Diverse drug-resistance mechanisms can emerge from drug-tolerant cancer persister cells

Michael Ramirez1,2,*, Satwik Rajaram1,2,*, Robert J. Steininger2,*, Daria Osipchuk1, Maike A. Roth1, Leanna S. Morinishi1, Louise Evans1, Weiyue Ji1, Chien-Hsiang Hsu1, Kevin Thurley1, Shuguang Wei3, Anwu Zhou3, Prasad R. Koduru4, Bruce A. Posner3, Lani F. Wu1,2 & Steven J. Altschuler1,2

Cancer therapy has traditionally focused on eliminating fast-growing populations of cells. Yet, an increasing body of evidence suggests that small subpopulations of cancer cells can evade strong selective drug pressure by entering a ‘persister’ state of negligible growth. This drug-tolerant state has been hypothesized to be part of an initial strategy towards eventual acquisition of bona fide drug-resistance mechanisms. However, the diversity of drug-resistance mechanisms that can expand from a persister bottleneck is unknown. Here we compare persister-derived, erlotinib-resistant colonies that arose from a single, EGFR-addicted lung cancer cell. We find, using a combination of large-scale drug screening and whole-exome sequencing, that our erlotinib-resistant colonies acquired diverse resistance mechanisms, including the most commonly observed clinical resistance mechanisms. Thus, the drug-tolerant persister state does not limit—and may even provide a latent reservoir of cells for—the emergence of heterogeneous drug-resistance mechanisms.
The emergence of diverse resistance mechanisms to targeted therapy is one of the foremost challenges in cancer today. Within the same patient or even tumour, multiple mechanisms of drug resistance can coexist. Random, resistance-conferring genetic events preceding drug treatment are an unquestionable means by which this diversity can occur. Yet, identifying alternate routes by which cancer cell populations can arrive at resistance mechanisms is of key interest.

One recently proposed alternative route for acquiring resistance is via a drug-tolerant persister state. Across multiple cell lines, in response to a variety of strong drug challenges, small subpopulations of cells have been reported to survive by initially entering a drug-tolerant (so-called) ‘persister’ state in which there is little-to-no population growth. Crucially, after long-term treatment (weeks to months) in drug without appreciable growth, a fraction of persisters gain the ability to expand in drug. It has been hypothesized, but never demonstrated experimentally, that survival and expansion through a drug-tolerant state could be part of an initial strategy that mediates the acquisition of bona fide, genetically driven, resistance mechanisms. However, the diversity of resistance mechanisms compatible with evolution from (or through) a persister bottleneck is unclear. Previous work examined pooled populations of drug-tolerant cells expanded from persisters and did not address this question. Does passage through the persister bottleneck in drug force cells into a single genetic/epigenetic state, or can multiple genetic resistance mechanisms eventually emerge?

Here we chose a strategy that allowed us to follow multiple instances of evolution in drug and focus on resistance mechanisms that emerged from a persister state. We began with a population of cancer cells of cancer cells that were recently clonally derived. Next, we ‘crashed’ the clonal population with a targeted therapy to reveal a small subpopulation of persisters, some of which eventually gained the ability to proliferate in drug. Finally, to avoid growth competition, we expanded surviving colonies in isolation from one another in drug over the course of a year. This process allowed us to establish a panel of 17 persister-derived, drug-resistant colonies that arose from a single cell. We used a combination of large-scale drug screening and whole-exome sequencing to show that our drug-resistant colonies exhibited diverse resistance mechanisms, including ones observed clinically. Our results suggest that the drug-tolerant persister state does not limit—and may even provide a latent reservoir of cells for—the emergence of heterogeneous drug-resistance mechanisms.

Results

Drug resistance emerges from drug-tolerant persisters. We chose as our model system the well-studied, epidermal growth factor receptor (EGFR)-addicted non-small cell lung cancer (NSCLC) cell line PC9 (ref. 15) for several reasons. It has been shown that a small fraction of PC9 cells (~0.5%) can enter a persister state to evade the strong selective pressure of high concentrations of the EGFR inhibitor erlotinib (2.5 \( \mu \)M, ~100 × IC50). In addition, known resistance-conferring genetic mutations can serve as a reference against which to evaluate any emergent genetic diversity.

