An Antibody That Locks HER3 in the Inactive Conformation Inhibits Tumor Growth Driven by HER2 or Neuregulin

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germs; and 4Genomics Institute of the Novartis Research Foundation,

Breast Cancer Research Program, Vanderbilt-Ingram Cancer Center, Van-

not be driven by HER2- or EGFR-targeted agents, may leverage their clinical

efficacy in ErbB-driven cancers. Cancer Res; 73(19); 6024–35. ©2013 AACR.

Introduction

In clinical practice, the HER2-targeted monoclonal anti-

body trastuzumab (Herceptin) is central to the treatment of

HER2-amplified breast cancer. Although trastuzumab has

well-established clinical benefit, responses are transient and patients frequently relapse with trastuzumab-resistant dis-
nase (1). A number of trastuzumab resistance mechanisms

have been proposed that most commonly center on sus-
tained phosphoinositide 3-kinase (PI3K) signaling (2, 3) either due to the presence of activating PI3K mutations
(4, 5), PTEN inactivation (4, 5), or persistent HER3 signaling
(6, 7). HER3 is the preferred dimerization partner of HER2
(8), acting as an allosteric activator of its partner kinase (9).

Activation of the HER2–HER3 complex results in trans-

phosphorylation of HER3 and initiation of downstream

signaling. HER2–HER3 activates PI3K signaling via HER3, which in contrast to other ErbB receptors contains multiple

phosphodependent binding sites for the regulatory p85 subunit of PI3K (10).

In HER2 amplified cancer, activation of HER3 may occur

through high-level expression of heterodimerization part-

ners such as HER2 (11). Consequently, in cases of HER2

amplification, HER2–HER3 heterodimer formation occurs in

a ligand-independent manner, resulting in unrestrained

HER3 signaling that is both necessary (12) and sufficient

(13) for transformation. Indeed, human HER2-amplified breast cancer samples harbor high levels of phosphorylated

HER3, indicative of HER3 activation and infrequent con-

comitant NRG1 expression (Supplementary Fig. S1A–1D and

Table S1; ref. 14). Continued HER3 signaling in the presence

of trastuzumab or PI3K inhibitors might also be driven by

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FOXO-dependent induction of HER3 expression (15–17) via the release of a PI3K/AKT-driven inhibitory feedback loop (7, 18).

The HER2-targeted antibody pertuzumab (Perjeta) reportedly inhibits ligand-induced HER3 activity by preventing HER2/HER3 dimerization (3, 19). The recent CLEOPATRA study (20) showed that the addition of pertuzumab to trastuzumab/docetaxel significantly prolonged progression-free survival when used as first-line treatment in HER2-overexpressing breast cancer. However, recent preclinical reports indicate that even dual HER2 blockade is unable to fully inhibit PI3K/AKT signaling, and superior benefit may be achieved with HER3-specific inhibition (21).

Elevated expression of NRG1 drives ligand-dependent HER3 signaling and functional NRG1/HER3 autocrine loops have been identified in models of squamous cell carcinoma of the head and neck (SCCHN; ref. 22) and ovarian cancer (23). Given that both ligand-dependent and independent HER3 activation appear to be of fundamental importance in multiple tumor types, a therapeutic agent capable of inhibiting both of these modes of HER3 activation may be efficacious in multiple indications.

Here we describe the discovery, biologic activity, and molecular mode of action of a fully human antibody (LJM716) currently in clinical testing. LJM716 is capable of neutralizing both ligand-dependent and independent HER3 signaling and data suggest this occurs by locking HER3 in the inactive conformation. In addition, we present in vitro and in vivo data that highlight the potential clinical benefit of combining LJM716 with both HER2- and EGFR-targeted agents.

Materials and Methods

Recombinant proteins

Recombinant monomeric HER3 extracellular domains (ECD) from human, rat, and cynomolgus monkey, as well as isolated HER3 domains (D1-2, D3-4, and D4) were cloned upstream of a C-terminal affinity tag, sequence-verified, expressed in HEK293-derived cells, and purified using an anti-tag antibody. Fc-tagged ECDs from three other ErbB-family proteins (EGFR, HER2, and HER4) were purchased from R&D Systems. Further details on all recombinant proteins used can be found in the Supplementary Materials.

Antibodies

HER3-targeted antibodies were selected from the Human Combinatorial Antibody Library (HuCAL GOLD) using phage-display technology (24). The affinity (KD) of the binding interaction between LJM716 and recombinant monomeric HER3 ECD was determined by solution equilibrium titration (SET; ref. 25).

