Dual mTORC1/2 and HER2 Blockade Results in Antitumor Activity in Preclinical Models of Breast Cancer Resistant to Anti-HER2 Therapy

Celina García-García1, Yasir H. Ibrahim1, Violeta Serra1, Maria Teresa Calvo1, Marta Guzmán1, Judit Grueso1, Claudia Aura2, José Pérez1, Katti Jessen3, Yi Liu3, Christian Rommel3, Josep Tabernero1, José Baselga4,5, and Maurizio Scaltriti4,5

Abstract

Purpose: The PI3K/Akt/mTOR pathway is an attractive target in HER2-positive breast cancer that is refractory to anti-HER2 therapy. The hypothesis is that the suppression of this pathway results in sensitization to anti-HER2 agents. However, this combinatorial strategy has not been comprehensively tested in models of trastuzumab and lapatinib resistance.

Experimental Design: We analyzed in vitro cell viability and induction of apoptosis in five different cell lines resistant to trastuzumab and lapatinib. Inhibition of HER2/HER3 phosphorylation, PI3K/Akt/mTOR, and extracellular signal-regulated kinase (ERK) signaling pathways was evaluated by Western blotting. Tumor growth inhibition after treatment with lapatinib, INK-128, or the combination of both agents was evaluated in three different animal models: two cell-based xenograft models refractory to both trastuzumab and lapatinib and a xenograft derived from a patient who relapsed on trastuzumab-based therapy.

Results: The addition of lapatinib to INK-128 prevented both HER2 and HER3 phosphorylation induced by INK-128, resulting in inhibition of both PI3K/Akt/mTOR and ERK pathways. This dual blockade produced synergistic induction of cell death in five different HER2-positive cell lines resistant to trastuzumab and lapatinib. In vivo, both cell line–based and patient-derived xenografts showed exquisite sensitivity to the antitumor activity of the combination of lapatinib and INK-128, which resulted in durable tumor shrinkage and exhibited no signs of toxicity in these models.

Conclusions: The simultaneous blockade of both PI3K/Akt/mTOR and ERK pathways obtained by combining lapatinib with INK-128 acts synergistically in inducing cell death and tumor regression in breast cancer models refractory to anti-HER2 therapy. Clin Cancer Res; 18(9); 2603–12. ©2012 AACR.

Introduction

Amplification of the receptor tyrosine kinase (RTK) HER2 has been reported in approximately one fourth of human breast tumors, and its overexpression is associated with a more aggressive phenotype and poor clinical outcome (1, 2). There are 2 main therapeutic strategies used to target HER2-positive cells: the first are monoclonal antibodies targeting the extracellular domain of the receptor (trastuzumab, pertuzumab); the second small-molecule ATP competitors that inhibit the phosphorylation of the HER2 intracellular kinase domain (lapatinib, neratinib). Trastuzumab, in combination with chemotherapy, has shown a robust improvement in progression-free and overall survival in cases of advanced disease (3, 4) as well as in the early (adjuvant) setting (5–7). Lapatinib, when given in combination with capecitabine, significantly improves time to progression in HER2-positive patients with breast cancer who have progressed on trastuzumab-based therapy (8). Moreover, lapatinib as monotherapy and in combination with paclitaxel has clinical activity as first-line treatment in HER2-positive patients with breast cancer (9, 10). Recently, our group and others reported that combinations of anti-HER2 agents with nonoverlapping mechanisms of action, so-called dual HER2 blockade, is superior to treatment with single-agent trastuzumab (11–14) and is active in patients who are resistant to trastuzumab-based therapies (15, 16).
PIK3CA gene or through loss of phosphatase and tensin (PTEN) and epidermal growth factor receptor (EGFR) by the cell lines have been previously described (17–19, 26). Targeting the PI3K/Akt/mTOR pathway in several models of resistance to anti-HER2 therapy. Because the activation of Akt requires mTORC2 (33), we chose to target both Akt and mTOR using INK-128, a potent and selective ATP competitor of both mTORC1 and mTORC2 currently in clinical development (34). To concomitantly inhibit HER receptor phosphorylation, we used lapatinib.

Here, we show that lapatinib in combination with INK-128 profoundly inhibits both the PI3K/Akt/mTOR and ERK pathways resulting in apoptosis in vitro and in vivo tumor shrinkage in all models tested. Importantly, no signs of toxicity were observed in our in vivo experiments. These data indicate that it is possible to achieve durable regressive responses in tumor models refractory to anti-HER2 therapy by inhibiting both the PI3K/Akt/mTOR and HER2 pathways without signs of toxicity.

