interaction with ETS1, a critical component of the complex that binds and activates Eα (Figure 1). To test the biological significance of this interaction on the enhancer element, the authors silenced TLX1/3 in human T-ALL cell lines and observed increases in differentiation and cell death, suggesting abortive differentiation and induction of apoptosis. Ectopic expression of rearranged TCRα caused identical effects. These findings therefore connect proper TCR rearrangement and expression to tumor differentiation state.

Altogether, the work of Dadi et al. (2012) presents a novel mechanism of differentiation arrest orchestrated by the TLX oncogenes in the induction and maintenance of T-ALL. To this end, it will be important to determine whether additional transcriptional targets that are potentially perturbed by TLX1/3 are also important for progression of the disease. This is an important question as the differentiation defects seen in *TLX1* transgenic mice are distinct from those caused by paucity of TCRα rearrangement in human leukemia. One such example could be the downregulation *BCL11B*, a target of TLX1 that is essential for T cell commitment. Further investigation on the potential synergistic role other factors play in the TCR recombination (Polycomb complex, the CTCF insulator protein, and others) and on the mechanisms leading to sustained expression of the TLX proteins will shed light on the intricacies of this leukemia. Ultimately, the most intriguing implication of this study is whether there are means of regulating TLX function using targeted therapies to enforce differentiation of TLX1/3$^+$ T-ALL. A similar approach of "differentiation therapy" has been extremely effective in the treatment of acute promyelocytic leukemia with all-trans retinoic acid (Kogan and Bishop, 1999). Given that their expression is normally restricted to embryonic development, TLX1/3 could prove to be ideal targets in the treatment of large fraction T cell leukemias with limited potential for adverse side effects.

REFERENCES

Aifantis, I., Raetz, E., and Buonamici, S. (2008). Nat. Rev. Immunol. *8*, 380–390.

Dadi, S., Le Noir, S., Payet-Bornet, D., Lhermitte, L., Zacarias-Cabeza, J., Bergeron, J., Villarèse, P., Vachez, E., Dik, W.A., Millien, C., et al. (2012). Cancer Cell *21*, this issue, 563–576.

De Keersmaecker, K., Real, P.J., Gatta, G.D., Palomero, T., Sulis, M.L., Tosello, V., Van Vlierberghe, P., Barnes, K., Castillo, M., Sole, X., et al. (2010). Nat. Med. *16*, 1321–1327.

Gutierrez, A., Kentsis, A., Sanda, T., Holmfeldt, L., Chen, S.C., Zhang, J., Protopopov, A., Chin, L., Dahlberg, S.E., Neuberg, D.S., et al. (2011). Blood *118*, 4169–4173.

Hatano, M., Roberts, C.W., Minden, M., Crist, W.M., and Korsmeyer, S.J. (1991). Science *253*, 79–82.

Kogan, S.C., and Bishop, J.M. (1999). Oncogene *18*, 5261–5267.

Mullighan, C.G., and Downing, J.R. (2009). Leukemia *23*, 1209–1218.

Schatz, D.G., and Swanson, P.C. (2011). Annu. Rev. Genet. *45*, 167–202.

Sleckman, B.P., Bassing, C.H., Bardon, C.G., Okada, A., Khor, B., Bories, J.C., Monroe, R., and Alt, F.W. (1998). Immunol. Rev. *165*, 121–130.

Van Vlierberghe, P., Palomero, T., Khiabanian, H., Van der Meulen, J., Castillo, M., Van Roy, N., De Moerloose, B., Philippé, J., González-García, S., Toribio, M.L., et al. (2010). Nat. Genet. *42*, 338–342.

Personalized Medicine: Patient-Predictive Panel Power

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Two recent papers published in *Nature* demonstrate the power of systematic high-throughput pharmacologic profiling of very large, diverse, molecularly-characterized human cancer cell line panels to reveal linkages between genetic profile and targeted-drug sensitivity. Known oncogene addictions are confirmed while surprising complexities and biomarker relationships with clinical potential are revealed.

The need to identify predictive biomarkers of tumor response has intensified with the era of molecularly-targeted therapies that exploit addictions and vulnerabilities in tumors with identifiable molecular traits, in contrast to the one-size-fits-all approach that dominated cytotoxic chemotherapy (Yap and Workman, 2012). Two recent *Nature* articles describe a systematic large-scale approach to this challenge by high-throughput profiling many targeted agents against hundreds of clinically-relevant human cancer cells lines with detailed genetic annotation (Garnett et al., 2012; Barretina et al., 2012).

There are three important general take-home messages from these two studies. (1) The articles provide the most extensive

validation to date for systematic large-scale analyses of human tumor cell panels that link drug sensitivity to cancer cell genetics. (2) They reveal examples of previously unsuspected genetic-pharmacologic relationships and complexities, some with immediate clinical potential. (3) They make available publicly the pharmacologic screening data alongside extensive genetic and other molecular characterization of the cancer cell panel (http://www.broadinstitute.org/ccle; http://www.cancerrxgene.org/), providing powerful tools and resources for use by the cancer community. Researchers can now mine the datasets to generate and test hypotheses, while new compounds can be submitted to the panel screens for pharmacogenomic interrogation.

