Pharmacological Profiling of Kinase Dependency in Cell Lines across Triple-Negative Breast Cancer Subtypes

Lauren S. Fink¹, Alexander Beatty¹, Karthik Devarajan², Suraj Peri², and Jeffrey R. Peterson¹

Abstract

Triple-negative breast cancers (TNBC), negative for estrogen receptor, progesterone receptor, and ERBB2 amplification, are resistant to standard targeted therapies and exhibit a poor prognosis. Furthermore, they are highly heterogeneous with respect to genomic alterations, and common therapeutic targets are lacking. Therefore, the development of novel therapeutic strategies based on gene expression and proposed to predict sensitivity to a variety of therapeutic agents including kinase inhibitors. To test this hypothesis, we screened a large collection of well-characterized small molecule kinase inhibitors for growth inhibition in a panel of TNBC cell lines representing all six subtypes. Sensitivity to kinase inhibition correlated poorly with TNBC subtype. Instead, unsupervised clustering segregated TNBC cell lines according to clinically relevant features including dependence on epidermal growth factor signaling and mutation of the PTEN tumor suppressor. We further report the discovery of kinase inhibitors with selective toxicity to these groups. Overall, however, TNBC cell lines exhibited diverse sensitivity to kinase inhibition consistent with the lack of common driver mutations in this disease. Although our findings support specific kinase dependencies in subsets of TNBC, they are not associated with gene expression-based subtypes. Instead, we find that mutation status can be an effective predictor of sensitivity to inhibition of particular kinase pathways for subsets of TNBC.

Introduction

Triple-negative breast cancers (TNBC) disproportionately affect younger women and African American women and exhibit a particularly aggressive phenotype. This disease remains a major therapeutic challenge due to its resistance to antihormonal and anti–ERBB2-targeted therapies, both of which are effective in treating breast cancers that overexpress key cellular receptors (1, 2). Although patients with TNBC respond well to chemotherapy initially, the frequency of relapse is high compared with other breast tumors (3), underscoring the need for the development of targeted therapies. However, studies have failed to identify tractable genomic alterations that consistently occur in a large fraction of TNBC (4–7), and consequently the molecular alterations that drive TNBC are still largely unclear. Functional viability screens represent an alternative approach to identify drivers of this disease by systematically assessing the consequences of eliminating expression of potential protein drivers (8, 9) or their catalytic activity (10) on cell viability.

Protein kinases represent attractive therapeutic targets due to their broad participation in pathways driving cell proliferation and survival and their readily targeted ATP-binding pocket. Dysregulated EGFR, PI3K pathway, and mTOR signaling have been associated with TNBC and represent potential therapeutic targets (5, 11–15), although the results of clinical trials using agents targeting these pathways have been disappointing (16, 17). In addition, the protein tyrosine phosphatase PTPN12 was recently identified as a tumor suppressor in TNBC, whereas another phosphatase, UBASH3B, is overexpressed in TNBC (18, 19). These findings suggest that kinase signaling may represent a therapeutic opportunity in TNBC. Furthermore, given the heterogeneity of TNBC, it would be valuable to identify predictors of kinase inhibitor sensitivity to increase therapeutic success.

Recent studies have aimed to identify subsets of breast cancer, including within TNBC, based on gene expression profiles or responses to gene knockdown (9, 20, 21). There has also been considerable focus on identifying predictive biomarkers for TNBC therapeutic response on a large scale (9, 10, 20, 21). Importantly, six subtypes of TNBC have recently been defined based on gene expression signatures, and these include two basal-like groups, mesenchymal-like, mesenchymal stem–like, immunomodulatory, and luminal androgen receptor types (20). The predictive value of these TNBC subtypes is still unclear, and additional studies to examine the key genetic events driving TNBC would be useful in understanding the molecular diversity of TNBC and predicting therapeutic benefit (22, 23).