Materials and Methods

Cell lines and treatments

Cell lines, except as otherwise indicated, were obtained from the American Type Culture Collection. The BT474 cell line was maintained in Dulbecco’s Modified Eagle’s Media (DMEM):F12 supplemented with 10% FBS and 2 mmol/L L-glutamine (Live Technologies, Inc. Ltd.). SKBR-3, HCC-1954, and JIMT1 cell lines were maintained in McCoy, RPMI (Live Technologies, Inc. Ltd.) and DMEM high glucose (PAA Laboratories GmbH) respectively, supplemented with 10% FBS. The KPL4 cell line, kindly provided by Prof. Kurebayashi (Kawasaki Medical School, Okayama, Japan), was maintained in DMEM (Sigma-Aldrich) supplemented with 5% FBS. MDA-MB361 and MDA-MB453 were maintained in L-15 (Live Technologies, Inc. Ltd.) supplemented with 20% and 10% FBS, respectively. All the cell lines were maintained at 37°C in 5% CO2, except for MDA-MB361 and MDA-MB453 that were maintained at 37°C in the absence of CO2. Mutational status of PI3K and expression of PTEN and epidermal growth factor receptor (EGFR) by the cell lines have been previously described (Cosmic Database; refs. 35, 36). The mTORC1/2 pathway

Despite the remarkable success of anti-HER2 therapies, patients with advanced HER2-positive breast cancer frequently display primary resistance and, in patients initially sensitive to these agents, acquired resistance may emerge over time.

To date, known mechanisms of resistance to anti-HER2 agents include aberrant activation of the downstream phosphoinositide 3-kinase (PI3K; refs. 17–19) and Src (20) pathways, coexpression of the truncated p95HER2 receptor (21), coexpression and dimerization with other RTKs (22, 23), and cyclin E amplification (24). These mechanisms are clinically relevant (17, 18, 20, 21, 24), therefore, specific therapeutic approaches are being investigated in patients (25).

The PI3K pathway lies directly downstream of HER2 and its aberrant activation, either because of mutations of the PIK3CA gene or through loss of phosphatase and tension homolog (PTEN) expression have been found to limit the sensitivity to either antibody or RTK inhibitor–based therapies (17–19, 26). Targeting the PI3K pathway in combination with anti-HER2 agents therefore seems to be a compelling therapeutic strategy.

It is well understood that PI3K signaling plays a critical role in cell growth, survival, and metabolism because of the numerous effectors regulated by this pathway (27). Moreover, recent studies conducted by both our group and others have elucidated feedback mechanisms of PI3K signaling that subvert inhibition of PI3K/Akt/mTOR by reactivating upstream PI3K pathway components as well as the extracellular signal–regulated kinase (ERK) pathway. In some breast cancer models, mTORC1/p70S6K1 activity regulates insulin—like growth factor-1 receptor (IGF-1R) function through negative feedback involving IRS-1 and Grb10 (28, 29). Inhibition of mTORC1 leads to disruption of this feedback loop and, ultimately, to hyperactivation of PI3K/Akt. A second compensatory mechanism occurs through the inhibition of PI3K/Akt in HER2-positive breast cancer activating ERK1/ERK2 via increased RTK expression and dimerization (30–32). These studies suggest that combined HER2 and PI3K/Akt/mTOR inhibition would be superior to single-agent therapy. However, whether this combinatorial strategy will be effective in diverse scenarios of trastuzumab and lapatinib resistance still remains to be elucidated. One limitation of this pharmacologic approach is predicted to be the increased toxicity observed in vivo (30).

In this study, we investigated the antitumor activity and feasibility of dual blockade of HER2 and the PI3K/Akt/mTOR pathway in several models of resistance to anti-HER2 therapy. Because the activation of Akt requires mTORC2 (33), we chose to target both Akt and mTOR using INK-128, a potent and selective ATP competitor of both mTORC1 and mTORC2 currently in clinical development (34). To concomitantly inhibit HER receptor phosphorylation, we used lapatinib.

