High-throughput identification of genotype-specific cancer vulnerabilities in mixtures of barcoded tumor cell lines

Channing Yu1–3, Aristotle M Mannan1, Griselda Metta Yvone1,6, Kenneth N Ross1,6, Yan-Ling Zhang1, Melissa A Marton1, Bradley R Taylor1, Andrew Crenshaw1, Joshua Z Gould1, Pablo Tamayo1, Barbara A Weir1, Aviad Tsherniak1, Bang Wong1, Levi A Garraway1–3, Alykhan F Shamji1, Michelle A Palmer1,6, Michael A Foley1,6, Wendy Winckler1, Stuart L Schreiber1,4,5, Andrew I Kung2,3,6 & Todd R Golub1–3,5

Hundreds of genetically characterized cell lines are available for the discovery of genotype-specific cancer vulnerabilities. However, screening large numbers of compounds against large numbers of cell lines is currently impractical, and such experiments are often difficult to control1–4. Here we report a method called PRISM that allows pooled screening of mixtures of cancer cell lines by labeling each cell line with 24-nucleotide barcodes. PRISM revealed the expected patterns of cell killing seen in conventional (unpooled) assays. In a screen of 102 cell lines across 8,400 compounds, PRISM led to the identification of BRD-7880 as a potent and highly specific inhibitor of aurora kinases B and C. Cell line pools also efficiently formed tumors as xenografts, and PRISM recapitulated the expected pattern of erlotinib sensitivity in vivo.

Cell lines have long served as tools for oncology drug discovery5. Their track record in predicting efficacy in patients, however, has been mixed. Cell line models often have been chosen more for their tractability than for their reflection of genetic diversity across human cancers. It is not surprising that generalizations from studies of few cell lines have met with limited success. With the ability to comprehensively characterize the genomes of tumors has come the recognition that the varied responses of patients to treatment are largely explained by the genetic diversity of cancer. Recent reports show that this diversity is reflected in established cell lines3. Although it is possible to profile the activity of compounds across large numbers of cell lines and then correlate response to specific genetic features3,4,6,7, explaining patterns of drug sensitivity observed in cell lines cultured alone, we reasoned that such concerns would be outweighed by the throughput advantage of a pooled approach. We also note that in vivo, tumors grow not as uniform masses of cells but rather as complex mixtures of genetically diverse tumor cells8 and nonmalignant cells of the tumor microenvironment.

To deconvolute pools of cancer cell lines, we used molecular barcoding because of the nearly limitless number of barcodes and the availability of a flexible, high-throughput (500-plex) barcode detection system based on Luminex microspheres, which have been used in multiplexed assays of gene expression9 and protein phosphorylation10, and in genetic screens of PI3-kinase inhibitor activity11.

We designed the PRISM assay using lentiviral vectors that permitted stable integration of 24-base-pair DNA barcode sequences engineered to have limited sequence homology to the human genome. Common primer sites flanking the barcode sequence allowed for amplification of barcodes with a single set of primers (including one biotinylated primer). We hybridized the amplicons to Luminex microspheres of distinct colors (each coupled to a different anti-barcode oligonucleotide) and stained with phycoerythrin-streptavidin. We quantified hybridization events on a Luminex detector, wherein the bead color denotes the barcode identity, and the phycoerythrin intensity reveals barcode abundance, a direct reflection of cell number (Fig. 1).

To establish feasibility and determine the sensitivity of PRISM, we analyzed five barcoded adenocarcinoma cell lines. We plated 1,000 cells each of four barcoded lines as a mixture and added the fifth line in varying number. The following day, we collected genomic DNA from the mixtures, and detected the barcodes. As predicted, the four invariant lines showed similar signals in all mixtures, whereas the
PRISM method. 24-base-pair DNA barcodes encoded within lentiviruses are stably integrated into individual tumor cell lines after blasticidin selection, and barcoded cell lines are individually frozen and later thawed to generate mixtures of equal numbers of barcoded cell lines, which are frozen again. thawed mixtures are plated and then rearrayed into tissue culture assay plates. Mixtures are treated with test compounds or vehicle (DMSO) controls. At assay conclusion, genomic DNA is harvested from the mixture of remaining viable cells. Barcode sequences are amplified using PCR and universal primers (one of which is biotinylated), and amplified sequences are hybridized to individual microbeads harboring antisense barcode sequences and then to streptavidin-phycoerythrin. A Luminex FlexMap detector quantitates fluorescence signal for each bead. To adjust for differing barcoding efficiencies and differing cell doubling, the signal for each barcoded cell line is scaled to that of vehicle-treated control, thus demonstrating relative inhibition profiles for specific test compounds across multiple cell lines in mixture.

varied fifth line gave a signal directly proportional to cell number (Fig. 2a). The assay was highly sensitive, enabling detection of as few as 10 cells in a mixture of 4,000 (i.e., <0.5% of the total cell number).

