Genome-scale signatures of gene interaction from compound screens predict clinical efficacy of targeted cancer therapies

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Summary
Identifying reliable drug response biomarkers is a significant challenge in cancer research. We present CARE, a computational method focused on targeted therapies, to infer transcriptomic signatures of drug efficacy from cell line compound screens. CARE outputs genome-wide scores to measure how the drug target gene interacts with other genes to affect drug efficacy in the compound screens. When evaluated using transcriptome data from clinical studies, CARE can predict the therapy outcome better than signatures from other methods. Moreover, the CARE signatures for the BRAF inhibitor are associated with an anti-PD1 clinical response, suggesting a common efficacy signature between targeted therapies and immunotherapies. When searching for genes in lapatinib resistance, CARE identified PRKD3 as the top candidate. PRKD3 inhibition, by both siRNA and compounds, significantly sensitized breast cancer cells to lapatinib. Thus, CARE should enable large-scale inference of response biomarkers and drug combinations for targeted therapies using compound screen data.
Introduction

Despite the rapid development of cancer treatment methods, many issues such as drug resistance and severe side effects are still limiting the effectiveness of most anticancer drugs (Holohan et al., 2013; Widakowich et al., 2007). Predictive biomarkers of clinical benefits are valuable components to assist personalized treatment decisions (Issaq et al., 2011). In an ideal scenario, a genomics biomarker could analyze the DNA sequences or gene expression profiles of a patient’s tumor and make a reliable prediction regarding therapy response to an anticancer drug. However, despite a few successful biomarkers from clinical genomics studies (Trifiletti et al., 2017), finding reliable prognostic biomarkers is still an open research area for most anticancer drugs in most cancer types (Mabert et al., 2014).

Many approaches have been adopted to identify gene biomarkers and regulators of response and resistance to anticancer therapies from clinical genomics data. For example, post-treatment tumors that are resistant to drugs could be profiled for recurrent genomic and transcriptomic alterations, with pre-treatment sensitive tumors as the reference (Hugo et al., 2015). When associated with patient therapy and clinical outcome, the gene expression or mutation profiles of pre-treatment tumors could also provide insight on prognostic biomarkers (Filipits et al., 2011; Guarneri et al., 2015; van ’t Veer et al., 2002). The ideal dataset from which to build predictive biomarkers would be systematic drug sensitivities and tumor molecular profiles (e.g., mutation, expression) across a large cohort of patients with clinical information. However, these data incur significant efforts and expenses and have limitations on the number of drugs tested.

A faster and cheaper alternative to profile clinical samples is to use cell lines as experimental systems. Genomic profiling and screening technologies, together with immortalized cell line models, are important experimental strategies to investigate the efficacy of therapeutic compounds. For example, molecular profiling of drug-resistant cell lines derived from chronic treatment of sensitive cell lines is widely used to identify cell-intrinsic gene signatures of drug resistance (McDermott et al., 2014; Nazarian et al., 2010). Genome-wide screens using clustered regularly interspaced short palindromic repeats (CRISPR) have been developed to investigate the impact of gene knockout on drug efficacy in cell line and mouse models (Manguso et al., 2017; Shalem et al., 2014). Additionally, CRISPR activation and open reading frame (ORF) screens can induce gene expression and reveal the associations between gene activation and drug resistance (Johannessen et al., 2013; Konermann et al., 2015).

With the rapid advance of automation technology, high-throughput compound screening is rapidly becoming a rich source of information about the mechanisms of drug response and resistance (Hu and Zhang, 2016). Early efforts in NCI60 cell lines correlated gene expression and drug activity patterns across a panel of 59 cancer cell lines to explore mechanisms of drug resistance (Scherf et al., 2000; Shoemaker, 2006). Several new consortiums have conducted both molecular profiling and drug efficacy measurements on hundreds of cancer cell lines. The Cancer Cell Line Encyclopedia (CCLE), Genomics of Drug Sensitivity in Cancer (GDSC), and Cancer Therapeutics Response Portal (CTRP) projects screened 24, 250, and 545 compounds, respectively, across almost 1000 cell lines (Barretina et al., 2012; Iorio et al., 2016; Seashore-Ludlow et al., 2015). These compound screen projects used statistical approaches such as
Elastic Net and ANOVA to identify drug efficacy signatures by testing the associations between gene mutation or expression status and drug efficacy across cell lines. Several studies also explored machine-learning approaches that incorporate high-dimensional nonlinear relationships to analyze compound screens (Costello et al., 2014). A limitation of these previous studies is that they applied the same analysis procedures to all types of compounds without distinguishing between targeted therapies on specific oncogenes and chemotherapies with general cytotoxicity. Since different drug types may follow distinct mechanisms of action, it is likely that customized approaches may achieve better predictive performance depending on the specific drug categories. Additionally, the clinical relevance of cell line models has been continuously questioned (Gillet et al., 2011; Gillet et al., 2013). It remains to be systematically tested whether the biomarkers identified from cell line data have sufficient relevance to the patient clinical response.

In this study, we use large-scale compound screening data to identify biomarkers that predict the clinical response to targeted therapies. We focused on targeted therapies and built a framework, Computational Analysis of REsistance (CARE). Different from previous methods analyzing compound screens, CARE evaluates how drug targets interact with other genes to affect drug efficacy by testing the interaction effects in multivariate models. Such interaction effects between drug targets and other genes were not considered in previous studies but are critical in identifying predictive biomarkers for targeted therapies. Additionally, CARE scores each gene in the genome and uses the correlation between the CARE scores and tumor expression to predict drug response and is therefore robust against noise on individual genes. In the following sections, we will introduce the algorithmic design of CARE and demonstrate its performance in predicting clinical outcomes of targeted cancer therapies. We will also show the better performance of our approach by systematic method comparisons and experimentally validate new synergistic drug combinations predicted by CARE. The source code and results of our study are available at http://care.dfci.harvard.edu.

Results

CARE identifies robust genome-wide signatures of targeted therapy efficacy

Compound screens on cancer cell lines, which measure the growth inhibition of drugs across many cell lines with diverse molecular characteristics, contain abundant information about factors determining drug efficacy (Hu and Zhang, 2016). We observed that many known biomarkers of targeted therapy efficacy had significant interaction associations with the drug target genes in the compound screening data. For example, CCLE screens show that the BRAF V600E mutation correlates with increased cell line sensitivity to the BRAF inhibitor PLX4720 (Barretina et al., 2012). However, this association between drug target and drug efficacy (gaps between the BRAF V600E mutant and other cell lines in Figure 1A) decreases with increased EGFR expression. This observation is consistent with the knowledge that EGFR activation promotes resistance to BRAF inhibitors (Sun et al., 2014). In contrast, the increased expression of \textit{LEFI}, a gene known to sensitize tumor cells to \textit{BRAF} inhibitors (Hugo et al., 2015), is associated with increased PLX4720 inhibition of \textit{BRAF} mutant cells in CCLE screens (Figure 1A). In statistics, an interaction between two variables happens if the effect of one variable (e.g., \textit{BRAF} mutation’s effect on PLX4720 efficacy) depends on the other
variable (e.g., EGFR or LEF1 expression). These observations on known targeted therapy biomarkers motivated us to develop a statistical method, Computational Analysis of REsistance (CARE), to systematically identify genes with similar behaviors.

To evaluate how genes impact targeted therapy efficacy, CARE examines how the expression or mutation state of a gene P interacts with the drug target gene status T to influence the drug inhibition efficacy in a multivariate linear model (Figure 1B). An interaction term is created as the product between the statuses of candidate gene P and drug target T and evaluated toward drug inhibition outcome through multivariate regression. A negative interaction coefficient “c” indicates activation of gene P to be associated with a decreased correlation between drug target and drug inhibition efficacy and thus with drug resistance (Figure 1B). In contrast, a positive coefficient “c” indicates activation of P to be associated with an increased correlation between drug target status and inhibition efficacy and thus with drug sensitivity.

The overall effect of gene P on drug efficacy is computed as a conditional effect when the drug target T is active, combining both interaction and base coefficients in a linear model (Figure 1C, STAR methods) (Brambor et al., 2006). To estimate the statistical significance of the conditional effect, CARE computes a t-value (coefficient/standard deviation) using the ordinary least square method (Figure 1C) (Freedman, 2009). For example, CARE correctly identified EGFR as a resistant gene for the BRAF inhibitor, as indicated by a negative CARE score (Table 1), and LEF1 as having a sensitizing role for the BRAF inhibitor, as shown by a positive CARE score (Table 2). CARE also identified AXL to be a resistant gene for lapatinib (a dual ERBB2 and EGFR inhibitor) through the negative CARE scores with ERBB2 as lapatinib target (Table S1), which is consistent with previous knowledge (Liu et al., 2009). Finally, instead of selecting specific genes, CARE outputs a vector with one score for every human gene (Figure 1C). This score vector allows us to identify potential biomarker genes, whose expression or mutation status has significant associations with drug efficacy. When predicting whether a tumor will respond to the drug, we calculate the genome-wide Pearson correlation between the tumor expression profile and CARE score (Figure 1D), which provides a robust estimation and avoids noise from individual genes.