For our study, we followed the previously established procedure with two crucial changes, which allowed us to focus on individual resistance solutions that emerged from persisters (Methods; Supplementary Fig. 1). First, to reduce pre-existing genetic heterogeneity, we established our persisters from a single, short-passage clonal parental cell line, PC9-1 (~20 doublings from the single, originating cell). Second, to search for diversity not evident from pooled-population studies, we isolated small, recently expanded colonies that emerged ~2 months after seeding and expanded them in separate culture wells to eliminate growth competition. (Except when noted otherwise, colonies were cultured and assayed in 2.5 \( \mu \)M erlotinib.)

Similar to previous observations, only a small fraction of cells survived drug treatment (2.5 \( \mu \)M erlotinib; Supplementary Fig. 2a); drug-tolerant cells were largely in a state of negligible growth during the first 6 weeks of observation; and drug tolerance could be abolished with co-treatment of erlotinib with trichostatin A (Supplementary Fig. 2b). Of the ~50 colonies originally isolated, 17 survived the expansion process, which took ~6–8 months to generate three confluent 10-cm plates each. We refer to these persister-derived erlotinib-resistant colonies as PERCs.

We tested whether our isolated PERCs were in the previously described, reversible state of drug resistance. A functional signature of this state is eventual reversion to erlotinib sensitivity after an extended (30 passages) ‘drug holiday’ (as opposed to non-reverting resistance due to clonal genetic events). We continuously cultured the 17 PERCs in erlotinib-free media for over 40 weeks and periodically restested for erlotinib sensitivity (Fig. 1b; for our PERCs, one passage ~1 week; Methods). During the long-term drug holiday, we observed that nearly all of the PERCs remained considerably more resistant to erlotinib than PC9, although to varying degrees. (We note that 50% viability of PERCs at 2.5 \( \mu \)M (Fig. 1b, red line) implies, by definition, an IC50 of 2.5 \( \mu \)M erlotinib, which is 100 × the IC50 of PC9; thus, 50% viability in this assay implies strong drug resistance compared with PC9.) To better understand the one apparent exception, PERC3, we used an image-based assay to profile PERC growth kinetics in erlotinib over a longer time period (2 weeks in Fig. 1c versus 72 h in Fig. 1b). As expected, PC9-1 cells experienced widespread cell death after treatment with erlotinib before settling into a persister state (~9–14 days) characterized by drug tolerance and negligible growth (Fig. 1c and Supplementary Fig. 2a-c). In contrast, all tested PERCs grew in drug; the apparent reversion to sensitivity of PERC3 simply reflected a relatively slower, but net positive growth rate. As our PERCs appeared not to have reverted to the original level of PC9-1 erlotinib sensitivity, we wondered what drug-resistance mechanisms they had acquired.

Evidence for diverse vulnerabilities via drug screening. To investigate erlotinib-resistance mechanisms present in our 17 PERCs, we performed a large-scale drug screen. This allowed us to scan for therapeutic vulnerabilities among our PERCs that were absent in PC9-1, and thereby identify pathway or target alterations that conferred resistance. We assayed the sensitivities of our PERCs to a panel of 560 anticancer compounds in combination with erlotinib (Methods; Supplementary Data 1; note that PC9-1 cells were assayed against the drug library in erlotinib-free media, as treatment with erlotinib potently kills these cells). To search broadly for potential vulnerabilities, the panel contained a diverse collection of compounds, including drugs targeted to the specific erlotinib-resistance-conferring T790M-EGFR mutation, kinase inhibitors affecting multiple cancer-related pathways and chemotherapy and epigenetic drugs. Each compound was assayed over a sixfold dosage range, in duplicate and for all 17 PERCs and control PC9-1.

We focused initially on identifying PERCs whose drug responses in combination treatment with erlotinib were strongly altered from PC9-1’s drug response without erlotinib. There are a number of approaches to assess drug sensitivity from dose–response curves. Here we chose to compute a sensitivity score based on signed-area differences between drug-response...
curves of PERCs versus PC9-1 that took into account replicate variability (Fig. 2a–d and Methods; an alternative scoring based only on signed-area differences gave similar results, Supplementary Figs 5–7). The large-scale drug screen allowed us to interrogate drug-resistance mechanisms across our collection of PERCs. A few broad trends were noticeable. As compared with PC9-1, PERCs were generally resistant to EGFR inhibitors (as might be expected, for example, Fig. 2b), Aurora kinase inhibitors and chemotherapeutics. Further, some PERCs developed broad resistance (for example, PERC3) or sensitivity (for example, PERC16) to drugs belonging to multiple drug classes (Fig. 2e,f).