We next treated a pool of 25 lung adenocarcinoma cell lines with compounds known to have genotype-specific patterns of killing. To account for different doubling times of cell lines, we compared each compound-treated cell line to vehicle-treated controls to compute the relative growth inhibition of each line. Whereas treatment with puromycin resulted in uniform killing across the pool (Fig. 2b), treatment with the EGFR inhibitor erlotinib (Tarceva) resulted in the dose-dependent killing of the four EGFR-mutant cell lines in the pool, concordant with previous studies (Fig. 2b and Supplementary Fig. 1). Similarly, we observed another expected pattern of cell killing12 with the ALK kinase inhibitor NVP-TAE-684 (Fig. 2b): the NCI-H3122 cell line, harboring an EML4-ALK translocation, exhibited sensitive to the drug, whereas NCI-H2228, with a different EML4-ALK translocation, exhibited intrinsic resistance.

To test whether PRISM can recapitulate the results observed in traditional cell line experiments, we created a panel of 100 barcoded cell lines comprising 18 lineages and challenged these in four pools of 25 cell lines with each of 43 anticancer compounds (including both targeted and cytotoxic agents), yielding 3,200 measurements per compound (Supplementary Table 1). We saw no evidence of PRISM performance varying as a function of tumor type or cell lineage, although larger panels of cell lines would be required to exclude this definitively. As expected, PRISM revealed similar patterns of activity across the 100 lines among functionally related compounds (e.g., microtubule binders, topoisomerase inhibitors or MEK inhibitors; Supplementary Fig. 2a). For 23 compounds, we had access to sensitivity data across the same 100 cell lines measured by others in individual cell line assays measuring either ATP content (using CellTiter-Glo) or enumeration of cell nuclei (using an optical fluorescent imaging method, Opera)13.14

Using area under the curve (AUC) as a measure, the traditional ATP content and number of nuclei (below referred as 'nuclei') readouts yielded similar global patterns of sensitivity (Pearson r = 0.80, P < 0.0001). PRISM yielded similar levels of global correlation (r = 0.72 compared to nuclei, P < 0.0001; r = 0.66 compared to ATP, P < 0.0001) (Fig. 2c and Supplementary Table 2). We note that the slightly stronger correlation between PRISM and nuclei data is expected because PRISM and nuclei are both direct readouts of cell number, whereas ATP measurement reflects a combination of cell number and metabolic activity.

For example, PRISM and nuclei similarly identified hypersensitivity of BRAF-mutant melanoma cell lines to the BRAF inhibitor PLX4720 (Supplementary Fig. 2b). We saw no significant differential sensitivity (two-tailed t-test, P > 0.05) to the RAF inhibitor sorafenib (Nexavar), now known to be only a weak inhibitor of the BRAF kinase14 (Supplementary Fig. 2b). Similarly, PRISM detected a trend (P = 0.054) between BRAF mutation and sensitivity to the MEK inhibitor AZD6244 (acting immediately downstream of BRAF), findings consistent with clinical activity in patients with BRAF-mutant melanoma15 (Supplementary Fig. 2b). PRISM and traditional methods yielded concordant results for 21/23 of compounds tested (91%), but two drugs (topotecan (Hycamtin) and paclitaxel (Taxol)) showed slightly discordant results (Supplementary Fig. 3). Whether these exceptions arise from the 3-d (nuclei) versus 5-d (PRISM) assay periods remains to be determined.

