Selective Inhibition of HER2-Positive Breast Cancer Cells by the HIV Protease Inhibitor Nelfinavir

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Background
Human epidermal growth factor receptor 2 (HER2)–positive breast cancer is highly aggressive and has higher risk of recurrence than HER2-negative cancer. With few treatment options available, new drug targets specific for HER2-positive breast cancer are needed.

Methods
We conducted a pharmacological profiling of seven genotypically distinct breast cancer cell lines using a subset of inhibitors of breast cancer cells from a screen of the Johns Hopkins Drug Library. To identify molecular targets of nelfinavir, identified in the screen as a selective inhibitor of HER2-positive cells, we conducted a genome-wide screen of a haploinsufficiency yeast mutant collection. We evaluated antitumor activity of nelfinavir with xenografts in athymic nude mouse models (n = 4–6 per group) of human breast cancer and repeated mixed-effects regression analysis. All statistical tests were two-sided.

Results
Pharmacological profiling showed that nelfinavir, an anti-HIV drug, selectively inhibited the growth of HER2-positive breast cancer cells in vitro. A genome-wide screening of haploinsufficiency yeast mutants revealed that nelfinavir inhibited heat shock protein 90 (HSP90) function. Further characterization using proteolytic footprinting experiments indicated that nelfinavir inhibited HSP90 in breast cancer cells through a novel mechanism. In vivo, nelfinavir selectively inhibited the growth of HER2-positive breast cancer cells (tumor volume index of HCC1954 cells on day 29, vehicle vs nelfinavir, mean = 14.42 vs 5.16, difference = 9.25, 95% confidence interval [CI] = 5.93 to 12.56, P < .001; tumor volume index of BT474 cells on day 26, vehicle vs nelfinavir, mean = 2.21 vs 0.90, difference = 1.31, 95% CI = 0.83 to 1.78, P < .001). Moreover, nelfinavir inhibited the growth of trastuzumab- and/or lapatinib-resistant, HER2-positive breast cancer cells in vitro at clinically achievable concentrations.

Conclusion
Nelfinavir was found to be a new class of HSP90 inhibitor and can be brought to HER2-breast cancer treatment trials with the same dosage regimen as that used among HIV patients.

J Natl Cancer Inst 2012;104:1576–1590
In addition, a heat shock protein 90 (HSP90) inhibitor, 17-AAG, which is undergoing phase I and II clinical trials for the treatment of lymphomas and solid cancers including metastatic breast cancers (9), has been shown to be effective in HER2-positive breast cancers. Recently, Vogelstein and colleagues systematically cataloged mutations in a number of breast and colorectal cancer cell lines through genome-wide sequencing of well-annotated human protein-coding genes (10). The knowledge on the genotypic status of each breast cancer cell line offered a unique opportunity to identify genotype-selective anti–breast cancer drugs from our established drug library (Johns Hopkins Drug Library [JHDL]) (11,12). In this study, we screened the JHDL for inhibitors of breast cancer lines and obtained a number of hits, including known anticancer drugs and new ones. Subsequently, we profiled the sensitivity of seven genotypically characterized breast cancer lines to a subset of drugs identified from the JHDL and analyzed intergroup similarity between the drug-sensitivity phenotypes and the defined genotypes of the seven lines, including the mutation status of BRCA1 and TP53 and the expression status (positive or negative) of ER and HER2. This approach led to the identification of an HIV protease inhibitor, nelfinavir, as an HER2-selective anti–breast cancer drug.

Nelfinavir (Viracept) is an HIV aspartyl protease inhibitor that was approved by the US Food and Drug Administration for the treatment of HIV in 1997 (13). It has been used in combination with other antiretroviral drugs, including HIV reverse-transcriptase inhibitors, for the treatment of HIV infection (14). In addition to its inhibitory effect on HIV, nelfinavir has been shown to inhibit phosphatidylinositol 3-kinase (PI3K) and AKT signaling pathway, a process that is thought to be associated with side effects such as hyperlipidemia (15). Nelfinavir has been reported to inhibit the growth of several types of cancer lines, including melanoma and non–small cell lung cancer (16,17), because of its inhibition of the PI3K and AKT signaling pathway. In addition, the PI3K and AKT pathway is known to be activated after ionizing radiation, conferring resistance to radiotherapy of cancer (18). As a result, inhibition of PI3K and AKT signaling enhances the efficacy of radiation therapy in many types of cancers (19,20). Nelfinavir has entered several clinical trials as either a chemotherapeutic agent or a radiosensitizer for cancer therapy. Promising phase I results have been reported recently for locally advanced pancreatic cancer (21). Despite extensive studies on the anticancer and radiosensitizing activity of nelfinavir, the precise molecular mechanism underlying its anticancer activity and its inhibitory effect on the PI3K and AKT signaling pathway remains unknown. It has been reported that nelfinavir inhibits 20% human proteosome activity (22,23), which may account for its inhibition of PI3K and AKT. However, this notion has been questioned (17). In addition, anticancer activity of nelfinavir did not always correlate with the inhibition of PI3K and AKT in vivo (24), suggesting that the PI3K and AKT signaling pathway may not be the only target of nelfinavir in cancer cells.

To further deconvolute the mechanism of action of nelfinavir in HER2-positive breast cancer cells, we screened the entire collection of haploinsufficiency yeast strains and identified HSP90 as a potential target.

### Materials and Methods

#### Cell Culture and Drug

HCC1143, HCC1395, HCC1937, HCC1954, HCC2218, MCF-7, BT474, and HCC38 breast cancer cells (kindly provided by Dr Bert Vogelstein, Johns Hopkins University School of Medicine, Baltimore, MD) were grown in Roswell Park Memorial Institute 1640 medium containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and 1% antibiotics (penicillin and streptomycin) solution (Invitrogen). HS578T cells were grown in Dulbecco’s modified Eagle medium with 10% fetal bovine serum and 1% antibiotics solution. MCF-10A cells were cultured in Dulbecco’s modified Eagle medium: nutrient mixture F12 (Invitrogen) containing 5% horse serum, 100 ng/mL cholesterin, 20 ng/mL epidermal growth factor (EGF). 0.5 μg/mL hydrocortisone, 10 μg/mL insulin, and 1% antibiotics solution. Trastuzumab-resistant cells were created by continuously exposing BT-474 cells to 4 μg/mL trastuzumab for 3 months, at which point cells regained morphology similar to the parental line (25). Cells per plate were then pooled together and tested for dose response to trastuzumab. Pools are routinely maintained in 4 μg/mL trastuzumab. The cells were maintained in a humidified incubator adjusted to 5% carbon dioxide. The genotypes of the cell lines were verified using the short tandem repeat profiling by Genetic Resources Core Facility (Johns Hopkins University School of Medicine) as reported previously (12). For drug screening, 10 mM stock solutions of the JHDL were arrayed in 96-well plates and screened at a final concentration of 10 μM. The cell growth was determined using a [3H]-thymidine incorporation assay (26). Briefly, cells at 5000 cells per well were seeded in 96-well plates containing 0.2 mL of growth media and allowed to adhere for 24 hours. The cells were then treated with drugs for 24 hours. Cells were pulsed with 0.5 μCi [3H]-thymidine (PerkinElmer, Waltham, MA) for 16 hours and harvested upon trypsin treatment onto glass fiber filters (Wallac, Turku, Finland), from which [3H] counts were determined using a MicroBeta plate reader (Perkin Elmer).

