Clinical Cancer Research

Phase I Study of an AKT Inhibitor (MK-2206) Combined with Lapatinib in Adult Solid Tumors Followed by Dose Expansion in Advanced HER2$^+$ Breast Cancer

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Abstract

Purpose: Preclinical data support combining AKT inhibitors with HER2-targeted therapies to overcome resistance to treatment. This phase I study combined the investigational AKT inhibitor, MK-2206, with lapatinib to determine the MTD.

Experimental Design: The dose escalation cohort enrolled adults with advanced solid tumors, who received MK-2206 dosed 30 to 60 mg every other day and lapatinib 1,000 to 1,500 mg daily continuously, escalated using a 3+3 design. Cycles were 28 days except cycle 1 (35 days, including an initial 8 days of MK-2206 alone to evaluate pharmacokinetic interactions). The dose expansion cohort enrolled adults with advanced HER2$^+$ breast cancer.

Results: Twenty-three participants enrolled in the dose escalation cohort. Dose-limiting toxicities were hyponatremia, fatigue, rash, hypocalcemia, and mucositis. Common toxicities included diarrhea, nausea, and rash. The MTD was reached at MK-2206 45 mg orally every other day and lapatinib 1,500 mg orally daily. Two participants maintained stable disease for >4 months, including a colorectal cancer participant with substantial carcinoembryonic antigen decrease. Of 5 participants in the dose expansion cohort, 2 maintained stable disease for >6 months, including one with prior progression on single-agent lapatinib. Plasma MK-2206 concentrations decreased after addition of lapatinib, but *in vitro* studies indicate lapatinib increases the intracellular levels of MK-2206.

Conclusions: MK-2206 combined with lapatinib can be tolerated with both drugs above biologically active single-agent doses. Overlapping toxicities result in significant diarrhea and rash, which can be managed medically. Antitumor activity was promising and supports evaluation of AKT inhibitors combined with HER2-targeted therapies. *Clin Cancer Res; 22(11); 2659–67. ©2016 AACR.*

Introduction

HER2 is an oncogene that is activated by amplification or overexpression in some cancers of the breast, stomach, bladder, and pancreas (1–3). Agents targeting HER2 increase response rates and improve survival when combined with chemotherapy in advanced HER2-positive (HER2$^+$) breast cancer and gastric cancer (4, 5). HER2-targeted therapies include the mAb trastuzumab and the small-molecule kinase inhibitor lapatinib. Lapatinib is an oral tyrosine kinase inhibitor, which potently and specifically inhibits HER2 and the epidermal growth factor receptor (EGFR) (6). When combined with capecitabine, lapatinib is approved for the treatment of advanced HER2$^+$ breast cancer (7). Lapatinib has modest single-agent activity in HER2$^+$ advanced or metastatic breast cancer (8–10). Mechanisms for primary and secondary resistance to lapatinib in HER2$^+$ cancer are driven in part by dynamic compensatory dysregulation of signaling within cancer cells, with inevitable development of resistance to HER2-targeted therapies in the metastatic setting. New therapeutic options are needed to overcome resistance to HER2-targeted agents.

The PI3K/AKT pathway lies downstream of growth factor tyrosine kinase receptors such as EGFR and HER2. It plays a vital role in cell growth regulation and differentiation and contributes to tumor progression in diverse cancer types (11). PI3K/AKT pathway activation drives malignant progression and chemoresistance (12) and is found in human solid tumors such as breast (13), colon (14), and lung (15). Efforts to identify mechanisms of primary resistance to HER2-targeted therapies have revealed the pivotal role of this pathway (16–21). Dysregulation of this pathway can occur through downregulation or loss of the PI3K antagonist PTEN or by the presence of activating mutations in the *PIK3CA* gene encoding the p110α catalytic subunit of the PI3K enzyme (19, 22–24). Downregulation of PTEN phosphatase or constitutive PI3K activation are demonstrated resistance mechanisms for anti-HER2 therapies, including primary resistance to lapatinib (25, 26). Decreased PTEN expression and activating mutations of *PIK3CA* are present in between 13%–52% and 13%–30% of HER2$^+$ breast cancers, respectively (27–31). This offers a

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Translational Relevance

The PI3K/AKT pathway lies downstream of growth factor tyrosine kinase receptors such as EGFR and HER2. It plays a vital role in cell growth regulation and differentiation and contributes to tumor progression in diverse cancer types. We conducted a phase I trial of AKT inhibitor MK-2206 and HER2-targeted therapy lapatinib to establish the MTD of the combination. This was followed by a dose expansion cohort in heavily pretreated metastatic HER2$^+$ breast cancer, which demonstrated clinical activity. Further investigation of AKT inhibitor and HER2-targeted combinations is warranted.

strong rationale for targeting the PI3K/AKT pathway to circumvent resistance to EGFR- or HER2-targeted therapies. AKT1/2 inhibition has been shown to abrogate proliferation of breast cancer cells harboring HER2 amplification, PI3K mutations, or PTEN loss (32). However, treatment with AKT inhibitors alone leads to upregulation of HER3 via feedback. HER3 is a key coreceptor for HER2, and may be an important driver of HER2$^+$ breast cancer (33). Thus, AKT inhibition might be more clinically efficacious if combined with simultaneously blockade of HER2 signaling via HER2-targeted therapies.

