

IN THE SPOTLIGHT

Large-Scale Drug Screens Support Precision Medicine

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Summary: The major challenge underlying the emerging precision medicine initiative is to make links between cancer subsets and drugs that can be used to guide treatment of individual patients, leading to improved outcomes and decreased toxicity. Seashore-Ludlow and colleagues support this effort by reporting measurements of responses of 664 adherent cancer cell lines to 70 FDA-approved drugs, 100 experimental compounds, and 311 small-molecule probes. They use a novel Annotated Cluster Multidimensional Enrichment algorithm to identify drug mechanisms of action, molecular markers of response, responsive cancer subtypes, and compounds that produce synergistic cell inhibition. *Cancer Discov*; 5(11); 1130–2. ©2015 AACR.

See related article by Seashore-Ludlow et al., p. 1210 (10).

The emerging precision medicine initiative is based on the concept that genetic and other molecular information about individuals and their diseases can be used to deploy effective, less toxic, patient-specific treatments. In cancer, this approach is enabled by recent advances in genome science that allow detailed molecular characterization of individual cancers quickly and inexpensively and by the increasing number of drugs that directly or indirectly target the molecular pathways that are deregulated by underlying aberrations in tumor cells and the host microenvironments. The Cancer Genome Atlas project and the International Cancer Genome Consortium combined with many smaller investigator-initiated programs have now provided detailed molecular compositions for over 10,000 cancers from at least 32 anatomic sites that can be used to define cancer subsets, either within or across cancer lineages, that could identify more homogeneous populations likely to benefit from similar interventions. In parallel, the pharmaceutical industry has developed almost 800 approved and experimental drugs and vaccines that can be considered for treatment of molecularly defined subsets of cancers (1).

The challenge is to make links between cancer subsets and drugs that can be used to guide treatment of individual patients. This is comparatively straightforward for drugs that target strong “driver” genomic aberrations. Early successful examples include imatinib mesylate for treatment of cancers with ABL kinase fusions and trastuzumab for treatment of cancers that overexpress the HER2 receptor tyrosine kinase. More recent examples include vemurafenib for treatment of cancers carrying *BRAF* mutations and crizotinib for treatment of tumors carry-

ing *ALK* or *ROS1* translocations or amplification of *MET*. Unfortunately, many patients with biomarkers indicative of response do not respond or responses are short. Most likely, drug combinations will be needed to convert the transient responses into durable responses that approximate cures. Furthermore, most tumors do not carry strong genome drivers that can be targeted by existing therapeutic agents. Instead, aberrant networks that are deregulated by combinations of genomic and epigenomic aberrations may be the only feasible target in many cancers. In addition, drugs are rarely highly specific for individual networks, and bypass and feedback loops may limit the efficacy of targeting single nodes, so responses may be driven by interactions against multiple regulatory processes. As a consequence, drug-subset links may need to be established experimentally by identifying the molecular properties of subsets of patients that show strong response (or resistance) to specific treatments. This would best be done in large clinical trials, such as MATCH, and the unusual responder initiative of the NCI. However, the large number of drugs now available and the extreme genomic and epigenomic diversity among tumors even from the same anatomic sites make comprehensive clinical association studies logistically and financially impossible. Assessing responses to combinations of drugs is even more difficult.

Associations between molecular features and responses to specific drugs are now being established by testing drugs in laboratory models of cancer that are designed to capture the molecular diversity of human cancers, because clinical evaluation of all drugs and in particular drug combinations in all tumors of interest is impossible. Models currently in use include collections of established cancer cell lines grown in 2-D and 3-D cultures, cell lines genetically engineered to carry specific aberrations, organoid cultures, mouse xenografts established from cell lines, and patient biopsies and genetically engineered animal models. All of these models have strengths and weaknesses. In general, logistical considerations make collections of cell lines grown in 2-D cultures the only practical system for large-scale drug testing today. Other, lower-throughput but perhaps more representative model systems can then be employed to further evaluate response-subtype associations.