Here, we show that lapatinib in combination with INK-128 profoundly inhibits both the PI3K/Akt/mTOR and ERK pathways resulting in apoptosis in vitro and in vivo tumor shrinkage in all models tested. Importantly, no signs of toxicity were observed in our in vivo experiments. These data indicate that it is possible to achieve durable regressive responses in tumor models refractory to anti-HER2 therapy by inhibiting both the PI3K/Akt/mTOR and HER2 pathways without signs of toxicity.
inhibitor INK-128 was kindly provided by Intellikine Inc. Lapatinib (Tykerb) was kindly provided by GlaxoSmithKline. Both compounds were dissolved in dimethyl sulfoxide for in vitro experiments.

**Western blotting**

Cells were grown in 60-mm dishes and treated with lapatinib, INK-128, or a combination of both for the indicated concentrations and times. Cells were washed with ice-cold PBS and scraped into ice-cold lysis buffer (20 mM Tris-HCl, pH 7.8, 137 mM NaCl, 2 mM EDTA, pH 8.0, 1% NP40, 10% glycerol), supplemented with 10 mM NaF, 10 μg/ml leupeptin, 200 μg/ml Na3VO4, 5 mM phenylmethylsulfonylfluoride, and aprotinin (Sigma-Aldrich). Lysates were cleared by centrifugation at 13,000 rpm for 10 minutes at 4°C, and supernatants removed and assayed for protein concentration using the Pierce BCA Protein Assay Kit (Thermo Scientific). Thirty micrograms of total lysate was resolved by SDS-PAGE and electrophoretically transferred to nitrocellulose membranes. Membranes were then hybridized using the following primary antibodies: p-Akt (Ser473), p-Akt (Thr308), Akt, p-β-catenin (Ser454), p-β-catenin (Thr410), p-β-catenin (Ser671), p-EGFR (Tyr1068), p-ERK (Thr202/Tyr204), ERK, p-HER3 (Tyr1289), HER3, p-HER2 (Tyr1221/1222), HER2, cleaved PARP, caspase-3, β-actin, β-actin (Clone 1A4, Santa Cruz), PRAS40, p-PRAS40 (Thr246), HER3, p-HER2 (Tyr1221/1222), HER2, cleaved PARP, cleaved caspase-3, cleaved caspase-7, tubulin (Cell Signaling), pan-cytokeratin (SIGMA-Aldrich) in 5% bovine serum albumin and HER2 (BioGenex Laboratories Inc.), and HER3 (Thermo Scientific), in 1% nonfat dry milk. Mouse and rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies (Amersham Biosciences) were used at 1:2,000 in TBS-T/1% nonfat dry milk. Protein–antibody complexes were detected by chemiluminescence with the Immobilon Western HRP Substrate (Millipore) and images were captured with a Fujifilm LASS-3000 camera system.

**Determination of IC50 and synergism**

Cells were seeded in 96-well plates and treated with escalating of lapatinib and INK-128 (100–10–0.01–0.001 nmol/L) as single agents or in 1:1 combinations. After 6 days of treatment, cell proliferation was analyzed with CellTiter-Glo Luminescent Cell Viability Assay (Promega) and cell viability was expressed as percentage of untreated control. Levels of release; Innovative Research of America) was implanted subcutaneously into each mouse 1 day before cell injection.

**Establishment of tumor xenografts in nude mice**

Mice were maintained and treated in accordance with Institutional Guidelines of Vall d’Hebron University (Barcelona, Spain) Hospital Care and Use Committee. Six-week-old female athymic nude HsdCpb:NMRI-Foxn1nu mice were purchased from Harlan Laboratories. Mice were housed in air-filtered laminar flow cabinets with a 12-hour light cycle and food and water ad libitum.

MDA-MB361 or JIMT1 cells were resuspended in sterile PBS and mixed 1:1 with Matrigel (BD Biosciences) before subcutaneous injection at a final concentration of 2 × 10⁶ cells per 200 μl per mouse. For MDA-MB361 xenografts, a 17β-estradiol pellet (0.72 mg/pellet, 60 days of release; Innovative Research of America) was implanted subcutaneously into each mouse 1 day before cell injection.

For patient-derived xenografts (PDX), patient consent for tumor use in animals was obtained under a protocol approved by the Vall d’Hebron Hospital Clinical Investigation Ethical Committee and Animal Use Committee. Tumors were subcutaneously implanted in 6-week-old female mice. Animals were supplemented with 1 μmol/L 17β-estradiol (Sigma) in their drinking water, an amount shown to be sufficient to reach serum levels and uterine growth in ovariectomized female mice similar to the ones obtained with other mechanisms of 17β-estradiol supplementation (39, 40). Upon xenograft growth, tumor tissue was reimplanted into recipient mice, which were randomized upon implant growth.