#### Drug Sensitivity Profiling

The half maximal inhibitory concentration (IC50) values of 70 drugs against seven breast cancer cell lines were determined using eight different concentrations (0.01, 0.1, 0.2, 0.5, 1, 2, 5, and 15 μM) of each drug by [3H]-thymidine incorporation assay. The IC50 values were calculated using the GraphPad Prism 4.0 software (GraphPad Software, San Diego, CA). The drug IC50 values equal to or less than 10 μM were set as 10 μM, and the drug IC50 values equal to or greater than 15 μM were set as 15 μM. Drug IC50 values (10–150000 nM) were then converted into log10 scale (1–4.17). The four genotypes of seven breast cancer cell lines shown in Supplementary Table 2 (available online) were obtained from the previous report (10) and the National Institutes of Health National Cancer Institute Integrative Cancer Biology Program (http://icbp.lbl.gov/breastcancer/celllines.php) (last accessed August 1, 2012). To examine selective anti–breast cancer drugs by genotype, we used the nonparametric Mann–Whitney U test because measured IC50 values were not normally distributed.

The IC50 values of trastuzumab, lapatinib, and nelfinavir against BT474 and three drug-resistant breast cancer cell lines were determined using eight different concentrations of each drug by...
[\text{[H]}\text{-thymidine incorporation assay}. The IC_{50} values of each drug against each cell line were determined as described above. The resistant index was calculated as a ratio of the drug IC_{50} against drug-resistant breast cancer cell lines to the IC_{50} against drug-sensitive cell line BT474.

**Screening of Haploinsufficiency Yeasts**

The wild-type (WT) yeast strain BY4743 was used for determining the IC_{50} value of nelfinavir. For the screening of drug-sensitive strains, the heterozygote yeasts grown in 96-well plates supplemented with the synthetic complete media (MP Biomedicals, Solon, OH) with glucose and 200 µg/mL G418 were treated with either dimethyl sulfoxide (DMSO) or 10 µM nelfinavir (IC_{50} of nelfinavir for WT yeasts) for 24 hours. The yeast growth was measured at an optical density of 600 nm using a BMG FLUOStar OPTIMA plate reader (BMG Labtech, Cary, NC) to obtain the cell survival rate (%) of each strain against nelfinavir. The cell survival rate of each strain was then converted to a Z score based on the following equation: 

\[ Z = \frac{x - \text{average}}{\text{standard deviation}} \]

where \( x \) is the survival rate of each strain against nelfinavir. To collect hits, we used the Z score cutoff of \(-1.96\) (type II error \( \alpha = 0.05 \)) for the screen. Z scores less than \(-1.96\) gave us 95% confidence, assuming normal distribution of the yeast viability screening data.

**Immunoblot and Immunoprecipitation**

For the immunoblot, cells were lysed by adding 1 volume of 2× Laemmlli buffer and then boiling for 10 minutes. The samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membrane (Bio-Rad, Hercules, CA). Proteins were detected using primary antibodies for HER2 (F-11, Santa Cruz Biotechnology), AKT (Santa Cruz Biotechnology), phospho-AKT (Ser473 of mouse AKT, Cell Signaling technology, Danvers, MA), phospho-ERK1 and -2 (Santa Cruz Biotechnology), extracellular signal-regulated kinase 1 and -2 (ERK1 and -2, Santa Cruz Biotechnology), cyclin-dependent kinase 6 (CDK 6) (Santa Cruz Biotechnology), CDK4 (Santa Cruz Biotechnology), activator of heat shock protein ATPase homolog 1 (AHA1) (Santa Cruz Biotechnology), eukaryotic initiation factor 2 (eIF2) (Cell Signaling Technology), phospho-eIF2α (Enzo Life Sciences), poly ADP ribose polymerase (PARP) (Cell Signaling Technology), and ubiquitin (Santa Cruz Biotechnology); this was followed by incubation with horseradish peroxidase–conjugated antimouse or antirabbit antibodies (Santa Cruz Biotechnology); this was followed by incubation with horseradish peroxidase–conjugated antimouse or antirabbit antibodies (Santa Cruz Biotechnology) and enhanced chemiluminescence (GE Healthcare, Pittsburgh, PA). For immunoprecipitation of HSP90 in rabbit reticulocyte lysate (Promega, Madison, WI), 50 µL of rabbit reticulocyte lysate were diluted in 450 µL of buffer containing 10 mM trisaminomethane hydrochloride, pH 7.5, 1 mM magnesium chloride, 0.2% Tween 20, and 10 mM sodium molybdate and incubated with 4 µg HSP90 antibody (H9010, Abcam) overnight at 4°C. Protein G sepharose was then added to the sample to precipitate the immunocomplexes for 2 hours. The beads were washed 3 times with the dilution buffer, and the bead samples were processed for SDS-PAGE and immunoblot.

**Proteolytic Footprinting of HSP90**

The full-length HSP90α–GST fusion construct (pGEX-4T-2-HSP90α) was kindly gifted by Dr Solomon Snyder at Johns Hopkins School of Medicine. Each domain of HSP90α was polymerase chain reaction amplified using primer pairs containing Sall:NotI restriction sites and was subcloned into pGEX-6P-2 vector (GE Healthcare). The GST fusion construct of each domain of HSP90α was expressed in BL21 and purified by glutathione beads and PreScission protease according to the manufacturer’s instruction (GE Healthcare). The full-length HSP90α (Enzo Life Sciences) or each purified domain was preincubated with drugs for either 30 minutes or 2 hours at room temperature. The reaction mixture was then brought on ice. The trypsin digestion was conducted by incubating the purified HSP90α and each domain with indicated concentrations of trypsin in an assay buffer containing 10 mM trisaminomethane chloride, pH 7.4, 150 mM sodium chloride, 4 mM calcium chloride, and 0.1 mM ethylenediaminetetraacetic acid for 10 minutes on ice. The reaction was then stopped by adding 1 volume of 2× Laemmli buffer, and the samples were boiled for 5 minutes. The proteolytic profile of HSP90 full-length was analyzed by immunoblots with antibodies specific for either the N-terminal (PA3-013) or C-terminal (H-114) of HSP90. The proteolytic profile of HSP90 subdomains were analyzed by SDS-PAGE, followed by Coomassie brilliant blue staining of the gels.