To systematically identify gene signatures associated with response and resistance to different targeted therapies, we applied CARE to three large-scale compound screen datasets. The CCLE (Barretina et al., 2012), GDSC (Iorio et al., 2016) and CTRP (Seashore-Ludlow et al., 2015) projects screened numerous compounds across hundreds (approximately 500 to 1000) of cancer cell lines (Figure S1A and Table S2). For each compound with annotated target genes, we first used a forward selection algorithm to ensure that the status (e.g., expression, mutation) of the annotated drug target gene has sufficient correlation with drug efficacy (Jiang et al., 2015) (STAR methods). There are 17, 118 and 190 compounds passing the selection criteria in the CCLE, GDSC and CTRP cohorts, respectively. The response measurements of these targeted therapies are reasonably consistent across different datasets (Figure S1B, C).

We applied CARE to identify genes associated with drug efficacy for many compounds across the three cohorts and focused on druggable genes with significant negative CARE scores for visualization (Figure 2). For example, AXL, a druggable kinase implicated as a resistance driver for many targeted therapies (Liu et al., 2009; Zhang et al., 2012), has
significant negative CARE scores for multiple compounds screened in three cohorts (Figure 2A, B). Another example is that SRC has negative CARE scores on most drugs (Figure 2A, C), which corroborates previous findings that SRC activation promotes resistance towards several targeted therapies (Wilson et al., 2014; Zhao et al., 2017). In contrast, CSK, a negative regulator of SRC (Okada et al., 1991), has significant positive CARE scores for many compounds (Figure 2C), suggesting that loss of CSK may promote drug resistance towards many targeted therapies. Notably, the interaction test in Figure 1B can evaluate both expression and mutation of gene P on drug efficacy. For example, the significant negative CARE scores of KRAS mutations in many compound screens (Figure S2) support the role of KRAS mutations in promoting drug resistance (Misale et al., 2012; Sameen et al., 2016).

**CARE outperforms other genomic signatures in predicting the clinical outcome of targeted therapies**

Encouraged by the consistent results of previous studies, we systematically tested the clinical utility of CARE by evaluating the accuracy of CARE scores in predicting the patient clinical outcome of targeted therapies. To this end, we used the expression profiles of pre-treatment tumors from 12 melanoma patients that had a BRAF V600E mutation and were treated with vemurafenib, each with progression-free survival (PFS) information (Hugo et al., 2015). Patients are predicted to be responders if their tumor expression profiles are positively correlated with CARE scores of PLX4720, a chemical analog of vemurafenib (Bollag et al., 2012), and non-responders if the profiles are negatively correlated (Figure 1D). The correlations between patient tumor expression profile and CARE scores were significantly associated with the PFS time, verifying CARE’s predictive power in clinical samples (Figure 3A).

To compare CARE with other experimental methods conducted on cell lines, we collected the gene scores associated with drug efficacy derived from CRISPR knockout, CRISPRa, shRNA, ORF screens, and expression profiles of drug-resistant cell lines (Table S3A). For each signature, we stratified the patients according to the correlation between tumor gene expression values and cell line gene scores (such as differential expression or screen selection level) published in these experiments. Then, we predicted positively correlated patients as the responders and negatively correlated patient as the non-responders. As an evaluation standard to test prediction accuracy, we defined patients with the top and bottom 50% PFS as the responders and non-responders, respectively. The prediction accuracy metrics are highest for CARE, followed by CRISPRa/CRISPR knockout screens and other experimental methods (Figure 3B). When evaluated with an alternative association metric computed by the Cox-PH model, CARE also demonstrated the highest association with PFS outcome among all methods (Figure S3A).

To further investigate the clinical utility of CARE, we next tested another drug, lapatinib, on HER2+ breast cancer. The CHER-LOB clinical trial released complete pathological response statuses of patients together with their gene expression profiles (Guarneri et al., 2015). We computed the Pearson correlation between gene expression values and CARE scores for lapatinib from the CCLE cohort and found that responders have significantly higher correlations with the CARE scores than non-responders (Figure 3C, one-tailed rank-sum test p-value = 0.047). We also collected gene signatures profiled by many experimental methods (Table S3B) and used the other signatures to correlate with patient gene expression profiles to predict response. When evaluating the fraction
of patients with correctly predicted response status, we found CARE prediction to give the highest accuracy over other signatures (Figure 3D).

The CARE signature for the PLX4720 BRAF inhibitor, computed from cell line screens, is also indicative of the clinical response to anti-PD1 immune therapy in melanoma (Figure 3E). The accuracy metric of CARE is even higher than several known biomarkers of anti-PD1 response (Figure 3F) (Hugo et al., 2016; Nishino et al., 2017), as well as a gene signature from an in vivo CRISPR screen (Table S3C) (Manguso et al., 2017). The clinical outcome of melanoma patients was known to be associated with the cytolytic activity (CYT), defined as the sum of GZMA, GZMB, and PRF1 expression in a bulk tumor as an infiltration estimation of cytolytic lymphocytes (Rooney et al., 2015). Among TCGA melanoma tumors, a higher CYT level predicts better patient overall survival but only in patients predicted to be responders by the CARE PLX4720 signature (Figure 3G). Thus, the cell-autonomous gene signature of CARE is associated with both immunotherapy response and tumor immune evasion. To explore the possible mechanisms, we applied gene set enrichment analysis on the PLX4720 CARE signature and found epithelial-to-mesenchymal transition (EMT) to be the most enriched category (Figure 3H). EMT was a known driver process in drug resistance and tumor immune evasion (Dongre et al., 2017; Shibue and Weinberg, 2017) and was implicated in resistance to anti-PD1 therapy (Hugo et al., 2016). We explored the prediction performance of other compound CARE signatures on the anti-PD1 response. In all screen cohorts, CARE signatures more correlated with the EMT signature show the better predictive power of anti-PD1 response (Figure S3B). Therefore, the CARE signature has predictive power on immune resistance through enrichment of EMT, a common resistance mechanism between targeted therapies and immunotherapies.

One limitation of our evaluation of clinical prediction accuracy on targeted therapies is that published studies only profiled a small number of patients. For example, there are only 12, 25, and 31 patients with complete clinical information and pre-treatment transcriptome for vemurafenib (Hugo et al., 2015), pembrolizumab (Hugo et al., 2016), and lapatinib (Guarneri et al., 2015), respectively. In contrast, there are published studies with data for hundreds of patients undergoing chemotherapy treatment. Even though the focus of our study is targeted therapy, we can also apply CARE to chemotherapies with known targets. For example, TUBB is the target gene of paclitaxel by the CCLE annotation (Barretina et al., 2012). There are three independent studies on paclitaxel treatment that include 193, 115 and 92 patients, respectively (Figure 3I) (Hatzis et al., 2011; Miyake et al., 2012; Popovici et al., 2010). We used the paclitaxel CARE scores, computed from CCLE data, to predict therapy response in the clinical cohorts and achieved approximately 70% accuracy (Figure S3C), a performance better than other genomics signatures (Figure 3 and Table S3D).

We also examined how well the paclitaxel response signatures derived from one clinical cohort predict responses in another cohort compared to the CARE signature derived from cell line compound screens. To this end, we computed associations between gene expression levels and therapy response using logistic regression in each clinical cohort. Then, we used the computed gene associations to predict responses in another clinical cohort and calculated the average accuracy among all six pairwise combinations across three paclitaxel studies. The CARE signature, although derived from cell line compound
screens, achieved comparable performance with signatures derived from independent clinical cohorts (“Clinical” in Figure 3J).

**CARE is more robust than other computational methods in finding biomarkers**

Previously, several computational methods were utilized by different studies to analyze compound screen data. Therefore, we compared CARE with other computational methods in predicting gene signatures of drug efficacy using the evaluation standards from both cell models and clinical studies. These methods include Correlation, Elastic Net, support vector regression (SVR), and the GDSC tool that uses the ANOVA test to identify response biomarkers (Iorio et al., 2016). Correlation refers to the Pearson correlation between the gene expression or mutation status and drug inhibition outcome. ANOVA estimates the difference among group means and variations of drug response according to the gene status. Elastic Net, which applies penalties to constrain the regression coefficients, is a popular method used in previous compound screen projects to identify gene alterations associated with compound efficacy (Barretina et al., 2012; Garnett et al., 2012). SVR is similar to the support vector machine (SVM) (Smola and Scholkopf, 2004), but the response variable modeled is continuous in SVM instead of categorical as in SVM. Both Elastic Net and SVR use regression coefficients on each gene as associations with drug efficacy.