**In vivo treatment study**

Cell line–derived xenografts: growth took approximately 2 to 3 weeks before animal randomization at a volume of 200 to 300 mm³. Animals were randomized to 4 groups, consisting of 6 to 9 mice per group. PDX: growth of subcutaneous implants took approximately 3 weeks before animal randomization at a volume of 100 to 200 mm³. Animals were randomized into 5 groups, consisting of 6 to 8 mice per group.

Animals were treated with INK-128 for 6 d/wk [0.3 mg/kg in 5% N-methyl-2-pyrrolidone (NMP), 15% polyvinyl pyrrolidone (PVP) in water, oral gavage], lapatinib for 6 d/wk [150 mg/kg in 0.5% hydroxypropyl methylcellulose (HPMC), 0.1% Tween-80 in water, oral gavage], or trastuzumab for 2 d/wk (10 mg/kg, in water, intraperitoneal injection). Tumor xenografts were measured with calipers and tumor volumes were determined using the formula: length × width² × (π/6). At the end of the experiment, animals were euthanized using CO2 inhalation. Tumor volumes are plotted as means ± SE.

For Western blotting, lysates from 2 different tumors derived from each treatment were processed as described earlier.

**Statistical analysis**

Two-way ANOVA with Bonferroni posttest was done using GraphPad Prism (GraphPad Software). Error bars represent the SE. All experiments were repeated at least 3 times.
Results

In vitro activity of anti-HER2 and mTORC1/2 therapy in HER2-positive cell lines

To test the effects of dual mTORC1/2 blockade in HER2-positive cells, we screened a panel of 7 HER2-positive breast cancer cell lines for both lapatinib and INK-128 sensitivity using an ATP-based cell viability assay (Fig. 1A and B). After 6 days of treatment, we observed varying sensitivity to lapatinib, with SKBR-3 and BT474 cells being significantly more sensitive than were MDA-MB361, MDA-MB453, KPL4, HCC-1954, and JIMT1 cell lines (Fig. 1A). Moreover, the latter 5 cell lines were completely refractory to the antiproliferative effects of trastuzumab (data not shown). Given these striking differences in anti-HER2 sensitivity, we defined BT474 and SKBR-3 cell lines as sensitive and MDA-MB361, MDA-MB453, KPL4, HCC-1954, and JIMT1 cell lines as resistant (Fig. 1A). Although we acknowledge that lower lapatinib IC50 values have been previously reported for MDA-MB361 cells (36, 41, 42), all 5 resistant cell lines tested were found to harbor activating alterations of the PI3K pathway (PIK3CA mutations, low/loss of expression of PTEN, or both) whereas the 2 sensitive cell lines did not [with the exception of the non-activating K111N PIK3CA mutation (ref. 43) present in BT474 cells]. A detailed biochemical analysis of the sensitive cell lines confirmed that both the PI3K/Akt/mTOR and ERK pathways were profoundly suppressed at low concentrations of lapatinib (Supplementary Fig. S1). In contrast, the same molecular markers, especially the ones related to the PI3K/Akt/mTOR axis, were only moderately affected by HER2 inhibition in the resistant cells (Supplementary Fig. S2). When treated with INK-128, the proliferation of all 7 cell lines was significantly inhibited at low nanomolar ranges (Fig. 1B).

In analyzing these data, we reasoned that the use of lapatinib in combination with a dual mTORC1/2 inhibitor, such as INK-128, would result in enhanced antiproliferative activity. Possible synergism between lapatinib and INK-128 in lapatinib-resistant cell lines was evaluated using the combination index (CI; ref. 37). In all 5 cell lines tested, the combination of INK-128 and lapatinib showed synergistic activity (CI < 1; Table 1 and Supplementary Fig. S3A). In particular, the best efficacy of this combination was observed at concentrations between 0.1 and 10 nmol/L, depending on the cell line (Supplementary Fig. S3B).