**Immunofluorescence**

HCC1954 cells were grown on a glass coverslip in a 24-well plate and were treated with drugs for 24 hours. The cells were fixed with 3.7% formaldehyde, permeabilized with 0.5% Triton X-100, and washed with phosphate-buffered saline before being incubated with the blocking solution containing 1% bovine serum albumin and 0.1% tween 20 in phosphate-buffered saline for 1 hour. After blocking, the cells were incubated with primary antibodies in the blocking solution overnight at 4°C, and then incubated with secondary antibodies, including antirabbit immunoglobulin G–cytine 3 and antimouse immunoglobulin G–cytine 2, for 1 hour. The cellular nuclei were stained with 4’,6-diamidino-2-phenylindole. The immunofluorescence images were obtained using the Zeiss 510 Meta multiphoton confocal microscope (Carl Zeiss, Thornwood, NY).

**In Vivo Breast Cancer Xenograft Assays**

Female athymic nude mice (BALB/c, nu/nu-NCr) aged 4–6 weeks and weighing 18–22 g were purchased from the National Cancer Institute (Frederick, MD) and treated in accordance with Johns Hopkins Animal Care and Use Committee procedures. For the xenograft experiment of HCC1954, HCC1937, and MDA-MB-231, approximately 2 million cells were implanted subcutaneously into mice (n = 6 per group for HCC1954; n = 4 per group for HCC1937; n = 5 per group for MDA-MB-231). For the BT474 xenograft, 17β-estradiol pellets (0.5 mg per pellet, 60-day release,
Innovative Research of America, Sarasota, FL) were implanted at 1 day prior to tumor cell injection. Approximately 6 million BT474 cells were implanted subcutaneously into mice (n = 5 per group). After tumors became palpable, the mice bearing HCC1954 and HCC1937 tumors were treated with either vehicle (saline with 5% DMSO) or nelfinavir via intraperitoneal injection every day. For BT474 and MDA-MB-231 tumors, mice were given orally either vehicle (saline with 5% DMSO) or nelfinavir every day. The drug delivery methods and doses of nelfinavir for the mouse experiment were selected based on the previous report (27). Nelfinavir was shown to be effective in growth inhibition of non–small cell lung cancer cell xenograft when mice were treated intraperitoneally with 50 mg/kg or orally with 100 mg/kg. We used a dose equal to or less than half (25 mg/kg for intraperitoneal injection and 40 mg/kg for oral administration) of the dose used for the non–small cell lung cancer cells. The tumor volume was measured periodically using a vernier caliper and calculated according to the modified ellipsoid formula: tumor volume (mm$^3$) = (short axis)$^3$ × (long axis) × $\pi$/6. After 30 days of treatment, the mice were killed, and the tumor tissues were extracted for immunoblots. The tumor volume index was calculated as a ratio of the tumor volume on a given day divided by the tumor volume of day 0.

**Statistical Analysis**

For in vitro studies, the differences between control and experimental groups were determined by two-sided Student’s $t$ test, and $P$ values less than 0.05 were considered statistically significant. To evaluate the effects of genotypes of the breast cancer cells on drug sensitivity, we performed the Mann–Whitney $U$ test. For in vivo tumor xenograft studies, a repeated mixed-effects regression analysis was used to evaluate the statistical significance between control and experimental groups over experimental days. The dependent variable was tumor volume index, and the independent variables were the treatment (nelfinavir vs vehicle) and time (experimental days). For each breast cancer xenograft (HCC1954, BT474, HCC1937, and MDA-MB-231), the repeated mixed-effects linear regression model was used to evaluate the effect of nelfinavir on changes in tumor volume indices with an interaction for the categorical variables of treatment (nelfinavir vs vehicle) and experimental days. An autoregressive covariance structure was used to model correlations between the repeated measurements within each treatment with a decay in the strength of correlation depending on the day between measurements. The Akaike information criterion indicated that the autoregressive covariance structure produced an adequate correlation matrix. The overall statistical significance of effects of nelfinavir on changes in tumor volume indices during experimental days was tested using type 3 $F$ tests. All statistical tests were two-sided.

**Results**

**Pharmacological Profiling to Identify Genotype-Selective Anti–Breast Cancer Drugs**

To identify genotype-selective anti–breast cancer drugs, we first screened the JHDL for drugs that inhibit breast cancer cell proliferation. Two cell lines, MCF-7, a transformed breast cancer line, and MCF-10A, an immortalized breast epithelial cell line, were used for the initial screening. Drugs that inhibited [H]-thymidine incorporation by more than 70% at 10 $\mu$M were designated hits. A total of 212 hits were identified from the JHDL, and a subset of 70 drugs was selected to profile seven genotypically characterized breast cancer cell lines in part based on the diversity of drug targets (Supplementary Figure 1, A and B; Supplementary Table 1, available online).

We determined the IC$_{50}$ value of each of the 70 drugs against the seven genotypically characterized breast cancer cell lines (10) using the [H]-thymidine incorporation assay. The IC$_{50}$ values (ranging 10–15 000 nM) were converted to the log$_{10}$ scale (ranging 1–4.17) prior to the identification of genotype-selective anti–breast cancer drugs. We were particularly interested in drugs that may have selectivity for mutations in TP53 and BRCA1 and -2, or overexpression of HER2 and ER, given their roles in defining the breast cancer genotype (Supplementary Table 2, available online). We found five drugs, including mercaptopurine, nelfinavir mesylate, gefitinib, triciribine, and 60-methylprednisolone, that showed larger inhibitory potential on cell proliferation with the HER2-positive breast cancer lines than with the HER2-negative breast cancer lines (Table 1). A similar result was obtained from a clustering analysis of standardized drug IC$_{50}$ values, in which nelfinavir mesylate, gefitinib, and triciribine were clustered in the same group and showed selectivity to HER2-positive breast cancer lines (HCC1954 and HCC2218) relative to HER2-negative ones (Supplementary Figure 1, C, available online). Another four drugs were found to be selective for TP53 WT or mutant cells from the test (Table 1). This result was confirmed again from the clustering analysis (Supplementary Figure 1, C, available online). However, no drugs were found to be selective for BRCA1 and -2 and ER genotypes.