Oral cancer regimens are preferred by cancer patients (34, 35). MK-2206 is an oral selective allosteric inhibitor of AKT, with a MTD of 60 mg every other day (36, 37). MK-2206 every other day has not previously been studied in combination with lapatinib. Single-agent MK-2206 toxicities include maculopapular rash and diarrhea, with the potential for significant overlap with known lapatinib toxicities of rash and diarrhea (8–10). Moreover, both drugs are metabolized by cytochrome P450 3A4 (CYP3A4), raising the possibility of substantial drug–drug interaction (38, 39).

We conducted a phase I study of MK-2206 every other day in combination with daily oral lapatinib. The primary objective was to determine the MTD and recommended phase II dose (RP2D) for this combination in adults with advanced or metastatic solid tumors. In a subsequent dose expansion cohort, we sought to confirm preliminary evidence of clinical activity, and safety, in participants with HER2$^+$ advanced or metastatic breast cancer. Pharmacokinetic and pharmacodynamic evaluations as well as assessments for downregulation/loss of PTEN and PI3K-activating mutations were also conducted.

Materials and Methods

This multicenter, phase I, open-label, nonrandomized study was approved by the institutional review boards at the UW and Sanford Cancer Centers and was conducted in accordance with the Declaration of Helsinki. Participants provided written informed consent prior to enrolling in the trial. A 3+3 design was used for the dose escalation cohort, and the MTD was defined as the dose level at which less than one-third of participants experienced a dose-limiting toxicity (DLT).

Eligibility

The study included a dose escalation cohort followed by a dose expansion cohort. Participants were 18 years or older and had Eastern Cooperative Oncology Group performance status 0–2 with adequate hematologic, renal, hepatic, and cardiac function. In addition, the study required evaluable or measurable disease from histologically or cytologically confirmed advanced or metastatic solid tumor for which no standard curative measure existed. Participants with any advanced and incurable solid malignancy were eligible for the dose escalation cohort.

Participants with HER2$^+$ breast cancer deemed to be locally advanced (unresectable and incurable) or metastatic were eligible for the dose expansion cohort. HER2$^+$ was defined as 3+ by IHC or ISH ratio $\geq$ 2.2. Participants who previously progressed on lapatinib were eligible as long as they did not demonstrate prior serious or life-threatening intolerance to doses of lapatinib exceeding 1,000 mg daily. Participants with treated, stable brain metastases or progressive but asymptomatic brain metastases who were not candidates for further local therapy were eligible.

All participants were required to have normal baseline QTc; diabetes mellitus was allowed if well controlled. Prior chemotherapy, radiotherapy, trastuzumab, and other targeted therapies excluding endocrine therapies were not allowed within 4 weeks of study drug. Tamoxifen was not allowed within 2 weeks and aromatase inhibitors within one day. In addition, participants were required to stop any strong/moderate inhibitors or inducers of CYP3A4; sensitive substrates of CYP3A4 with a narrow therapeutic index were disallowed. Medications prolonging QTc were not allowed. Pregnant or lactating women and individuals with HIV were not eligible.

Treatment plan

Lapatinib was started at 1,000 mg orally daily and MK-2206 at 30 mg orally every other day, with dose escalation as outlined in Results (Table 2). To evaluate pharmacokinetic interactions, cycle

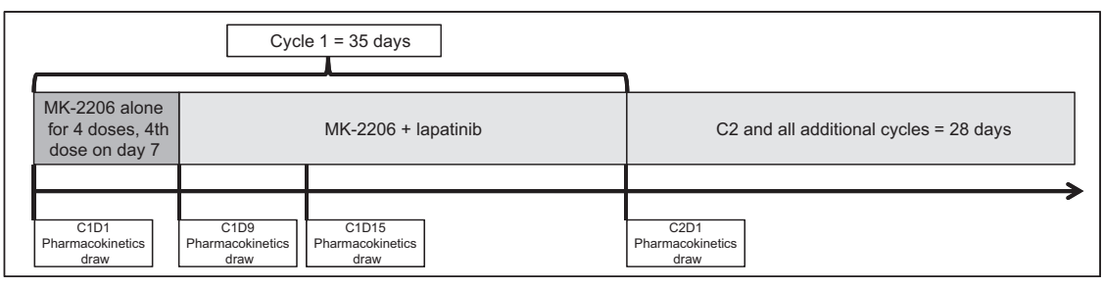

Figure 1.
Schema of cycle 1 drug administration, pharmacokinetic draws, and subsequent cycles for dose escalation and dose expansion cohorts.

