Molecular Target Class Is Predictive of in vitro Response Profile

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Abstract

Preclinical cellular response profiling of tumor models has become a cornerstone in the development of novel cancer therapeutics. As efforts to predict clinical efficacy using cohorts of in vitro tumor models have been successful, expansive panels of tumor-derived cell lines can recapitulate an “all comers” efficacy trial, thereby identifying which tumors are most likely to benefit from treatment. The response profile of a therapy is most often studied in isolation; however, drug treatment effect patterns in tumor models across a diverse panel of compounds can help determine the value of unique molecular target classes in specific tumor cohorts. To this end, a panel of 19 compounds was evaluated against a diverse group of cancer cell lines (n = 311). The primary oncogenic targets were a key determinant of concentration-dependent proliferation response, as a total of five of six, four of four, and five of five phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway, insulin-like growth factor-I receptor (IGF-IR), and mitotic inhibitors, respectively, clustered with others of that common target class. In addition, molecular target class was correlated with increased responsiveness in certain histologies.

Introduction

The expanding development and use of targeted therapies for cancer treatment reflects an increasing understanding of key oncogenic pathways and how the targeted perturbation of these pathways corresponds to clinical response. Difficulties in predicting efficacy to targeted therapies is likely a consequence of the limited global knowledge of causal mechanisms for pathway deregulation (e.g., activating mutations and amplifications). Preclinical translational research studies for oncology therapies focus on determining what tumor type and genotypes are most likely to benefit from treatment. Treating selected patient populations may help maximize the potential of a therapy. The successful transition of preclinical tumor response profiles (i.e., those tumor cells having maximal efficacy) to the subsequent clinical development is now well established. Numerous specific molecular markers are now used to identify patients most likely to benefit in a clinical setting. For example, in vitro, imatinib selectively kills cells with the activated BCR-ABL gene fusion (1), whereas lapatinib preferentially inhibits proliferation of ERBB2-overexpressing cells (2). Both have achieved commercial success, benefiting patients with tumors harboring these genetic aberrations. Although inhibition of activated oncogenic pathways (normally via mutation/amplification) is thought to be the predominant mechanism of action for most novel cancer therapies, the diversity of compound selectivity for different targets obscures the logical association between compound target activity and efficacy in cancer cells. For example, the in vitro assessment of kinase inhibitory profiles of 38 unique kinase inhibitors revealed great diversity in selectivity even for those in the same molecular target “class” (3). The consequences of a single molecule inhibiting multiple kinases are complex and poorly understood in the context of clinical response. Further, the inhibition of multiple kinases not associated with the primary therapeutic target may reveal unexpected relationships in cellular response patterns between tumors. This is likely due to the existence of many more contributing cancer-promoting pathways than previously thought—the result of the unique genomic profile of each tumor. For example, comprehensive
scans for somatic mutations in breast and colon cancers indicate that each tumor harbors an average of 90 nucleotide changes, ~10% of which are predicted to be functionally relevant (4). All functionally significant somatic aberrations likely influence therapeutic response to some degree.

Although the specific "signature" of genetic lesions in each tumor likely results in a unique but predictable cellular response profile to therapeutics, to date, few have investigated patterns of cancer cell response between different therapeutics. There is some indication that predictable patterns may exist. Response of the JFCR39 tumor cell line panel to a diverse set of 130 chemicals found that a majority of compounds segregate by shared mechanisms of action (5). Early analyses of the NCI-60 tumor cell line set identified correlated patterns of proliferation response between topoisomerase inhibitors and doxorubicin (6). Identifying negative relationships is equally as important for implementing pharmacogenomics in cancer treatment. Specific breast tumors responding to 5-fluorouracil are resistant to Adriamycin and docetaxel (7). High-throughput screening of a set of tumor-derived cell lines of diverse origin is a system for studying relationships in cellular efficacy among a set of cancer therapeutics. Here, we present a meta-analysis of tumor cell line responsiveness to several general classes of targets, including (a) signal transduction pathway inhibitors, (b) mitotic inhibitors, and (c) antiangiogenics (Supplementary Table S1). When taken as a whole, response profiles are highly predictive of compound therapeutic target class. In the case of inhibitors of signaling pathways, response profiles correspond to the oncogenic cascade that it targets. Additionally, this study addresses how the relative specificity of individual therapies (e.g., a lack of specificity in kinase inhibition) affects response profiles. Finally, both individual tumor types and specific genotypes (e.g., those harboring specific mutations) seem to contribute to the degree of responsiveness to compounds of different therapeutic target class.