**Effect of Nelfinavir on HER2 Signaling Pathway**

Among the five HER2-selective anti–breast cancer drugs identified from the Mann–Whitney $U$ test, 60a-methylprednisolone showed the least selectivity for HER2-positive breast cancer cells (Table 1; Supplementary Figure 1, C, available online). Mercaptopurine showed a relatively good selectivity for HER2-positive breast cancer cells, but it also showed a decent inhibitory potential against HS578T, an HER2-negative cell line (Supplementary Figure 1, C, available online). Therefore, we did not pursue these two drugs further. It is not surprising that gefitinib and triciribine were found to be selective inhibitors for HER2-positive breast cancer cells because gefitinib is an EGFR tyrosine kinase inhibitor (28) and triciribine is an inhibitor of the AKT pathway, which lies downstream of HER2 (29). To determine if other inhibitors of the HER2 signaling pathway also showed selectivity for HER2-positive breast cancer cells, we examined inhibitors of PI3K, ERK1 and -2, and p38 MAPK in several breast cancer cell lines. All the HER2 signaling pathway inhibitors tested here showed a selectivity for HER2-positive breast cancer cells (Supplementary Figure 2, A, available online). Expression levels of HER2 and its downstream pathway proteins in those breast cancer cells were verified by immunoblot analysis (Supplementary Figure 2, B, available online).

Next, we determined the effect of nelfinavir on the HER2 signaling pathway in either HER2-positive (HCC1954 and HCC2218) or HER2-negative (HCC1937; control) breast cancer cells. Nelfinavir (10 $\mu$M) strongly inhibited all downstream HER2 signaling events examined, including phosphorylation of AKT, ERK1
and -2, and the HER2 protein level itself in HER2-positive cells, whereas it had negligible effects on the same events in HER2-negative cells (Supplementary Figure 2, C, available online). We noted that the phosphorylation of AKT and ERK1 and -2 eventually succumbed to inhibition by higher concentrations of nelfinavir in HER2-negative breast cancer cells (Figure 1, A). These results suggested that inhibition of AKT and ERK1 and -2 signaling by nelfinavir is, in part, independent of its inhibitory effect on HER2. In addition, nelfinavir activated caspases and induced apoptosis in HCC1954 cells. But no caspase activation or apoptosis occurred in HCC1937 (HER2-negative) or in HCC2218 (HER2-positive), implying that induction of apoptosis by nelfinavir is also independent of its effect on HER2 (Figure 1, A). Supplementary Figure 3, available online). In a time-course experiment with HCC1954 cells, nelfinavir slightly increased the protein level of HER2 in early time points (2–4 hours post-treatment) and then decreased the level of the protein and its phosphorylated form (Figure 1, B). Similar to its effect on HER2, nelfinavir decreased the protein level of AKT as well as its phosphorylated form. Inhibition of HER2 and AKT by nelfinavir was followed by the inhibition of ERK1 and -2 and the induction of apoptosis in HCC1954 cells. These data suggested that nelfinavir might affect the function of a common upstream regulatory protein that is crucial for maintaining the protein levels as well as activities of HER2 and AKT.

The inhibitory effect of nelfinavir on AKT signaling and cancer cell growth has been attributed to its inhibition of the 20S proteasome (23). We thus compared the effects of nelfinavir and two well-characterized proteasome inhibitors, MG132 and bortezomib (Velcade), on HER2-positive breast cancer cells. Both nelfinavir and the proteasome inhibitors diminished the HER2 protein level and activated the caspase cascade (Figure 1, C). However, the proteasome inhibitors generated a 23-kD fragment, a putative caspase-8 cleaved product of HER2, which was not present in cells treated with nelfinavir. In addition, AKT and ERK1 and -2 phosphorylation was greatly inhibited by nelfinavir but not by the proteasome inhibitors. Moreover, caspase inhibitors did not reverse the inhibitory effect of nelfinavir on AKT phosphorylation, whereas the proteasome inhibitors did reverse the nelfinavir effect. In contrast, caspase inhibitors reversed the inhibitory effect of nelfinavir on ERK1 and -2 phosphorylation, whereas the proteasome inhibitors did not. These results demonstrated that inhibition of the proteasome activity cannot account for the biological activity of nelfinavir in breast cancer cells. Given that the proteasome inhibitors prevented certain nelfinavir effects, the actual molecular target of nelfinavir may lie upstream of the proteasome.

### Identification of a Molecular Target of Nelfinavir

We employed the diploid heterozygous deletion mutant yeast collection to screen for synthetic lethal or hypersensitive strains with nelfinavir treatment. Prior to screen, we first analyzed the dose response curve of nelfinavir on the WT diploid yeast (BY4743) (Supplementary Figure 4, A, available online). For the yeast haploinsufficiency screen, we used a suboptimal dose of nelfinavir, which generated only approximately 10% of maximum inhibition on WT yeast growth (IC_{50} = 10 µM). This way, we were able to screen a broad range of synthetic lethal strains that showed a hypersensitivity to the low concentration of nelfinavir. From the initial screen, 133 strains were identified to be more sensitive to nelfinavir than WT yeast (Supplementary Figure 4, A and C, available online). We then conducted a secondary screen of the hits and found 17 strains that showed at least two-fold higher sensitivity to nelfinavir than WT yeast showed (Supplementary Table 3, available online). The corresponding gene products include four proteins related to heat shock proteins, five proteins related to Golgi complex and vacuolar proteins, six ribosomal proteins, and two unclassified proteins. Notably, all those proteins are known to interact physically or genetically with HSP82, a yeast ortholog of mammalian HSP90 (Figure 2, A). It is known that deficiency in HSP82 causes synthetic growth defect with those sensitive strains, suggesting that HSP82 might be a potential nelfinavir target in budding yeast and raising the possibility that HSP90 is the target of nelfinavir in mammalian cells.