To set up the first evaluation standard, we collected several differential gene expression profiles between drug-resistant cell lines derived from chronic drug treatment and their parental cell lines. For PLX4720 and lapatinib screened in all three cohorts, we collected expression profiles from several studies (Bailey et al., 2014; Giles et al., 2013; Liu et al., 2009; Nazarian et al., 2010; Zhang et al., 2012). We defined the positive set in evaluation standard as the upregulated genes in a resistant cell line compared to its parental line and the negative set as the down-regulated genes. We then compared the computational methods using a receiver operating characteristic (ROC) curve (Figure 4A and Figure S4A, B) in which a reliable prediction should curve toward the upper left corner. Elastic Net predicted a small number of genes and missed most efficacy-associated genes in the evaluation standard (Figure S4C, D and Table S4A) (Barretina et al., 2012; Garnett et al., 2012). Although GDSC, Correlation, and SVR all have better performance than Elastic Net, CARE outperformed them all (Figure 4A and Figure S4A, B). We also tested the performance of different model variations. The current form of CARE score combined both interaction and base coefficients (Figure 1C). Alternatively, we can compute a t-value for only the interaction coefficient (“c” in Figure 1B, C) or the base coefficient (“b” in Figure 1B, C) without an interaction term in the linear model. Similarly, for other statistical methods, we also fitted model variations considering the combination, interaction, and base effects (STAR methods). On average, the combination form of CARE score outperformed all the other variations (Figure 4B).

To set up the second evaluation standard of comparison, we utilized the metrics for predicting clinical response to targeted therapies (Figure 3B, D, F, J) and compared the drug efficacy signatures predicted from different computational methods (Figure 4C). Among all targeted therapies, CARE achieved robust prediction performance based on various accuracy metrics (Figure 4C). Thus, based on the comparisons above with evaluation standards from both cell-line models and clinical studies, CARE outperformed other computational approaches in predicting gene signatures of drug response and resistance from compound screening data.
For all the comparisons above, we only focused on drugs with high correlation between drug target status and drug efficacy. We further tested the CARE performance for drugs whose efficacy has low correlation with gene status of annotated targets, since the target gene status may not be the feature most correlated with drug efficacy. For example, the Pearson correlations between topotecan effectiveness and \textit{TOP1} expression levels are 0.099 and 0.105 in the CCLE and CTRP data, respectively, which all rank below 70\% among all gene feature correlations. Using a ROC curve, we found that the CARE statistics and Pearson correlation achieved similar performance and that the interaction-only statistic has the worst prediction performance (Figure S4E, F). Thus, CARE may not have better performance than correlation when the annotated target gene does not correlate well with drug efficacy.

\textbf{CARE predicts synergistic drug targets in treating HER2+ breast cancer cells}

Finally, we evaluated whether CARE can identify synergistic drug targets to enhance the effectiveness of targeted therapies through combination medicine. We focused on the gene signatures for the \textit{ERBB2} inhibitor lapatinib, an orally active drug for HER2+ breast cancer and other solid tumors (Geyer et al., 2006; Janjigian, 2016). To this end, we selected genes with significantly negative CARE scores, indicating that high gene expression levels correlate with lapatinib resistance. We further selected genes upregulated in lapatinib-resistant cell lines (Bailey et al., 2014; Liu et al., 2009) and negatively associated with patient response in the CHER-LOB trial (Guarneri et al., 2015). Among the 47 genes meeting these criteria, only PRKD3 and AKT3 have compound inhibitors available (Table S5A). AKT3 has been reported to promote resistance to anti-HER2 therapy in breast cancer (Moody et al., 2015). PRKD3 was predicted by CARE to have a stronger association with the clinical outcome than the known lapatinib resistance drivers AKT3 and the previously discussed AXL (Figure S5A) and yet has never been reported to promote lapatinib resistance. Thus, we decided to validate PRKD3 experimentally.

We first knocked down PRKD3 using a pooled mixture of 30 different siRNAs targeting PRKD3 to reduce off-target effects and observed significantly increased lapatinib sensitivity in the HER2+ breast cancer cell line SKBR3 (Figure 5A, B). Encouraged by this result, we searched publicly available inhibitors and found three pan-PRKD kinase family (\textit{PRKD1}, \textit{PRKD2}, and \textit{PRKD3}) inhibitors, KBNB14270, CRT0066101, and CID2011756. Using the Bliss independence model (Bliss, 1939) to evaluate the synergy between two compounds, we tested the effect of each PRKD inhibitor in combination with lapatinib. The Bliss independence model assumes no drug interaction and defines the co-treatment additive inhibition as $I_A + I_B - I_A \ast I_B$, where $I_A$ and $I_B$ represent the inhibition by compound A and B alone, respectively. The drug combination is synergistic if the measured co-treatment inhibition is higher than the expected additive effect and antagonistic if the measured inhibition is lower than the additive effect. At varying lapatinib doses, the inhibition effect of co-treatment with 4 \text{\mu M} PRKD inhibitor KBNB14270 is consistently better than the additive effect, indicating a consistent synergy between KBNB14270 and lapatinib (Figure 5C and Figure S5B). We also observed a similar synergy for CRT0066101 (Figure S5B, C). We next tested the drug synergy by varying the dose of different PRKD inhibitors. KBNB14270 and CRT0066101 consistently showed positive synergy with lapatinib (Figure 5D, E), and CID2011756 showed synergy at low lapatinib doses (Figure S5D). Besides lapatinib, trastuzumab is
another targeted therapy used in HER2+ breast cancer. We also observed significant synergy between PRKD inhibitors and trastuzumab on the SKBR3 cell line (Figure S5C, D). Thus, our results suggest PRKD inhibitors as potentially synergistic therapies with anti-HER2 treatment in breast cancers.

During the gene prioritization process for lapatinib resistance, we only explored those genes with consistently high CARE scores across all three screen cohorts. However, some genes prioritized without this stringent threshold may still have very significant CARE scores in a subset of cohorts. For example, SERPINE1 has very significant CARE scores in CCLE and CTRP cohorts, but not GDSC, therefore not included in our previous prioritization result (Table S5B). While other genes may not have consistently significant CARE scores (Table S5B), which indicates that their association with drug resistance may not be valid across a broad range of cell lines in the compound screens.

**Discussion**

We developed a computational method, CARE, to systematically identify genes associated with response and resistance to targeted therapies based on compound screens. Comparisons based on multiple clinical datasets demonstrated the superior performance of CARE over other computational and experimental methods. Notably, we predicted PRKD3 as a potential regulator of anti-HER2 resistance and validated it as a promising target to increase lapatinib and trastuzumab efficacy through combinatorial treatment with PRKD inhibitors. Further experiments on animal models are needed to increase the clinical potential for these drug combinations.

CARE outperformed the gene signatures from CRISPR screens in predicting the patient clinical outcome (Figure 3B, F). One possible explanation is that the quality of CRISPR screens awaits further improvement at this early stage of technology development, as indicated by the low agreement between replicates (Figure S3D). Another possibility is that CARE analysis is based on drug sensitivity measurements in many cell lines with diverse characteristics, whereas current CRISPR/CRISPRa (or shRNA) screens on drug treatment were only available in individual cell lines.

For standard drugs in clinics, the data from drug-resistant cell models and human clinical studies could help to identify the response biomarker genes independently from CARE signatures. However, the molecular profiles of drug-resistant models are typically available on the scale of one or two cell lines. Additionally, most drug clinical studies only profiled a small number of patients (e.g., 12 patients for vemurafenib, 32 for lapatinib). In contrast, the CARE signature is trained using the genomics and pharmacological data from approximately 1,000 cell lines with distinct characteristics; thus, it is generalized from a broad genetic background. Second, for most compounds that are not used in clinics, the molecular profiles of drug-resistant cell lines and clinical response do not exist. However, CARE signatures would still be available to understand potential drug resistance mechanisms for these compounds.

A limitation of CARE is that some driver mutations of drug resistance may not exist in sufficient frequency in the screened cell lines. For example, there is only one CCLE cell line harboring the EGFR T790M mutation, the dominant mechanism of resistance to EGFR inhibitors (Holohan et al., 2013). As a result, CARE cannot make statistical
inferences on this mutation. Meanwhile, although current compound screening projects have tested almost 1000 cell lines, the number of cell lines in each cancer type is still limited (Figure S4G). CARE has better performance in pan-cancer analysis than lineage-specific analysis due to the sample size limitation in each cancer lineage (Figure S4H, I). Another limitation is that CARE currently utilizes only cell line data that describe cell autonomous behavior. However, the efficacy of many targeted therapies not only involves direct cytotoxic effects but also relies on the activation of tumor-targeting immune responses (Galluzzi et al., 2015). Thus, future computational methods should model the impact of the tumor microenvironment in predicting therapy response.