1 was 35 days with a lead-in of MK-2206 in both cohorts (Fig. 1). All subsequent cycles were 28 days. Treatment was continuous until disease progression, unacceptable toxicity or withdrawal of consent. Dose modification of either or both drugs was based on grade and attribution of any adverse events (AE). The dose expansion cohort was treated at the MTD identified in the dose escalation cohort.

Assessments

Participants were monitored with history, physical exams, laboratory testing, and electrocardiograms at baseline and every 4 weeks during study. Cardiac function by echocardiogram or Multi Gated Acquisition Scan was monitored at baseline and every third cycle. AEs were graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. DLTs were those that occurred during cycle 1 and at least possibly related to MK-2206 and/or lapatinib. Hematologic DLTs included grade 3 or higher neutropenia regardless of duration. Hematologic DLT included grade 4 thrombocytopenia or any grade if associated with a clinically significant or life-threatening bleed. Nonhematologic DLTs included any grade 3–4 toxicities, except nausea, vomiting, diarrhea, or rash not yet treated with maximal medical therapy. Because of concerns of hyperglycemia, systemic steroids were not permitted in the dose escalation cohort and not used to premedicate for the maculopapular rash common with MK-2206 treatment. A DLT was also defined as any toxicity preventing delivery of > 75% of the protocol specified cycle 1 treatment or dose delay of > 14 days starting prior to cycle 2 day 1 when at least possibly related to study drugs, given the planned chronic, continuous, oral nature of study drug administration (40).

Pharmacokinetic, pharmadynamic, and PTEN/PI3K analyses

Lapatinib inhibits and is primarily metabolized via CYP3A4 (38), which also metabolizes MK-2206 (39). Therefore, we evaluated potential interactions. Pharmacokinetic samples were collected at days 1, 9, and 15 of cycle 1 (5 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 24 hours) and day 1 of subsequent cycles. MK-2206 and lapatinib plasma concentrations were analyzed using a validated LC/MS-MS.

To evaluate P-glycoprotein (Pgp) inhibition as mechanism for drug interaction, HCT-15, a Pgp-expressing human colorectal adenocarcinoma cell line was obtained from the NCI (Bethesda, MD). Cells were cultured in RPMI1640 medium (HyClone) supplemented with 10% FBS at 37°C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells at a concentration of 2×10^6/mL were incubated under the following conditions in triplicate: (i) no treatment, (ii) MK-2206, (iii) pretreatment with 5 μmol/L of lapatinib for 2 hours followed by MK-2206, (iv) pretreatment with 10 μmol/L of verapamil hydrochloride, a Pgp inhibitor, for 2 hours followed by MK-2206. All conditions used 1 μmol/L of MK-2206 for 2 hours. Posttreatment, supernatant media and cell pellets were collected for analysis of MK-2206 levels by LC/MS-MS.

Peripheral blood mononuclear cells (PBMC) were collected before MK-2206 on cycle 1 day 1, before lapatinib on cycle 1 day 9 and cycle 1 day 15 and cycle 2 day 1 for all participants. While MK-2206 and lapatinib inhibit AKT and HER2 proteins, respectively, measuring changes in protein activity/quantity was not feasible in this study and we hypothesized protein inhibition could result in RNA expression changes via indirect mechanisms. In addition, in a prior study at our institution, gene expression demonstrated improved sensitivity and was associated with activity (41). On the basis of the gene expression changes identified, the remaining PBMCs were spun down to form a cellblock for quantitative analysis of p70S6k. Automated quantitative analysis was performed in the Translational Research Initiatives in Pathology lab using the Vectra platform and interpreted by a single pathologist (C. Flynn). The slides were processed using the Roche Ventana Medical System's Discovery XT Automated platform and Roche Ventana reagents. In brief, the slides were deparaffinized, exposed to EDTA buffer at 100°C for 20 minutes (antigen retrieval), incubated with the primary antibody for 20 minutes at room temperature followed by the horseradish-conjugated secondary antibody, and finally incubated with DAB substrate kit and counterstained with hemotoxylin.

Archived tumor tissue (metastatic samples preferred) was collected from all dose expansion cohort participants to evaluate for downregulation/loss of PTEN and activating mutations of PI3K. Macrodissection of tumor tissue was performed, followed by DNA isolation, and PI3K mutation analysis using the IonTorrent AmpliSeq panel as described previously (42). Loss of PTEN was evaluated using IHC with previously described antibodies (42–44). As above, the slides were processed using a similar protocol employing the Roche Ventana Medical System's Discovery XT Automated platform and Roche Ventana reagents.