We next searched for functional evidence of PERC resistance/vulnerability in different mechanistic drug categories. It is not to be expected that a PERC would respond similarly to every drug within the same category. Therefore, we developed a drug-category-response score to search within each defined category for evidence of PERC sensitivities to a larger-than-expected fraction of drugs (Fig. 2e and Supplementary Fig. 3; scores were normalized per PERC across all drugs; Methods). There was no single category for which all 17 PERCs were vulnerable. However, we identified specific vulnerabilities (Fig. 2e–f): PERC17 to MET drugs (including SGX-523, INCB28060, JNJ-38877605 and crizotinib); PERCs 10, 13 and 16 to MEK inhibitors (including selumetinib, PD0325901 and pimasertib); and PERCs 4, 5, 10, 12 and 17 to mechanistic target of rapamycin (MTOR) drugs (including rapamycin and everolimus). Taken together, our drug screen identified putative, mechanistically distinct vulnerabilities, suggesting that our PERCs evolved multiple strategies to escape erlotinib treatment.

Evidence for diverse resistance mechanisms via sequencing. We next sought to use genetics as a way to corroborate predicted vulnerabilities as well as to identify mechanisms that were not detected by our initial analysis of the drug screen. From our exome-sequencing data, we identified genetic changes between each PERC and the parent PC9-1 (ref. 25; Fig. 3a, Methods; only amplifications > 2.5 × compared with PC9-1 are reported below). The derivation of the PERCs from a single, clonal parent offered a unified basis to identify and interpret genetic changes.

We first searched for mechanisms of erlotinib resistance that are most commonly observed in the clinic. On the basis of the sequence data, we found the T790M mutation in EGFR present in PERCs 1, 4–9 (Fig. 3a, Supplementary Fig. 5 and Supplementary Table 1). This caused us to re-evaluate our analysis of the drug screen. While all PERCs became more resistant to EGFR drugs when compared with PC9-1, comparison of PERCs with each other revealed that those harbouring a T790M mutation showed increased, albeit partial, sensitivity to T790M-targeting drugs (including afatinib, dacomitinib and WZ3146 (ref. 18); Figs 2b and 3b and Supplementary Figs 4c and 5). In addition, we found genetic evidence for MET amplification26 in PERC17 (Fig. 3a). This observation is consistent with recent findings from Engelman and colleagues27 (published whilst our work was under
consideration) demonstrating that acquisition of EGFR-T790M mutation by drug-tolerant cells may result in a diminished apoptotic response of EGFR-T790M targeted drugs. This was confirmed at the single-cell level using fluorescence in situ hybridization (Supplementary Fig. 6c). PERC17 exhibited an exquisite sensitivity to MET inhibitors (Figs 2 and 3c and Supplementary Figs 4 and 6a) and apoptosis from short interfering RNA (siRNA) knockdown of MET (Fig. 3d). Thus, MET amplification is a bona fide resistance mechanism for PERC17. To our knowledge, a MET amplification has never previously been reported for the parent PC9 line. Together, T790M and MET have been implicated in over half of all clinically reported cases of EGFR-addicted NSCLC with resistance to first-generation EGFR inhibitors14 (for example, erlotinib and gefitinib).

We next examined genetic changes in the MAPK pathway, one of the most frequently mutated pathways associated with erlotinib resistance28. We observed point mutations in NRAS for PERCs 10, 13, 14 (Q61K) and PERC15 (E63K), two mutational events that have been implicated in erlotinib resistance in preclinical models29 (Fig. 3a and Supplementary Fig. 7). PERC16 exhibited an amplification of RAF1, a genetic alteration that has not been reported in lung cancer but has been characterized as a driver mutation in other cancer types30 (Fig. 3a and Supplementary Fig. 7). We used our genetic data to revisit our drug screen, and found that PERCs 10, 13, 16 were sensitive to drugs targeting MEK (for example, selumetinib), which is a downstream member of the MAPK pathway (Figs 2 and 3e and Supplementary Figs 4, 7 and 9)31. PERCs 14 and 15 did not display this sensitivity across all drugs in our initial analysis of the drug screen (Fig. 2); however, re-examination of response curves, overlaid with genetic data, revealed that all NRAS and RAF1 mutants had relatively higher MEK sensitivities than the other PERCs (Fig. 3e and Supplementary Fig. 7).
Supplementary Fig. 7). Further testing revealed that all PERCs carrying NRAS and RAF1 mutations had higher sensitivity to co-treatment with erlotinib and MEK inhibitors than to either alone, suggesting a role for MEK in 'bypass' signalling32 (Fig. 3f).