Despite these limitations, CARE has demonstrated reliable performance in inferring gene biomarkers to predict the efficacy of targeted therapy, as well as synergistic drug combinations. Recent years have seen the advances in high-throughput technologies and increased data volumes for cancer drug research (Chen and Butte, 2016). We foresee that CARE will provide future assistance in the development of anticancer drug biomarkers and combination therapies.

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Author Contributions
P.J., and X.S.L. designed the research, analyzed data and wrote the paper. P.J. performed both computational analysis and experimental validation. J.C.A provided the experimental platforms and reagents. X.L. collected and preprocessed the compound screen data. J.C.A., M.B., and J.S.L discussed with P.J. about the study. W.L., C.J., M.B., and J.C.A. advised P.J. on the experimental techniques.

Declaration of Interests
The authors declare no competing interests.

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Figure titles and legends

Figure 1. Inference of genome-scale gene signatures for targeted therapy efficacy
(A) Association between BRAF mutation and inhibitor efficacy conditioned on other
gene variables. All CCLE cell lines were divided into three categories ranked by EGFR or
LEF1 expression (Low: below 25%, Mid: between 25% and 75%, High: above 75%). In
each category, the PLX4720 inhibition values are shown for cell lines with a BRAF
V600E mutation and others without the mutation, with the median value as a thick bar
in the box-plot. The bottom and top of the boxes are the 25th and 75th percentiles
(interquartile range). Whiskers on the top and bottom represent the maximum and
minimum data points within 1.5 times the inter-quartile range.
(B) Interaction test in multivariate regression. Variables T and P represent the statuses
of drug target and candidate gene in the test, respectively. The coefficient of product
covariate (T*P) in regression represents the interaction effect between T and P on drug
inhibition outcome. The slopes between drug target status and drug inhibition efficacy
are shown by arrows, with red and black colors indicating the direction of change when
variable P increases.
(C) CARE score definition. The effect of variable P on drug inhibition efficacy is
computed as a t-value conditioned on drug target status T as one. The standard error
(s.e.) is estimated through the coefficient covariance.
(D) CARE framework of clinical response prediction. For each targeted therapy, CARE
will first calculate a genome-wide vector of scores with one value per gene using
compound screen data. To predict drug responses, CARE computes Pearson
correlations between the tumor gene expression profiles and CARE scores on genome-
scale, with positively correlated patients labeled as responders and negatively
correlated ones labeled as non-responders.

Figure 360: Available as Movie S1. See also Figure S1 and Table S2.

Figure 2. CARE scores for genes associated with resistance to multiple drugs
(A) CARE scores of druggable genes associated with resistance to more than one
compound. To uniformly compare scores across different cohorts, we normalized all
CARE scores within each cohort to zero mean and unit variation.
(B) Statistically significant CARE scores of AXL for compounds analyzed in each cohort.
The median value in each group is shown as a thick bar in the box-plot. The bottom
and top of the boxes are the 25th and 75th percentiles (interquartile range).
Whiskers on the top and bottom represent the maximum and minimum data points
within the range represented by 1.5 times the inter-quartile range.
(C) Statistically significant CARE scores for SRC and its inhibitor CSK for compounds
analyzed in each cohort. All values are shown in the same way as panel B.

See also Figure S2.
Figure 3. CARE can reliably predict clinical outcome of targeted therapies

(A) CARE prediction results on the anti-\textit{BRAF} clinical outcome. For 12 patients treated with vemurafenib (Hugo et al., 2015), CARE computed the Pearson correlations between PLX4720 CARE scores and tumor gene expression profiles. The progression-free survival (PFS) was compared between positively and negatively correlated patients by the Kaplan-Meier curves, with \textit{p}-values from the two-sided Wald test in Cox-PH regression.

(B) Performance comparison on predicting anti-\textit{BRAF} vemurafenib response. For each gene signature (Table S3A), the response was predicted for patients by correlating between tumor expression profiles and signature gene scores. The prediction accuracy metrics were compared. AUC: area under ROC curve.

(C) CARE prediction results on the anti-\textit{HER2} clinical outcome. For 32 patients treated with lapatinib (Guarneri et al., 2015), the patient-wise correlations with CARE signature were shown according to the clinical response status, with the \textit{p}-value computed by one-sided Wilcoxon rank-sum test.

(D) Performance comparison on predicting anti-\textit{HER2} lapatinib response among several signatures (Table S3B) as panel B.

(E) Prediction results on the anti-\textit{PD1} clinical outcome using the anti-\textit{BRAF} CARE scores. For 25 patients treated with pembrolizumab (Hugo et al., 2016), the patient-wise correlations with PLX4720 CARE signature were shown according to the response status, with the \textit{p}-value computed by one-sided Wilcoxon rank-sum test.

(F) Performance comparison on predicting anti-\textit{PD1} pembrolizumab response. \textit{PDL1}: \textit{CD274} expression; \textit{CD8}: expression sum of \textit{CD8A} and \textit{CD8B}; \textit{BRCA2}: \textit{BRCA2} mutation status; Mutation: non-synonymous mutation load; CRISPR: anti-\textit{PD1} resistance signature determined by in-vivo CRISPR screen (Table S3C).

(G) Association between CARE signature and tumor immune evasion. CARE predicted all TCGA melanoma patients as either responders or non-responders using the PLX4720 signature computed from CCLE data. In each category, the overall survival (OS) was shown for patients with positive and negative values of immune cytolytic activity (\textit{CYT}, computed as expression sum of \textit{GZMA}, \textit{GZMB}, and \textit{PRF1}). The \textit{p}-values were computed by two-sided Wald test in Cox-PH regression.

(H) Gene set enrichment analysis for the PLX4720 CARE signature. The epithelial-to-mesenchymal transition (EMT) was the highest enrichment term. All CARE gene scores are shown in ascending order in the bottom. The genes with EMT function are labeled with black bars in the middle. The enrichment score of EMT signature at each score rank is shown on the top. NES: normalized enrichment score. \textit{P}-value: permutation test \textit{p}-value with 1000 shuffles.

(I) CARE prediction results on the paclitaxel response. For paclitaxel, we identified three studies with both tumor gene expression data and pathologic complete response (pCR) status. There are 193, 115 and 92 patients in the studies of Pusztai, Noguchi, and Symmans, respectively. The correlation between patient expression profiles and CARE scores of paclitaxel was shown according to the response status. The two-sided rank-sum \textit{p}-values, testing the correlation difference between responders and non-responders, are 7.25e-4, 4.95e-5, and 2.25e-2 for the Pusztai, Noguchi, and Symmans studies, respectively.

(J) Performance comparison on predicting paclitaxel response among signatures (Table S3D) as panel B, with accuracy metrics averaged among three cohorts in panel I.

See also Figure S3 and Table S3.
Figure 4. CARE outperforms other computational methods in finding drug efficacy biomarkers

(A) Performance comparison on predicting transcriptome signatures of drug resistant cell lines. Receiver operating characteristics (ROC) curves were used to compare different computational methods for their performance in predicting gene expression signatures of PLX4720 and lapatinib resistant cell lines.

(B) Performance comparison among model variations. For each method, we computed several variations of scores and compared their performance in predicting drug resistance associated genes as panel A. The mean of the area under ROC curve (AUC) across three screen cohorts are compared among all methods with standard deviations as error bars. Combination: the association calculated for the sum of both interaction and base coefficients. Interaction: only the interaction coefficient. Partial: only the coefficient of each gene P in a model without interactions.

(C) Performance comparison on predicting clinical outcome. For each method, the response was predicted for patients through the correlation between patient gene expression values and the result scores from each model. The accuracy metrics are compared in the same way as Figure 3B.

See also Figure S4 and Table S4.

Figure 5. Inhibition of PRKD3 increases lapatinib sensitivity

(A) The siRNA pool knockdown efficiency against PRKD3 measured by qPCR and western blot (WB). (NC: negative control siRNA of scrambled sequences)

(B) The inhibition effects on SKBR3 growth from both PRKD3 knock-down and negative control conditions on a series of lapatinib doses by twofold dilution from 5 µM. Each inhibition fraction is the median value from three replicate experiments, with standard deviations as the error bars.