In this study, we used systematic pharmacologic inhibition of kinases using a highly characterized collection of kinase inhibitors in a panel of 12 TNBC cell lines to characterize kinase dependencies in TNBC. Importantly, the TNBC cell lines we screened included representatives of each of the six subtypes of TNBC.
recently defined by gene expression, and we sought to determine if these subtypes predicted response to our kinase inhibitor panel. We found that TNBC subtypes based on gene expression were poor predictors of response to our inhibitor panel, implying that gene expression alone may be insufficient to predict response to kinase-targeted therapies in TNBC cell line models. Instead, we identified subgroups of cell lines with similar responses to EGFR and PI3K pathway inhibitors, and demonstrated that PI3K inhibitor sensitivity can be predicted by PTEN mutation status in TNBC.

Materials and Methods

Cell lines

BT20, BT549, HCC38, HCC70, HCC1143, HCC1187, HCC1806, Hs578T, and MDA-MB231 human TNBC cell lines were obtained from the ATCC. CAL148 and MFM223 TNBC cell lines were obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures. MDA-MB468 cells were obtained from the ATCC as part of the NCI-60 panel via the Cell Culture Facility at Fox Chase Cancer Center. All cell lines were purchased from suppliers that routinely authenticate cell lines using short tandem repeat profiling. The MDA-MB468 cells have not been authenticated by our group since their purchase from the ATCC in 2003. All cell lines were cultured according to the supplier’s recommendations. All cell lines were amplified and frozen at low passage number within two months of receipt, and all experiments were performed within 20 passages of thawing. For cell viability assays, between 200 and 3,000 low-passage cells were plated per well in 384-well microplates using the Matrix Wellmate automated plate filler (Thermo Scientific). Optimal cell plating density was determined before screening for each cell line to allow for at least two population doublings and to avoid confluence by the end of the one-week assay period (Supplementary Table S1).

Kinase inhibitors

A kinase inhibitor library containing 160 well-characterized kinase inhibitors was purchased from EMD Millipore. Neratinib, afatinib, PD153035, and GS2126458 were purchased from Selleck Chem. RAD001 and GDC0980 were provided by Joseph Testa (Fox Chase Cancer Center), and BEZ235 was supplied by Timothy Yen (Fox Chase Cancer Center). The following compounds were purchased from LC Labs: bosutinib isomer, dasatinib, dovitinib, erlotinib, gefitinib, imatinib, lapatinib, masitinib, muttinib, nilotinib, pazopanib, roscovitine, sorafenib, sunitinib, tadalafil, tofacitinib, tozasertib, vandetanib, vatalanib, and VX702. All compounds were solubilized and stored at −80°C in 100% dimethyl sulfoxide (DMSO).

Kinase inhibitor screen

Twenty-four hours after cell seeding in 384-well plates, cells were treated with DMSO or one of eight doses of kinase inhibitors (final concentration range, 64 pmol/L–5 μmol/L). Inhibitors were added by pin transfer, and the final concentration of DMSO was <0.3% for all inhibitor concentrations. This DMSO concentration was determined to have minimal effect on cell viability in each cell line. Cell viability was assessed one week later using the CellTiter Glo luminescent cell viability assay (Promega). Screening was performed in duplicate on separate days. Duplicate dose-response data were averaged and fit using a sigmoidal dose-response curve in GraphPad Prism 6.0 to generate half maximal effective concentration values ($EC_{50}$).

Statistical methods

Heat map. A reordered heat map of $EC_{50}$ values for all inhibitor-cell line pairs was obtained using two-way hierarchical clustering based on Manhattan distance and complete linkage. No scaling was applied to these data. These choices of distance metric and linkage method are robust to outlying observations and identify compact clusters of cell lines associated with subgroups of kinase inhibitors based on their $EC_{50}$ values. In addition, this approach effectively handles the presence of a large number of observations in the dataset with an $EC_{50}$ value of 20 μmol/L. For the identification of group-selective inhibitors, $EC_{50}$ values across the three groups identified by hierarchical clustering were compared using the Kruskal–Wallis test to identify inhibitors where $EC_{50}$ values were significantly lower in a particular group compared with the other groups.

Identification of potential cell line–specific kinase drivers. Each kinase inhibitor in the panel was first classified as either toxic ($EC_{50} < 5$ μmol/L) or nontoxic ($EC_{50} ≥ 5$ μmol/L) to a given cell line. Next, we used published in vitro specificity data for these kinase inhibitors against 300 kinases (kir.fccc.edu and ref. 24) to binarize the target spectrum of each inhibitor such that each of the 300 kinases was classified as either “targeted” (inhibited by > 50% in vitro) or “nontargeted” (inhibited by ≤ 50% in vitro) by a given inhibitor. Frequency data for each kinase across all inhibitors were summarized in the form of a $2 \times 2$ contingency table, and a one-sided Fisher exact test of association between compound toxicity and kinase targeting was performed.