ELISA binding assays

Maxisorp plates (Nunc) were coated with the appropriate recombinant protein and blocked before incubating with the relevant test antibody for two hours at room temperature. Plates were washed and human antibody detected using peroxidase linked goat anti-human antibody (Pierce).

Immunoblotting

For immunoblots, cell lysates were prepared in 1% NP-40 buffer including protease and phosphatase inhibitors (Roche) and analyzed by Western blot using the Odyssey detection system (Licer) or by enhanced chemiluminescence after incubation with horseradish peroxidase-conjugated secondary antibodies (Promega). Details of antibodies used are in the Supplementary Material.

Cell lines

For information on cell lines used in this study please see Table 1. Cell lines were acquired, maintained, and authenticated by single-nucleotide polymorphism (SNP) fingerprinting (Sequenom) as previously described (26).

Table 1. Summary table of cell lines used in this study

<table>
<thead>
<tr>
<th>Cell line name</th>
<th>Supplier</th>
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<tbody>
<tr>
<td>SkBr3</td>
<td>ATCC</td>
</tr>
<tr>
<td>MDA-MB-453</td>
<td>ATCC</td>
</tr>
<tr>
<td>BT474</td>
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<td>ATCC</td>
</tr>
<tr>
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<td>ATCC</td>
</tr>
<tr>
<td>EFM192A</td>
<td>DSMZ</td>
</tr>
<tr>
<td>KPL4</td>
<td>J. Kurebayashi*</td>
</tr>
<tr>
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<td>ATCC</td>
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<tr>
<td>HCC202</td>
<td>ATCC</td>
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<td>ECACC</td>
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<td>ATCC</td>
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<td>ATCC</td>
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<tr>
<td>HCC1569</td>
<td>ATCC</td>
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<td>JimT1</td>
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<td>ECACC</td>
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<td>ATCC</td>
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<tr>
<td>SCC-9</td>
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</table>

Abbreviations: ATCC, American Type Culture Collection; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; ECACC, The European Collection of Cell Cultures; HSRRB, Health Science Research Resources Bank. *Kawasaki Medical School, Kurashiki, Japan.
**pHER3 and pAKT assays**

pHER3 and pAKT were measured by Mesoscale Discovery (MSD) assay. Briefly, cell or tumor lysates were prepared in NP-40 lysis buffer supplemented with protease and phosphatase inhibitors, followed by capture of the analyte on an MSD plate and detection with a phosphospecific primary antibody and Sulfo-tagged secondary immunoglobulin G (IgG). Supplementary Materials contain details.

**In vitro proliferation assays**

For single-agent growth assays, cells were seeded onto 96-well plates (Costar #3904) at 5,000 cells/well, treated with the appropriate concentration of the indicated drug, and incubated for three to five days at 37°C. Cell viability was quantified by the addition of CellTiterGlo reagent (Promega). For drug combination treatments, the procedure was the same except that cells were plated into 384-well plates (Greiner #781091) at 1,000 cells/well. Percent growth inhibition was calculated by normalizing raw luminescence values to that of untreated wells.

**FACS and cellular NRG1-blocking assays**

Binding of HER3 antibodies and NRG1 blocking was determined using flow cytometry. Details are presented in the Supplementary Materials.

**HER3 crystallography**

The MOR09825–HER3 complex was prepared by mixing tagged HER3 ECD (residues 20-640) with *Escherichia coli*-expressed MOR09825 Fab and then purifying the complex on a Superdex 200 10/300 column (GE Healthcare). Data were collected on a crystal cooled to 100K using a Dectris Pilatus 6M detector and synchrotron radiation (λ = 1.0000Å) at the IMCA-CAT beam line 17-ID of the Advanced Photon Source at Argonne National Laboratory. For details on data processing, refer to Supplementary Materials.

**In vivo studies**

For xenograft studies, female athymic nu/nu Balb/C (Harlan Laboratories) or NOG SCID Gammas NSG; Jackson Labs) mice were implanted with BT-474, BxPC-3, FaDu, KPL4, L3.3, N87, or Harca mice. Mice were treated with 20 mg/kg trastuzumab (every second day, 20 mg/kg LJM176 (every other day), 20 mg/kg pertuzumab (every second day), cetuximab 20 mg/kg (every second week), and 100 mg/kg lapatinib (daily). Further details on the in vivo efficacy and pharmacodynamic (PD) studies can be found in the Supplementary Methods.