(C) The inhibition effects on SKBR3 growth from lapatinib mono-treatment and co-treatment with PRKD inhibitor KBNB14270 in 4 µM. The additive effect of co-treatment is estimated from the Bliss independence model. Each inhibition fraction is the median value from three replicate experiments, with standard deviations as the error bars.

(D) The synergy scores (the difference between co-treatment and Bliss additive inhibition) for dose combinations between lapatinib (Lap) and KBNB14270 (KBNB). Each score is the median value from three replicate experiments.

(E) The synergy scores for combinations between lapatinib and CRT0066101 (CRT).

See also Figure S5 and Table S5.
Tables and legends

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Table 1. Negative interaction between *BRAF* mutation and *EGFR*
The interaction between *BRAF* mutation and *EGFR* gene expression is evaluated by linear regression with PLX4720 efficacy as the outcome. The t-value is defined as regression coefficient divided by the standard error, and the p-value is calculated by the two-sided Student’s t-test.

See also Table S1.

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Table 2. Positive interaction between *BRAF* mutation and *LEF1*
The interaction between *BRAF* mutation and *LEF1* gene expression is evaluated by linear regression with PLX4720 efficacy as the outcome. The t-value is defined as regression coefficient divided by the standard error, and the p-value is calculated by the two-sided Student’s t-test.

See also Table S1.
STAR Methods

Contact for Reagent and Resource Sharing
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Xiaole Shirley Liu (xsliu@jimmy.harvard.edu). All cell lines, chemicals, and reagents can be attained directly from the original supplier.

Experimental Model and Subject Details
The SKBR3 is a female breast cancer cell line of HER2+ subtype purchased from ATCC. All models are regularly tested for being free of mycoplasma, and their identities are verified.

Method Details

Pharmacological screen data collection
Compound inhibition, gene expression, and mutation profiles were available from the websites of CCLE (Barretina et al., 2012), GDSC (previously named CGP) (Iorio et al., 2016) and CTRP (Seashore-Ludlow et al., 2015) with data version until 07/10/2016. All compound names are converted to the standard names in PubChem database (Kim et al., 2016). The CARE framework uses a sigmoid function to fit drug inhibition values at different doses into a response curve, and compute the drug inhibition as the area under the dose-response curve, ranging from 0% to 100%. The source code of response curve fitting is released as program “nls_logsig” (Key Resources Table).

Clinical data collection
The gene expression profiles of pre-treatment tumors and patient clinical information were collected for vemurafenib in melanoma (Hugo et al., 2015), lapatinib in HER2+ breast cancer (Guarneri et al., 2015), pembrolizumab in melanoma (Hugo et al., 2016), and paclitaxel in breast cancer (Hatzis et al., 2011; Miyake et al., 2012; Popovici et al., 2010). In each dataset, the expression value of each gene is normalized by subtracting the mean value across all samples. Only genes with top 50% expression variance in each study were considered in response prediction. In the pembrolizumab study, 28 patients were profiled in the original study (Hugo et al., 2016). However, one patient is profiled on-treatment, one patient has duplicated profiles, and one patient does not have survival information. Therefore, only the pre-treatment expression profiles of 25 patients with complete clinical information are included in further analysis.

Selection of drug target gene statuses
For each compound, we used the drug target gene annotation provided by each screen projects. Among annotated targets, we ran a forward feature selection algorithm RABIT (Jiang et al., 2015) to minimize the Mallow’s Cp and make sure that included target gene status can jointly predict the drug efficacy without redundancy. Mallow’s Cp estimates the prediction precision of a linear model with drug target features as covariates and drug efficacy as the response (Mallows, 1973). The stepwise selection procedure stops
at the minimum Mallow’s Cp value (James et al., 2013). Our analysis only included reliable drug target gene statuses with positive coefficients in the final linear model. There are 17, 190, and 118 compounds with reliable targets selected in the CCLE, CTRP, and GDSC cohorts, respectively.

The drug target selection process above may not select the primary drug target status. For example, our procedure selected \( EGFR \) gene expression for erlotinib and gefitinib through high correlation with drug efficacy (Figure S1D), but \( EGFR \) mutation is known as the primary drug target status. The reason is that the cell line collection screened in three projects have a very low frequency of \( EGFR \) mutation. The most common \( EGFR \) activating mutations are in-frame deletions of exon 19 and L858R point mutation in exon 21, which account for ~90% of all \( EGFR \) mutations in lung cancer (Ladanyi and Pao, 2008). Among the CCLE cell lines screened by both CCLE and CTRP project, only five cell lines harbor these mutations (Figure S1E upper). In the COSMIC cell collection screened by GDSC project, only four cell lines harbor these mutations (Figure S1E bottom). In both CCLE and COSMIC cohorts, only one cell line has T790M mutation that drives \( EGFR \) inhibitor resistance (Figure S1E). Among about 1000 cancer cell lines in each collection, less than five cell lines in total cannot lead to any reliable statistical inference. We only included mutation variables with more than 10% frequency among tested cell lines. Thus, we excluded \( EGFR \) mutation in our analysis and used the expression as alternative target status. Consistent with the results from cell screens, several clinical studies also reported that \( EGFR \) gene level as a strong predictor of erlotinib and gefitinib efficacy (Cappuzzo et al., 2005; Haas-Kogan et al., 2005; Jazieh et al., 2013; Tsao et al., 2005).

For \( CDK4/6 \) inhibitor palbociclib, we noticed that the status of \( RB1 \), but not \( CDK4/6 \), is used as the patient selection criterion in palbociclib clinical trials (O’Leary et al., 2016). Indeed, the correlations between \( RB1 \) expression and palbociclib efficacy rank above 98% among all gene correlations in both CCLE and GDSC screens (Figure S1F). Thus, in our current analysis, we included \( RB1 \) as the target.

**Interaction test in multivariate linear regression**

For each targeted therapy, the drug efficacy associations of all genes are tested by an interaction test in linear regression (Figure 1B). Variable \( T \) and \( P \) represent the statuses of the drug target gene and a candidate gene in the test, respectively. For gene mutation status, a value of zero on variable \( T \) or \( P \) indicates an absence of mutation; and a value of one indicates a presence of the mutation. For gene expression status, our analysis normalized all values to zero mean and one standard deviation. Thus, a value of zero on variable \( T \) or \( P \) represents the average expression level of a gene across all cell lines; and a value of one represents one standard deviation higher than average. We solve a linear model “Inhibition = a*T + b*P + c*T*P + Intercept + e” using the ordinary least square method (OLS) (Freedman, 2009). Since we have selected compounds whose inhibition efficacy positively correlates with the drug target status \( T \), the coefficient “a” is always positive. The slope between drug target status and drug inhibition efficacy is “a + cP” (Figure 1B). If the coefficient “c” is negative, increasing \( P \) will decrease the slope. In contrast, if the coefficient “c” is positive, increasing \( P \) will increase the slope.

To estimate the overall association between gene \( P \) and drug inhibition efficacy, we computed the sum of coefficients “b + c” (Figure 1C), defined as the conditional effect
(Brambor et al., 2006). The linear model of CARE has the form “Inhibition = a T + b P + c T P + Intercept + e”. The covariates T and P represent gene statuses. For mutation status, value “0” represents the absence of mutation, and value “1” represents the presence of the mutation. For expression status, we have transformed the values for each gene to zero-mean and one standard deviation. Thus, value “0” represents the average expression level among all cell lines, and value “1” represents the expression level of one standard deviation higher than average. The association between drug inhibition response and candidate gene P when target gene T is activated could be represented as “Inhibition = a + b P + c P + Intercept + e | T = 1”. Thus, “b + c” represents the association between drug inhibition and candidate gene P when drug target is activated (i.e., T = 1).

For coefficient sum b + c, the t-value is calculated following the procedure of general linear hypothesis testing (Figure 1C) (Hothorn et al., 2008). If a linear model has the form y = X β + e, following OLS conditions, we could estimate coefficient β and its covariance matrix Σ as β̂ and Ŝ. For any linear combinations of parameters cβ (c is a linear vector), we can estimate it as c β̂ and its covariance matrix as c′Σc (Freedman, 2009). Under the OLS assumptions, the t-statistics \( \frac{c \hat{\beta}}{\sqrt{c \hat{\Sigma} c}} \) follows student t-distribution with n-p-1 as the degree of freedom (Hothorn et al., 2008). In the hypothesis test, the null hypothesis is cβ = 0 and alternative hypothesis is cβ≠0. In our CARE method, we use the linear combination of two coefficients b and c as a special case of t-statistics described above. CARE computes a two-sided p-value for the t-statistics through the student t-test. For all p-values of tested genes in each screen profile, the Benjamini Hochberg procedure can convert p-values to false discovery rates (FDR) with 0.05 as result selection threshold.