Dual PI3K/mTOR and ERK suppression combining INK-128 with lapatinib

It has been previously described by our group and others that PI3K/Akt/mTOR pathway inhibition may result in the activation of compensatory pathways that could reduce the antiproliferative activity of these inhibitors (28, 30, 31, 44, 45). As expected, we observed reproducible increases in HER2/HER3 phosphorylation following INK-128 treatment in all the tested cell lines (Fig. 2 and data not shown). This phenomenon coincided with ERK activation, which was more evident at the 2-hour time point.

The combination of lapatinib with INK-128, tested in 3 lapatinib/trastuzumab-resistant cell lines, was shown to effectively prevent HER phosphorylation, to suppress PI3K/Akt/mTOR signaling, and to limit ERK phosphorylation consequent to INK-128 treatment (Fig. 2).
Cell-cycle analysis and apoptosis in trastuzumab/lapatinib-resistant cell lines treated with lapatinib and INK-128

The in vitro synergistic effects summarized in Table 1 prompted us to investigate both cell-cycle progression and apoptosis after treatments with lapatinib, INK-128, and the combination of both. Cell-cycle progression of HCC-1954, KPL4, and MDA-MB453 cell lines was affected by both lapatinib and INK-128 (Supplementary Fig. S4A–S4C).

Each inhibitor alone was sufficient to delay the cell cycle (increased G1 phase) of these cells and the combination of both treatments further enhanced these effects. No major changes in cell-cycle progression were observed in the other 2 resistant cell lines tested (Supplementary Fig. S4D and S4E).

We then analyzed the proapoptotic effects of these compounds, as single agents and in combination. Treatments with either lapatinib or INK-128 variably increased the percentage of apoptotic cells in lapatinib-resistant cells (Fig. 3A and Supplementary Figs. S5 and S6). The combination of both agents, however, exacerbated the induction of cell death. In Fig. 3B, we confirm that this biologic effect is accompanied by higher activation of both caspase-3 and -7 along with increased PARP cleavage. Therefore, whereas cell-cycle arrest is observed in some models treated with lapatinib and INK-128, enhanced cell death is a common feature of this combination in vitro.

Antitumor activity of lapatinib and INK-128 in cell-based trastuzumab/lapatinib-resistant xenograft models

To expand our findings in vivo, we determined the activity of INK-128, lapatinib, or the combination in different animal models. We first analyzed the tumor growth of MDA-MB361 xenografts under the therapeutic conditions shown in Fig. 4A. As expected from the in vitro findings,

Table 1. Combination index of the combination of lapatinib and INK-128 in resistant cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CI</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB361</td>
<td>0.052 ± 0.018</td>
<td>Strong synergism</td>
</tr>
<tr>
<td>MDA-MB453</td>
<td>0.183 ± 0.187</td>
<td>Synergism</td>
</tr>
<tr>
<td>KPL4</td>
<td>0.090 ± 0.073</td>
<td>Strong synergism</td>
</tr>
<tr>
<td>HCC-1954</td>
<td>0.202 ± 0.087</td>
<td>Strong synergism</td>
</tr>
<tr>
<td>JIMT1</td>
<td>0.224 ± 0.054</td>
<td>Strong synergism</td>
</tr>
</tbody>
</table>

NOTE: Trastuzumab/lapatinib-resistant HER2-positive breast cancer cell lines were treated with escalating concentrations of lapatinib, INK-128, or the combination. Viability was measured after 6 days of treatment with a cell viability assay (CellTiter-Glo; Promega), and CI was calculated using CompuSyn computer program (Fa = 0.9).
MDA-MB361 xenografts scarcely responded to lapatinib. Although an initial response to INK-128 was observed, the xenografts eventually gained resistance after 20 days of treatment. In contrast, the combination of lapatinib and INK-128 induced long-lasting tumor regression. Specifically, 40% of the mice in this combination arm experienced complete tumor shrinkage. To evaluate the degree of signaling inhibition in these tumors, we conducted pharmacodynamic studies, treating the tumors for 3 days with lapatinib, INK-128, and the combination of both agents. In Fig. 4B, we show by Western blotting that lapatinib resulted in marked inhibition of ERK phosphorylation but only moderately affected the PI3K/mTOR pathway. Conversely, treatment with INK-128 resulted in a reduction of p-Akt and downstream p-S6 and p-4EBP1 compared with lapatinib, with no demonstrable effects on the ERK pathway. Importantly, significant inhibition of both pathways was observed in the samples treated with the combination.