Additional statistical methods. All other tests were two-sided and used a Type I Error of 5%. Because of the exploratory nature of this study, whose primary goal is to generate new hypotheses, no correction for multiple hypothesis testing was performed. All computations were performed in the R statistical language and environment (25).

For statistical analysis of gene mutation frequency, data were derived from the Cancer Cell Line Encyclopedia and the COSMIC database (10, 26), and the Fisher exact test was used to associate the presence of mutations with group membership.

Results

TNBC molecular subtype is a poor predictor of kinase inhibitor sensitivity

To gain insight into kinases important for growth and survival of TNBC, we screened a well-characterized library of 180 known kinase inhibitors (24) for growth inhibition of a panel of 12 TNBC cell lines, selected to represent each of the recently defined gene expression subtypes (molecular subtypes) of TNBC (20). Importantly, this inhibitor set has been shown to target the majority of human protein kinases (24) and includes FDA-approved drugs as well as commercially available research compounds. We treated cells with 8 doses of each kinase inhibitor (64 pmol/L–5 μmol/L) for one week and monitored cell viability, relative to solvent-treated control wells, using the CellTiter Glo assay to measure ATP released from cell lysates. The one-week treatment time was chosen to increase the sensitivity of the viability assay for slower-growing cell lines. Viability data were fit to a sigmoidal
dose–response curve, and EC50 values were determined for each inhibitor–cell line pair (Fig. 1). Seven inhibitors had no effect on any of the tested cell lines, and these inhibitors were excluded from further analysis. The complete dataset is presented in Supplementary Table S1.

Overall, we observed significant heterogeneity in the effect of the compounds across the cell line panel (Fig. 2), consistent with the genomic heterogeneity observed in TNBC (5, 20). For example, some TNBC cell lines, including MDA-MB468, HCC1806, CAL148, and BT20, were highly sensitive to kinase inhibition, exhibiting median EC50 values in the low micromolar range (black bars in Fig. 2). Other cell lines, particularly HCC38, HCC1143, Hs578T, MDA-MB231, and MFM223, were strikingly resistant to the majority of kinase inhibitors tested. Notably, general sensitivity to kinase inhibition did not strictly correlate with TNBC molecular subtype (Fig. 2).

Sensitivity of TNBC cell lines to individual compounds was also highly variable, suggesting that individual cell lines may exhibit diverse kinase dependencies (Supplementary Table S1). We used two-way, unsupervised hierarchical clustering to group TNBC cell lines with similar responses to the kinase inhibitors. Likewise, kinase inhibitors were clustered based on similarities in their toxicity profiles (Fig. 3; Supplementary Fig. S1). This analysis revealed three major subgroups within the cell line panel (Groups 1, 2, and 3), representing cell lines with most similar responses to the set of kinase inhibitors tested (Fig. 3). As a singlet cell line, the CAL148 cell line was included in Group 1, given its proximity to Group 1 cell lines after clustering, to increase the number of representatives of this group. As discussed below, though, CAL148 cells exhibit features that distinguish them from other Group 1 cell lines. Strikingly, cell lines of the same molecular subtype did not generally cocluster, suggesting that these subtypes do not strongly predict inhibitor sensitivity. To test this rigorously and quantitatively, we used the adjusted Rand index (ARI) and normalized mutual information (NMI) indices to assess whether there is an association between molecular subtype and the three groups determined by kinase inhibitor profiling (27, 28). The majority of TNBC cell lines in our panel fall into one of three major gene expression–based subtypes: basal-like (BL), mesenchymal-like (ML), and luminal androgen receptor (LAR; ref. 20). Two additional subtypes with only one representative cell line each (HCC1187, immunomodulatory subtype, and BT20, unclassified) were excluded from the analysis. Next, we calculated the ARI and NMI for the association between BL, ML, and LAR.
subtypes and Groups 1, 2, and 3 as \(-0.079\) and \(0.22\), respectively, indicating poor correlations between the two groupings. A test of significance of the null hypothesis that there is no association between the classes (ARI or NMI = 0) against the one-sided alternative was performed using permutation testing. The realized \(P\) values based on 1,000 random permutations were 0.84 (ARI) and 0.61 (NMI), indicating that there is no evidence for association between BL, ML, and LAR subtypes and Groups 1, 2, and 3. Consistent with this finding, we found no kinase inhibitors with statistically significant toxicity specifically against cells of any one molecular subtype (not shown). Thus, our results indicate that molecular subtype is not a strong predictor of kinase inhibitor sensitivity.