**Interaction test for compounds with multiple drug targets**

Certain drugs may have multiple annotated drug targets (e.g., Lapatinib targets both ERBB2 and EGFR). For such cases, we solve a single linear model with all covariates: Inhibition = \( \sum_1^N a_i \cdot T_i + b \cdot P + \sum_1^N c_i \cdot T_i \cdot P + \text{Intercept} + \epsilon \), where N is the number of drug targets. Then, for each target gene T_i, CARE computes the t-value as \( \frac{b+c_l}{\sqrt{\hat{\sigma}^2 (b+c_l)}} \) (Table S1). For lapatinib, the CHER-LOB trial only considered the ERBB2 level in patient selection. Thus, we used the CARE scores associated with ERBB2 to predict therapy response.

**Difference between CARE and other regression, correlation, and ANOVA methods**

Most computational approaches tested in this study involved computing products among different variables. However, the products among these methods are different. The product “T*P” in CARE is a covariate for testing the interaction effects “c” in linear model “Inhibition = a*T + b*P + c*T*P” (Figure 1B). After computing “T*P”, we still need to use the ordinary least square approach to calculate all coefficients (e.g., b, c) as the final output (Freedman, 2009). In contrast, for correlation, the product of normalized variables “Inhibition * P” is directly the final output. ANOVA is different from correlation in that ANOVA is testing the difference among group variations associated with categorical variables. We used the ANOVA implemented in the GDSC package (Iorio et al., 2016), which requires the gene expression level of P as discrete values of either high
or low with a cutoff on the average value among all patients. In contrast, both correlation and CARE work on continuous variables without any cut-off threshold.

**Annotation of druggable genes**

For all genes with drug score larger than 5 in the Pfizer OASIS database (Fernandez-Banet et al., 2016), we further searched their gene name on Selleck website (http://www.selleckchem.com), and only keep genes with inhibitors commercially available.

**Experimental validation on PRKD3 as synergistic target of anti-HER2 therapies**

The siRNA pool targeting PRKD3 and non-target control siRNAs were purchased from the siTools Biotech company (http://www.sitoolsbiotech.com). The antibody to PRKD3 was from Cell Signaling Technology (D57E6, 1:500 dilution). The antibody to ACTB was from Sigma Aldrich (A1978, 1:5000 dilution). Secondary antibody HRP anti-mouse was from Cell Signaling Technology (#7076S, 1:2500). HRP anti-rabbit was from Cell Signaling Technology (#7074S, 1:2500). Lapatinib was from SelleckChem, and trastuzumab was from Dana-Farber Pharmacy store. All PRKD inhibitors (KBNB14270, CRT0066101, and CID2011756) were purchased from Sigma Aldrich.

The SKBR3 cell line (ATCC, Mycoplasma tested free) was cultured in McCoy 5A medium (Fisher Scientific) with 10% FBS. On day 1, siRNA pools (siTools Biotech) targeting PRKD3 and control were reverse transfected at a concentration of 20nM with Lipofectamine RNAiMax (Invitrogen), and seeded in 96 well plates at 10,000 cells per well. We examined the knockdown efficiency at 24 hours with qPCR and 72 hours with western blot. ACTB is the control gene for both qPCR and western blot. On day 2, the cell line was treated with lapatinib with ten concentrations, starting from 5uM with a dilution factor of two. After three days, we used the Cell Titer Blue assay (Promega) to test the cell viability.

In the drug co-treatment experiments, different dose combinations of PRKD inhibitors and lapatinib or trastuzumab are seeded together with the SKBR3 cell at 10,000 cells per well. The inhibition effects are measured after three days using Cell Titer Blue assay.

**Quantification and Statistical Analysis**

**Gene biomarkers from different computational methods**

We trained each statistical model on the full range of screen data. The coefficient or statistical metric associated with each gene feature is the genome-wide biomarker to predict drug efficacy. Specifically, the CARE biomarker is the genome-scale score vector of t-values computed from interaction test (Figure 1C). For correlation, the biomarker is the genome-scale vector of Pearson correlation values between molecular gene status and drug inhibition values across cell lines. The ANOVA analysis was implemented in the GDSC software package (Iorio et al., 2016), and the biomarker is the vector of delta values comparing the difference of drug response by gene mutation status, or discrete status of gene expression.
For support vector regression (SVR), we trained linear epsilon-SVM using Python model scikit-learn. The input matrix $X$ is composed of gene expression and mutation status. The response $Y$ consists of drug inhibition values across cell lines. For CCLE screen, the dimension of $X$ matrix is 18,293 (gene variables of expression and mutation) x 493 (cell lines). For CTRP screen, the dimension of $X$ matrix is 18,293 x 821. For GDSC screen, the dimension of $X$ matrix is 31657 x 962. For each compound, the SVM training is done together with ten-fold cross validation (CV) with the high dimensional $X$ matrix. Using the linear kernel, we tuned the cost parameter among 1, 10, 100, 1000, and selected an optimal value with best CV R2 score. The biomarker from the linear SVM consists of the coefficients of all genes.

For elastic net, we trained one linear model for each compound, using ten-fold cross validation by R glmnet package with the same SVR input (Friedman et al., 2010). The parameter “alpha” controls the combination between the L1 penalty of lasso and the L2 penalty of ridge regression. We explored alpha values from 0 to 1 with 0.1 as the step and took the value with the best CV R2 score. The biomarker from elastic net consists of the coefficients of all genes.

Gene biomarkers from different genomics experiments
The gene biomarker from each genetic screen (CRISPR, CRISPRa, shRNA, siRNA, and ORF) consist of all gene log-fold-change values that test the difference of median guide RNA frequency between drug treatment and control conditions. The biomarker from each chronic cell line experiment consists of the gene differential expression t-values computed by Limma between the drug resistant cell line and parental sensitive line (Ritchie et al., 2015). For clinical studies (Hatzis et al., 2011; Miyake et al., 2012; Popovic et al., 2010), we tested the association between gene expression values and patient clinical response with logistic regression, and the biomarker comes from the t-values of gene coefficients.

Clinical response prediction through genome-scale biomarkers
For each genome-scale biomarker, the Pearson correlation between gene expression values and biomarker gene scores predicts the treatment response for each patient in a clinical study (Figure 1D). Positively correlated patients are responders, and negatively correlated patients are nonresponders. For each clinical study, we ranked all human genes according to the standard deviation of expression values across patients and only used the top 50% genes in a correlation analysis. Certain genomic screen studies did not release their data (Table S3) (Eichhorn et al., 2008; Moody et al., 2015). For these studies, we extracted the top gene hits reported in the publication and computed the average gene expression value over these top gene hits as therapy response prediction.

Comparison of methods for finding genes associated with drug efficacy
As the evaluation standard of gene signatures associated with response and resistance to targeted therapy, we utilized gene expression data from drug resistant cell lines derived from chronic treatment on sensitive cell lines. For microarray data, we used Limma to extract the differential expressed genes between drug resistant cell line and parental sensitive cell line (Ritchie et al., 2015). The evaluation standard positive set consists of the upregulated genes selected by Limma, and the evaluation standard negative set consists of those down-regulated genes.
For *BRAF* inhibitor, we used the data from a previous study that derived drug resistant cell lines from chronic drug treatment and compared the gene expression profiles between drug resistant cell lines and their parental lines (Nazarian et al., 2010). Among three drug resistant cell lines, only M229-R5 and M238-R1 (but not M249-R4) have more than ten differential upregulated genes with default Limma parameters. We used the intersection of differential expressed genes between M229-R5 and M238-R1 as the evaluation standard set for *BRAF* inhibitor. Comparing to the union, the overlap of differentially expressed genes could give a more consistent result in a third drug resistant cell line SKMel28 (Figure S4J, K) (Hugo et al., 2015). Many previously known genes involved in anti-*BRAF* resistance are differentially expressed in the M229-R5 and M238-R1 cell lines (Table S4B, C).

For lapatinib, we found microarray data of drug resistant cell lines derived from BT474 (Liu et al., 2009) and SKBR3 (Bailey et al., 2014), and used the overlap of differentially expressed genes between these two cell lines as evaluation standard. For topotecan, we found microarray data of drug resistant cell line derived from A2780 (Januchowski et al., 2014), and used the overlap of differentially expressed genes between two independent resistant clones as the evaluation standard.

Based on the evaluation standard defined above, we used receiver operating characteristic (ROC) curve to compare computational methods in predicting drug efficacy gene scores from compound screen data (Figure 4A). The ROC curve plots the true positive rate against the false positive rate at various thresholds of gene scores. If a method makes random predictions, the ROC curve will stay on diagonal. If a method makes perfect predictions, the ROC curve will stay on upper left corner. The area under ROC curve (AUC) can measure the overall performance of a method (Figure 4B).