Study objectives and statistical analysis

The dose escalation cohort's primary objective was to determine the MTD of MK-2206 + lapatinib. The dose expansion cohort's primary objective was to evaluate the safety of MK-2206 + lapatinib in participants with locally advanced and unresectable or metastatic $HER2^+$ breast cancer previously treated with trastuzumab. Secondary objectives included describing the DLTs, safety, pharmacokinetics, and clinical activity of the combination and to assess inhibition of the HER2–PI3K–AKT pathway via PBMCs and to assess $HER2^+$ cancers for mechanisms of lapatinib resistance. The dose expansion cohort was initially planned for 10 participants with lapatinib-naïve $HER2^+$ breast cancer. Because of changes in the treatment of metastatic $HER2^+$ breast cancer following study conception, the study was amended to allow prior lapatinib for the dose expansion cohort prior any participant enrollment to this cohort. The study closed in January 2014 due to limited supply of MK-2206 with 5 participants enrolled in the dose expansion cohort.

All participants who received at least one dose of study drug(s) were assessed for safety and tolerability. Participants were evaluable for the MTD determination if they received $\geq$75% of planned therapy for cycle 1. If a participant received <75% of planned therapy due to toxicity unrelated to study therapy or for other reasons, the participant was considered unevaluable and replaced. A participant was evaluable for efficacy determinations if s/he received $\geq$75% of the planned therapy over the first two cycles. Confirmed antitumor response rate was assessed by the RECIST version 1.1. Descriptive summaries of demographic and toxicity data are presented. The pharmacokinetic parameters were calculated via noncompartmental analysis. All pharmacokinetic parameters were summarized in terms of means ± SD. AUC and C_{max} were tested for dose proportionality using ANOVA; all pharmacokinetic parameters are summarized by using means, SD, and ranges. Changes in pharmacokinetic parameters from day 1 to day 9 and day 15 assessments were evaluated using the nonparametric

Table 1. Participant demographic and baseline characteristics

	Dose escalation cohort ($N = 23$)	Dose expansion cohort ($N = 5$)
Age, median (range)	59 (22–72)	43 (33–56)
Gender, *n* (%)		
Female	15 (65)	5 (100)
Male	8 (35)	
Race, *n* (%)		
Caucasian	21 (91)	5 (100)
Asian	1 (4)	
Unknown	1 (4)	
Primary cancer site, *n* (%)		
GI: colorectal, esophageal	9 (39)	
Lung	4 (17)	
Breast	3 (13)[a]	5 (100)
Gyn: uterine, ovarian	2 (9)	
Other[b]	5 (22)	
Median lines prior therapy, *n* (range)[c]	3 (0–9)	4 (1–12)
Median time on study, *n* weeks (range)	8 (3–35)[d]	8 (4–28)

[a]One participant had HER2$^+$ breast cancer.
[b]$n = 1$ each of adrenal, nasopharyngeal, chondrosarcoma, peritoneal, and salivary cancer.
[c]Includes adjuvant and metastatic setting; combination therapies were counted as one line.
[d]Includes only the 19 participants evaluable for MTD.

Wilcoxon signed rank test. A multilevel linear mixed effects model was used to examine changes in gene expression levels of HER2 or AKT from day 1 to day 9 and day 15 assessments. All *P* values were two-sided and $P < 0.05$ was used to determine statistical significance. Statistical analyses were conducted using SAS software (SAS Institute), version 9.3.

Results

Participant and disease characteristics

Twenty-eight participants enrolled between February 2011 and January 2014. In the dose escalation cohort, 23 participants started study treatment and 19 were evaluable for MTD determination. In the dose expansion cohort, 5 women with HER2$^+$ breast cancer enrolled. The dose expansion participants were previously treated for metastatic disease on trastuzumab, 3 had progressed on pertuzumab, 4 had progressed on ado-trastuzumab emtansine (T-DM1), and 2 had progressed on lapatinib. Table 1 includes demographics and other baseline characteristics.

Dose escalation and DLTs

Table 2 outlines the doses of study drugs received and the DLTs experienced by participants on study by dose level. Four participants were unevaluable for MTD determination—one had progression of malignancy in cycle 1 and three requested to stop study or decrease dosing due to grade 1–2 toxicities (primarily nausea, vomiting, diarrhea, rash, and fatigue). Because 2 of 4 evaluable participants in dose level 4 experienced DLTs, additional participants were accrued to dose level 3. Four additional participants were enrolled at dose level 3, one unevaluable due to withdrawal based on participant wishes. No further DLTs were experienced in the 3 evaluable participants, resulting in defining the MTD and RP2D as MK-2206 45 mg every other day combined with lapatinib 1,500 mg daily given continuously.