Analysis of the clustering of kinase inhibitors revealed the presence of two major subgroups. Strikingly, the lower inhibitor cluster in Fig. 3 included a disproportionate number of compounds that potently inhibited growth of all cell lines. We hypothesized that these highly toxic compounds might include compounds that inhibit a larger number of kinase targets (more promiscuous inhibitors). Indeed, we found that the mean Gini coefficients, a measure of inhibitor selectivity ranging from 0 (promiscuous) to 1 (perfectly selective), of the two clusters were modestly different but showed strong statistical significance (Supplementary Fig. S2; 0.67 vs. 0.60 for inhibitor clusters 1 and 2, respectively; \(P = 2.293 \times 10^{-5}\); refs. 24, 29).

**Identification of TNBC subgroups with similar kinase dependency**

We next examined our data to identify kinase inhibitors that exhibited group-selective toxicity. We performed a Kruskal–Wallis comparison of the mean \(EC_{50}\) values for each inhibitor across Groups 1, 2, and 3. Supplementary Table S2 presents the ranked list of compounds according to \(P\) value. We observed that several of the compounds most selectively toxic for Group 1 cell lines were reported previously to inhibit EGFR catalytic activity (24). Consistent with this finding, the BT20 Group 1 cell line has been previously shown to have increased EGFR expression and activity and has increased sensitivity to genotoxic drugs following pretreatment with EGFR inhibitors (14, 30, 31). To validate dependence on EGFR catalytic activity for the growth of Group 1 cells, we tested three additional EGFR inhibitors (afatinib, neratinib, and PD153035) in cell viability assays in each cell line. We calculated the mean \(EC_{50}\) for the 20 EGFR inhibitors from our screening set and afatinib, neratinib, and PD153035 in each cell line (Fig. 4A; complete dataset in Supplementary Table S3). Consistent with EGFR dependence, Group 1 cell lines BT20 and HCC1806 were 7- to 15-fold more sensitive to treatment with...
EGFR inhibitors compared with cell lines of Groups 2 and 3. Not surprisingly, CAL148 cells, which segregate from other Group 1 cell lines (Fig. 3), did not show increased sensitivity to EGFR inhibitors. Figure 4B presents examples of the most selective compounds for Group 1, with additional compounds significantly toxic to Group 1 shown in Supplementary Fig. S3. In addition to inhibitors of EGFR (PD174265 and EGFR/ErbB2/ErbB4 inhibitor), these include inhibitors of Rho kinase and the CDK2 cell-cycle kinase. Notably, an additional inhibitor of Rho kinase, Y-27632, was also more toxic to Group 1, although this selectivity did not reach statistical significance (Supplementary Table S2). These data suggest that Rho kinase might also promote the growth of Group 1 cell lines.

Examples of kinase inhibitors preferentially toxic for Group 2 are shown in Fig. 5A. These include AGL2043, an inhibitor of PDGFR and other type III receptor tyrosine kinases and SU9516, a cyclin-dependent kinase inhibitor. Intriguingly, all three Group 2 cell lines were previously shown to be highly sensitive to siRNA-mediated depletion of the CDK2/5/6 partner cyclin D1 (32). In addition, the Aurora kinase inhibitors tozasertib and an additional Aurora kinase/CDK inhibitor were selectively toxic to Group 2 cells. Other compounds selectively toxic to Group 2 include the VEGFR inhibitor vatalanib, in clinical development, and an inhibitor of the breakpoint cluster region-Ab1 kinase (BCR-ABL) fusion protein.