As might be expected, not every putative genetic mechanism was found to correspond to a drug vulnerability. Our drug screen identified as a vulnerability with our drug data. Conversely, not every drug vulnerability was found to correspond to an obvious genetic basis (Methods). Further, not all NSCLC erlotinib-resistance mechanisms reported in the literature were exhibited by the PERCs; we found no compelling evidence of transformation to small cell lung cancer, epithelial-to-mesenchymal transition or kinase-inhibitor resistance. For example, in PERC9, in addition to the T790M mutation, we observed a mutation in PIK3CA (E542K); this mutation is implicated in driving constitutive signalling through AKT33, but was not corroborated with drug sensitivities (Figs 2 and 3a). This is consistent with growing evidence that PIK3CA mutations may be bystander mutations in NSCLC34. In addition, the mutation in BRAF (G466A) for PERC11 was not identified as a vulnerability with our drug data. Conversely, not every drug vulnerability was found to correspond to an obvious genetic mechanism. For example, we observed mTOR sensitivity in PERC 10 for which we could not find any obvious genetic basis (Figs 2 and 3a). Further, not all NSCLC erlotinib-resistance mechanisms reported in the literature were exhibited by the PERCs; we found no compelling evidence of transformation to small cell lung cancer, epithelial-to-mesenchymal transition or activation of IGFR1, AXL or NFK-B. We were unable to determine the erlotinib-resistance mechanisms for PERCs 2, 3, 11 and 12, suggesting that the diversity of resistance mechanisms...
compatible with the persister state could be even greater than what we have found. Of these, PERC3 stood out as having nearly threefold more mutations than any other PERC, potentially because of mutation in the DNA polymerase gene PolN (ref. 35; Supplementary Fig. 8). Finally, some of our PERCs (for example, with multiple concurrent genetic alterations and/or drug vulnerabilities) may themselves be heterogeneous, comprising multiple subpopulations with different resistance mechanisms; if true, then there is even more diversity emerging from the persister state than we described. Nevertheless, in total, we discovered pharmacological and/or genetic evidence (as well as corroborating reverse phase protein array (RPPA) data)\textsuperscript{36}; Methods; Supplementary Figs 5–7) for mechanisms of erlotinib resistance in 13 of our 17 PERCs (Supplementary Fig. 9).

**Discussion**

Cancer therapy has traditionally been focused on eliminating fast-growing cells. Here we focused on drug-resistant cancer populations that emerge from a persister state in which cells show little-to-no growth for weeks to months in drug treatment. Our work is a proof-of-principle study that demonstrates for the first time that diverse drug-resistance mechanisms can emerge from persisters, derived from a single, recent ancestor cell and grown under the same selective pressure (Fig. 1a). This heterogeneity presents considerable clinical challenges for ‘personalized’ therapy: even if an effective therapy is selected for one PERC, there is no guarantee (and indeed it is not true in our data) that this drug would be effective for other PERCs, which in practice may have been undetected. Persisters, which are a small subpopulation of the bulk cancer population, are currently difficult to study in a clinical setting, and there is no known molecular signature of having passed through this state clinically. The diversity of resistance mechanisms we observed suggests that passage through the persister state is not a limiting factor in the emergence of drug-resistance heterogeneity (Fig. 1a). Although our study was not focused on when or how resistance arose, we believe it unlikely that our diverse resistance mechanisms were all pre-existing at the time of drug treatment: resistant cells would have had to emerge de novo within 20 generations from a single cell without selective pressure and then not expand appreciably for ~6 weeks in drug. We suspect, as previously conjectured\textsuperscript{3}; that persisters provide a drug-tolerant reservoir of cells from which drug-resistance mechanisms can eventually emerge\textsuperscript{37}. Our study raises a number of interesting questions. Would different initial cells, passing through the persister bottleneck, give rise to the same resistance landscape? Are persisters themselves in diverse molecular states and, if so, would different persister states favour different sets of resistance mechanisms? These unanswered questions provide motivation for further studies of the timing, diversity and mechanisms by which drug resistance can arise from (or through) the persister bottleneck in different selective pressures and cancer types.