Safety and tolerability

Common AEs of any grade at least possibly related to the study drugs, and experienced by at least 10% of participants, are included in Table 3. Diarrhea, nausea, and rash were experienced by the majority of participants, although these were typically manageable grade 1–2 toxicities.

Antitumor activity

In the dose escalation cohort, no complete or partial responses were seen among the 19 participants treated for more than one cycle. However, a participant with colon cancer experienced stable disease for 20 weeks with a substantial CEA decline (599 at baseline; nadir 228 on cycle 3 day 1). In addition, one patient with adrenal cortical carcinoma experienced stable disease for 24 weeks. Seventeen participants (89%) had progressive disease.

In the dose expansion cohort, no complete or partial responses were seen among the 5 participants. However, one participant with prior progression on lapatinib, had response in nonmeasurable skin disease (Fig. 2) and experienced stable disease until progression at 28 weeks. Her disease eventually progressed after repeated dose reductions required for lapatinib-associated rash. Another participant had stable disease for 24 weeks. One participant was not evaluable for response, due to discontinuation after only 21 days on study drugs for rash, pruritus, and diarrhea. No unexpected or new long-term concerns were noted in this cohort.

Table 2. Lapatinib and MK-2206 dosing and DLTs by cohort

	Participants (*n*)	Lapatinib (mg, qDay)	MK-2206 (mg, qoDay)	DLTs
Dose escalation cohort				
Level 1	7[a]	1,000	30	Participant 2: Gr 3 fatigue and hyponatremia
Level 2	3	1,500	30	None
Level 3	8[b]	1,500	45	None
Level 4	5[c]	1,500	60	Participant 15: received < 75% of drug due to Gr 2 intolerable mucositis Participant 18: Gr 4 hyponatremia, Gr 3 rash and hypocalcemia
Dose expansion cohort				
R2PD	5	1,500	45	Not applicable in dose expansion

Abbreviations: qDay, daily; qoDay, every other day.
[a]Six evaluable participants.
[b]Six evaluable participants.
[c]Four evaluable participants.

Table 3. AEs (worst grade) at least possibly related to study drugs experienced by any participants in either the dose escalation or dose expansion cohort

AE	Grade 1–2, *n* (%)	Grade 3, *n* (%)	Grade 4, *n* (%)
Diarrhea	17 (60.7%)	4 (14.2%)	1 (3.5%)
Nausea	15 (53.5%)	3 (10%)	0
Rash	15 (53.5%)	4 (14.2%)	0
Vomiting	10 (35.7%)	1 (3.5%)	0
Anorexia	9 (32.1%)	1 (3.5%)	0
Fatigue	8 (28.5%)	2 (7%)	0
Weight loss	6 (21.4%)	1 (3.5%)	0
Dehydration	5 (17.8%)	1 (3.5%)	0
Dry skin	4 (14.2%)	0	0
Fever	5 (18%)	0	0
Pruritus	5 (18%)	1 (3.5%)	0
QTc prolongation	6 (21%)	0	0
Bradycardia	3 (10%)	0	0
Dizziness	5 (18%)	0	0
Stomatitis/mucositis	5 (18%)	0	0
Bradycardia	3 (10%)	0	0
Hypokalemia	2 (7%)	1 (3.5%)	0

Pharmacokinetic analysis

Both MK-2206 and lapatinib are metabolized by CYP3A4 (38). We hypothesized that competition for metabolism via this pathway might result in higher concentrations of one or both agents when given in combination. MK-2206 plasma concentrations were evaluated on day 1 (baseline prior to study drug), day 9 [MK-2206 at steady state (C_{ss}) as single agent], and day 15 (MK-2206 at C_{ss} with lapatinib). Lapatinib plasma concentrations were evaluated on day 9 and day 15. Mean pharmacokinetic parameters can be found in Table 4, and dosing is shown in Fig. 1.

The lapatinib dose-adjusted AUC and C_{max} were significantly greater at on day 15 when compared with day 9. The ratio of day 15/day 9 for the AUC was 1.73 ± 0.69 ($P = 0.0016$), whereas the dose-adjusted C_{max} ratio was 1.65 ± 0.80 ($P = 0.0005$), which is most likely explained by accumulation occurring at Css. This was also observed for MK-2206, where the dose-adjusted AUC and C_{max} were significantly greater on day 9 at steady state compared with day 1 after the first dose, with a day 9/day 1 ratio of 5.90 ± 5.92 ($P = 0.0020$) and 2.16 ± 0.72 ($P < 0.001$) for the AUC and C_{max}, respectively. When comparing day 15 and day 9 to day 1, the dose-adjusted MK-2206 AUC increases on day 9, but then decreases on day 15 (day 15/day 9 ratio of 0.73 ± 0.39, $P = 0.005$) while the C_{max} remains constant (day 15/day 9 ratio of 0.98 ± 0.23). This indicates a potential drug interaction between MK-2206 and lapatinib.