We next asked whether the PRISM approach could be extended to the in vivo setting, where the ability to multiplex cell lines in a single xenotransplant might accelerate translational research. One theoretical concern was that a small number of cell lines within a pool might rapidly overtake the others in vivo. To test this, we injected a pool of 24 barcoded lung adenocarcinoma cell lines subcutaneously into each of ten recipient NSG (NOD-SCID-IL2Rgamma null) mice. Several weeks later, we euthanized the mice and resected the tumors. PRISM detected 23/24 cell lines (96%) in each of ten vehicle-treated mouse tumors. The 23 detectable lines grew at different rates, but their relative abundances within the tumors were nearly identical across the ten vehicle-treated xenografts (Fig. 2d). Similarly, we sampled four different portions of each tumor, and found little variation in the contribution of particular cell lines in different parts of the tumor (e.g., Supplementary Fig. 4). We also passaged an aliquot of the initial mixture of cell lines used for injection in vivo weekly for 3 months. As with the in vivo experiments, the vast majority of the initial cell lines were detectable after 98 d in culture, and the abundance of each line remained stable over time (Supplementary Fig. 5). These results...
suggest that despite differences in growth rates, cell mixtures may reach an equilibrium state.

Next, we investigated whether PRISM could detect the expected sensitivity of EGFR-mutant cell lines when treated with erlotinib in vivo. We treated cohorts of ten mice daily for 16 d by gavage with erlotinib versus vehicle. Erlotinib treatment caused a marked reduction in relative abundance of 4/4 EGFR-mutant lines (by 22%, 88%, 75% and 76%), whereas the 15/19 detectable wild-type EGFR lines were either minimally affected or increased in proportional abundance (Fig. 2d). As a group, mutant EGFR lines were significantly different from wild-type EGFR lines (Kolmogorov-Smirnov test, D = 0.84, P = 0.0079; Fig. 2e). Notably, the EGFR-mutant line showing the smallest in vivo response to erlotinib (HCC2935) was also less sensitive to erlotinib in vitro (cf. Fig. 2b). These experiments demonstrate the feasibility of PRISM to assess drug sensitivity in vivo.

We next examined whether PRISM could be used not only to elucidate the differential cytotoxic activity of optimized compounds (drugs) but also to discover new anticancer agents. We tested 102 barcoded lines (90 non-small cell lung adenocarcinoma (NSCLC) (drugs) but also to discover new anticancer agents. We tested 102 candidate the differential cytotoxic activity of optimized compounds to reveal genotype–phenotype relationships. We observed expected relations-
We next turned to the results of the 8,000 DOS compounds screened against 102 cell lines. One 199 of the DOS compounds (2.5%) scored as hits in the primary screen (defined as at least one cell line being inhibited > 80% relative to control) and 139/199 (69.8%) compounds validated in a 8-point PRISM dose-response assay (Supplementary Table 6). Of the 139 compounds, 49 (24.5%) killed > 70% of the cell lines, suggesting that they were nonspecific cytotoxic agents. One of the 90 selective compounds, we examined BRD-7880 in detail.

First, we asked whether the PRISM sensitivity profile across 102 cell lines could be used to gain insight into BRD-7880’s mechanism of action by comparing its sensitivity profile to those of 400 tool compounds profiled in these same lines. BRD-7880 showed markedly similar activity to the aurora kinase inhibitor tozasertib (VX-680; Spearman $r = 0.77$, Fig. 3a,b and Supplementary Table 7), suggesting that despite the lack of structural similarity between the two compounds (Fig. 3c), BRD-7880 might be an aurora kinase inhibitor. Treatment of HCT-116 cells with BRD-7880 resulted in polyploidy (Supplementary Fig. 10a) and decreased phosphorylation of serine 10 in histone H3 (Supplementary Fig. 10b), supporting its functioning as an inhibitor of aurora kinase B (AURKB)$^{21,23}$.

Biochemical kinase activity assays showed that BRD-7880 is a potent inhibitor of AURKB and AURKC (half-maximal inhibitory concentration (IC$_{50}$) of 7 nM and 12 nM, respectively) with less activity against AURKA (IC$_{50}$ = 2,153 nM) (Fig. 3d), a profile resembling that of barasertib (AZD1152-HQPA; Supplementary Fig. 11). Kinetic measurements of in vitro AURKB activity suggested that BRD-7880 functions in an ATP-competitive manner (Supplementary Fig. 12). To assess the specificity of BRD-7880, we profiled kinase activity for 308 kinases, and this analysis showed that BRD-7880 is far more selective than tozasertib, substantially inhibiting (to < 25% control activity) only AURK and AURKC (Supplementary Table 8). Similarly, in a screen of kinase-binding selectivity across 98 kinases BRD-7880 showed highly specific binding to AURKB and AURKC (Fig. 3e and Supplementary Table 9). We are unaware of any other aurora kinase that of barasertib (AZD1152-HQPA; Supplementary Fig. 11). Kinetic measurements of in vitro AURKB activity suggested that BRD-7880 functions in an ATP-competitive manner (Supplementary Fig. 12). To assess the specificity of BRD-7880, we profiled kinase activity for 308 kinases, and this analysis showed that BRD-7880 is far more selective than tozasertib, substantially inhibiting (to < 25% control activity) only AURK and AURKC (Supplementary Table 8). Similarly, in a screen of kinase-binding selectivity across 98 kinases BRD-7880 showed highly specific binding to AURKB and AURKC (Fig. 3e and Supplementary Table 9). We are unaware of any other aurora kinase
inhibitor with this degree of specificity. The result also demonstrates the utility of PRISM for rapidly identifying a molecular target: the target of BRD-7880 was revealed simply by virtue of its pattern of activity across a large panel of cell lines. Such activity would not have been obvious had the compound been tested on only a small number of cell lines.