No kinase inhibitors tested were selectively toxic to Group 3 cell lines, consistent with their general resistance to the kinase inhibitor panel (Fig. 3). To identify potential kinase drivers of individual cell lines in Group 3, we performed a statistical test for association between compound toxicity and the inhibition of particular kinases. This approach takes advantage of previous comprehensive target characterization of the kinase inhibitors in our library (24). Inhibitors were classified as either toxic (EC_{50} < 5 \mu M) or nontoxic (EC_{50} > 5 \mu M) for a cell line, and kinases were classified as targeted by an inhibitor if they were inhibited by > 50% in the in vitro analysis. Fisher exact tests were then used on a kinase by kinase basis to assess whether cellular toxicity was associated with inhibition of that particular kinase. The analysis produced numerous significantly enriched kinase targets for each of the Group 3 cell lines (Supplementary Table S4). Among the kinases most significantly associated with cellular toxicity for any member of Group 3 (P < 1 \times 10^{-3}) were the EGFR and CDK2 in MFM223 cells. This finding is consistent with data showing FGFR2 gene amplification and a dependence on FGFR2 activity for survival in this cell line (33, 34). Although analysis of existing gene expression data failed to generally associate kinase expression with sensitivity to inhibitors of those kinases (data not shown), increased expression of FGFR2 in MFM223 cells is consistent with FGFR dependence in this cell line. We also identified CDK6 and CDK1 as putative kinase drivers in HCC38 cells, consistent with previous evidence of their sensitivity to CDK4/6 inhibition (32). In addition, inhibition of the tyrosine kinase FES/feline sarcoma oncogene (FPS) was identified as highly significantly associated with toxicity to HCC1143 cells. FES/FPS has been previously associated with tumor growth and metastasis in breast cancer (35). These and the other significantly associated kinases identified from our statistical analysis warrant further investigation to examine their roles in growth and survival of these TNBC cell lines.

Our findings demonstrate that Group 1 TNBC cell lines exhibit a dependence on EGFR for growth or survival, consistent with a plethora of data supporting a role for this receptor in a subset of TNBC (14, 15, 31). In addition to EGFR, we have shown that inhibitors of Rho kinase and CDK2 are preferentially toxic to Group 1 cell lines. We have also identified kinase inhibitors selectively toxic to Group 2 cell lines, including those targeting CDKs, PDGFR, VEGFR, and Aurora kinase, all of which have been previously associated with TNBC (32, 36–38). Finally, we have identified a group of TNBC cell lines (Group 3) that appear generally resistant to kinase inhibition, and while as a group they do not appear to show a common dependence on a particular kinase pathway, we have identified potential dependencies for individual cell lines.

Features of TNBC cell lines predict kinase inhibitor response

To gain insight into the molecular features of TNBC cell lines that drive group membership, we examined whether any mutations are overrepresented in any particular group. The mutation status of 623 genes was determined for each cell line using data from the COSMIC database and the Cancer Cell Line Encyclopedia (10, 26), and the Fisher exact test was used to determine whether any particular gene was disproportionately mutated in any of the three groups. Mutations in the lipid phosphatase and
tumor suppressor PTEN were significantly associated with Group 2 cell lines (\( P = 0.01 \)), but no mutations reached significance in Groups 1 or 3 (\( P < 0.05 \)). Indeed, all three Group 2 cell lines, BT549, HCC70, and MDA-MB468, have PTEN mutations that render the cells null for PTEN protein expression (39, 40).

PTEN opposes the activity of PI3 kinases (PI3K), suggesting that elevated PI3K pathway activity may contribute to the growth and survival of Group 2 TNBC cells. To test this hypothesis, we calculated the mean EC\(_{50}\) value for inhibitors of PI3K pathway kinases for all cell lines, including 18 inhibitors from our screening panel (Supplementary Table S5). These inhibitors include compounds that target PI3K directly as well as the downstream kinases Akt and mTOR. Generally, we found increased sensitivity of Group 2 cell lines to inhibition of PI3K signaling relative to cell lines in Groups 1 and 3 (Fig. 5B). An additional PTEN null cell line, CAL148 from Group 1, was also highly sensitive overall to PI3K pathway inhibition. This observation is consistent with the role of PTEN loss inactivation of the PI3K pathway and subsequent sensitivity to agents targeting this pathway. The BT20 Group 1 cell line was also quite sensitive to PI3K pathway inhibition, likely due to the presence of the H1047R-activating mutation in PI3K (41).