![Figure 1.](image-url) Identification of LJM176, a HER3-binding monoclonal antibody capable of blocking ligand-dependent and -independent HER3 signaling and cell proliferation. A, SKBR-3 were treated with increasing concentrations of IgG, MAB348, or AF234 for five days followed by cell viability assessment using the CTG assay. B, SKBR-3 cells were treated with 10 μg/mL IgG, MAB348, and AF234 for one or 24 hours. Cell lysates were harvested and immunoblotted with antibodies directed against the indicated proteins. C, HER3-targeted antibodies were probed for their ability to inhibit HER3 tyrosine phosphorylation and cell growth in HER2-amplified SKBR-3 cells. Maximal growth inhibition was calculated relative to that achieved with AF234. pHER3 was measured via MSD assay and Family 15 members are highlighted in red. D, MCF7 cells were treated with LJM176 (red) or IgG (black) for one hour and the level of pHER3 quantified via MSD assay. E, MCF7 cells were treated with LJM176 (red) or IgG (black) for 30 minutes before NRG1 stimulation for 10 minutes. F, pHER3 levels were measured by MSD assay. G, SKBR-3 cells grown in serum-free medium were treated with LJM176 (red) or IgG (black) for five days and cell viability determined by CTG. H, serum-starved MCF7 cells were treated with LJM176 (red) or IgG (black), stimulated with NRG1 (50 ng/mL), and cell viability determined after five days using CTG. Cell proliferation values relative to unstimulated cells are plotted.

**Table 2. Equilibrium dissociation constants (K_D) for binding of LJM716 to human, monkey, mouse, and rat HER3**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>K_D (pmol/L) ± SD</th>
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<tbody>
<tr>
<td>Human HER3</td>
<td>32 ± 10</td>
</tr>
<tr>
<td>Cynomolgus monkey HER3</td>
<td>43 ± 17</td>
</tr>
<tr>
<td>Mouse HER3</td>
<td>37 ± 7</td>
</tr>
<tr>
<td>Rat HER3</td>
<td>57 ± 7</td>
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</tbody>
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**Results**

**Discovery of an anti-HER3 antibody that inhibits both ligand-dependent and -independent HER3 activity in vitro**

Previous short-hairpin RNA (shRNA) data have shown that loss of HER3 expression in HER2-amplified models is sufficient to inhibit cell proliferation (14). Treatment of the HER3 shRNA-sensitive cell line SKBR-3 with commercially available HER3-targeted antibodies further validates the ability to modulate cell growth in this setting from the extracellular compartment. AF234 (a HER3-specific goat polyclonal) induced significant inhibition of growth, whereas MAB3481, a ligand-blocking mouse monoclonal, had little to no effect (Fig. 1A). The greater activity observed with AF234 was correlated with its more robust downregulation of HER3 and prolonged inhibition of pAKT (Fig. 1B). Thus, antibodies that simply block ligand binding to HER3 and fail to inhibit AKT phosphorylation are unlikely to be active in this setting.

To identify a HER3 therapeutic antibody capable of inhibiting both ligand-dependent and ligand-independent HER3 signaling, we isolated HER3-targeted Fabs using MorphoSys HuCAL phage-display technology (24) against a number of recombinant HER3 antigens. Corresponding full IgGs were screened for their ability to neutralize HER3 tyrosine phosphorylation and cell proliferation driven by either HER2 (SKBR3 cells) or NRG1 (MCF7 cells). From all confirmed hits, only four antibodies were capable of effectively inhibiting HER2-driven HER3 phosphorylation and proliferation in SKBR-3 cells (Fig. 1C). In contrast, a large number of antibodies efficiently inhibited HER3 phosphorylation and cell proliferation in NRG1-stimulated MCF7 cells (Fig. 1D) due to their ability to prevent ligand binding (data not shown). Three antibodies were active in both settings (Fig. 1C and D).
Figure 2. LJM716 inhibits HER3 signaling in vivo and is efficacious in multiple ligand-dependent and independent xenograft tumor models. A, BT474 xenografts were dosed intravenously with a single 20 mg/kg dose of LJM716, and tumors were harvested at 0, 4, or 24 hours followed by analysis of lysates for pHER3 and pAKT levels by MSD assay. B, BxPC-3 xenografts were dosed intravenously with a single 20 mg/kg dose of LJM716, and tumors were harvested at 0, 4, or 72 hours followed by analysis of lysates for pHER3 and pAKT levels by MSD assay. C, BT474 xenografts were dosed intravenously every other day with 20 mg/kg LJM716 (red) or IgG (black). D, HBCx-13A primary human xenografts were dosed intravenously every other day with 20 mg/kg LJM716 (red) or IgG (black). E, BxPC-3 xenografts were dosed intravenously every other day with 20 mg/kg LJM716 (red) or IgG (black). F, FaDu xenografts were dosed intravenously every other day with 20 mg/kg LJM716 (red) or IgG (black). Two-tailed non-paired *t* test was conducted for A–B. Data in C–F are presented as mean tumor volume ±SEM. All δ volumes subjected to One Way ANOVA and Tukey post hoc analysis. *, *P < 0.05; **, *P < 0.01; ***, *P < 0.001.
suggesting they may be uniquely capable of inhibiting multiple modes of HER3 activation. The sequence of these antibodies differed only in residues contained within hCDR2 and, thus, these antibodies were highly related and are referred to herein as Family 15.