Materials and Methods

Human cancer cell line screening panel

A total of 311 unique cancer cell lines were purchased from several vendors (American Type Culture Collection; Developmental Therapeutics Program, National Cancer Institute; German Resource Centre for Biological Material; and European Collection of Animal Cell Cultures) and grown to standard culture media recommended by the vendor (Supplementary Table S2). Typically, this included RPMI 1640 supplemented with a final concentration of 10% fetal bovine serum, 2 mmol/L glutamax, and 1 mmol/L sodium pyruvate. The cell line panel was compiled to effectively represent a diverse set of 130 chemicals found that a majority of compounds segregate by shared mechanisms of action (5). Early analyses of the NCI-60 tumor cell line set identified correlated patterns of proliferation response between topoisomerase inhibitors and doxorubicin (6). Identifying negative relationships is equally as important for implementing pharmacogenomics in cancer treatment. Specific breast tumors responding to 5-fluorouracil are resistant to Adriamycin and docetaxel (7). High-throughput screening of a set of tumor-derived cell lines of diverse origin is a system for studying relationships in cellular efficacy among a set of cancer therapeutics. Here, we present a meta-analysis of tumor cell line responsiveness to several general classes of targets, including (a) signal transduction pathway inhibitors, (b) mitotic inhibitors, and (c) antiangiogenics (Supplementary Table S1). When taken as a whole, response profiles are highly predictive of compound therapeutic target class. In the case of inhibitors of signaling pathways, response profiles correspond to the oncogenic cascade that it targets. Additionally, this study addresses how the relative specificity of individual therapies (e.g., a lack of specificity in kinase inhibition) affects response profiles. Finally, both individual tumor types and specific genotypes (e.g., those harboring specific mutations) seem to contribute to the degree of responsiveness to compounds of different therapeutic target class.

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the growth of control cells treated with DMSO at 72 hours. The gIC50 value serves as a metric for measuring the inhibition of proliferation in cancer cells. The curves for each seeding density for every cell line were manually inspected for both data quality and appropriateness of curve fitting. Where the starting data were poor, the curve and subsequent gIC50 values were excluded from further analysis. Cell line seedings were considered of poor quality when the r² value of the fitted curve was <0.75. Data of poor quality were removed from all subsequent analysis.

**DNA copy number analysis**

DNA copy number alterations were calculated for each cell line using the Affymetrix 500 K "SNP chip" (Affymetrix, Inc.). DNA was extracted from each line using Mini DNeasy kit (Qiagen, Inc.) and purified. Two aliquots (250 ng each) were digested with the restriction enzyme Nsp or Sty (New England Biolabs). Digested DNA was subsequently ligated to an adaptor and amplified by PCR using Platinum Pfx DNA Polymerase (Invitrogen), yielding a product of approximately 250 to 2,000 bp. For each enzyme digest, PCR was carried out in four 100 μL aliquots, pooled, purified, quantified, normalized to 40 μg/45 μL, and fragmented with DNease I to yield a size range of approximately 25 to 200 bp. The fragmented products were labeled, denatured, and hybridized to the Affymetrix 500 K chip. On completion of hybridization, each assay was washed and stained using Affymetrix fluidics stations. Image data were acquired using the GeneChip Scanner 3000. Genome-wide estimates of DNA copy number were calculated as described by Greshock and colleagues (9). A panel of lymphoblastic cell lines (n = 20) was used to calibrate diploid for all cancer cell lines. Additionally, to ensure the unique genetic identity of each cell line, SNP chip genotypes were calculated using the BRLMM algorithm (10) and compared between all cell lines. The genetic fingerprint (i.e., each genotype) was compared for every cell line to all other cell lines in the set. Each comparison resulted in a "percent identity" that represents the genetic similarity of each cell line, where the potential values range from 0% to 100%. Cell lines having >80% identity were considered of a similar genetic origin. Additionally, the genotype profiles of this cell line set were compared with that published by the Wellcome Trust Sanger Institute’s Cancer Genome Project (11). Those cell lines with similar genetic profiles are noted in Supplementary Table S2. Details about the genotype comparisons can be found in Supplementary Materials and Methods.