Despite sensitivity to PI3K pathway inhibitors, CAL148 and BT20 cells did not cluster with Group 2, perhaps because this dependence is overwhelmed by a general sensitivity to the majority of compounds tested (Fig. 2). Overall, these results show that PTEN mutations predict sensitivity to PI3K pathway inhibitors in TNBC cell line models and highlight the relevance of performing functional assays in TNBC cell lines in demonstrating kinase inhibitor sensitivity.

**Discussion**

Both the aggressiveness of TNBC and the lack of therapeutic success with targeted agents have prompted studies to better characterize the disease and to predict which patients will benefit from which therapies (9, 20, 21). Here, we have sought to further characterize the relevance of TNBC molecular subtypes defined by gene expression and their value for predicting sensitivity to kinase inhibition. We found that cell lines from the same molecular subtype were differentially sensitive to kinase inhibitors in our panel (Fig. 3). We also found that no kinase inhibitors showed toxicity selectively to a particular molecular subtype (data not shown). These findings suggest that TNBC molecular subtypes may not be strong predictors of response to kinase inhibition. This finding can be understood based on the diverse potential mechanisms for dysregulation of kinase signaling other than alterations in expression at the mRNA level, including mutation or alteration in upstream regulators. Indeed, we found that although molecular subtypes did not strongly predict kinase inhibitor sensitivity, PTEN mutation status correlated well with sensitivity to inhibitors of the PI3K pathway (Fig. 5B). Thus, gene expression signatures on their own are unlikely to be generally effective predictors of kinase inhibitor sensitivity and highlight the advantages of functional profiling to identify kinases critical for TNBC growth.

Functional pharmacologic profiling, though a powerful approach for characterizing cultured cell lines, is poorly suited to guiding personalized therapies for patient tumors. Consequently, clinically relevant biomarkers such as gene mutation or overexpression that predict kinase inhibitor sensitivity must first be identified in cell line panels and subsequently validated through clinical trials. Because our data and others’ suggest that TNBC may exhibit a broad and heterogeneous dependence on diverse kinase pathways, functional profiling of very large and diverse arrays of cell lines will likely be required to identify dependencies on specific individual kinases that may be present in only a small proportion of patients with TNBC. Nevertheless, even the modestly sized cell line panel examined here revealed expected dependencies on EGFR and PI3K pathways in a subset of TNBCs as well as additional subtype-selective agents (Figs. 4 and 5).

Although we found that kinase inhibitor sensitivity was not generally well predicted by molecular subtype, sensitivity to other anticancer agents has been linked to molecular subtype both in cell lines and clinically (20, 42, 43). For example, chemotherapeutic agents, such as cisplatin, taxanes, and anthracyclines, are more toxic to basal-like TNBCs, whereas the androgen receptor antagonist bicalutamide is selectively toxic to cell lines of the LAR subtype (20, 43). Interestingly, the SRC/ABL kinase inhibitor dasatinib was previously reported to exhibit preferential toxicity for mesenchymal-like TNBC cell lines (20). Although it did not
reach statistical significance, our mesenchymal-like cell lines, BT549, Hs578T, and MDA-MB231, were more sensitive to dasatinib than cells of other TNBC subtypes (P = 0.12).

Our unsupervised hierarchical clustering of cell lines based on their similarity of kinase inhibitor sensitivity identified three major groups of TNBC cell lines. We identified a significant feature of Group 1 as sensitivity to EGFR inhibition, and all EGFR inhibitors that we tested were toxic to BT20 and HCC1806 Group 1 cell lines, pointing to a subset of TNBC that is highly dependent on this kinase for growth and/or survival. The CAL148 cell line, included in Group 1 to increase the number of cell line representatives for this group, was not more sensitive to EGFR inhibitors, and we suggest that CAL148 cells rely more on alternate kinases for survival. We observed that CAL148 cells were the most sensitive to kinase inhibitors overall (Figs. 2 and 3), suggesting the possibility that the specific kinases critical to the growth of CAL148 cells are overshadowed by a general sensitivity to kinase inhibitors (Figs. 2 and 3).