We further characterized LJM716, an antibody derived from Family 15. LJM716 binds to human, mouse, rat, and cynomolgus monkey HER3 with high affinity as determined by solution equilibrium titration (Table 2) and flow cytometry (Supplementary Table S2). LJM716 potently inhibited phosphorylation of HER3 driven by either HER2 overexpression or NRG1 stimulation (Fig. 1E and F, Supplementary Table S3, Supplementary Fig. S2A) in SKBR-3 and MCF7 cells. The inhibitory activity of LJM716 similarly led to inhibition of PI3K pathway signaling including downregulation of AKT phosphorylation (pAKT S473; Supplementary Fig. S2B and 2C and Table S3) and inhibition of cell proliferation (Fig. 1G and H, Supplementary Table S4) in both SKBR-3- and NRG1-stimulated MCF7 cells. Despite their high degree of sequence homology, no binding of LJM716 to other ErbB family members was observed (Supplementary Fig. S3A). Together, these data suggest that LJM716 is a specific and...
Figure 4. LJM716 displays in vitro and in vivo combination activity with trastuzumab and cetuximab. A, HER2-amplified cell lines grown in full-serum were treated with 10 nmol/L LJM716 or trastuzumab for one hour and the impact on both pHER3 (Y1197) and pAKT (S473) determined by MSD assay. Percentage inhibition relative to control IgG-treated cells is visualized in the form of a heat map colored from blue (0% inhibition) to red (100% inhibition). Cell lines harboring hotspot PI3K mutations are highlighted in red. B, the HER2-amplified cell lines UACC812, MDA-MB-453, NCI-N87, and SKBR-3, grown in full serum, were treated with increasing doses of LJM716 or trastuzumab for one hour and the impact on pHER3 (Y1197) was determined by MSD assay. C, HER2-amplified cell lines were dosed with LJM716 (33 nmol/L), trastuzumab (33 nmol/L), or LJM716 plus trastuzumab. Cells were grown for five days and cell viability determined by CTG. Percentage of inhibition relative to untreated cells is visualized in the form of a heat map. D, BT474 tumor xenografts were grown in NSG mice and treated with IgG (20 mg/kg, q2d), LJM716 (20 mg/kg, q2d), or LJM716/trastuzumab. (Continued on the following page.)
potent inhibitor of ligand-dependent and -independent HER3 signaling and growth.

**LJM716 displays single-agent activity in HER2- and NRG1-driven in vivo models**

Having established that LJM716 inhibits multiple modes of HER3 activation in vitro, we next asked whether this activity was maintained in vivo. SCID mice bearing HER2- amplified BT474 breast cancer xenografts were treated with a single-dose of 20 mg/kg LJM716, resulting in maximal reduction of pHER3 by 52% and pAKT by 84% over a 24-hour period when compared with an equivalent dose of an isotype control antibody (Fig. 2A). Similarly, in SCID mice bearing ligand-expressing BxPC-3 pancreatic xenografts, intravenous dosing of 20 mg/kg LJM716 resulted in 86% maximal inhibition of pHER3 and 74% inhibition of pAKT compared with isotype-matched treated controls (Fig. 2B). These pharmacodynamic studies show that LJM716 is capable of inhibiting both HER2- and ligand-mediated HER3 signaling in vivo as evidenced by decreased pHER3/pAKT in both xenograft models.

