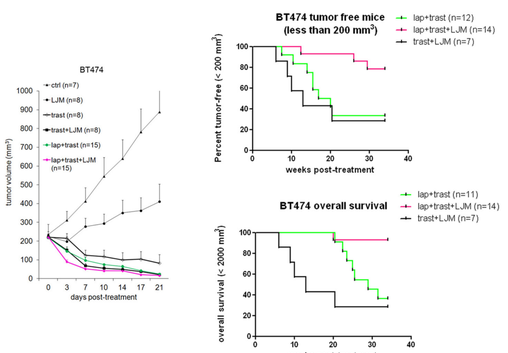
HER2-HER3 heterodimer assay format

PLoS One. 2011 Jan 28:6(1):e16443.



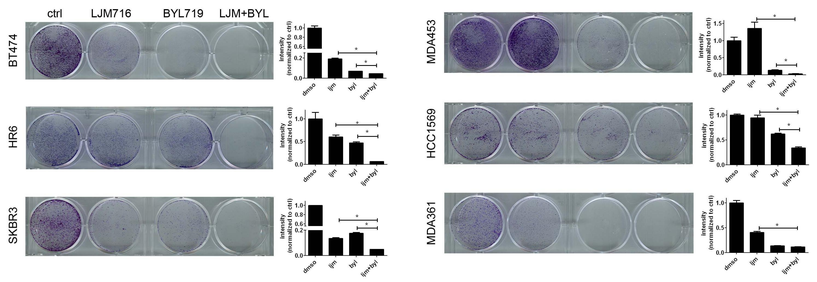
Lysates from BT474 and SKBR3 cells treated with LJM716 for 0-4 h were precipitated with a HER3 antibody followed by immunoblot analysis with HER2 and HER3 antibodies.

HER3 antibody in combination with dual blockade of HER2 improves survival *in vivo*

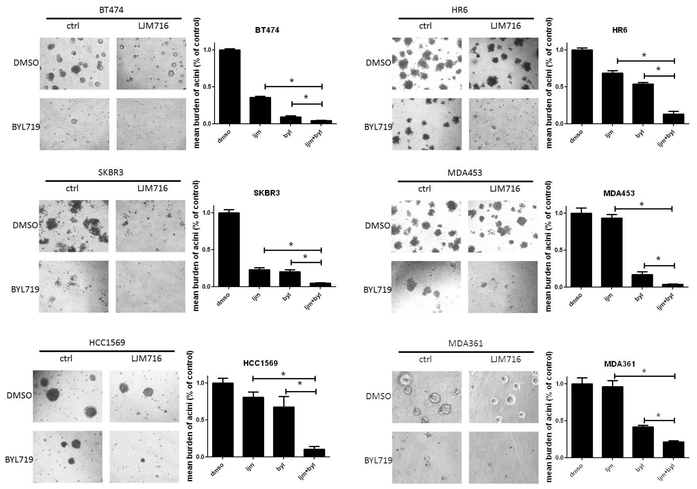


Female athymic mice were injected with BT474 cells and randomized to vehicle or the indicated combinations of 20 mg/kg LJM716, 20 mg/kg trastuzumab and 100 mg/kg lapatinib. Treatment was administered for 21 days. Tumors were measured two to three times a week with calipers. Each data point represents the mean tumor volume +SEM. Right panel: at the end of 3 weeks of treatment, mice from the lap+trast, lap+trast+LJM and trast+LJM were monitored for tumor re-growth. The x-axis indicates weeks after drug treatment stopped. The upper graph indicates tumor-free mice (tumor bearing mouse is defined as tumor larger than 200 mm³). The lower graph indicates overall survival. Mice were sacrificed once tumor burden was larger than 2000 mm³.

HER3 antibody sensitizes cells to PI3Kα inhibitor

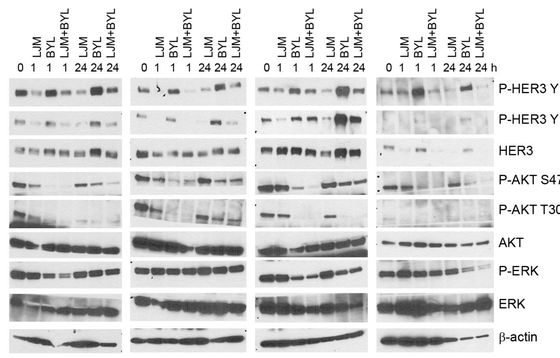


Cells were plated at 10,000 to 50,000 cells per well in 6-well plates and treated in triplicate with DMSO, 10 µg/ml LJM716, and/or 1 µM BYL719. Media was replenished every 3-4 days with replenishment of LJM716. Cells were stained with crystal violet when control treated cells were confluent, ranging from 14-21 days. Representative images and quantification of integrated intensity (% control) are shown. *, $P < 0.05$, *t* test.