A growing number of cell line-based studies have now been published, showing associations between molecular features

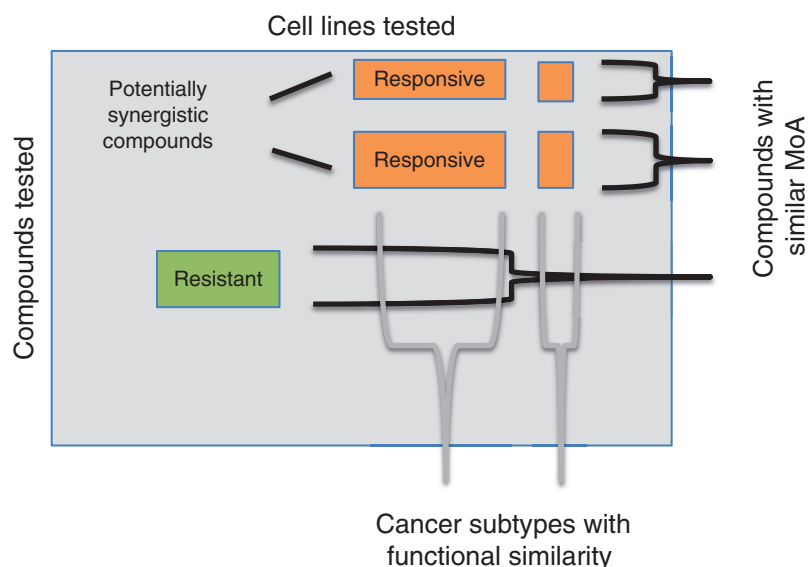
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Figure 1. Schematic illustration of Annotated Cluster Multidimensional Enrichment (ACME) analysis. MoA, mechanisms of action.



and therapeutic responses or perturbations (e.g., response to RNAi; refs. 2–8). In most cases, these data have been made publicly available in an effort to support community development of strategies to identify molecular features that predict response to therapeutic agents, identify new therapeutic targets, and enable comparison of predictors. Concordance between studies is reasonable, but, not surprisingly in large high-throughput datasets, some discrepancies have been identified (9).

Seashore-Ludlow and colleagues (10) now take large-scale cancer cell line–based screening to a new level by reporting responses of 664 adherent cancer cell lines from the Cancer Cell Line Encyclopedia to a 481-drug “informer set” comprised of 70 FDA-approved drugs, 100 experimental compounds, and 311 small-molecule probes, about half of which had no identified protein targets. All compounds were tested at 16 different concentrations in duplicate so that response assessments are particularly robust. DNA and RNA profiles and mutation calls from targeted DNA sequencing are available for these lines from the Cancer Cell Line Encyclopedia project (6). Proteomics data (Reverse Phase Protein Arrays) for many of the cell lines are available (TCPAportal.org) and will be released for the complete cell line set in the near future. The new data, including responses (reported as area under the curve values < AUC), will be available from the NCI CTD2 Data Portal.

The key features of this study, in addition to the powerful new dataset, are that it presents new robust strategies to identify molecular markers that define cancer subsets that are likely to respond well to specific drug classes, provides information on mechanisms of action (MoA) for new compounds, identifies cancer subtypes with common therapeutic liabilities, and—most importantly—suggests a way to identify combinations of compounds that may produce synergistic results when combined. This information comes from an Annotated Cluster Multidimensional Enrichment (ACME) analysis algorithm in which the results of hierarchical clustering of cell lines and compound responses are integrated to identify sensitivity hotspots (Fig. 1).

ACME analysis automatically reveals cell lines that respond similarly to many different compounds and collections of compounds that produce similar responses in many different cell lines. Linking these sensitivity clusters to genetic or lineage features establishes molecular feature–response relationships. This approach clearly identifies known relationships (e.g., sensitivity of *BRAF*^{V600} mutant cells to MEK and BRAF inhibitors and *HER2*-amplified cells to ERBB2 inhibitors). However, it also identifies response associations not previously appreciated (e.g., sensitivity of cells overexpressing EGFR to EGFR inhibitors). Several of these were validated in wet-bench studies.

ACME analysis provides insights into compound MoA because compounds that cluster together are likely to have similar MoA (e.g., PI3K and AKT pathway inhibitors cluster together). This allows tentative assignment of MoA for novel compounds and allows reclassification of compound MoA in some cases. These predictions can then be tested by experimental interventions. For example, the authors used ACME analysis to show that a compound designed as an inhibitor of leucine-rich repeat kinase 2 (LRRK2), designated LRRK2-in-1, clustered with BRD4 inhibitors. Subsequent studies showed that LRRK2-in-1 did indeed inhibit BRD4-acetylated peptide interaction *in vitro* and diminished MYC levels as expected for BRD4 inhibitors.