In non–small cell lung cancer, increased efficacy of EGFR inhibitors can be seen in patients with high EGFR copy number, increased EGFR expression, or mutated EGFR (44, 45). Although EGFR mutations in TNBC are rare, EGFR overexpression or amplification has been reported in some TNBCs and basal breast cancers, making it an attractive therapeutic target for TNBC (12, 46–49). Our results suggest that EGFR expression or amplification, as seen in BT20 and HCC1806 cells (30, 49), is associated with response to small molecule inhibitors of EGFR (Fig. 4A). Interestingly, though, the MDA-MB468 cell line also expresses high levels of EGFR (49) but is not highly sensitive to EGFR inhibitors (Fig. 4A). This suggests that for some high EGFR-expressing TNBCs, additional features may attenuate sensitivity to EGFR inhibition and is consistent with low patient response rate of anti-EGFR antibody cetuximab treatment in TNBC (17). Thus, a more complete understanding of the role of the EGFR pathway and its response to EGFR inhibitors in TNBC is needed to improve the prognostic value of EGFR overexpression for response to EGFR-targeted therapies.

A second group (Group 2) of TNBC cells showed increased sensitivity to PI3K pathway inhibition associated with mutations in the tumor suppressor PTEN. In fact, three of four PTEN-mutant cell lines in our TNBC panel clustered in Group 2. PI3K pathway activation, either through mutational activation of PIK3CA or loss of the negative regulator PTEN, has been associated with TNBC, and our results confirm that a subset of TNBCs is dependent on PI3K signaling for growth (5, 10, 26, 50, 51). Interestingly, CAL148 and BT20 cells, which are both highly sensitive to PI3K inhibitors (Fig. 5B), have documented activating PIK3CA mutations (H1047R; refs. 10, 26). Our results suggest that routine sequencing for PTEN and PIK3CA mutations in patients with TNBC would predict sensitivity to PI3K pathway inhibitors, and, consistent with this, recent evidence demonstrates increased efficacy of a highly selective Akt inhibitor in PTEN-mutant cancer cell lines grown in culture and as xenografts (52).

Interestingly, we found no inhibitors significantly more toxic to Group 3 cell lines than to the other groups, and no mutations that we tested were enriched in Group 3. Group 3 cell lines appear overall to be more resistant to the kinase inhibitor panel, as evidenced by higher EC50 values generally (median EC50 values: 20 μmol/L for Group 3; 15 μmol/L for Group 2; and 5.33 μmol/L for Group 1; Figs. 2 and 3). It has previously been shown that kinase reprogramming occurs following treatment of TNBC cells with MEK inhibitors, suggesting plasticity of the kinome in response to single-agent kinase inhibitors (53). The increased resistance of Group 3 cell lines might be due to a more inherent ability to rewire kinase signaling in response to kinase inhibition, and therefore combination therapies may be required to target the growth of these cell lines. Upon further analysis of potential kinase dependencies in individual Group 3 cell lines, we found that CDKs, FGFR, and FES/FPS, which have previously been associated with breast cancer (32–34, 36), were most significantly associated with toxicity to specific cell lines of Group 3 (Supplementary Table S4). More broadly, the statistical method we have used to identify these associations provides an unbiased and widely applicable approach to identify candidate driver kinases from pharmacologic profiling data in cell lines using compound libraries with defined target spectrums.

There have been several recent efforts focused on large-scale pharmacologic studies of cancer cell lines though none have focused exclusively on TNBCs (10, 21, 54). The heterogeneity of this disease and the lack of predominant driver mutations suggest that the success of this approach for TNBC will depend on large and diverse, well-characterized cell line panels with adequate annotation of mutation status and gene expression. Indeed, our analysis indicates that sensitivity to existing kinase inhibitors can be predicted based on clinically obtainable markers. Functional profiling of the diversity of TNBC remains a promising approach to defining these actionable kinase dependencies. Although RNAi-mediated kinase inactivation is a successful alternative approach (9), pharmacologic kinase inactivation provides a simpler and less expensive approach better suited to the large cell line panels required to capture the diversity of TNBC.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: L.S. Fink, J.R. Peterson
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