LJM716 displayed significant antitumor activity in BT474 (17% T/C) and HBCx-13A (7.7% T/C) HER2-amplified xenografts (Fig. 2C and D). Treatment of SCID mice bearing NRG1-expressing xenografts with 20 mg/kg LJM716 induced tumor regression in FaDu (SCCHN), tumor stasis in Hara (lung), and expressing xenografts with 20 mg/kg LJM716 induced tumor regression in FaDu (SCCHN), tumor stasis in Hara (lung), and expressing xenografts with 20 mg/kg LJM716 induced tumor regression in FaDu (SCCHN), tumor stasis in Hara (lung), and expressing xenografts with 20 mg/kg LJM716 induced tumor regression in FaDu (SCCHN), tumor stasis in Hara (lung), and expressing xenografts with 20 mg/kg LJM716 induced tumor regression in FaDu (SCCHN), tumor stasis in Hara (lung), and expressing xenografts with 20 mg/kg LJM716 induced tumor regression in FaDu (SCCHN), tumor stasis in Hara (lung), and expressing xenografts with 20 mg/kg LJM716 induced tumor regression in FaDu (SCCHN), tumor stasis in Hara (lung), and expressing xenografts.

LJM716 traps HER3 in the inactive conformation

In an effort to understand the mechanism by which LJM716 inhibits HER3, the epitope was identified by determining the three-dimensional (3D) structure of the Fab fragment of MOR09825 bound to the HER3 extracellular domain. MOR09825, the parent Fab molecule of LJM716, has identical CDR regions and an identical biologic profile to LJM716 (data not shown). The 3.4Å crystal structure reveals that MOR09825 binds to HER3 in the tethered (inactive) ErbB conformation and recognizes a nonlinear epitope formed by two distinct domains D2 and D4 (Fig. 3A). The epitope is centered over the D2/D4 interface with a similar occluded surface area for each domains D2 and D4 (Fig. 3A). The epitope is centered over the D2/D4 interface with a similar occluded surface area for each domains D2 and D4 (Fig. 3A). The epitope is centered over the D2/D4 interface with a similar occluded surface area for each domains D2 and D4 (Fig. 3A). The epitope is centered over the D2/D4 interface with a similar occluded surface area for each domains D2 and D4 (Fig. 3A). The epitope is centered over the D2/D4 interface with a similar occluded surface area for each domains D2 and D4 (Fig. 3A). The epitope is centered over the D2/D4 interface with a similar occluded surface area for each domains D2 and D4 (Fig. 3A). The epitope is centered over the D2/D4 interface with a similar occluded surface area for each domains D2 and D4 (Fig. 3A). The epitope is centered over the D2/D4 interface with a similar occluded surface area for each domains D2 and D4 (Fig. 3A). The epitope is centered over the D2/D4 interface with a similar occluded surface area for each domains D2 and D4 (Fig. 3A).

The crystallographically defined epitope is corroborated by biochemical characterization of the anti-HER3/HER3 interaction. ELISA characterization of MOR09825 and other Family 15 members binding to recombinantly expressed HER3 domains and ECD shows that binding is only detected for the full ECD construct. No binding is observed for constructs containing isolated domains or domain pairs, D1-D2, D2, D3–4, or D4 (Supplementary Fig. S3C). In addition, inspection of the crystal structure indicated that HER3 residues Lys267 and Leu268 located within the D2 β-hairpin dimerization arm are involved in numerous interactions with both the heavy and light chains of the antibody (Fig. 3C), suggesting they may be fundamentally important for binding. Mutation of Lys267 and/or Leu268 to alanine abolishes LJM716 binding (Fig. 3D), whereas binding of a D3-targeted antibody is largely unaffected by these mutations (Supplementary Fig. S3D). These data show that residues within the β-hairpin dimerization arm are an integral part of the LJM716 epitope. We conclude that binding of LJM716 locks HER3 in the inactive conformation, thus preventing the large-scale structural rearrangements required for transition of HER3 to the extended conformation characteristic of activated ErbB receptors (30–32).