To assess the possibility that nelfinavir acts on HSP90, we determined the effect of nelfinavir on the interaction between HSP90 and other co-chaperones in rabbit reticulocyte lysates using a coimmunoprecipitation assay. We used rabbit reticulocyte

### Table 1. Nonparametric Mann–Whitney U test of drug sensitivity profiles and breast cancer genotypes

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Mean IC_{50}, µM (95% CI)</th>
<th>Mean IC_{50}, µM (95% CI)</th>
<th>Pt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TP53 WT cell lines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toremifene</td>
<td>13.00 (9.27 to 16.72)</td>
<td>15.00 (15.00 to 15.00) 1</td>
<td>.0003</td>
</tr>
<tr>
<td>Phenformin hydrochloride</td>
<td>2.15 (0.01 to 4.49)</td>
<td>14.66 (14.20 to 15.12)</td>
<td>.003</td>
</tr>
<tr>
<td>Proscillaridin A</td>
<td>0.05 (0.01 to 0.08)</td>
<td>0.01 (0.01 to 0.02)</td>
<td>.006</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>0.05 (0.01 to 0.09)</td>
<td>0.01 (0.01 to 0.02)</td>
<td>.007</td>
</tr>
<tr>
<td><strong>TP53 MT cell lines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toremifene</td>
<td>13.00 (0.61 to 2.88)</td>
<td>12.75 (10.16 to 15.33)</td>
<td>.002</td>
</tr>
<tr>
<td>Nelfinavir mesylate</td>
<td>3.10 (2.45 to 3.74)</td>
<td>13.00 (11.91 to 14.08)</td>
<td>.002</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>0.13 (0.01 to 0.28)</td>
<td>5.46 (4.63 to 6.28)</td>
<td>.002</td>
</tr>
<tr>
<td>Tricirine</td>
<td>0.21 (0.01 to 0.41)</td>
<td>4.84 (3.01 to 6.67)</td>
<td>.002</td>
</tr>
<tr>
<td>6α-methylprednisolone</td>
<td>0.29 (0.01 to 0.56)</td>
<td>2.47 (0.57 to 4.38)</td>
<td>.006</td>
</tr>
</tbody>
</table>

* CI = confidence interval; IC_{50} = half maximal inhibitory concentration.
† P values less than.01 were considered statistically significant in the drug sensitivity difference between two opposite genotypes.
‡ Toremifene did not inhibit the proliferation of TP53 MT cells up to 15 µM treatment (see Materials and Methods).
Lysate system for this experiment to avoid any possibility of changes in protein expression levels by drugs. Geldanamycin, an N-terminal binding inhibitor of hSP90, was used as a positive control. Like geldanamycin, nelfinavir enhanced the interaction between hSP70 and hSP90 (Figure 2, B). Unlike geldanamycin, however, nelfinavir had no effect on the interaction between hSP90 and p23. We also examined the effect of nelfinavir on the homodimerization of hSP90, which is known to be inhibited by novobiocin, a C-terminal binding inhibitor of hSP90. Neither nelfinavir nor geldanamycin inhibited hSP90 dimerization and/or oligomerization (Supplementary Figure 5, available online). These results suggested that nelfinavir affects hSP90 function in a way that is distinct from either geldanamycin or novobiocin.

Effect of Nelfinavir on Conformational Changes of HSP90

HSP90 contains several trypsin cleavage sites (three major and two minor cleavage sites) (Figure 3, A), and trypsin digestion profiling (proteolytic footprinting) of HSP90 has been widely used to characterize its drug binding sites as well as its conformational changes (30–32). To determine the effects of nelfinavir and other HSP90 inhibitors on the HSP90 conformational changes, purified HSP90 was incubated with each drug for 2 hours at room temperature before the reaction mixtures were subject to digestion with trypsin for 10 minutes on ice. The reactions were stopped, and the resultant mixtures of HSP90 fragments were analyzed by immunoblot with antibodies directed towards the N- and C-terminal domains of HSP90, respectively. Both nelfinavir and novobiocin generated increasing amounts of a C-terminal 50 kD (C50) fragment of HSP90 in a dose-dependent manner, whereas DMSO or geldanamycin did not (Figure 3, B). In contrast with novobiocin and geldanamycin, only nelfinavir gave rise to two N-terminal fragments, N78 and N40 of HSP90 (Figure 3, C). These effects of nelfinavir were further confirmed with different concentrations of nelfinavir.
interaction between heat shock protein 90 (HSP90) and co-chaperones in rabbit reticulocyte lysates. Rabbit reticulocyte lysates were preincubated with drugs (NFV, 5 µM and 20 µM; MG132, 5 µM; geldanamycin [GA], 5 µM; novobiocin [NB], 0.2 mM) for 2 hours on ice and were diluted in a buffer for the immunoprecipitation assay with HSP90 antibody. DMSO = dimethyl sulfoxide; HOP = HSP70–HSP90 organizing protein; HSP70 = heat shock protein 70; IgG = immunoglobulin G; IP = immunoprecipitation; WB = immunoblot.

To explore the effect of nelfinavir on HSP90 conformational changes in greater detail, two domains of HSP90 (N+M and M+C, depicted in Figure 3, A) were generated and subjected to the trypsin proteolytic footprinting. Neither novobiocin nor nelfinavir affected the proteolytic profile of N+M domain (Figure 3, E). Nelfinavir, however, enhanced the trypsin digestion of the M+C domain, with slight increases in the 50- and the 35-kD fragments, whereas novobiocin delayed the trypsin digestion of M+C domain, as judged by an increase in the 50-kD fragment and the corresponding decrease in the 35-kD fragment (Figure 3, F). A time course of the trypsin digestion experiment with the M+C domain showed that nelfinavir enhanced the trypsin cleavage of M+C domain, with an accumulation of the 50- and 35-kD fragments in a time-dependent manner (Figure 4, A and B). The identities of both the 50- and 35-kD fragments were assigned based on the results from the full-length HSP90 and the novobiocin proteolytic profiles of M+C domain (Supplementary Figure 7, available online). Because nelfinavir has no effect on the trypsin digestion of the N+M domain, it is highly likely that nelfinavir binds to the C-terminal domain of HSP90.

The HSP90 C-terminal fragment contains a dimerization domain that is known to be targeted by novobiocin (33). To determine whether nelfinavir and novobiocin share the same binding site in HSP90, it was sequentially treated with nelfinavir and novobiocin (or vice versa), followed by trypsin digestion. As shown in Figure 4, C, nelfinavir alone significantly enhanced the first cleavage of HSP90 by trypsin, as confirmed by the increase in the ratio of the amount of the tryptic fragments to that of the full-length HSP90. However, regardless of the order of addition, novobiocin completely blocked the effect of nelfinavir on the trypsin digestion profile of HSP90, suggesting that nelfinavir-induced conformational change of HSP90 requires dimerization of HSP90. These results indicate that nelfinavir and novobiocin do not share the same binding site in HSP90 and novobiocin has a dominant effect on the conformational change in the HSP90 over nelfinavir. The overall effects of nelfinavir, geldanamycin, and novobiocin on each trypsin cleavage site are summarized in Figure 4, D. As reported previously, geldanamycin has no effect on the cleavage of HSP90 by trypsin. Nelfinavir seems to bind to the C-terminal region of HSP90 and induce a conformational change that enhanced the initial trypsin cleavage at 282 and 616 and/or 621 sites on the full-length HSP90 and reduced the cleavage at the 401 site. In contrast, novobiocin binds to C-terminal dimerization domain and induces a conformational change that decreased the trypsin cleavage at 401 and 616 and/or 621 and increased the cleavage at 282 and 227 and/or 250 sites. These observations suggest that although nelfinavir interacts with HSP90 via its C-terminal domain like novobiocin, its binding site differs from that of novobiocin.
Systematic Comparison Between the Effect of Nelfinavir and Known HSP90 Inhibitors on Breast Cancer Cells