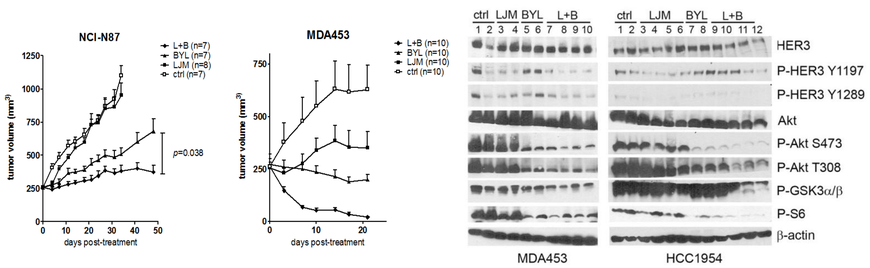
Cells were seeded in Matrigel and allowed to grow in the absence or presence of 10 µg/ml LJM 716 and/or 1 µm BYL719 as indicated. Medium was subsequently changed every 3 days. Images shown were recorded 15-19 days after cell seeding. Acini burden was quantified using the GelCount system. Each bar graph represents the mean + S.E.M. of triplicate samples. *, $P < 0.05$, *t* test.

HER3 antibody and PI3Kα inhibitor in combination have enhanced inhibition of PI3K



Cells were treated with 10 µg/ml LJM 716 and/or 1 µm BYL719 for 1 or 24 hours. Whole cell lysates were prepared and separated in a 7% SDS gel followed by immunoblot analysis with indicated antibodies.

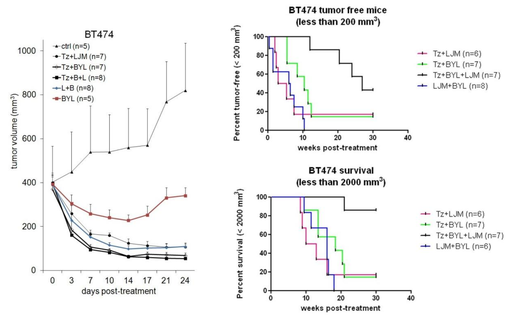
Combination of HER3 antibody and PI3K inhibitor completely eliminate PI3K mutant tumors *in vivo*



Left panel: Female athymic mice were injected with NCI-N87 or MDA453 cells and randomized to vehicle or 20 mg/kg LJM716 and/or 12.5 to 30 mg/kg BYL719 . Treatment was administered for 21 to 48 days. Tumors were measured two to three times a week with calipers. Each data point represents the mean tumor volume +SEM.

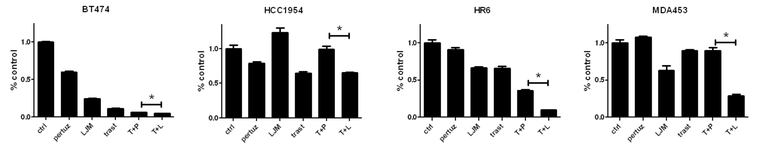
Right panel: Mice bearing HCC1954 or MDA453 xenografts were treated over a 72 hour period with two doses of 20 mg/kg LJM716 and three doses of 30 mg/kg BYL719. Mice received BYL719 1h before sacrifice and LJM716 24h before sacrifice. Tumor cell lysates were prepared and separated in a 7% SDS gel followed by immunoblot analysis with the indicated antibodies.

Trastuzumab plus HER3 antibody plus PI3K inhibitor demonstrate superior anti-tumor properties *in vivo*

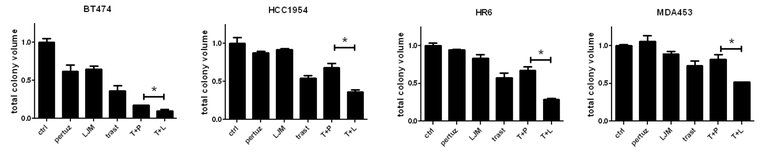


Far left: Female athymic mice were injected with BT474 cells and randomized to vehicle or the indicated combinations of 20 mg/kg LJM716, 20 mg/kg trastuzumab and 30 mg/kg BYL719 . Treatment was administered for 24 days. Tumors were measured two to three times a week with calipers. Each data point represents the mean tumor volume +SEM. Near left: At the end of 24 days of treatment, mice from the combination groups were monitored for tumor re-growth. The x-axis indicates weeks after drug treatment stopped. The upper graph indicates tumor-free mice (tumor bearing mouse is defined as tumor larger than 200 mm³). The lower graph indicates overall survival. Mice were sacrificed once tumor burden was larger than 2000 mm³.