**LJM716 does not prevent neuregulin binding to HER3**

The crystal structure indicated that the ligand-binding site of HER3, which has been mapped to D1 and D3 by analogy to EGFR (31, 32), was not occluded by Fab binding (Fig. 3A and B). We explored the impact of LJM716 upon the HER3–NRG1 interaction. LJM716–HER3 complexes were preformed and passed over a surface plasmon resonance biosensor chip previously coated with NRG1. LJM716/HER3 complexes were capable of binding immobilized NRG1 whereas a complex comprised of HER3 and a ligand-blocking HER3 antibody (MOR09624) prevented binding to NRG1 (Fig. 3E). Furthermore, the presence of LJM716 had no significant impact on the binding affinity of HER3 for NRG1 (Supplementary Fig. S4), indicating that LJM716 does not influence NRG1 binding in biochemical assays. The affinity of NRG1 was consistent with that previously determined using a mutant form of HER3 that is in the locked conformation (33). To confirm these results in a cell-based system, we tested whether MCF7 cells pre-bound with LJM716 were capable of binding NRG1. Using flow cytometry we found that pre-binding of MCF7 with LJM716 had no impact on subsequent binding by NRG1 (Fig. 3F). These findings strongly suggest that the LJM716 epitope is not essential for ligand binding and that LJM716 and NRG1 can bind concurrently.

**LJM716 effectively combines with HER2 and EGFR inhibitors**

The inability of trastuzumab to fully inhibit HER2/3 complexes is thought to limit its therapeutic benefit, owing to...
The HER2-targeted antibody pertuzumab, which blocks dimerization of HER2 with other ligand-activated ErbB members, was recently approved for treatment of metastatic breast cancer. We sought to compare the combination activity of trastuzumab–pertuzumab (T+P) with that of trastuzumab–LJM716 (T+L) in a set of HER2-amplified breast cancer cell lines. Although T+P and T+L combinations were both highly potent at inhibiting colony, as well as 3D spheroid formation in the trastuzumab-sensitive cell line BT474, the LJM716-containing combination was significantly more active in trastuzumab-insensitive cell line HR6 (36) and MDA-MB-453 cells (Fig. 5A and B, Supplementary Fig. S6). Increased activity of LJM716 containing treatments was accompanied by enhanced inhibition of HER2 and AKT (Fig. 5C). In trastuzumab-sensitive BT474 xenografts in vivo, T+P and T+L combinations are equally active at inducing tumor regressions (Fig. 5D, Supplementary Fig. S5C). When monitoring overall survival following a 35-day dosing period, both combinations equally prolong survival compared with either T or P alone (Fig. 5E). These studies show that addition of LJM716 to trastuzumab is at least as active as the T+P combination in trastuzumab-sensitive settings, but enables enhanced inhibition of oncogenic signaling and proliferation in trastuzumab-resistant settings.

Discussion

Clinical and preclinical data suggest that resistance to ErbB-targeted therapies occurs frequently and can arise through a variety of mechanisms. Mounting evidence has implicated HER3 in resistance to multiple targeted agents via both ligand-dependent and -independent mechanisms (37, 38). Through both structural and biochemical studies, we have shown that LJM716 binds a novel epitope that specifically locks HER3 in the inactive conformation. LJM716 binding ensures that the HER3 D2 dimerization arm remains inaccessible even in the presence of NRG1 or high-levels of HER2. Therefore, once bound to LJM716, HER3 is unable to transition to the active conformation and interact with partner receptors.