ACME analysis of the cell lines representing many different tumor types in this study also has the potential to guide the selection of tumor subsets in which to initiate proof-of-concept clinical trials. Drugs showing sensitivity hotspots in one cancer subtype may also be considered for assessment in other cancer subtypes showing comparable sensitivity hotspots. This may be particularly powerful as a guide to identify new indications for drugs already showing efficacy in one cancer subtype. However, this is complicated by the difficulty in directly relating transcriptional profiles and other patterns in cell lines to patient samples due to effects of tissue culture for the cell lines as well as patient tumor contamination by stromal cells and intratumoral heterogeneity. Indeed, a “tumor like mine” approach of matching a

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single patient to a drug-responsive cell line cluster remains an elusive goal.

Perhaps the most exciting feature of the ACME analysis is identification of compounds that will produce synergistic responses in subsets of cancer cells. Potentially synergistic drugs are those that appear effective in the same cell subpopulations but that differ in MoA, as illustrated in Fig. 1. Seashore-Ludlow and colleagues (10) used this approach to identify synergistic combinations in CCLs that harbored *KRAS* mutations. They identified multiple compound clusters that intersected with cell lines carrying *KRAS* mutations, suggesting sensitivity of this CCL subpopulation to inhibitors of MEK, IGF1R, SRC, EGFR, MTOR, PIK3CA, and HDACs. They then tested all pairwise combinations of 10 compounds targeting these pathways and found 10 synergistic combinations and 2 antagonistic combinations. They explored a predicted synergism between the IGF1R inhibitor BMS-754807 and the MEK inhibitor selumetinib and found a particularly strong synergy in a subset of lung and large intestine CCLs harboring *KRAS*^{G12} mutations, suggesting that both the driving mutation and co-mutations present in these tumor lines or the intrinsic gene expression patterns of the lineages contribute to the ultimate sensitivity or resistance to the combination therapy. This illustrates the concept that ACME analysis also reveals predictive markers to guide use of potentially synergistic drug combinations. Importantly, all compounds predicted to be synergistic had at least one synergistic pair, and no combinations of drugs selected at random showed synergism even though they were individually toxic.

Of course, the *in vitro* cell line screening approach has limitations that are inherent in most cell line studies. The number of cell lines is still too small to capture all of the important molecular subtypes in human cancers, the cell lines may have drifted during extended culture, and some cancer subtypes are not represented in the collection because they do not perform well in high-throughput assays or do not adapt well to cell culture. For example, *IDH1/2* and *EGFRVIII* aberrations are greatly underrepresented in cell lines. In addition, effects from the microenvironment are not well modeled so that the translation of results to the clinic will not be perfect. However, these limitations are well recognized and likely will be remedied in future work or by using other model approaches. Indeed, perhaps the most cogent use of predictions from ACME of cell lines in 2-D cultures would be to direct and limit the number of permutations to be tested in more complex and less tractable conditions, such as 3-D culture, organoids, or *in vivo* models.

Overall, the present study by Seashore-Ludlow and colleagues (10) illustrates the power of large-scale *in vitro* drug response analysis in general and the ACME analysis in particular in identifying subsets of patients that have the highest potential to benefit from specific drugs or drug combinations, developing predictive markers to guide drug delivery, and identifying drug MoAs. The authors have reported only a fraction of associations that exist in the present dataset, so it is likely that many additional insights will be revealed as the community explores the rich dataset and applies the

approaches espoused in these studies to existing datasets and drug screening datasets that may be generated in the future.

Disclosure of Potential Conflicts of Interest

J.W. Gray reports receiving commercial research grants from Cepheid, FEI Corporation, Murdock Trust, PDX Pharmaceuticals, Prospect Creek Foundation, and Susan G. Komen for the Cure; has ownership interest (including patents) in Abbott, Amgen, Becton Dickinson, Cepheid, Intel, PDX Pharmaceuticals, and Urology Diagnostics; and is a consultant/advisory board member for Cepheid, FEI Corporation, Intel, Nanotech, New Leaf Ventures, KromaTiD, Merrimack Pharmaceuticals, Samsung, and Urology Diagnostics. G.B. Mills reports receiving commercial research grants from Adelson Medical Research Foundation, AstraZeneca, Critical Outcome Technology, Komen Research Foundation, and Nanostring; has received speakers bureau honoraria from AstraZeneca, ISIS Pharmaceuticals, Nuevolution, and Symphogen; has ownership interest (including patents) in Catena Pharmaceuticals, Myriad Genetics, PTV Ventures, and Spindletop Ventures; and is a consultant/advisory board member for Adventist Health, AstraZeneca, Blend, Catena Pharmaceuticals, Critical Outcome Technologies, HanAl Bio Korea, ImmunoMET, Millennium Pharmaceuticals, Nuevolution, Precision Medicine, Provista Diagnostics, Signalchem Lifesciences, and Symphogen.

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