PRISM will facilitate oncology drug discovery by making it feasible to rapidly test chemical analogs across an entire cell line panel, thus assuring that the expected on-target pattern of activity is retained. The facile expansion of a single vial of pooled, barcoded cells provides a practical solution for extending traditional compound screening to hundreds of individual cell lines. Furthermore, our demonstration of the feasibility of using PRISM in vivo suggests that cost-effective xenograft studies are possible. The bead-based barcode quantitation method used here has proven reliable and inexpensive, but further cost reductions will likely be achievable with massively parallel sequencing.

PRISM may facilitate cancer therapeutic discovery. Entire small-molecule libraries could be screened across large panels of cell lines, and compounds selected for their differential killing (e.g., selectively killing cells harboring ‘undruggable’ targets). We believe that the cancer research community would benefit from the creation of thousands of genetically characterized, barcoded cell lines. With such a resource, large-scale testing of compounds across the diversity of human cancer types could become a routine activity.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We thank S. Kim, G. Bonamy, J. (J.) Che, J. Thibault, T. Haynah, I. Engels and A. Shipway at Novartis for sharing data before publication; A. Christie and T. Davis for technical assistance in animal studies; C. Hartland, S. Donovan, E. Rubin and E. Winchester for technical assistance in compound assays; J. Bittker, J. McGrath and G. Wendel for assistance in compound management; S. Le Quement and E. Winchester for technical assistance in compound assays; J. Gale for technical assistance in enzyme kinetic assays; S. Howell for assistance in curation of computational data sets; A. Koehler, S. Dandapani, B. Muñoz, C. Scherer, D. Gray, D. Bachovchin, S. Santaguida and J. Elkins for expert scientific guidance; J. Barretina, N. Stransky, S. Nijman, B. Julian, W. Read-Button, J. Davis and D. Peck for technical advice; and S. Santaguida and J. Elkins for expert scientific guidance; J. Barretina, N. Stransky, S. Nijman, B. Julian, W. Read-Button, J. Davis and D. Peck for technical advice; and members of the Golub laboratory for critical review of the manuscript. This work was supported in part by the US National Institutes of Health (NIH) Genomics Based Drug Discovery consortium grants R01-CA133834, R01-GM084437 and UL1DE019585 (administratively linked to NIH grant R01-HG004671), US National Cancer Institute Integrative Cancer Biology Program grant U54CA112962, the Howard Hughes Medical Institute, the Claudia Adams Barr Program in Cancer Research Innovative Basic Science Research Program Grant, the American Society of Clinical Oncology Conquer Cancer Foundation Young Investigator Award and the Prostate Cancer Foundation.