**Data and Software Availability**

The open source CARE package is available under GNU Public License v3 on our website: [http://care.dfci.harvard.edu/download/](http://care.dfci.harvard.edu/download/). The source code for our Bliss synergy analysis is also available on the download page. Users can query our analysis results with drug or gene names on our website ([http://care.dfci.harvard.edu](http://care.dfci.harvard.edu)). Also, all of our processed input data, analysis output data, and an example script to repeat our major results are available at [http://care.dfci.harvard.edu/download/](http://care.dfci.harvard.edu/download/).

**Supplemental Movie Legends**

**Movie S1. Methodology of CARE framework, Related to Figure 1.**
Supplementary Information Inventory

1. Supplementary Figures and Tables.pdf: This file contains supplementary figures and tables related with figures and tables in the main manuscript.

2. Figure1_360.mp4: Figure 360 for Figure 1 in main manuscript.
Supplementary Figures

Figure S1. Compound screen data and drug target definition, Related to Figure 1.
(A) Overlap of compounds among three screen cohorts shown with Venn diagram. The numbers of compounds within sub-regions are also labeled.
(B) Consistency of PLX4720 response measurements between different cohorts. The drug response values, measured by area under dose response curve, are shown for PLX4720 across different cancer cell lines with CTRP results on X-axis and CCLE results on Y-axis. The consistency is computed with a Pearson correlation value.
(C) Consistency of drug response measurements among three cohorts. Between each combination of two cohorts, the Pearson correlations of drug response are shown for all targeted therapy compounds, with correlation 0.5 as the dotted reference line.
(D) Ordered correlations between gene features and EGFR inhibitor efficacy, with the relative position of EGFR expression marked as a black dot. Top: Erlotinib results in CCLE, Bottom: Gefitinib results in GDSC.
(E) EGFR inhibitor efficacy in different EGFR status groups. Top: Erlotinib results in CCLE, Bottom: Gefitinib results in GDSC. There are only five and four cell lines with gain-of-function mutations (Mut.GOF, exon 19 in-frame and L858R) for CCLE and GDSC cohorts, respectively. There is one cell line with T790M mutation driving EGFR inhibitor resistance in both cohorts. For EGFR wild-type cell lines, the category “WT.High” consists of samples with EGFR expression two-fold greater than average, and “WT.Low” consists of samples with EGFR expression lower than average. The p-value is computed from the two-sided Wilcoxon rank-sum test.
(F) Ordered correlations between gene features and palbociclib efficacy, with the relative ranks of RB1, CDK4 and CDK6 labeled as black dots. Top: results in CCLE; Bottom: results in GDSC.

Figure S2. Significant CARE scores for KRAS mutation, Related to Figure 2.
Among all compounds screened in three cohorts, the statistically significant CARE scores are shown for KRAS mutation, with the median value as a thick bar in the box-plot. The bottom and top of the boxes are the 25th and 75th percentiles (interquartile range). Whiskers on the top and bottom represent the maximum and minimum data points within 1.5 times the inter-quartile range.

Figure S3. CARE can predict clinical outcome of targeted therapies, Related to Figure 3.
(A) Association between CARE signature and anti-BRAF clinical outcome. Using the clinical data of 12 patients treated with vemurafenib (Hugo et al., 2015), we applied the Cox-PH regression to test the association between each gene signature (Table S3A) and progress-free survival without any cutoff. The z-score of each signature (hazard coefficient/standard deviation) is used as a performance metric.
(B) Prediction performance on anti-PD1 response correlates with the enrichment of EMT signature. For the CARE signature of each compound, its performance of predicting anti-PD1 response was measured with area under ROC curve (AUC) (Figure 3). The enrichment of epithelial-to-mesenchymal (EMT) transition was the normalized enrichment score (NES) from the GSEA analysis software (Subramanian
et al., 2005). In each screening cohort, the AUC and EMT enrichment of each compound are plotted on X and Y axes, with Pearson correlation between two axes as the title. The case of BRAF inhibitor on predicting anti-PD1 response in Figure 3 was labeled with red dot in the CCLE plot.

(C) Prediction accuracy on paclitaxel response. All patients are classified into four subgroups according to the status of pathological complete response (pCR) and predicted response (Pred), followed by the percentage of patients whose response status is consistent with CARE prediction.

(D) Consistency between replicates in CRISPRa screen. For the CRISPRa genome-wide screen (Konermann et al., 2015), the gene-wise fold change scores are shown for Puromycin selected replicate and Zeocin selected replicate. Pearson correlation between two replicates is shown as title.

Figure S4. Performance comparison between CARE and other computational methods, Related to Figure 4.

(A) Performance comparison based on variations of evaluation standards. For each drug, the evaluation standard gene sets are selected with different thresholds of false discovery rate (FDR) computed by Limma (Ritchie et al., 2015). The area under ROC curve (AUC) of results from different computational methods are compared.

(B) Performance comparison as panel A with different thresholds of log-fold change (logFC) that tests the differential gene expression between drug-resistant and parental cell lines.

(C) Gene features selected by Elastic Net regression. The non-zero coefficients of Elastic Net are plotted for each drug in CCLE. Coefficients of drug target genes are highlighted with thick dots.

(D) Performance of Ridge and LASSO regression in predicting the gene expression signature of drug-resistant cell lines for PLX4720 and lapatinib.

(E) Performance of different models in predicting genes associated with topotecan resistance. The performance of CARE, Interaction and Correlation are compared using ROC curves.

(F) Performance AUC metrics for both interaction effects and base effects (Correlation normalized by its standard deviation) in CARE model on predicting genes associates with topotecan resistance. The mean values of three screen cohorts are shown, with standard deviations as error bars.

(G) Fractions of cell lines in different lineages of CCLE and GDSC (COSMIC) collections.

(H) CARE performance of lineage-specific analysis. PLX4720’s chemical analog vemurafenib is primarily for skin cancer treatment, and lapatinib is mainly for breast cancer treatment. For each drug, the AUC metrics were shown for results using all cell lines and results using lineage-specific cell lines, with the mean and standard deviation (error bar) averaged among three screen cohorts.

(I) Performance of CARE after sub-sampling data. For each drug, the cell lines were sub-sampled, and the AUC metrics were computed under each sample rate. The mean and standard deviation were computed across three cohorts. A significant deterioration of performance happens after a down-sampling rate of 20%, which is higher than the fractions of most abundant lineage in panel G.

(J) Quality comparison between evaluation standard variations. Evaluation standard gene sets are created by taking either the union or overlap between two sets of differentially expressed genes in the drug-resistant M229-R5 and M238-R1 cell lines...
over their respective parental sensitive lines M229 and M238. To compare the quality of different evaluation standards, we used the differential gene expression profile of a third drug-resistant cell line SKMel28-R1 compared to its parental line SKMel28. The average log-fold changes (logFC) between SKMel28-R1 and its parental line are plotted for both positive and negative evaluation standards. (K) Prediction performance of SKMel28-R1 expression on the “overlap” or “union” evaluation standard. In the ROC curves, the prediction performance on “overlap” set is consistently higher than the performance on “union” set.

**Figure S5. Inhibition of PRKD3 increases anti-HER2 efficacy, Related to Figure 5.**

(A) Association between gene expression level and lapatinib clinical response. For each gene, the expression value is shown according to the pathological complete response status of patients, with median value in each group as a black bar. The bottom and top of the boxes are the 25th and 75th percentiles (interquartile range). Whiskers on the top and bottom represent the maximum and minimum data points within the range represented by 1.5 times the inter-quartile range.

(B) Inhibition effects on SKBR3 growth for PRKD inhibitors KBNB14270 and CRT0066101 at different concentrations. Each inhibition fraction was the median value from three replicate experiments with standard deviation as the error bar. N: none treatment control.

(C) Inhibition effects on SKBR3 growth for drug co-treatment compared with mono-treatment. The additive inhibition effect of co-treatment is estimated using Bliss independence model. Left: CRT0066101 in 3uM with lapatinib in different doses (2x dilution from 5uM); Middle: KBNB14270 in 5uM with trastuzumab in different doses (2x dilution from 20ug/mL); Right: CRT0066101 in 2 uM with trastuzumab in different doses. N: none treatment control.

(D) Bliss synergy scores between combinations of anti-HER2 drugs and PRKD inhibitors. Left: Lapatinib and CID20111756, Middle: Trastuzumab and KBNB14270, Right: Trastuzumab and CRT0066101.
Supplementary Tables

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<th>p-value</th>
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Table S1. Interaction test for multiple target genes of lapatinib, Related to Table 1 and Table 2.
The interactions between expression variables of lapatinib dual targets ERBB2, EGFR and the partner AXL are evaluated by linear regression with lapatinib efficacy as the outcome. The t-value is defined as regression coefficient divided by the standard error, and the p-value is calculated by the two-sided Student’s t-test.