**Gene expression analysis**

Transcript abundance was quantified by using the Affymetrix U133 Plus2 GeneChips in triplicate. Briefly, cell lines were plated in triplicate and lysed in Trizol (Invitrogen Life Technologies Co.). Lysates were captured with chloroform.

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**Table 1. Response profiles of 19 compounds in a panel of 311 cell lines**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Primary target</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
<th>Minimum</th>
<th>Maximum</th>
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<tr>
<td>pazopanib</td>
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<td>263</td>
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<td>2,975</td>
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<td>6,751</td>
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<td>lapatinib</td>
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<td>3,261</td>
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<td>9,842</td>
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<td>49</td>
<td>2,911</td>
<td>0</td>
<td>5,000</td>
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<td>AURKB</td>
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<td>21</td>
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<td>359</td>
<td>1,537</td>
<td>26</td>
<td>32</td>
<td>56</td>
<td>6</td>
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<tr>
<td>paclitaxel</td>
<td>Tubulin</td>
<td>273</td>
<td>149</td>
<td>939</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>0</td>
<td>10,000</td>
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<td>PLK1</td>
<td>266</td>
<td>71</td>
<td>655</td>
<td>6</td>
<td>9</td>
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<td>GSK661637</td>
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<td>foretinib</td>
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<td>1,446</td>
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<td>512</td>
<td>832</td>
<td>1,296</td>
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<td>PI3K</td>
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<td>250</td>
<td>2,091</td>
<td>34</td>
<td>65</td>
<td>140</td>
<td>7</td>
<td>29,326</td>
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<td>9,317</td>
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<td>10,000</td>
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<td>AKT</td>
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<td>9,206</td>
<td>4,568</td>
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<td>14,663</td>
<td>638</td>
<td>14,663</td>
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<tr>
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<td>9,891</td>
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<td>751</td>
<td>5,586</td>
<td>2</td>
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</table>

NOTE: Response profiles for all compounds are represented by gIC50 values (nmol/L). Where cells were assayed at multiple plating densities, gIC50s were averaged. Clustering analysis was done with gIC50 scaled by the overall median for each compound to account for differences in potency. Q1 and Q3 represent the 25th and 75th percentiles, respectively, whereas n represents the total number of cell lines assayed for each compound.
and purified using RNeasy Mini kit (Qiagen). cDNA was prepared from 5 μg of total RNA using the SuperScript Double-Stranded cDNA Synthesis kit (Invitrogen) and amplified using the Enzo BioArray High-Yield RNA Transcript Labeling kit (Enzo Biochem, Inc.). Finally, the samples were fragmented and hybridized to the HG-U133 Plus2 GeneChips, stained, and scanned according to the manufacturer’s protocols. Transcript abundance was estimated by normalizing all probe signal intensities to a value of 150 using the mas5 algorithm in the Affymetrix Microarray Analysis Suite 5.0. Both gene expression and SNP chip data are publicly available (12).

Compound clustering analysis

For each compound, gIC50s were first normalized to the overall median for that compound and transformed to a log10 scale. This produced a scaled proliferation score that is a potency-independent means of comparing response profiles. All subsequent clustering analysis used Pearson’s distance as a metric and was based on the average distance between nodes [using code provided by de Hoon and colleagues (13)]. Tree plotting was done with the Phylodendron software package (14).

Results/Discussion

Although all compounds elicited some degree of growth inhibition in at least a subset of the cell line panel, response profiles across the compound set were diverse (Supplementary Table S2; summary gIC50 values in Table 1). For example, BEZ-235 showed relatively strong potency to induce proliferation inhibition, where 96% (188 of 196) of lines had gIC50s of <500 nmol/L. In contrast, few cell lines showed such response levels to pazopanib (9 of 263; 3%). Overall, the antimitotic compound group induced comparatively high growth inhibition among compound classes. In every case, the overall median (Q2; 50th percentile) was <150 nmol/L. In addition, the antimitotics exhibited response profiles distinct from the other compounds, where the highly responsive majority of cell lines strongly deviate from the few less responsive lines. For example, the mean gIC50 for all lines <Q3 (75th percentile) for the PLK1 inhibitor GSK461364 is 7.6 nmol/L, whereas those >Q3 was 258 nmol/L (Fig. 1A and C). By contrast, despite a dynamic range similar to GSK461364, the dual ERBB1/2 RTK inhibitor lapatinib has few responsive lines, where the mean gIC50 for those <Q1 was 1,888 nmol/L, and just 5.2% (13 of 267) had gIC50s of