AUTHOR CONTRIBUTIONS

C.Y. and T.R.G. designed the PRISM method and wrote the manuscript. C.Y., G.M.Y. and A.M.M. performed the experiments in the study. L.A.G. provided cell lines and drug response validation data from the Cancer Cell Line Encyclopedia Project. B.A.W. performed cell line genotype verification analyses. K.N.R. and P.T. contributed to statistical analyses of PRISM validation data. J.Z.G. and C.Y. created data processing and data visualization tools. M.A.P, W.W., A.C., M.A.M., B.R.T., G.M.Y., A.M.M. and C.Y. performed the large-scale PRISM screen. A.T. and C.Y. performed genomic correlation analyses in the large-scale PRISM screen. A.F.S. and S.L.S. contributed to compound creation and curation and design of experiments with BRD-7880. Y.-L.Z. performed kinetic kinase inhibition experiments with BRD-7880. A.L.K., C.Y. and T.R.G. contributed to design and execution of in vivo PRISM experiments. B.W. contributed to data visualization tools and to manuscript figures. All authors discussed the results and commented on the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. McDermott, U. et al. Identification of genotype-correlated sensitivity to selective kinase inhibitors by using high-throughput tumor cell line profiling. Proc. Natl. Acad. Sci. USA 104, 19936–19941 (2007).
2. Kos, M. et al. Predicting drug susceptibility of non-small cell lung cancers based on genetic lesions. J. Clin. Invest. 119, 1727–1740 (2009).
3. Barretina, J. et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 483, 603–607 (2012).
4. Garnett, M.J. et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature 483, 570–575 (2012).
5. Sharma, S.V., Haber, D.A. & Settleman, J. Cell line-based platforms to evaluate the therapeutic efficacy of candidate anticancer agents. Nat. Rev. Cancer 10, 241–253 (2010).
6. Abaan, O.D. et al. The exomes of the NCI-60 panel: a genomic resource for cancer biology and systems pharmacology. Cancer Res. 73, 4372–4382 (2013).
7. Basu, A. et al. An interactive resource to identify cancer genetic and lineage dependencies targeted by small molecules. Cell 154, 1151–1161 (2013).
8. Turner, N.C. & Reis-Filho, J.S. Genetic heterogeneity and cancer drug resistance. Lancet Oncol. 13, e178–e185 (2012).
9. Peck, D. et al. A method for high-throughput gene expression signature analysis. Genome Biol. 7, R61 (2006).
10. Du, J. et al. Bead-based profiling of tyrosine kinase phosphorylation identifies SRC as a potential target for glioblastoma therapy. Nat. Biotechnol. 27, 77–83 (2009).
11. Mueller, M.K. et al. A chemical-genetic screen reveals a mechanism of resistance to PI3K inhibitors in cancer. Nat. Chem. Biol. 7, 787–793 (2011).
12. Komuveni, J.P. et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin. Cancer Res. 14, 4275–4283 (2008).
13. http://www.broadinstitute.org/ccle
14. Wilhelm, S.M. et al. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol. Cancer Ther. 7, 3129–3140 (2008).
15. Flaherty, K.T. et al. METRIC Study Group. Improved survival with MEK inhibition in BRAF-mutated melanoma. N. Engl. J. Med. 367, 107–114 (2012).
16. Comer, E. et al. Fragment-based domain shuffling approach for the synthesis of pan-kinase inhibitors. Science 332, 651–656 (2011).
17. Lowe, J.T. et al. Synthesis and profiling of a diverse collection of azetidine-based scaffolds for the development of CNS-focused lead-like libraries. J. Org. Chem. 77, 7187–7212 (2012).
18. Darcareille, L.A. et al. An aldol-based build/couple/pair strategy for the synthesis of medium- and large-sized rings: discovery of macrocyclic histone deacetylase inhibitors. J. Am. Chem. Soc. 132, 16962–16976 (2010).
19. Schreiber, S.L. et al. Cancer Target Discovery and Development Network. Towards patient-based cancer therapeutics. Nat. Biotechnol. 28, 904–906 (2010).
20. Zhang, X.D. Illustration of SSMD, z score, SSMD*, z* score, and t statistic for hit selection in RNAi high-throughput screens. J. Biomol. Screen. 16, 775–785 (2011).
21. Andrews, P., Knatko, E., Moore, W.J. & Swedlow, J.R. Mitotic mechanics: the role of the Aurora kinases. Nat. Rev. Mol. Cell Biol. 12, 904–906 (2011).
22. Carmena, M. & Earnshaw, W.C. The cellular geography of aurora kinases. Nat. Rev. Mol. Cell Biol. 4, 482–484 (2003).
23. Ditchfield, C. et al. Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and CenP-E to kinetochores. J. Cell Biol. 161, 267–280 (2003).
24. David, D.M. et al. Comprehensive analysis of kinase inhibitor selectivity. Nat. Biotechnol. 29, 1046–1051 (2011).
25. Karaman, M.W. et al. A quantitative analysis of kinase inhibitor selectivity. Nat. Biotechnol. 26, 127–132 (2008).
ONLINE METHODS