LJM716 is at least equivalent to the HER2 antibody Pertuzumab in combination with Trastuzumab against HER2+ tumors

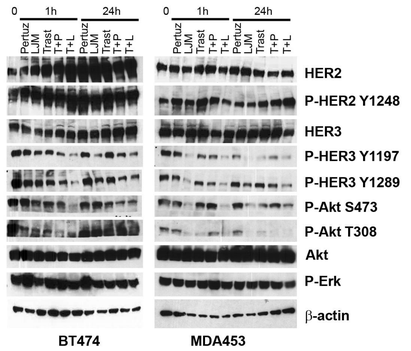


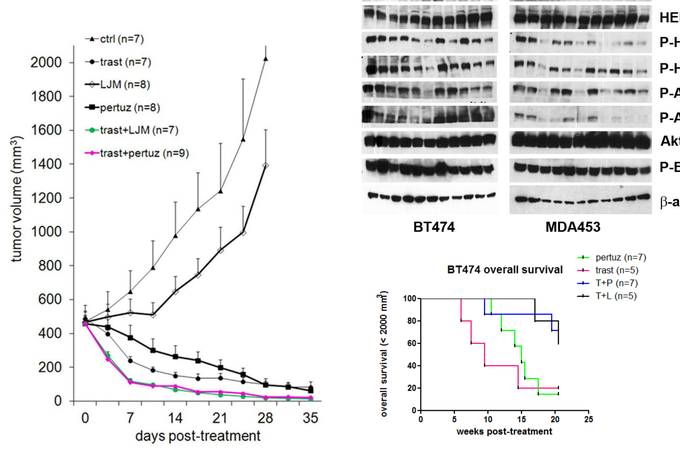
Cells were plated at 10,000 to 50,000 cells per well in 6-well plates and treated in triplicate with DMSO, 10 µg/ml LJM716, pertuzumab, and/or trastuzumab. Media was replenished every 3-4 days with replenishment of antibody. Cells were stained with crystal violet when control treated cells were confluent, ranging from 14-21 days. Representative images and quantification of integrated intensity (% control) are shown. *, $P < 0.05$, *t* test.



Cells were seeded in Matrigel and allowed to grow in the absence or presence of 10 µg/ml LJM716, pertuzumab, and/or trastuzumab as indicated. Medium was subsequently changed every 3 days. Images shown were recorded 15-19 days after cell seeding. Acini burden was quantified using the GelCount system. Each bar graph represents the mean + S.E.M. of triplicate samples. *, $P < 0.05$, *t* test.

(Right) BT474 and MDA453 cells were treated with 10 µg/ml LJM 716, 10 µg/ml pertuzumab and/or 10 µg/ml trastuzumab for 1 or 24 hours. Whole cell lysates were prepared and separated in a 7% SDS gel followed by immunoblot analysis with indicated antibodies.





(Left) Female athymic mice were injected with BT474 cells and randomized to vehicle or the indicated combinations of 20 mg/kg LJM716, 20 mg/kg trastuzumab and 20 mg/kg pertuzumab . Treatment was administered for 35 days. Tumors were measured two to three times a week with calipers. Each data point represents the mean tumor volume +SEM. (Right) At the end of 35 days of treatment, mice were monitored for tumor re-growth. The x-axis indicates weeks after drug treatment stopped. Mice were sacrificed once tumor burden was larger than 2000 mm³.

Conclusions

- Treatment with LJM716 inhibited HER2-HER3 dimers, P-HER3 and P-Akt in HER2+ breast cancer cells with PI3K pathway mutations
- As a single agent the HER3 antibody markedly inhibited HER2+ xenograft growth. Treatment with LJM716 in combination with lapatinib and trastuzumab improved survival of mice with HER2+ xenografts compared to lapatinib and trastuzumab
- LJM716 sensitized breast cancer cells and xenografts to a p110α-specific inhibitor
- The HER3 antibody in combination with trastuzumab inhibited PI3K/Akt and xenograft growth as well as the combination of pertuzumab and trastuzumab