Figure 1. Each of the 19 compounds had unique proliferation profiles. This is illustrated by plotting the gIC50 values in order of increasing resistance. A, the PLK1 inhibitor GSK461364 potently inhibits proliferation in a majority of cell lines (median gIC50 = 9 nmol/L). B, comparatively, the ERBB1/2 inhibitor lapatinib has few sensitive cell lines. C, a comparison of the PLK1 inhibitor GSK461364 and paclitaxel shows how the antimitotic profiles were highly correlated. The small number of highly resistant cell lines (defined as those >Q3) was common between this class of compounds. D, a quartile comparison for GSK461364 and paclitaxel shows how the antimitotic profiles were highly correlated. The small number of highly resistant cell lines (defined as those >Q3) was common between this class of compounds. D, a quartile comparison for GSK461364 and paclitaxel shows how the antimitotic profiles were highly correlated.
<150 nmol/L (Fig. 1B). This suggests that inhibiting mitosis is more uniformly effective in slowing cellular proliferation for *in vitro* tumor models. Conversely, relative to other classes, inhibitors of the PI3K/AKT/mTOR pathway showed more diverse potency, where Q3 ranged from <150 nmol/L (BEZ-235) to >10,000 nmol/L (perifosine). This profound disparity is likely the result of unique targeting strategies (i.e., where in the pathway) and differential potency between inhibitors of this pathway.

Compounds of similar target classes associate by the hierarchical clustering of their median-scaled proliferation inhibition scores (Fig. 2). In other words, specific compound target classes are predictive of which specific cell lines are most responsive to increasing drug concentrations. The overall degree of correlation between compounds of similar target classes is, in part, likely a manifestation of the variable levels of growth and survival dependence each cell line has on the activation of different oncogenic pathways. This is shown by the fact that compounds of a common molecular target class cluster together due to similar subsets of cell lines showing high levels of dose-dependent response. For example, all four inhibitors of IGF-IR cluster together due to strongly associated *in vitro* profiles [mean Pearson’s distance of 0.34 (± 0.10; n = 6) compared with an overall mean distance of 0.88 (± 0.19; n = 145); Fig. 2, cluster A]. In contrast, although four of five drugs targeting the PI3K/AKT/mTOR pathway do cluster together (Fig. 2, cluster B), their association is considerably weaker than that of IGF-IR inhibitors (mean distance = 0.63 ± 0.15; n = 10). This likely reflects the more diverse means of targeting this pathway by this set of compounds or that each of these targets (PI3K/AKT/mTOR) has slightly unique signaling outcomes. Selective inhibitors of AKT and mTOR target this pathway downstream of those targeting PI3K directly. The two pan-isoform PI3K inhibitors in this study, BEZ-235 and GSK1059615, have notable and direct inhibition of mTOR kinase, which makes the relationships of their response profiles with other pathway inhibitors more complex. The correlation between these inhibitors was not driven by structural similarity. For example, two more strongly correlated members of this panel of inhibitors, the AKT inhibitor GSK690693 and the PI3K inhibitor GSK1059615 (Pearson’s distance = 0.54), are structurally distinct (Supplementary Table S3). The mitosis-inhibiting compounds (PLK/AURK/CENPE/tubulin) also strongly correlate, where five of five members of cluster C (Fig. 2) are of this class. The mean Pearson’s distance for the pairwise comparison of these is 0.56 (± 0.11; n = 10). The specific mitotic targets of these compounds are largely unique, and their clustering is consistent with previous observations that the biological process targeted (mitosis in this case) can drive similarities in cellular response (15). The few cell lines whose proliferation is not affected by treatment with an antimitotic help define this compound cluster. For example, the high correlation of those lines >Q3 contributes to the correlation between GSK461364 and paclitaxel (Fig. 1C), a statistically significant relationship by χ² analysis when binning cell lines by quartile (γ = 0.34, P = 0.002; Fig. 1D; ref. 16). It is likely that the medium conditions conducive to high rates of cellular proliferation produce an artificially high response rate to those compounds targeting mitosis (17).