Lentiviral barcoding vector. A 6.4-kb Miul-Clal fragment was isolated from pLent6.2/VSD34 (Invitrogen) and ligated to a linker comprising oligonucleotides 5’-CGATAACTGCAGACCCATGCATGGA-3’ and 5’-CGCGTCAATGCTTGTTCTGCAGATTAT-3’. A library of Miul-Pst linkers was constructed using 24-bp Lumines DNA barcodes2 placed within oligonucleotides 5’-CGCGTXXXXXgXXXXXXgXXCGCA-3’ and 5’-gXXXXXXXXXXgXXXXXgX-3’, where the X tract includes the sense barcode sequence and x tract includes the antisense barcode sequence. Each of these linkers was individually ligated into the Miul-Pst backbone of the above vector to generate lentiviral barcoding plasmids. Lentivirus was generated from lentiviral barcoding plasmids as previously described (at http://www.broadinstitute.org/maiz/public/resources/protocols choosing shRNA/sgRNA/ORF Low Throughput Viral Production (10cm dish/6 well)) using pCMV-dR8.2 dvpr and pCMV-NSG packaging vectors in FuGENE6-transfected (Roche Corporation) HEK-293T cells; viral supernatant was collected after 72 h, passed through a sterile 0.45-μm syringe filter (VWR cat. 28144-007), and stored at ~80 °C.

Cell lines. Cell lines were obtained through the American Type Culture Collection (ATCC) or provided by the Broad-Novartis Cancer Cell Line Encyclopedia4 and cultured in HEPES-buffered RPMI medium (ATCC cat. 30-2001) containing 10% heat-inactivated FBS (Sigma cat. F5410) and penicillin-streptomycin G (Invitrogen cat. 10378-016). Drug sensitivity data of CCLE lines, using SNP fingerprint analysis were used as profiles to query previously reported genome-wide features (gene expression and copy number) of cell lines in the Cancer Cell Line Encyclopedia13. Among the pairs of duplicate cell lines, the cell line with the higher baseline PRISM signal was selected for genomic correlation analysis. Three cell lines which showed markedly decreased baseline signals in control wells (COR-L23 (994), NC1-H228 (029) and NC1-H661 (051)) were excluded from correlation analyses. Spearman’s rank correlation was computed using the PRISM AUC measurement from each compound versus either gene expression or copy number, and the significance of correlation was calculated using permutation testing with 106 iterations. All genomic data for these cell lines are available at http://www.broadinstitute.org/ccle.

Correlation of PRISM profiles with genomic features. PRISM viability measurements in cell lines verified to be identical to Cancer Cell Line Encyclopedia lines using SNP fingerprint analysis were used as profiles to query previously reported genome-wide features (gene expression and copy number) of cell lines in the Cancer Cell Line Encyclopedia13. Among the pairs of duplicate cell lines, the correlation of PRISM profiles with genomic features was calculated. For each compound versus either gene expression or copy number, the significance of correlation was calculated using permutation testing with 106 iterations. All genomic data for these cell lines are available at http://www.broadinstitute.org/ccle.

PRISM compound assays. Frozen mixtures containing randomly chosen assortments of 25–27 barcoded cell lines were thawed (day −2) and replated (day −1) into 384-well microtiter plates at 50 cells per well in per well. On day 0, compounds suspended in DMSO were pipetted into cultures to achieve 8–16 concentrations. On day 5, cells were washed with phosphate-buffered saline and lysed for 60 min at 60 °C in 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.45% IGEPEL CA-630 (Sigma cat. 18896), 0.45% Tween-20 (Sigma cat. P9416) and 10% proteinase K (Qiagen cat. 19133). Proteinase K activity was achieved by incubation at 37 °C for 2–4 weeks until no sham-infected cells survived. Barcoded lines were frozen individually and later frozen as frozen pools.

SNP fingerprinting of cell lines. For confirmation of cell line identity, we used 4 HX Fluidigm IPC chip loaders and 4 FC1 cyclers for the 96.96 dynamic array. The reference set of SNP genotypes was derived from the Affymetrix SNP6.0 array Birdseed genotypes from the Cancer Cell Line Encyclopedia (CCLE)12,13. Birdseed genotypes for 42 SNPs were used as references for cell line identity. Fingerprints (genotypes for those same SNPs) assayed by Fluidigm after fingerprinting were extracted and compared to the reference set of SNPs across all CCLE lines, using the GenePattern FPmatching module at http://genepattern.broadinstitute.org/gpp.