Given the known competition for CYP3A4, lapatinib was expected to increase MK-2206 plasma concentrations. Thus, the observed decrease in plasma MK-2206 was surprising. We hypothesized that lapatinib, a Pgp inhibitor (45), was blocking the Pgp-mediated cellular extrusion of MK-2206, resulting in lower plasma concentration and elevated intracellular concentrations. To evaluate this possibility, we performed *in vitro* studies using the Pgp-expressing cell line, HCT-15, to test whether lapatinib-mediated intracellular accumulation of MK-2206 through Pgp inhibition which could explain the accelerated MK-2206 pharmacokinetics in plasma (Fig. 3). Indeed, compared with control cells, MK-2206 intracellular concentrations were increased in the presence of lapatinib (1347.1 ± 30 vs. 314.8 ± 12.5 ng/mL), but not with verapamil (321 ± 32.7 ng/mL). This data support the clinical pharmacokinetic findings, but suggests the lapatinib enhances intracellular MK-2206 by a mechanism distinct from classic Pgp inhibition.

Pharmacodynamic analysis

PBMC studies showed no significant changes in the gene expression levels of HER2 or AKT over time (days 1 vs. 9 vs. 15). There was a trend toward significant decrease in p70S6K expression observed from day 1 to day 15 (mean decrease of 55% ± 31%, $P = 0.0927$). Evaluation with Vectra for p70S6K expression revealed no difference in protein expression between days 1, 9, and 15. All cases contained moderate staining for p70S6K at all time points examined.

PI3K mutations and PTEN loss

As noted in Table 5, one sample (participant 24) from the dose expansion cohort yielded sufficient DNA for AmpliSeq analysis. This participant did not have *PIK3CA* mutation, but IHC revealed loss of PTEN. Another participant had a previously identified activating mutation of *PIK3CA*.

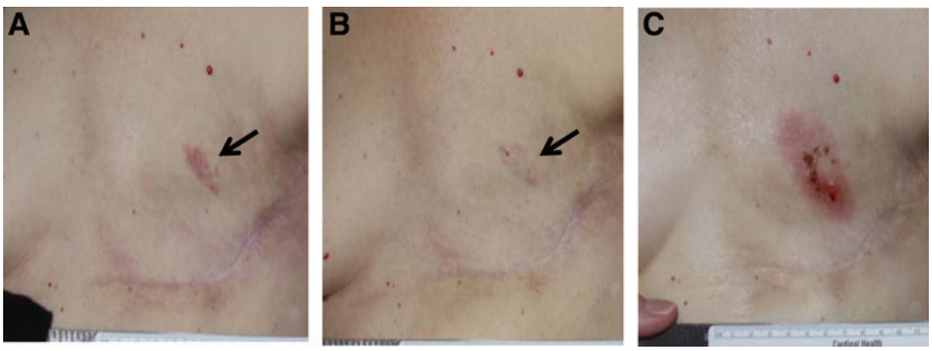

Figure 2.
Nonmeasurable skin lesion response for participant in dose expansion cohort. This participant's locally advanced, unresectable HER2$^+$ breast cancer developed 2 months after completing adjuvant radiation and while still receiving adjuvant trastuzumab. Biopsy at recurrence revealed an activating mutation at PI3K-α [NM_006218.2(PIK3CA):c.3140A>G p.H1047R]. She started study 13 months after recurrence following progression on docetaxel/pertuzumab/trastuzumab, lapatinib single agent (unable to tolerate capecitabine/lapatinib), capecitabine/trastuzumab, and T-DM1. A, baseline (prior to any MK-2206 or lapatinib) biopsy-proven progression of skin lesions on T-DM1; B, skin lesion best response on study; C, skin lesion progression at 28 weeks following multiple dose reductions and holds due to rash.

Table 4. Pharmacokinetic parameters by day (Mean ± SD)

	C_{max}/dose (ng/mL/mg)	$AUC_{0-\infty}$ (hour*ng/mL/mg)	Cl/F (L/hour)	V/F (L)	Half-life (hours)
MK-2206 (*n* samples)					
Day 1 (28)	0.42 ± 0.23	26.58 ± 31.77	119.8 ± 62.2	15.63 ± 0.95	43.51 ± 39.26
Day 9 (27)	0.86 ± 0.50	100.55 ± 78.27	54.11 ± 62.2	15.40 ± 0.99	95.64 ± 76.16
Day 15 (24)	0.75 ± 0.45	62.83 ± 51.17	35.70 ± 28.19	15.11 ± 0.53	59.05 ± 25.97
Lapatinib (n)					
Day 1 (28)	—	—	—	—	—
Day 9 (27)	1.59 ± 0.87	38.16 ± 29.08	63.1 ± 43.2	14.1 ± 0.89	18.5 ± 14.2
Day 15 (24)	2.21 ± 1.20	58.29 ± 36.58	39.5 ± 28.4	13.6 ± 0.63	18.5 ± 10.3