Because nelfinavir has been reported as a human proteasome inhibitor, we verified the effect of nelfinavir on both 26S and 26S proteasome activity in breast cancer cells. Nelfinavir strongly inhibited the chymotrypsin-like activity of the 20S proteasome with an IC50 of 1.5 µM (Supplementary Figure 8, A, available online). In contrast, nelfinavir did not affect the 26S proteasome activity at all at 20 µM concentration in hCC1954 cell lysates. As a control, mG132 completely inhibited the chymotrypsin-like activity and peptidylglutamyl peptide hydrolysing activity of 26S proteasome in the lysate (Supplementary Figure 8, B–D, available online). These data demonstrated that nelfinavir has no effect on the 26S proteasome activity.

Next, we compared the effects of nelfinavir, some known hSP90 inhibitors, and a proteasome inhibitor on the morphology of HER2-positive breast cancer cells. Both nelfinavir and novobiocin induced a unique morphological change in HCC1954 cells at low drug concentrations and an extensive cellular vacuolization at high concentrations (Supplementary Figure 9, available online). Geldanamycin and MG132 caused quite different morphological changes in the cells. In regard to the hSP90 client proteins, nelfinavir, geldanamycin, and novobiocin decreased the levels of all client proteins examined, whereas mG132 increased the levels of CDKs (Figure 5, A, upper panel). Both nelfinavir and novobiocin reduced the amount of two co-chaperones of hSP90, AhA1 and sHSP70, whereas geldanamycin increased the level of those proteins (Figure 5, A, lower panel). As predicted from the induction of the cellular vacuolization, nelfinavir and novobiocin as well as mG132 strongly enhanced PArP cleavage, a hallmark of apoptosis induction, whereas geldanamycin had a negligible effect. We also analyzed

Figure 3. Proteolytic footprinting to assess heat shock protein 90 (HSP90) conformational changes under the nelfinavir (NFV) treatment. A) A schematic illustration of HSP90 domain structures and trypsin cleavage sites. Major peptide fragments of HSP90 generated by trypsin digestion were labeled based on the observed molecular size of each peptide. N+M and M+C domains of HSP90 were cloned and purified. B and C) Effect of drugs on trypsin digestion profile of the full-length HSP90. Purified full-length (FL) HSP90 (150 ng) was incubated with NFV (100 µM), geldanamycin (GA) (50 µM), or novobiocin (NB) (10 mM) for 2 hours at room temperature prior to trypsin digestion. The cleavage fragments were then detected using a C-terminal (B) or an N-terminal (C) HSP90 antibody. D) FL HSP90 (600 ng) was incubated with NFV (100 µM) or NB (10 mM) for 30 minutes at room temperature prior to trypsin digestion. The cleavage fragments were then analyzed using an N-terminal HSP90 antibody. E and F) Effect of drugs on trypsin digestion profile of HSP90 truncation domains. Two micrograms of HSP90 N+M domain (E) or M+C domain (F) were incubated with GA (50 µM), NFV (100 µM), and NB (10 mM) for 1 hour at room temperature prior to trypsin digestion. The cleavage products were then analyzed by Coomasie brilliant blue staining after separation with sodium dodecyl sulfate polyacrylamide gel electrophoresis. CL = charged linker domain; DMSO = dimethyl sulfoxide; EEVD = glutamic acid, glutamic acid, valine, aspartic acid.
the ubiquitination status of cellular proteins after treatment with those inhibitors. Interestingly, both nelfinavir and novobiocin partially induced ubiquitination of proteins, mainly in lower half of all proteins. mG132 strongly increased the ubiquitination of all proteins (Figure 5, B, lower panel). Geldanamycin also slightly increased protein ubiquitination. To confirm the above results visually, we determined the level and subcellular localization of Her2 and ubiquitin in HCC1954 cells by immunofluorescence. As shown in Figure 5, C, Her2 is mainly localized in the cytoplasmic membrane and there is very low basal level of ubiquitinated proteins in the cells. Both nelfinavir and novobiocin reduced the Her2 fluorescence on the cytoplasmic membrane and increased the ubiquitin fluorescence in nuclei with some particles throughout the cytosol, which are presumably preaggregative particles. Geldanamycin strongly reduced the amount of cytoplasmic Her2 and increased protein ubiquitination in nuclei with little cytosolic particles. mG132 reduced the Her2 level and strongly increased ubiquitin fluorescence in nuclei with numerous particles in the cytosol. Finally, we determined the effects of nelfinavir and novobiocin on the interaction between HSP90 and HER2 in HCC1954 cells. In a 3–5-hour treatment during which the HER2 protein level was not significantly reduced, both nelfinavir and novobiocin inhibited the interaction between HSP90 and HER2 (Figure 5, D and E).

**Effect of Nelfinavir on HER2-Positive and HER2-Negative Breast Cancer Growth in Mice**

We assessed the anti-breast cancer activity of nelfinavir in vivo by paired mouse xenograft experiments with HER2-positive (HCC1954, n = 6 per group; BT474, n = 5 per group) and HER2-negative (HCC1957, n = 4 per group; MDA-MB-231, n = 5 per group) breast cancer cells. Nude mice bearing tumors were given daily vehicle or nelfinavir for 30 days, and tumor volumes were measured periodically. A longitudinal data analysis showed that intraperitoneal injection of nelfinavir significantly inhibited the xenograft tumor growth of HCC1954 cells over the treatment period (tumor volume index on day 29, vehicle vs nelfinavir, mean = 14.42 vs 5.16, difference = 9.25, 95% confidence interval [CI] = 5.93 to 12.56, \( P < .001 \), two-sided \( F \) test), whereas HCC1937 tumor growth was not statistically significantly affected (Figure 6, A and B). Similarly, oral administration of nelfinavir strongly inhibited the tumor growth of BT474 over the treatment period (tumor volume index on day...
vehicle vs nelfinavir, mean = 2.21 vs 0.90, difference = 1.31, 95% CI = 0.83 to 1.78, \( P < .001 \), two-sided \( F \) test), whereas the growth of mDA-mB-231 was not statistically significantly affected (Figure 6, C and D). At the end of the xenograft experiment, the levels of her2, phospho-her2, AKt, and phospho-AKt in the tumor tissue extracts were analyzed by immunoblot analysis. Total protein and the phosphorylated levels of her2 were statistically significantly reduced by nelfinavir in the tumor tissues of Bt474 xenografts (Figure 6, E). No detectable her2 was found in mDA-mB-231 tumor extracts. These results demonstrated that nelfinavir selectively inhibited the growth of her2-positive breast cancers in vivo and the selective inhibition of her2-positive breast cancer growth by nelfinavir was attributable to its inhibitory effect on HER2 protein level. Unexpectedly, the levels of AKt and its phosphorylated form were not decreased, but rather, they were increased by nelfinavir in BT474 tumor extracts (Figure 6, E). Also, the levels of AKt and phospho-AKt were not decreased in MDA-MB-231 tumors treated with nelfinavir (Figure 6, F). These results suggest that the inhibitory effect of nelfinavir on PI3K and AKT pathway is not a common mechanism of in vivo anticancer activity of nelfinavir.