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<td>24</td>
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<tr>
<td>CTRP</td>
<td>824</td>
<td>545</td>
</tr>
<tr>
<td>GDSC</td>
<td>990</td>
<td>250</td>
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Table S2. Number of cell lines and compounds in each cohort, Related to Figure 1.
The numbers of cell lines and compounds screened in each pharmacological cohort.
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<th>Treatment</th>
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<th>Genes</th>
<th>Pubmed</th>
<th>Data</th>
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<td>25494202</td>
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<tr>
<td></td>
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<td>shRNA screen</td>
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- **A. BRAF**

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<td>siRNA screen</td>
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- **B. ERBB2**

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<td>In-vivo CRISPR screen in mice</td>
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- **C. PDCD1**

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<td>siRNA.2 screen</td>
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<td>Clinical: Symmans</td>
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</table>

- **D. TUBB**

Table S3. Genomics signatures for clinical response prediction, Related to Figure 3.

For each targeted therapy, we collected published data related to drug response and resistance profiling. Column “Treatment” represents the drug used in an experiment. Column “Experiment” includes the brief description of experiments. CRISPR, shRNA, and siRNA are gene loss of function screens. ORF and CRISPRa are gene gain of function screens. For the in-vivo CRISPR screen on mice treated with immunotherapies, we translated all mouse top gene hits in supplementary table 1 of the publication (Manguso et al., 2017) into their human orthologs for further analysis. Chronic treatment represents differential gene expression profiling of drug resistant cell lines derived from chronic drug treatment compared with its parental sensitive cell lines. Column “Genes” tells the number of genes covered in each signature. Column “Data” indicates the availability of data with the study. If the data is not released, we extract the top gene hits reported in the publication as a gene set signature.

(A) **BRAF** inhibitors.

(B) **ERBB2** inhibitors.

(C) Anti-**PD1** antibody and GVAX vaccine.

(D) **TUBB** inhibitor paclitaxel.
### A. Computational results

<table>
<thead>
<tr>
<th>Role</th>
<th>CCLE</th>
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<th>GDSC</th>
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<td>CARE</td>
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<tr>
<td>Resistant</td>
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### B. M229-R5 differential expression.

<table>
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<th>p-value</th>
<th>adj.p-value</th>
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### C. M238-R1 differential expression.

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<th>p-value</th>
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**Table S4. Method comparison based on known genes associated with BRAF inhibitor efficacy, Related to Figure 4.**

Two previous studies have identified regulator genes of anti-BRAF resistance (Hugo et al., 2015; Sun et al., 2014). The type of association with drug efficacy is shown in the first column of each sub-table. If the activation of a gene is associated with a gain of drug resistance, the gene will be classified as "Resistant" markers. In contrast, if the loss of a gene is associated with a gain of drug resistance, the gene will be classified as "Sensitive" markers.

(A) Computational results from CARE and Elastic Net are shown across three cohorts. For CARE t-value, a negative score indicates the gene feature as a resistant marker, and a positive score indicates the gene feature as a sensitive marker.

(B) Differential gene expression between the M229-R5 drug resistant cell line and parental line M229. For these regulator genes, their gene expression values are compared between drug resistant cell lines and sensitive parental lines using the data from a previous study (Nazarian et al., 2010). The differential expression analyses were done with Limma (Ritchie et al., 2015). logFC represents the log-fold change of mRNA level between the drug resistant cell line and its parental sensitive cell line. The t-value was computed as logFC divided by standard deviation.
estimated in the linear model. The p-value represents the statistical significance for each t-value, followed with the adjusted p-value after multiple test correction.

(C) Differential gene expression between the M238-R1 drug resistant cell line and parental line M238 as panel B.
### Table S5. Druggable genes associated with lapatinib resistance, Related to Figure 5.

The first group of CARE score was computed using data from three compound screen cohorts. The second group ("Expression") of t-values are from Limma results, testing the differential gene expression between Lapatinib resistant cell line and parental line. The third group of t-values was calculated using Limma, testing the association between gene level and patient pathological complete response (pCR) status. All genes shown have commercial inhibitors available.

(A) Genes with consistently significant CARE scores.

(B) Other genes without the requirement of consistently significant CARE scores, with genes shown in panel A excluded.

<table>
<thead>
<tr>
<th>ID</th>
<th>CARE CCLE</th>
<th>CARE CTRP</th>
<th>CARE GDS</th>
<th>Expression BT474</th>
<th>Expression SKR6</th>
<th>PCR</th>
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</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>CARE CCLE</th>
<th>CARE CTRP</th>
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Graphical Abstract

Compound screen

CARE model
Inhibition = \( (a + c*P) \frac{T}{\text{slope}} + b*P \)

CARE score
Tumor expression

Find Drug Combinations

Predict Therapy Response

Inhibition

Survival Fraction

Drug against T

Drug against P

Drug

Compounds

Cell lines

Inhibition

Survival Fraction

Day

100 200 300

Neg

Pos
Figure 1

A

- **BRAF.V600E**
- **Others**


B

\[
\text{Inhibition} = a^*T + b^*P + c^*T^*P
\]

\[
= (a + c^*P)^*T + b^*P
\]

\[
c < 0
\]

\[
c > 0
\]

Inhibition

Target

Inhibition

Target

C

\[
\text{Inhibition} \mid T=1 = a + (b + c)^*P
\]

**CARE score**

\[
t_p = \frac{\hat{b} + \hat{c}}{s.e.(\hat{b} + \hat{c})}
\]

\[
s.e.(\hat{b} + \hat{c}) = \frac{\sqrt{\text{var}(\hat{b}) + \text{var}(\hat{c}) + 2\text{cov}(\hat{b}, \hat{c})}}
\]

D

**Drug against T**

**Response**

Yes

No

**Model**

**Correlation**

**CARE score**

**Tumor expression**

**Compound screen**

**genome-wide**
Figure 3

A. Vemurafenib

B. Vemurafenib

C. Lapatinib

D. Lapatinib

E. Pembrolizumab

F. Pembrolizumab

G. Predicted Responder

H. Predicted Non-responder

I. Paclitaxel

J. Paclitaxel

- Correlation
- PFS (day)
- Metrics (%)
- Accuracy
- AUC
- CARE
- CRISPRa
- CRISPR
- shRNA
- ORF
- Chronic
- Cyto
- -
- +
- YES
- NO
- Enrichment
- CARE score
- Gene score rank
- ESV: 3.121
- p-value < 0.001
- EMT genes
Figure 4

A

PLX4720

CARE Correlation GDSC SVR Elastic

Lapatinib

B

AUC

PLX4720

CARE Elastic SVR

Lapatinib

Vemurafenib Pembrolizumab Paclitaxel

C

Vemurafenib Lapatinib Pembrolizumab Paclitaxel

metrics (%) Accuracy AUC

CARE Correlation GDSC SVR Elastic

CARE Correlation GDSC SVR Elastic

CARE Correlation GDSC SVR Elastic
**Figure 5**

A. Expression of PRKD3 and NC.

B. Plot showing inhibition (%) of PRKD3 and NC with KBNB dose (uM) as x-axis and -log2(Lap dose/5uM) on the y-axis.

C. Plot showing inhibition (%) with CRT dose (uM) as x-axis and -log2(dose/5uM) on the y-axis.

D. Heatmap showing synergy (%) with KBNB dose (uM) on the x-axis and -log2(Lap dose/5uM) as the y-axis.

E. Heatmap showing synergy (%) with CRT dose (uM) on the x-axis and -log2(Lap dose/5uM) as the y-axis.

WB images showing anti-PRKD3 and anti-ACTB.
Figure S5

A

Expression

PRKD3  AKT3  AXL

+ pCR  ● No

B

KBNB14270

CRT066101

Dose (μM)

-10 10

-10 10

C

CRT + Lapatinib

KBNB + Trastuzumab

CRT + Trastuzumab

Inhibition (%)

0 20 40 60 80

0 20 40 60 80

0 20 40 60 80

Dose (μM)

-log2(Lapatinib dose/5μM)

-log2(Trastuzumab dose / 20ug/mL)

-log2(Trastuzumab dose / 20ug/mL)

Co-treatment  Additive  Mono-treatment

D

Synergy (%)

Synergy (%)

Synergy (%)

0 20 40 60 80

0 20 40 60 80

0 20 40 60 80

-log2(Lapatinib dose/5μM)

-log2(Trastuzumab dose / 20ug/mL)

-log2(Trastuzumab dose / 20ug/mL)