Our work suggests yet a new layer of complexity for treating cancer. Diverse drug-resistance mechanisms can arise from pre-existing mutations before treatment (as has been extensively studied\textsuperscript{36–7}) as well as from slow-growing persisters after long-term treatment (which we study here). In fact, it is possible that both mechanisms contribute to drug-resistance heterogeneity in the clinic\textsuperscript{8}. Certainly, eliminating, modulating or even anticipating the range of drug-resistance solutions that can emerge from the persister state will help guide the treatment of cancer.

**Methods**

**Medium conditions.** We made use of two types of media in our experiments. First, ‘erlotinib-free media’ is composed of RPMI 1640 (Corning #10 040 CM) supplemented with 5% fetal bovine serum (Life Technologies #16140-071) and 1% Antibiotic-Antimycotic (Life Technologies #15240-062). Second, ‘erlotinib media’ is composed of erlotinib-free media and 2.5 μM erlotinib HCl (Selleckchem, Cat.#S1023). Unless otherwise stated, all experiments were performed in ‘erlotinib-free media’ and experiments with PERCs were performed in ‘erlotinib media’.

**Generation of clonal line PCP-1 in erlotinib-free media.** We used make use of the ‘EGFR-addicted’ NSCLC cell line PC9 acquired from the Minna Laboratory at UT Southwestern; single-nucleotide polymorphism cell line fingerprinting was performed to confirm the cell identity. Overall, 10,000 PC9 cells were seeded on a 10-cm plate, sonicated for 10 s, and most cells were isolated from one another. PC9 clonal colonies were selected (we chose colonies that were well separated from others to maximize the chance of being clonally derived) and transferred to a new 6-well plate. These clones were then rapidly expanded from a 6-well plate to one 10-cm plate. The process of generating a confluent plate for each of the clonal populations took ~2 weeks. Four vials of each clone were frozen down using all cells at the single confluent 10-cm plate. We designated one of these clones PC9-1 and used it for all subsequent experiments.

**Generation of PC9-1-derived PERCs in erlotinib media.** PERCs were derived by performing the following steps (Supplementary Fig. 1a). We note that erlotinib media was used for the whole duration of the PERC generation time (~2 months before isolation and ~7 ± 1.5 months after isolation) and changed regularly (~every 2–3 days). Five 10-cm plates were each seeded with 100,000 PC9-1 cells, allowed to adhere overnight and then treated with erlotinib media. Most cells died, leaving a few, isolated, drug-tolerant and slow-growing cells (‘persisters’) on the plates. Clearly separated colonies (~50) were isolated and transferred to 96-well plates between 6 and 8 weeks of drug treatment. Colonies were expanded from 96-well plates to 24-well plates to 6-well plates to one confluent 10-cm plate, and then finally to three confluent 10-cm plates. Plate transfers were performed only when the cells were grown to confluence. Nine vials of these persister-derived erlotinib-resistant colonies (‘PERCs’) were frozen down using all cells from the three confluent 10-cm plates. To obtain sufficient cells for our large-scale experiments (drug screening, exome-sequencing and RPPA assays), we expanded the PERCs and PC9-1 cells ~6 further passages. Cell lines were periodically examined and found negative for mycoplasma contamination during the course of this work.

**Persister time course.** PC9-1 cells were plated in triplicate in six-well plates with erlotinib-free media at 40,000 cells per well (Supplementary Fig. 2a). Media was changed to erlotinib media after allowing cells to adhere overnight. The cells were grown for 14 days, with erlotinib media being changed every 3 days. Cells were imaged every 2 days at 10 × magnification with phase-contrast on a Nikon Ti Eclipse.

**Persister drug response.** PC9-1 cells were plated in duplicate in six-well plates with erlotinib-free media at 40,000 cells per well (Supplementary Fig. 2b). Media was changed to drug-containing media (described below) after allowing cells to adhere overnight. Plates were treated with various drugs either singly in erlotinib-free media or in combination with erlotinib media. The following drugs were used: 0.1 μM WZ0040 (Selleckchem, Cat.#S1179), 0.1 μM WZ3146 (Selleckchem, Cat.#S1170), 0.1 μM SGX-523 (Selleckchem, Cat.#S1112), 0.0316 μM Crizotinib (PF-02341066; Selleckchem, Cat.#S1068) and 0.02 μM trichostatin A (Aldrich, Cat.#S1045). One pair of wells was used as controls with no drug in either erlotinib-free media or erlotinib media. The cells were grown for 14 days, with media being changed every 3 days. Cells were imaged every 3 days at ×10 magnification with phase-contrast on a Nikon Ti Eclipse.