LJM716 inhibitory properties result in significant tumor growth inhibition in several different xenograft models that represent ligand-dependent and -independent modes of HER3 activation. The LJM716 mechanism of action offers several potential advantages when attempting to tackle clinical resistance to targeted agents. Inhibiting multiple modes of HER3 activation may prevent tumors from circumventing LJM716 activity by simply switching between ligand-dependent and ligand-independent HER3 activation as the tumor adapts in response to treatment. Evidence for an enrichment of HER2 amplification was recently shown in cetuximab-refractory colorectal tumors (39), suggesting that plasticity in the expression of ErbB-family members known to activate HER3 is clinically relevant. The ability of LJM716 to lock HER3 in the inactive conformation may also prevent HER3 activation through the upregulation of alternate heterodimer partners (40). A solely HER2-targeted therapy, like the trastuzumab–pertuzumab combination is unable to
Figure 5. The addition of LJM716 to trastuzumab enables more potent HER3–PI3K pathway signaling and growth inhibition than trastuzumab–pertuzumab combination, particularly in trastuzumab-insensitive models. A, cells were plated at 10,000 to 50,000 cells per well in six-well plates and treated in triplicate with dimethyl sulfoxide (DMSO), 10 μg/mL LJM716, pertuzumab, and/or trastuzumab. Media containing antibodies was replenished every three to four days. Cells were stained with crystal violet when control-treated cells were confluent, ranging from 14 to 21 days. Representative images and quantification of integrated intensity (% control) are shown. *P < 0.05, t test. B, 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 three days. Images shown were recorded 15 to 19 days after cell seeding. Acini burden was quantified using the GelCount system. Each bar graph represents the mean ± SEM of triplicate samples. *P < 0.05, t test. C, BT474 and MDA-MB-453 cells were treated with 10 μg/mL LJM716, 10 μg/mL pertuzumab, and/or 10 μg/mL trastuzumab for one or 24 hours. Whole-cell lysates were prepared and separated in a 7% SDS gel followed by immunoblot analysis with antibodies directed against the indicated proteins. D, BT474 xenografts were treated with either IgG (20 mg/kg), trastuzumab (20 mg/kg), pertuzumab (20 mg/kg), LJM716 (20 mg/kg), q2d, trastuzumab + pertuzumab, or trastuzumab + LJM716 for 35 days. Data are presented as mean tumor volume ± SEM. E, Kaplan–Meier survival analysis following the end of 35 days of treatment. Mice were monitored for tumor regrowth and sacrificed when tumor burden was larger than 2,000 mm³. q2d, dosed every other day; q2w, dosed every second week.
suppress HER3-driven PI3K pathway signaling resulting from a switch from HER2/3 heterodimers to alternate signaling, such as EGFR/HER3 heterodimers (41). Our data show that NRG1_EGFR_HER3-driven cell lines are sensitive to LJM716 inhibition and that dual targeting of EGFR and HER3 can prevent the emergence of resistance in vivo (Fig. 4E and F). One might speculate that consolidated HER family inhibition using a triple combination of HER2, LJM716 (HER3), and EGFR-targeted agents, if tolerated, could provide additional benefit by restricting possible routes to resistance through adaptive switching between HER-family dimers.

HER3 is a central component of many interdependent signaling pathways activated in cancer. We hypothesize that combination of LJM716 with either HER2, EGFR, or PI3K-targeted agents may yield a more effective treatment strategy. Indeed, addition of LJM716 to trastuzumab resulted in increased inhibition of pAKT and improved in vitro efficacy exceeding that achieved by the trastuzumab–pertuzumab combination (Figs. 4B and 5A and C). Interestingly, the presence of PIK3CA hotspot mutations, a clinically validated mechanism of trastuzumab resistance (2), appears to limit the added activity contributed by trastuzumab in LJM716–trastuzumab combinations (Supplementary Fig. S8). Furthermore, the solely HER2-targeted pertuzumab–trastuzumab combination was less effective than LJM716/trastuzumab in PIK3CA mutant cells, suggesting that PIK3CA mutations more dominantly impact sensitivity to HER2 inhibition compared with HER3 blockade. The importance of having multiple methods of attacking oncogenic drivers is further underscored by the recent identification of cetuximab-resistant EGFR mutants that remained sensitive to panitumumab (42). Because HER2 mutations located within the pertuzumab binding site have already been identified in multiple tumor types (43, 44), it is possible that a similar resistance mechanism may arise in pertuzumab-treated patients. In these cases, LJM716 may present a complementary or superior approach for combination therapy with trastuzumab in both breast and gastric cancers.

In summary, we show that trapping HER3 in the inactive conformation is a highly effective therapeutic strategy that may be extended to other receptors, which are also activated in both a ligand-dependent and -independent manner. Within the ErbB-family, both HER1 and HER4 can adopt a similar inactive conformation and, thus, may be targeted using an analogous approach. As a result of its novel mechanism, LJM716 displays the unique potential to treat HER2/HER3 as well as NRG1_EGFR/HER3-driven tumors either as a single agent or in combination with HER2, EGFR, and PI3K-targeted agents. LJM716 is currently undergoing clinical evaluation in HER2-positive breast and gastric cancers, as well as SCCHN.

Disclosure of Potential Conflicts of Interest

A Huang is employed as Senior Director of Novartis. N. Haubst and C. Kunz have ownership interests including patents. A.B. Heidt received commercial research grants from and has ownership interest (including patents) in Novartis. W.R. Sellers is employed as a Vice-President/Glbal Head of Oncology and has ownership interest (including patents) in Novartis. S.A. Ettenger has an ownership interest (including patents). No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


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