Discussion

HER2-targeted therapies are effective in the treatment of advanced HER2$^+$ breast and gastric cancers (4, 5). Although these cancers may be "addicted" to HER2 oncogenic signaling, primary and secondary resistance results in progression of these cancers despite HER2 signaling blockade. The PI3K/AKT signaling pathway plays a significant role in this resistance (17, 32, 46–48) and may serve as a second targetable signaling node to intervene pharmacologically. However, single-agent AKT-directed therapy is insufficient likely due to circumventing pathways such as upregulation of HER3 (33). Combinations of HER2-targeted therapies with PI3K/AKT pathway inhibitors have promising activity in preclinical and clinical studies (49, 50). This provided the rationale for this phase I study of MK-2206 plus lapatinib. The dose escalation cohort established the MTD of the combination as MK-2206 45 mg every other day and lapatinib 1,500 mg daily, both given continuously. A 45-mg dose of MK-2206 exceeds the clinical monotherapy efficacy trough target (37, 51), suggesting that this is a biologically active dose despite being lower than the single-agent MTD. In addition, we established the safety and identified pharmacologic interactions of this combination as well as preliminary evidence of efficacy in a heavily pretreated population with advanced HER2$^+$ breast cancer.

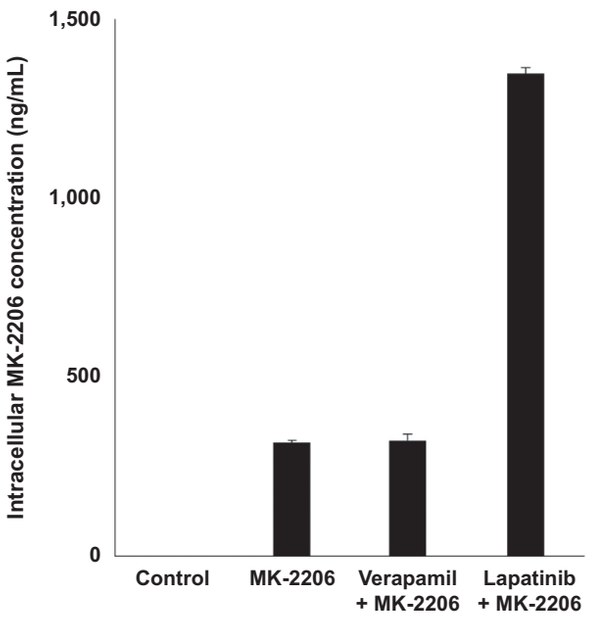

Figure 3.
Intracellular MK-2206 concentration in HCT-15 cells with no treatment (A), MK-2206 alone (B), pretreatment with 10 μmol/L of verapamil hydrochloride, a Pgp inhibitor, for 2 hours followed by MK-2206 (C), pretreatment with 5 μmol/L of lapatinib for 2 hours followed by MK-2206 (D). All conditions used 1 μmol/L of MK-2206 for 2 hours. Each condition performed in 3 replicates and error bars indicate SEM.

The overlapping toxicities of MK-2206 and lapatinib resulted in high rates of diarrhea and rash, although this was primarily grade 1–2 and medical management was feasible. The maculopapular rash associated with MK-2206 was described in the phase I studies (37) and is distinct from the acneiform rash associated with lapatinib and other EGFR-targeted agents (38). On protocol, determination was made by the treating oncologists regarding the likely causal drug, with subsequent management per protocol (steroids were not permitted during dose escalation for MK-2206 rash). A recent study of MK-2206 combined with trastuzumab (51) reported 17% grade 3 rash and no grade 3–4 diarrhea compared with 14% grade 3 rash and 19% grade 3–4 diarrhea in our trial, suggesting overlapping gastrointestinal (GI) toxicities of MK-2206 and lapatinib. Aside from these toxicities, the combination was well tolerated. Notably, there was no evidence of clinically significant hyperglycemia. The dose expansion cohort did not reveal unexpected cardiac risk in a population treated with prior cardiotoxic therapies such as anthracyclines and trastuzumab. Future MK-2206 and HER2-targeted therapy combinations will need to weigh the benefits of an all-oral regimen versus the potential for more rash and diarrhea when considering which HER2-targeted therapy to use.