Effect of Nelfinavir on the Growth of Drug-Resistant, HER2-Positive Breast Cancer Cells

We next asked whether nelfinavir as an inhibitor of HSP90 can overcome the drug-resistance of her2-positive breast cancer cells, which can often occur during treatment with trastuzumab and lapatinib in clinic. We examined both acquired and intrinsic drug-resistant breast cancer cell lines, including BT474- TrastR (acquired trastuzumab resistance), MDA-MB-43 (intrinsic resistance to trastuzumab and lapatinib), and JIMT-1 (intrinsic resistance to lapatinib).
to trastuzumab). BT474 parental cell line was used as a control for drug-sensitive, HER2-positive breast cancer cell line. Trastuzumab inhibited the proliferation of BT474 cells with an $IC_{50}$ of 1.18 µg/mL (95% CI = 0.89 to 1.57). However, it didn’t inhibit the proliferation of three drug-resistant cell lines up to 50 µg/mL treatment (resistant index > 50) (Figure 7, A; Supplementary Table 4, available online). Lapatinib showed an $IC_{50}$ value of 0.083 µM (95% CI = 0.073 to 0.093) for BT474 cells, but it showed $IC_{50}$ values of 1.35 µM (95% CI = 1.08 to 1.67, resistant index = 16.26) and 1.07 µM (95% CI = 0.74 to 1.55, resistant index = 12.89) for MDA-MB-453 and JIMT-1 cells, respectively (Figure 7, B; Supplementary Table 4, available online). Nelfinavir inhibited the proliferation of BT474 cells with an $IC_{50}$ of 5.83 µM (95% CI = 5.46 to 6.22). It also inhibited the proliferation of all three drug-resistant

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cell lines within a concentration range that was effective in BT474 (IC\textsubscript{50} values for BT474-TrastR cells: 6.44 µM, 95% CI = 5.14 to 8.07, resistant index = 1.1; for MDA-MB-453 cells: 4.74 µM, 95% CI = 4.17 to 5.38, resistant index = 0.81; for JIMT1 cells: 6.88 µM, 95% CI = 6.18 to 7.66, resistant index = 1.18) (Figure 7, C; Supplementary Table 4, available online).

**Discussion**

Identification and characterization of new pharmacological activities from existing drugs represents an effective way to accelerate the translation of discoveries at the bench to clinical applications; it also facilitates the subsequent target identification and validation. Under these premises, we established the JhDL. To date, a number of interesting and potentially applicable hits have been identified, including the immunosuppressive drug mycophenolic acid, the antifungal drug itraconazole, and the urinary tract antibiotic nitroxoline as novel antiangiogenic agents; the antileprosy drug clofazimine as a novel inhibitor of Kv1.3 channel and immunomodulator; and the antihistamine astemazole as novel antimalarial agent (11,12,26,34–36). In the present study, we performed a two-stage screen for new anti–breast cancer agents. In stage 1, we screened the entire JhDL against two representative breast cell lines for inhibitors and identified over 200 hits. In stage 2, a subset of the hits was profiled against seven genotypically characterized breast cancer cell lines. We identified nelfinavir as a selective inhibitor of HER2-positive breast cancer cells. Selectivity of nelfinavir for HER2-positive breast cancer cells was attributable to its inhibitory effects on HER2 protein and phosphorylation levels as well as AKT and ERK1 and -2 signaling pathways. In vivo anti–breast cancer activities of nelfinavir were verified using mouse xenograft models with both HER2-positive and -negative breast cancer cells. Either intraperitoneal or oral administration of nelfinavir selectively suppressed the growth of HER2-positive breast cancer cells over the HER2-negative ones. Immunoblot analysis confirmed that nelfinavir had a similar effect on HER2 protein level in vivo.

To unravel the mechanistic basis of the selectivity of nelfinavir for HER2-positive breast cancer cells, we initially entertained the possibility that nelfinavir worked through inhibition of the human proteasome, as previously reported (23). Although we were able to confirm the inhibitory effect of nelfinavir on purified 20S proteasome, several lines of evidence argued against the proteasome as the relevant target for nelfinavir for its selective inhibition of HER2-positive breast cancer cells. First, pretreatment with proteasome inhibitors blocked certain nelfinavir effects. Second, nelfinavir did not inhibit 26S proteasome activity at all in cell lysates. Third, whereas MG132 increased the protein level of CDK4 and CDK6, nelfinavir enhanced degradation of those CDKs, whose stability is known to be regulated.
by the proteasome-dependent pathway. Lastly, morphological changes of the breast cancer cells caused by nelfinavir were distinct from those induced by MG132. These results suggested that nelfinavir has a different target than the 20S proteasome.

We employed a chemical genetic screening of haploinsufficiency yeast collection to seek clues on the potential nelfinavir target. This approach can identify not only the drug target gene deletion strain, which could be more sensitive to the drug if the target gene is essential, but also the synthetic lethal strains to the drug if the deleted gene product interacts with the drug target either genetically or physically. Among the 17 strains with more than two-fold increase in sensitivity to nelfinavir, the majority of them are known to directly interact with HSP82, the yeast ortholog of HSP90. All of the above genetic interactions are synthetic growth defect or synthetic lethal with HSP82 (Saccharomyces Genome Database, http://www.yeastgenome.org/) (last accessed August 1, 2012), suggesting that HSP90 is a likely molecular target of nelfinavir in eukaryotic cells. Recently, another HIV protease inhibitor, ritonavir, has been reported to inhibit the functions of HSP90 (37), supporting an idea that a certain class of HIV protease inhibitors is a new HSP90 antagonist.