Previous experiments combining lapatinib with other inhibitors of the PI3K/mTOR pathway were run for shorter times or were limited by in vivo toxicity (30, 31, 42). In our hands, the antitumor activity of INK-128 plus lapatinib was accompanied by an absence of toxicity (defined as weight loss or lethargy) after 80 days of continuous administration with the drugs (Fig. 4C).

The in vivo efficacy of dual HER2 and PI3K/Akt/mTOR inhibition was also confirmed in xenografts derived from JIMT1 cells (Supplementary Fig. S7). Despite the absence of complete tumor shrinkage in this trastuzumab/lapatinib-resistant model, we reproducibly observed superior efficacy of the combination of lapatinib and INK-128 as compared with treatment with a single agent.

Antitumor activity of lapatinib and INK-128 in a trastuzumab-resistant PDX

To better recapitulate the clinical setting, we tested the activity of lapatinib and INK-128 in an HER2-positive PDX model. This was derived from a biopsy of a patient who relapsed after trastuzumab-based therapy (see Materials and Methods). We have previously shown that both morphologic and gene expression signatures of the original surgical samples are maintained in our PDX models (46). Deriving from a patient who became refractory to trastuzumab-based therapy, we first confirmed that this PDX model was unreponsive to trastuzumab therapy (Supplementary Fig. S8). We then tested the tumor activity of lapatinib, INK-128, and the combination of both. As shown in Fig. 5A, patient-derived tumors showed initial response to INK-128 but became partially resistant after approximately 20 days of treatment, recapitulating the results obtained with the MDA-MB361 xenograft model (Fig. 4A). Conversely, tumor regression induced by lapatinib was maintained over time, although this regimen never achieved complete tumor shrinkage. Despite the strong antitumor effects exhibited by lapatinib when used as single agent, its activity was enhanced by the addition of INK-128. Superior (and more rapid) tumor regression was achieved by dual HER2 and PI3K/Akt/mTOR blockade, compared with lapatinib as a solo agent.
single agent, and complete tumor shrinkage was observed in 2 of 6 mice. By Western blot analysis, we confirmed that only tumor samples exposed to the combination therapy showed significant inhibition of both PI3K/Akt/mTOR and ERK pathways (Fig. 5B).

Discussion

Hyperactivation of the PI3K/Akt/mTOR pathway has previously been reported to negatively correlate with sensitivity to anti-HER2 therapy in breast cancer (17, 18, 47). Direct inhibition of PI3K/Akt/mTOR signaling is therefore an attractive clinical strategy for this disease. Insufficient target inhibition and/or activation of compensatory pathways that diminish the efficacy of these agents are, however, predictable limitations to the clinical development of PI3K/Akt/mTOR inhibitors. Combination therapies in which both the PI3K/Akt/mTOR pathway and key reactivated targets are inhibited together may result in improved efficacy. Rapalogs, for example, lead to the hyperactivation of Akt by relieving p70S6 kinase–driven suppression of the IGF-IR signaling (28). Dual mTORC1 and IGF-IR receptor blockade has been tested in estrogen receptor (ER)-positive breast cancer with encouraging results (48). Some light has been shed recently on cross-talk between the PI3K/Akt/mTOR pathway and ER signaling. Supplementation of estradiol seems to protect from the proapoptotic effects of PI3K/Akt/mTOR inhibition (49), whereas mTORC1 was described to activate ER in a ligand-independent fashion (50). Moreover, hyperactivation of the PI3K/Akt/mTOR pathway was observed in endocrine-resistant breast cancer cells (51). These findings provided the rationale to test combined mTORC1 and ER blockade in the clinic, with significant improvement of progression-free survival in ER-positive patients who recurred after anti-estrogen therapy (52). In addition, inhibition of mTORC1 and/or Akt has been described to increase the phosphorylation of both EGFR and HER3 in both trastuzumab-sensitive and -resistant cell lines (53). In their work, Miller and colleagues showed that a combined HER2 and mTORC1 blockade was more effective than single agents at inducing tumor regression and inhibiting cell growth. In 3 independent studies, trastuzumab and the mTORC1 inhibitor everolimus showed encouraging antitumor activity in heavily pretreated trastuzumab-resistant patients (54–56). In a similar fashion, an RTK-dependent activation of ERK1 and ERK2 in response to PI3K/Akt/mTOR inhibition has also been described in preclinical models of HER2-positive breast tumors (30–32). In these cases, the combination of PI3K/Akt/mTOR inhibitors with either an anti-HER2 agent or a mitogen–activated protein (MAP)/ERK (MEK) inhibitor was superior to single agents.