No objective responses were noted, but the decline in CEA in one participant with colorectal cancer (previously treated with 5-fluorouracil, oxaliplatin, irinotecan, and cetuximab) is interesting. Although not tested in this participant, mutations in the PI3K pathway are relatively common in colorectal cancer (52) and may have mediated the clinical activity. An important limitation of this study is the limited number of HER2$^+$ breast cancer patients studied. The original dose expansion cohort was intended to explore clinical activity in 10 lapatinib-naïve HER2$^+$ patients. However, with changes in the landscape of HER2-directed therapies, the protocol was amended to allow lapatinib-naïve and -treated patients with evaluable or measurable disease and the primary objective changed to safety. Thus, this study had limited power to evaluate clinical activity in a HER2$^+$ cohort (in whole study, total $n = 6$). However, one participant in the dose expansion cohort had improvement in skin disease (Fig. 2), despite prior progression of HER2$^+$ breast cancer on lapatinib. Notably, she had a documented activating mutation of PI3K based on AmpliSeq testing performed at time of incurable recurrence.

Our pharmacokinetic analyses suggest a potential drug–drug interaction, with lapatinib reducing the plasma AUC of MK-2206. The MK-2206 plasma AUC at steady state was decreased when comparing day 9 (MK-2206 alone) and day 15 (MK-2206 and lapatinib). This was not the anticipated effect given the

Table 5. Dose expansion participants and HER2-AKT pathway analysis

Participant	AmpliSeq analysis	IHC analysis	Other known analyses	Best response to study drugs	Prior HER2 therapy for MBC
24	(1) Wild-type for *PTEN* (2) *TP53* c. 744 G>C; p.R248R (3) *MET* c.3029C>T; p.T1010I	PTEN loss		SD; PD after cycle 6	Trastuzumab
25	Insufficient sample	Normal		SD, off study after cycle 2	Trastuzumab, T-DM1
26	Insufficient sample	Normal		Not evaluable	Trastuzumab, pertuzumab, T-DM1
27	Insufficient sample	Normal		PD after cycle 2	Lapatinib, trastuzumab, pertuzumab, T-DM1
28	Insufficient sample	Normal	(1) [a]*PIK3CA*:c.3140A>G; p.H1047R (2) [a]*TP53*:c.839G>C; p.R280T	Skin response; PD after 6 cycles	Trastuzumab, pertuzumab, T-DM1, lapatinib

Abbreviations: MBC, metastatic breast cancer; PD, progressive disease; SD, stable disease.
[a]Ion Torrent 46 gene Ampliseq panel done at MD Anderson, see Fig. 2 for additional details.

shared metabolism of both agents via CYP3A4 (38, 39). Evaluation of Pgp inhibition by lapatinib demonstrated that lapatinib increases the intracellular levels of MK-2206. However, we have preliminarily ruled out Pgp as the primary mediator as verapamil did not increase the intracellular levels of MK-2206. As lapatinib also inhibits ABCG2 and MRP1, it is possible that MK-2206 is a substrate of either of these transporters. Regardless of the mechanism, this is a potentially synergistic interaction, as increased MK-2206 intracellular concentrations may be another means of overcoming resistance. Measurement of intracellular concentrations of MK-2206 in subsequent clinical trials combining these two agents is warranted. Lapatinib pharmacokinetics were similar to those reported previously (53). Protein expression of p70S6K, measured by IHC, was not significantly different between PMBCs collected on days 1, 9, and 15 of treatment. All patients had a moderate level of expression in all of their PMBCs in all time points examined. IHC of PTEN was lost in the metastatic cancer of a patient that contained mutations in *TP53*, *MET*, and an intron of *PIK3CA* in the same tissue. Although the PTEN in this tissue was not mutated, the other mutations, especially of PIK3CA, may be altering the expression of the cognate protein.

Further work is ongoing with MK-2206 and anti-HER2 combinations. The phase I trial of MK-2206 combined with trastuzumab demonstrated preliminary findings of efficacy in heavily pretreated breast and gastroesophageal cancer, and did not demonstrate pharmacokinetic interactions (51). A trial of trastuzumab combined with MK-2206 with trastuzumab and lapatinib in $HER2^+$ solid tumors has completed accrual, but has not yet reported results (NCT00963547). In addition, a phase I trial of MK-2206 dosed every week combined with daily lapatinib is ongoing in women with metastatic $HER2^+$ breast cancer (NCT01281163). Future study designs should consider emerging data regarding which populations may benefit most from combinations. Benefit was only seen in the hormone receptor–negative cohort of $HER2^+$ patients in BOLERO-1 (54), and results have differed between first line compared with later line use of drugs such as everolimus (50, 54). On the basis of our results, we believe that such combinations warrant further investigation in $HER2^+$ breast cancer as well as further evaluation of novel AKT inhibitors combined with HER2-targeted therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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