HSP90 is a molecular chaperone that facilitates the folding of proteins after translation or under stress conditions and has a stabilizing effect on a number of client proteins. Several specific inhibitors of HSP90 have been developed to date (38). We systematically compared the effects of nelfinavir and two known HSP90 inhibitors in a number of biochemical and cellular assays. Geldanamycin is known to bind to the N-terminal ATP-binding pocket of HSP90 and affect interaction between HSP90 and some other co-chaperones (39). Similar to geldanamycin, nelfinavir enhanced the interaction between HSP90 and HSP70. Unlike geldanamycin, which reduced the interaction of p23 with HSP90, nelfinavir had no effect on the same interaction. Moreover, geldanamycin has no effect of the HSP90 conformation changes, as reflected in trypsin digestion profiles, whereas nelfinavir substantially changed the stability and the proteolytic profile of HSP90, further distinguishing nelfinavir from geldanamycin. Novobiocin is known to bind to the C-terminal dimerization domain of HSP90, thereby inhibiting HSP90 dimerization and inducing its conformational changes (31,33). Unlike novobiocin, nelfinavir had no effect on the dimerization of HSP90. Interestingly, the effects of nelfinavir on the trypsin cleavage profiles were completely abrogated by novobiocin. Although the phenotypic changes elicited by nelfinavir are largely identical to those caused by novobiocin at the cellular level, the observed difference between the two inhibitors suggest that they act differently on HSP90, likely through proximal but distinct binding sites. Although the lack of proper affinity probe of nelfinavir hampered the determination of the precise binding site of nelfinavir on HSP90, these results clearly suggest that nelfinavir interacts with a site of HSP90 distinct from the binding sites of all known inhibitors and thus represents a new type of HSP90 inhibitor.

HER2 and AKT are known client proteins of HSP90, which is required for their stability and functions (40,41). Inhibition of HSP90 functions by pharmacological inhibitors leads to degradation of HER2 and AKT or a decrease in their phosphorylation (41,42). In this study, nelfinavir was found to decrease the levels of total protein and phosphorylated form of HER2 and AKT in breast cancer cells, though short-term treatment (1–4 hours) showed an opposite effect. These effects of nelfinavir are attributable to its inhibition of HSP90. However, nelfinavir increased the levels of total protein and phosphorylated form of AKT in BT474 cells in animals in vivo. These results are consistent with a previous report showing that nelfinavir increased AKT phosphorylation in melanoma cells, but it inhibited the cancer cell growth (17). In a separate study, short-term treatment with nelfinavir increased AKT phosphorylation in lung cancer cells and had no inhibitory effect on AKT phosphorylation in animal tumor xenograft model, though it had a significant anticancer activity (27). These results suggest that the PI3K and AKT pathway may not be the common mechanism of anticancer activity of nelfinavir in vivo. Rather, nelfinavir exhibited anticancer activity through inhibition of multiple pathways, including Cdc25A, CDKs (17), and hypoxia inducible factor-1α (43) as well as HER2 (shown in this study), all of which are known client proteins of HSP90. These results suggest that the mechanism underlying the anticancer activity of nelfinavir is the inhibition of HSP90, followed by the dysfunction of HSP90 client proteins. It is unclear, however, why AKT was increased by nelfinavir in the tumor mouse model. It is interesting to note that both nelfinavir and novobiocin increased the level of HER2 in early time points of treatment (1–4 hours) but decreased the levels after longer exposure. In contrast, geldanamycin quickly (within an hour) decreased the level of HER2 in the breast cancer cells upon treatment (data not shown). These results suggest that there is a difference between N-terminal and C-terminal HSP90 inhibitors in the kinetics of client protein stabilization and destabilization.

In addition to HER2 and AKT, a number of other client proteins of HSP90, including EGFR, HIF-1α, androgen receptor, Bcr-Abl, and CDKs, are known to be key players of cancer cell survival and proliferation. It has been suggested that inhibition of HSP90 function can cause simultaneous inhibitory effects on multiple pathways of cancer cell signaling, which may decrease drug resistance (44). The development of drug resistance against trastuzumab has been one of the biggest hurdles in the treatment of HER2 breast cancer. It has been shown that activating mutations in PIK3CA is the major cause of the drug resistance against HER2 targeting agents, including trastuzumab and lapatinib (45). PIK3CA encodes the p110α catalytic subunit of PI3K, which is a direct downstream effector kinase of HER2. Thus, constitutively active PI3K could allow cancer cells to bypass the effect of HER2 targeting agents. Nelfinavir, indeed, was effective in inhibiting the growth of drug-resistant, HER2-positive breast cancer cell lines in large part because a large number of signaling proteins downstream of HER2 are dependent on HSP90 to function, allowing nelfinavir to overcome resistance to both trastuzumab and lapatinib.

This work does have a few limitations. First, the precise mode of interaction between nelfinavir and HSP90 remains to be further elucidated using such techniques as x-ray crystallography. Second, we were not blinded to the control and treatment groups of animals, which may affect the objectivity of the interpretation of the experimental outcome.

Nelfinavir is a first-generation HIV protease inhibitor approved by the US Food and Drug Administration for an oral dose regimen of 750mg three times daily for AIDS patients. However, it was modified to a regimen of 1250mg twice daily, as recommended.
by US Food and Drug Administration in 1999. The efficacy of nelfinavir was proven to be equal in both regimens in a large, randomized trial (46). Extensive pharmacokinetics studies have shown that nelfinavir has an average peak plasma level of 8–10 µM, which is approximately twice as high as its IC50 for the HER2-positive breast cancer cell proliferation (3–6 µM), suggesting that it may be effective in breast cancer patients with the current dosage regimen. With a relatively low toxicity profile and much available information on its drug–drug interactions and on pharmacokinetics, nelfinavir is ready for clinical testing in HER2 breast cancer patients. In conclusion, the discovery of HER2 selective inhibition of breast cancer cells by nelfinavir and the elucidation of its unique mode of action through binding to a new site on HSP90 have important implications in the development of nelfinavir and its analogs as new anticancer agents.

References


**Funding**
National Cancer Institute (CA122814); Flight Attendant Medical Research Institute (FAMRI); the Commonwealth Foundation (to JOL); the National Institutes of Health (NIH) (R01AI065983 to RR); National Center for Research Resources, a component of the NIH and NIH Roadmap for Medical Research (UL1 RR 025005 to Johns Hopkins School of Medicine).

**Notes**
We are grateful to Dr Bert Vogelstein for kindly providing the breast cancer cell lines. We thank Dr Solomon Snyder for providing HSP90α construct. The authors are solely responsible for the design of the study, the analysis and interpretation of the data, the writing of the manuscript, and the decision to submit the manuscript for publication.

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