Although there is evidence that both HER2 and PI3K/Akt/mTOR signaling need to be suppressed to achieve optimal therapeutic activity, this combinatorial strategy has not been extensively tested in tumors resistant to anti-HER2 therapy. In this study, we show the validity of this approach, clearly showing that the combination of lapatinib with INK-128 results in synergistic reduction of cell viability in 5 trastuzumab/lapatinib-resistant cell lines, along with strong antitumor activity in 3 different in vivo models of breast cancer refractory to anti-HER2 therapy. Despite the...
In previous preclinical studies conducted in nude mice, the combination of lapatinib with inhibitors of the PI3K/Akt/mTOR pathway proved to be difficult to manage (or impractical) because of skin toxicity and excessive weight loss (30, 31). Conversely, in our hands, the combination of lapatinib and INK-128 in the same animal models did not lead to any sign of toxicity even after 60 days of treatment. It is still unclear whether this significant improvement was because of the high activity of INK-128 despite the low dosage used in our experiments (0.3 mg/kg), or to the optimal pharmacokinetic characteristics of this compound.

There are currently 3 clinical trials of dose-escalation studies with INK-128–recruiting patients. One of them is being conducted in HER2-positive patients with breast cancer evaluating the safety and preliminary antitumor activity of the combination of INK-128, paclitaxel, and weekly trastuzumab (http://clinicaltrials.gov/ct2/show/NCT01351350?term=INK128&rank=1). It would be interesting to know whether the same patient population, who eventually relapse when treated with trastuzumab-based therapy, would benefit by combining lapatinib with INK-128.

In conclusion, we propose that the simultaneous blockade of the PI3K/Akt/mTOR and ERK pathways achieved by combining lapatinib with INK-128 acts synergistically in inducing cell death and tumor regression in breast cancer refractory to anti-HER2 therapy.

Disclosure of Potential Conflicts of Interest
K. Jessen, Y. Liu, and C. Rommel are employees of Intellikine. K. Jessen has employment in Intellikine as Biology Group Leader. J. Baselga is a consultant/advisory board member for Aragon, AstraZeneca, Sanofi, Bayer, Onyx, Chugai, Constellation, Exelixis, Intellikine, Merck, Novartis, and Roche Genentech. No potential conflicts of interest were disclosed by other authors.

Authors’ Contributions
Conception and design: C. Garcia-Garcia, Y.H. Ibrahim, V. Serra, Y. Liu, J. Tabernero, J. Baselga, M. Scaltriti
Development of methodology: C. Garcia-Garcia, Y.H. Ibrahim, V. Serra, M. T. Calvo, M. Guzman, J. Grueso, C. Aura
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Garcia-Garcia, Y.H. Ibrahim, V. Serra, M.T. Calvo, M. Guzman, J. Grueso, J. Perez, J. Tabernero
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Garcia-Garcia, Y.H. Ibrahim, V. Serra, C. Rommel, J. Tabernero, J. Baselga, M. Scaltriti
Writing, review, and/or revision of the manuscript: C. Garcia-Garcia, Y.H. Ibrahim, V. Serra, K. Jessen, Y. Liu, C. Rommel, J. Tabernero, J. Baselga, M. Scaltriti
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Garcia-Garcia, Y.H. Ibrahim, V. Serra, C. Aura, J. Perez, Y. Liu, C. Rommel
Study supervision: C. Garcia-Garcia, Y.H. Ibrahim, V. Serra, K. Jessen, M. Scaltriti

Grant Support
This work was supported by Breast Cancer Research Foundation and European Research Council (AdG09 250244).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 25, 2011; revised January 12, 2012; accepted February 28, 2012; published OnlineFirst March 8, 2012.
References

Dual mTORC1/2 and HER2 Blockade Results in Antitumor Activity in Preclinical Models of Breast Cancer Resistant to Anti-HER2 Therapy


Updated version
Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-11-2750

Supplementary Material
Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2012/03/22/1078-0432.CCR-11-2750.DC1

Cited articles
This article cites 52 articles, 23 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/18/9/2603.full.html#ref-list-1

Citing articles
This article has been cited by 19 HighWire-hosted articles. Access the articles at:/content/18/9/2603.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.