**Reversion.** Long-term reversion experiments were performed by maintaining our established PERCs in erlotinib-free media. PERCs and PC9-1 were then probed for their responses to erlotinib periodically over the course of 40 weeks (Fig. 1b, and Supplementary Figs 1b and 2c). We tested for per cent viability (relative to vehicle-treated cells) of PERCs and PC9-1 treated with 2.5 μM erlotinib for 72 h using CellTiter-Glo assays. Bright-field images of PERCs at ×10 magnification were captured daily after long-term (46 weeks) growth in drug-free media and subsequent re-treatment with 2.5 μM erlotinib (Supplementary Fig. 2c). Image analysis was used to identify the portions of these images occupied by cells. A ‘% confluence’ for each image was quantified by the fraction of pixels occupied by the identified cellular areas.

**Drug screen.** The primary screen (Fig. 2) was performed at the UT Southwestern High-Throughput Screening (UTSW-HTS) Core Facility. For the primary screen, a custom library was constructed using the following libraries: Kinase Inhibitor Screening Library (96-well; Selleckchem, Cat.#L1200), Epigenetic Compounds Library (96-well; Selleckchem, Cat.#L1900), Apoptosis Compounds Library (96-well; Selleckchem, Cat.#S3300), InhibitorSelect 384-Well Protein Kinase Inhibitor Library 1 (EMD Calbiochem, Cat.#F539743, Batch#DD0015831) and the NCI Oncology Set (Plates 4,762 and 4,763). Cells lines were each seeded in 384-well plates at an empirically-determined optimal seeding density (defined as ~70–80% confluence at the end of the experiment) and allowed to adhere overnight. Compounds and negative

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controls were added using a BIOMEK liquid-handling robot on the second day, resulting in a final DMSO concentration of 0.5%, and six, tenfold dilutions of compounds then titrated from 10 PM to 100 PM. Cells were then incubated for 72 h at 37 °C and 5% CO2. Next, media was removed and 25 µl of CellTiter-Glo diluted 1:5 with passive lysis buffer (Promega) was added using a Multidrop Reagent Dispenser. Plates were incubated for 10 min at room temperature with shaking and read on an Envision plate reader (Perkin Elmer). Per cent viability calculation was performed by UTSW-HTS using the following formula for single-point normalization: Per cent viability = 100 × Sample Raw Values/C176

A small-scale "bypass" experiment (Fig. 3f and Supplementary Figs 6b and 7b) was performed at the Small Molecule Discovery Center at UCSF using the same protocol described above. Selected PERCs were treated with ± erlotinib conditions and either SGX-523 (MET drug) or Selumetinib (MEK drug). Drug-response curves were measured at six doses, with multiple technical replicates (n = 3 + erlotinib; n = 4 – erlotinib) per drug dose.

Exome-seq. Genomic DNA was extracted from confluent 15-cm plates using the QIAamp DNA Kit (Qiagen). Metastasis-knockout PC9-1 cells with known exome mutational background were used as a "parent" line for the development of PC9-1r1 and PC9-1r2 PERCs. PERCs with a single allele deletion common to all PERCs was omitted. Metastasis-knockout PC9-1 alleles were dropped in Fig. 3a (where only a single NRAS variant changes). The following antibodies were used: MET (Cell Signaling #4560; 1:1,000 dilution), GAPDH (Santa Cruz #sc-47724; 1:3,000 dilution) and Cleaved Caspase 3 (Cell Signaling #9662; 1:1,000 dilution). RIPA buffer supplemented with phenylmethyl sulphonyl fluoride, sodium ortho- vanadate and a protease inhibitor cocktail (Santa Cruz #sc-24948). SDS–PAGE immunoblots were performed, and data were collected using the LI-COR Odyssey system. Images were captured using the Applied Spectral Imaging system. Probes used are developed by MD Anderson's Functional Proteomics Core Facility. Images were analyzed using standard fluorescence microscope and Applied Spectral Imaging system. Probes used are developed by MD Anderson's Functional Proteomics Core Facility. Images were analyzed using standard fluorescence microscope and Applied Spectral Imaging system.