Clustering analysis also suggests logical relationships between the response profiles of the compounds not in one of these major classes. For example, the high correlation of those lines >Q3 contributes to the correlation between GSK461364 and paclitaxel (Fig. 1C), a statistically significant relationship by χ² analysis when binning cell lines by quartile (γ = 0.34, P = 0.002; Fig. 1D; ref. 16). It is likely that the medium conditions conducive to high rates of cellular proliferation produce an artificially high response rate to those compounds targeting mitosis (17).
cellular response to lapatinib (2), can activate PI3K signaling. Conversely, direct activation of PI3K via mutations to PIK3CA confers resistance to trastuzumab, another upstream inhibitor of ERBB2 (18). In other words, some, but not all, cell lines that respond to PI3K/AKT inhibition do not respond to upstream inhibition of the ERBB1/2 RTKs. The clustering of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK) inhibitor GSK1120212 remains separate from those compounds targeting the PI3K/AKT/mTOR pathway and lapatinib. This relationship is likely the result of the high importance of activated RAS/RAF in those cells that are more responsive to MEK inhibition (19).

The reclustering of the 19 compounds from the initial study with additional proliferation inhibition data from 12 compounds presented by McDermott and colleagues (8) further illustrates that therapeutic target selectivity is predictive of cell line response profiles (Fig. 3). For example, the node containing the ERBB1 inhibitor erlotinib and the ERBB1/2 inhibitor HKI-272 cluster with the other RTK inhibitor lapatinib (cluster D) in this histologically diverse panel of cell lines (n = 117). In addition, the lone additional mitotic inhibitor MK0457, which targets Aurora kinases (AURK), clusters with the five other mitotic inhibitors. Similarly, the c-MET inhibitor PHA665752 clusters with GSK1363089 (foretinib), whereas the RAF inhibitor AZ628 clusters with the MEK inhibitor GSK1120212. Although these results show that the primary target is the dominant driver of gross cell line response profiles, the potent inhibition of additional targets and degree of selectivity play a role in these relationships. Only two of four compounds known to potently inhibit vascular endothelial growth factor (VEGF) components (pazopanib, foretinib, sunitinib, and sorafenib) cluster together (Fig. 3, cluster E). Sunitinib, a potent inhibitor of VEGF receptor (VEGFR) and platelet-derived growth factor receptors (20), clusters with pazopanib. Sorafenib, a potent inhibitor of VEGFR, RAF, and numerous other kinases (21), does not cluster with other inhibitors of VEGFR. This is not surprising, as a direct comparison of these three inhibitors of tumor vasculature reveals that each potently inhibits a
unique set of kinases known to be involved in tumorigenesis and progression (22). It is likely that inhibiting these additional kinases drives proliferation response, as cell lines do not serve as faithful models of tumor vasculature in two-dimensional cell culture. In the case of sorafenib, the inhibition of kinases unique to this compound (compared with that of foretinib and sunitinib) is more critical in determining cellular response in vitro. In addition, although sunitinib and sorafenib show comparable benefit as second-line treatment of cytokine-refractory kidney cancers (23), it is not known if the same patients would benefit from each therapy.

Relationships between cluster groups also show rational patterns of cell line response that are not directly related to the target but rather the cascading of cellular signaling. For example, inhibitors of the RTKs ERBB1/2, in addition to the SRC inhibitor AZD0530 [also shown to selectively inhibit growth of epidermal growth factor receptor (EGFR)–activated cells (8)], cluster with inhibitors of PI3K/AKT/mTOR. Although it is established that the RTKs inhibited by these compounds (ERBB1/2) modulate PI3K/AKT/mTOR in numerous human cancers, the relationship between PI3K/AKT/mTOR activity and clinical response to RTK inhibition is complex and only partially understood. For instance, activating mutations of the PIK3CA gene confer resistance to EGFR inhibition (24) while correlating with sensitivity to compounds targeting PI3K directly (25).