PRISM detection. Detailed protocols of the PRISM method are available in Supplementary Methods. Genomic DNA from cell lysates was amplified by PCR using primers Biotin pLENT4R (5’-biotin-CGTCATTACTAAAC00GTAACG-3’) and pLENT4F1 (5’-GGAAATAAGAAGAAGAGGTGC-3’). PCR product was hybridized to Lumines beads with covalently attached antisense barcodes, and streptavidin–phycocerythrin addition, washing and detection on Lumines FlexMap machines was performed as previously described9. PCR without genomic DNA was hybridized with beads to serve as background control; signal for each bead was subtracted from each sample measurement. DMSO-treated cell mixtures were used as reference control for scaling of each cell line signal at the conclusion of each experiment (viability = 100 for each cell line). Thus the signal from each treated cell line was calculated as 100 × (median Lumines measurement across replicates) – (median Lumines measurement of no DNA control)/(median Lumines measurement of DMSO control).

In vitro kinase inhibition assays. Incorporation of radioactivity from 10 μM γ-32P-ATP was measured in in vitro kinase assays across eight doses in duplicate by the EMD Millipore KinaseProfiler service under published standard conditions with 10 nM ATP. Full-length human AURKA was assayed with 200 μM LRRASLG (Kemptide); full-length human AURKB with 30 μM AKRRLLSLRA; and full-length human AKURC with 30 μM AKRRLLSLRA. IC50 values were modeled using least-squares and variable slope with Prism 6.0 software (GraphPad).

KinaseProfiler profiling. Specificity of in vitro kinase inhibition by BRD-7880 (30 nM) or tozasertib (30 nM) was performed by EMD Millipore using standardized protocols.

KinomeScan profiling. Kinase binding was performed by DiscoveRx using their KinomeScan method, using the scanEDGE profile (97 kinases) plus the inclusion of AKURC (total 98 kinases). Images were generated using TREESpot Software Tool and reprinted with permission from KINOMEScan, a division of DiscoveRx Corporation.

AURKB in vitro kinase assays. Enzyme kinetic experiments were performed at pH 7.0 in 8 mM MOPS buffer with 0.2 mM EDTA and 10 mM magnesium acetate. Reactions were assembled in 384-well plate wells by adding 400 ng/ml of AURKB (EMD Millipore cat. no. 14-835) into separate reaction mixtures containing 1.5 μM fluorescently labeled Caliper peptide substrate (FL-peptide 1, 5-FAM-AKKRLLSLRA-COOH, Perkin-Elmer cat. no. 760345) with various concentrations of ATP and compound (BRD-7880, tozasertib, barasertib). The final ATP concentrations varied from 6.25 μM to 200 μM and compound varied from 0 nM to 200 nM. Plates were immediately placed into a
Perkin-Elmer Caliper LabChip EZ Reader and wells were sampled periodically throughout a 1-h reaction period for initial reaction rate. The fluorescent product and substrate were separated and monitored on the Caliper microfluidic instrument. The conversion of substrate was calculated with Caliper software. $K_m$ and $k_i$ values were determined from the double reciprocal Lineweaver-Burk plot by linear regression with GraFit 6 software (Erithacus Software Ltd.) using competitive inhibition equation modeling.

**DNA content analysis.** HCT-116 cells were treated with 10 µM of DMSO, barasertib, GSK1070916, MLN8054, BRD-7880 or tozasertib. 24 h or 48 h following treatment, cells were stained with propidium iodide and DNA content per cell was assessed using a BD LSR II flow cytometer (BD Biosciences).

**Immunoblotting.** HCT-116 cells were treated with 10 µM of DMSO, barasertib, GSK1070916, MLN8054, BRD-7880 or tozasertib. Cells lysates were probed on immunoblot using antibodies (diluted 1:1,000) to histone H3 (Abcam cat. 24834, 1Degree Bio no. 1DB-001-0000112469), phosphoserine10-histone H3 (Cell Signaling Technology cat no. 3377, 1DegreeBio no. 1DB-001-0000808394), aurora kinase B (Millipore cat. no. 04-1036, 1DegreeBio no. 1DB-001-0000847547) or beta-actin (Santa Cruz Biotechnology cat. no. sc-47778, 1DegreeBio no. 1DB-001-000003510), and detected using a LI-COR Odyssey analyzer (LI-COR Biosciences).

26. Korn, J.M. et al. Integrated genotype calling and association analysis of SNPs, common copy number polymorphisms and rare CNVs. *Nat. Genet.* **40**, 1253–1260 (2008).