Fluorescence in situ hybridization. Cells were harvested and fixed in methanol-acetic acid (3:1) and air-dried slides were prepared (Supplementary Fig. 6c). Probe and nuclear DNA were co-denatured at 72 °C in formamide and hybridized overnight at 37 °C. After washing and counterstaining with 4,6-diamidino-2-phenylindole, analysis was performed using standard fluorescence microscopy and images were captured using the Applied Spectral Imaging system. Probes used are CEP-7 (green) and cMET (red; Abbott Molecular, Downers Grove, IL).

RPPA. Cells were seeded onto six-well plates at a density of 150,000 cells per well and cultured for 24h (Supplementary Figs 5, 6a and 7a). Cells were lysed using the protocol outlined by the MD Anderson Functional Proteomics Core Facility, where RPPA was performed. Total protein concentration in lysates was determined using the Bio-Rad Protein Assay (Sigma-Aldrich, H-7021). A total of 50 µg of protein was denatured using 2× Laemmli buffer and loaded onto a 4–12% Bis-Tris Gel and electrophoresed for 90 min at 200 V. After electrophoresis, gels were stained with Coomassie Blue G-250 (Sigma-Aldrich, C-5250) and destained with 10% methanol and 10% acetic acid. Image acquisition was performed using a Fluorchem System 8000 (Alpha Innotech). Densitometric analysis was performed using ImageJ software. The relative concentration of each protein of interest was defined using the ‘Super Curve Fitting’ method developed by MD Anderson’s Functional Proteomics Core Facility.

Drug-response score. We use the notation \( V_{r,d}^{n,c} \) to denote the percentage viability of PERC \( n \) (n = 1–17) treated with drug \( d \) (d = 1–560) at concentration \( c \) (1–16) and replicates treatment \( r \) (1–2). We sought to identify PERCs whose drug response is strongly and reproducibly from that of PC9-1. We made use of the drug response in terms of the area under the curve (AUC) to quantify changes:

\[
A_{r,d} = \sum_{c} V_{r,d}^{n,c}.
\]

Our score for change reflected the degree to which AUCs for response curves of PC9-1 and PERCs were distinguishable, given a notion of experimental variability. Our measure of variability was constructed by considering the distribution of AUCs when systematically sampling from the replicate measurements; at each of the six concentrations \( c \), we chose one of two replicates, giving rise to \( 2^6 = 64 \) possible response curves and 32 corresponding AUCs. For every PERC and drug and we constructed the AUC set:

\[
S(n,d) = \{A_{r,d}^0, ..., A_{r,d}^{31}\}.
\]

The parent line PC9-1 was assayed twice for each drug (each with two replicates per concentration). We denote these two replicate assays here by PC9-1r1 and PC9-1r2 and define a corresponding AUC set for PC9-1 by combining the corresponding AUC sets:

\[
S(PC9-1,d) = \{A_{r,d}^{1+1,1,1}, ..., A_{r,d}^{1+12,12,12}\}.
\]

Then, the drug response for PERC \( n \) and drug \( d \) was characterized in terms of its difference from the PC9-1 response as quantified by the test statistic of the Student’s t-test (two sample, unequal sample sizes and variances):

\[
\text{Response}(n,d) = t(n,d,S(PC9-1,d)).
\]

Smoothed drug-response curves. The mean (across replicates) dose–response curves were fit to the sigmoidal form using a least-squares unweighted model (lognormal in Matlab v2014a).
RPPA analysis. The output of the RPPA experiment was a matrix of values, each row corresponding to a cell line (PERC or PC-1) and each column corresponding to an antibody. These data were analyzed as follows:

1. Normalization: the data (which are in log 2) were linearized (by raising to power 2), each row (cell line) was divided by its median, each column (antibody) was divided by its median and the values were then converted back to log 2.
2. Replicate averaging: replicates for each cell line were averaged to generate cell line profiles.
3. For each cell line and antibody, the level of the corresponding antibody for PC-1 was subtracted.
4. Quality: antibodies marked as Validated (V), Caution (C) or QC by MD Anderson RPPA Core Facility were used. (V): Pearson correlation coefficient between in-house RPPA western blot data and internal RPPA data is >0.7. (C): Pearson correlation coefficient between in-house RPPA data and western blot data is <0.7. (QC): antibody is suitable for cell line analysis but not tissue sample analysis.

Code availability. Computer code used to generate Figs 1–3 is available as Supplementary Software 1–3.

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