The observed compound class clustering associations are consistent with previous pharmacogenomic studies, showing that cell line response to therapeutics is dependent on both genotype and cellular origin (8). When considering the response data for cells of breast, colon, lung, and hematopoietic origin in the original panel of 19 compounds (those tissue types represented by at least 15 cell lines), inhibitors of the PI3K/AKT/mTOR pathway are selectively active in breast cancer cells, whereas those compounds targeting IGF-IR more potently inhibit proliferation in colon cancer cells. This is shown by a direct comparison of gIC50 values of breast, colon, lung, and hematopoietic tumor cells (n > 15 cell lines per tumor type). Bars in this graph represent the average-scaled gIC50 value for each compound by tumor type. Among these four tumor types, breast cancer cells have the lowest median-scaled gIC50 values for four of five PI3K/AKT/mTOR inhibitors, whereas colon cancer cell had the lowest scores for all four IGF-IR inhibitors tested.
subtypes \((P = 0.0009)\). Notably, luminal cells preferentially respond to the AKT inhibitor GSK690693 and the PI3K inhibitor GSK1069615, whereas the MEK inhibitor GSK1120212 selectively inhibits proliferation in basal breast cancers. This result is not surprising because PI3K activation can mediate resistance to MEK inhibition\((31)\). Although, collectively, this suggests a strongly divergent clinical path for targeting these pathways in breast cancers, the coinhibition of MAPK and PI3K may be particularly efficacious in the basal subtype\((32)\). Mutations in \(\text{PIK3CA}\) and \(\text{PTEN}\) do not seem to associate with compound clusters \((P > 0.05)\), suggesting that these alterations are of secondary importance to ER and \(\text{ERBB2}\) in determining what cell lines are more responsive across this diverse panel of compounds. Similarly, when analyzing tumor types with frequent \(\text{KRAS}\) mutations (colon and pancreatic cancers), those cell lines harboring mutant \(\text{KRAS}\) tended to cluster together \((P = 0.14, \text{Fisher's exact test}; \text{Supplementary Fig. S1})\). This trend is even more profound when distinguishing those harboring \(\text{KRAS}\) mutations with wild-type \(\text{PIK3CA}\) \((P = 0.008, \text{Fisher's exact test})\). The unique

Figure 5. The clustering of median-scaled \(gIC_{50}\) values shows that cellular genotype is a strong driver of organization of 16 breast cancer cell lines (columns). Luminal breast cancer cells, most of which associate with ER overexpression, segregate from the basal breast cancer lines \((P = 0.0009, \text{Fisher's exact test})\). Green and red cells represent cell lines with lower and higher \(gIC_{50}\) values, respectively, whereas gray cells indicate missing data. The organization of compounds (rows) is similar to that when all tumor types are analyzed (e.g., inhibitors of PI3K/AKT and IGF-IR cluster together).
sensitivity profile of KRAS-mutated cell lines is an expected result, as mutations to this gene correlate with clinical response patterns, such as resistance to inhibitors of EGFR (33). The fact that not all KRAS-mutant tumors cluster together is also concordant with previous observations that mutations that co-occur with KRAS can drive cellular response to therapeutics, such as the association of cooperating LKB1/KRAS mutations with more exquisite response to MEK inhibition (34).

This study shows that although kinase selectivity of cancer therapeutics can vary greatly (3), the predominant targets of these therapies play a major role in determining inhibition of cell proliferation in a dose-dependent manner in vitro. The similarity in concentration-response profiles among multiple inhibitors targeting a common oncogenic pathway emphasizes how inhibition of that specific pathway is a key cellular response mechanism. In addition, the unique but consistent profiles from distinct therapeutic target classes (e.g., PI3K/AKT/mTOR versus IGF-IR) are suggestive of divergent clinical strategies for these compounds. Large panels of tumor-derived cell lines show response affinities for molecular target classes with specific tumor types (as was the cases with PI3K/AKT/mTOR and IGF-IR inhibitors for breast and colon cancer cells, respectively). Further, cellular response profiles among classes show genotype specificity, exemplified by the congregation of luminal/basal breast cancer cells across compounds of diverse target classes. These results also show that cell culture is a largely faithful model of primary tumors with respect to drug response, particularly for those compounds targeting signal transduction pathways. In contrast, hyperphysiologic growth conditions may hinder the accurate modeling of drug response of some compound target classes, such as those designed to inhibit mitosis. This notion may become increasingly important in the future of cancer drug development as target classes expand to include those selectively obstructing tumor metabolism because specific cell culture conditions can drastically affect metabolic activity (35). Collectively, the high-throughput screening of cancer therapeutics across a histologically and genetically varied cell line population can simulate clinical response profiles and identify both tumor types and genotypes that may benefit most from specific treatment regimens.

Disclosure of Potential Conflicts of Interest

All authors are GlaxoSmithKline stock owners.

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