A caveat of these studies is that both use the artificial two-dimensional adherent growth of established cancer cell lines on plastic and also lack the environmental and cellular complexity of clinical cancers. Nevertheless, very large diverse cancer cell line panels provide the only means currently available with which to model the enormous genetic and epigenetic heterogeneity present in human cancers and hence to identify predictive response biomarkers that address the molecular diversity seen in the clinic (Caponigro and Sellers, 2011).

The systematic large-scale pharmacologic and genetic analysis approach has its origins in the pioneering NCI60 tumor cell line panel (Weinstein et al., 1997). This led to use of larger cancer cell line panels that identified genotype-correlated sensitivity to kinase inhibitors within and between cancer histologies. However, it became clear that hundreds of cancer cell lines must now be used to mimic clinical heterogeneity and to account for pathogenic driver mutations present in only, say, 5% or even less of cancers (Caponigro and Sellers, 2011; Garnett and McDermott, 2012).

Figure 1A summarizes the workflow used for the two studies. Several clear high-level trends emerge from the analysis. Certain agents, e.g., the microtubule stabilizer paclitaxel and the pan-histone deacetylase inhibitor panobinostat, show broad activity whereas a few, including p53-MDM2 antagonists, are active in a small minority of cancer cell lines. Outlier cell lines with unusual sensitivity are often informative. Sensitivity to most agents is associated with at least one gene mutation, importantly demonstrating the very broad potential applicability of predictive biomarkers. Known oncogene addiction paradigms are reconfirmed in the large scale panels for several approved or developmental kinase inhibitors, including those targeting BCR-ABL, BRAF, MEK, ALK, ERBB2, EGFR, FLT3, and PIK3CA. Interestingly, however, gene expression features often correlate as well or even better with sensitivity than kinase mutation. Furthermore, mutation-sensitivity relationships are frequently modified by tissue-specific or gene expression biomarkers, suggesting the need for multigene signatures of sensitivity and reinforcing the importance of mechanistic drug combinations.

Some specific examples are illustrative (Figure 1B). Both studies conclusively demonstrate that expression of the quinone reductase metabolizing enzyme NQO1 was the strongest predictor of sensitivity to the benzoquinone ansamycin HSP90 molecular chaperone inhibitor 17-AAG (tanespimycin), as initially discovered using the NCI60 panel (Kelland et al., 1999), but not to the non-quinone chemotype NVP-AUY922, thus exemplifying differences in predictors between chemotypes hitting the same target.

The now clinically-validated synthetic-lethal relationship between inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP) and *BRCA* mutations could not be revealed in the short, 3 day drug exposures used. Thus other synthetic lethal relationships might also be missed. However, Garnett et al. (2012) do confirm the very recent discovery that PARP inhibitors (here olaparib and AG-014699) are highly active against Ewing's sarcoma cells with the characteristic *EWS-FLI1* rearrangement (Brenner et al., 2012), analogous to the sensitivity to PARP inhibition of prostate cancer cells with related *ETS* gene fusions (Brenner et al., 2011). Sensitivity may relate to PARP1 acting as a cofactor for the transcriptional activity of *ETS* family proteins or to a known EW1-FLI1-PARP positive feedback loop in transcriptional activation. Although sensitivity does not appear to be due to a defective DNA damage response per se, PARP inhibition may potentiate EST-mediated DNA damage. Thus there is exciting potential for evaluating PARP inhibitors in Ewing's sarcoma patients.

An unexpected and unexplained finding is that expression of *SLFN11*, encoding a member of the schlafen family of proteins with unknown function, is predictive of sensitivity to two camptothecin-based topoisomerase I inhibitors in several cancer cell lineages including, again, Ewing's sarcoma lines (Barretina et al., 2012). Clinical trials with topoisomerase inhibitors are already underway in Ewing's sarcoma.

BRAF and *NRAS* mutations are reconfirmed in both large-scale studies as single-gene predictors of sensitivity to MEK inhibitors. Expression of a group of genes, including those encoding known regulators of MAP kinase signaling, is found to modulate MEK inhibitor sensitivity. A novel observation is that expression of the aryl hydrocarbon receptor gene (*AHR*) strongly correlates with sensitivity to MEK inhibition in cancer cells with *NRAS* mutations and may be a predictive biomarker for increased MEK sensitivity in such cancers (Barretina et al., 2012). The causal link was confirmed by *AHR* silencing, which suppresses the growth of high but not low *AHR* expressing *NRAS* mutant cancer lines. Interestingly, Garnett et al. (2012) find that resistance to MEK inhibitors is also associated with the driver *EWS-FLI1* translocation.

Our own preliminary analysis of these data sets reveals, with a few notable exceptions, a surprising lack of correlation between the cellular effects of groups of drugs that supposedly have the same primary molecular mechanism, indicating polypharmacology and complex response drivers other than the declared targets. Interestingly, among the exceptions that are tightly correlated, the highly selective MEK inhibitors AZD-6244 and PD-0325901 show identical effector gene associations. In contrast, PARP and HSP90 inhibitors exhibit different effector gene associations that suggest a more complex basis for their action (Figure 1B).

For most drugs, sensitive cell lines are distributed across multiple cancer types. However, both articles show examples of cell lineage, rather than genetics, being the predominant predictive feature. Multiple myeloma cell lines with elevated IGF1 and IGF1R expression exhibit enhanced sensitivity to an IGF-1 receptor inhibitor, renal cell carcinomas to SRC inhibitors, and gliomas to a ROCK inhibitor. A hematologic lineage was predictive

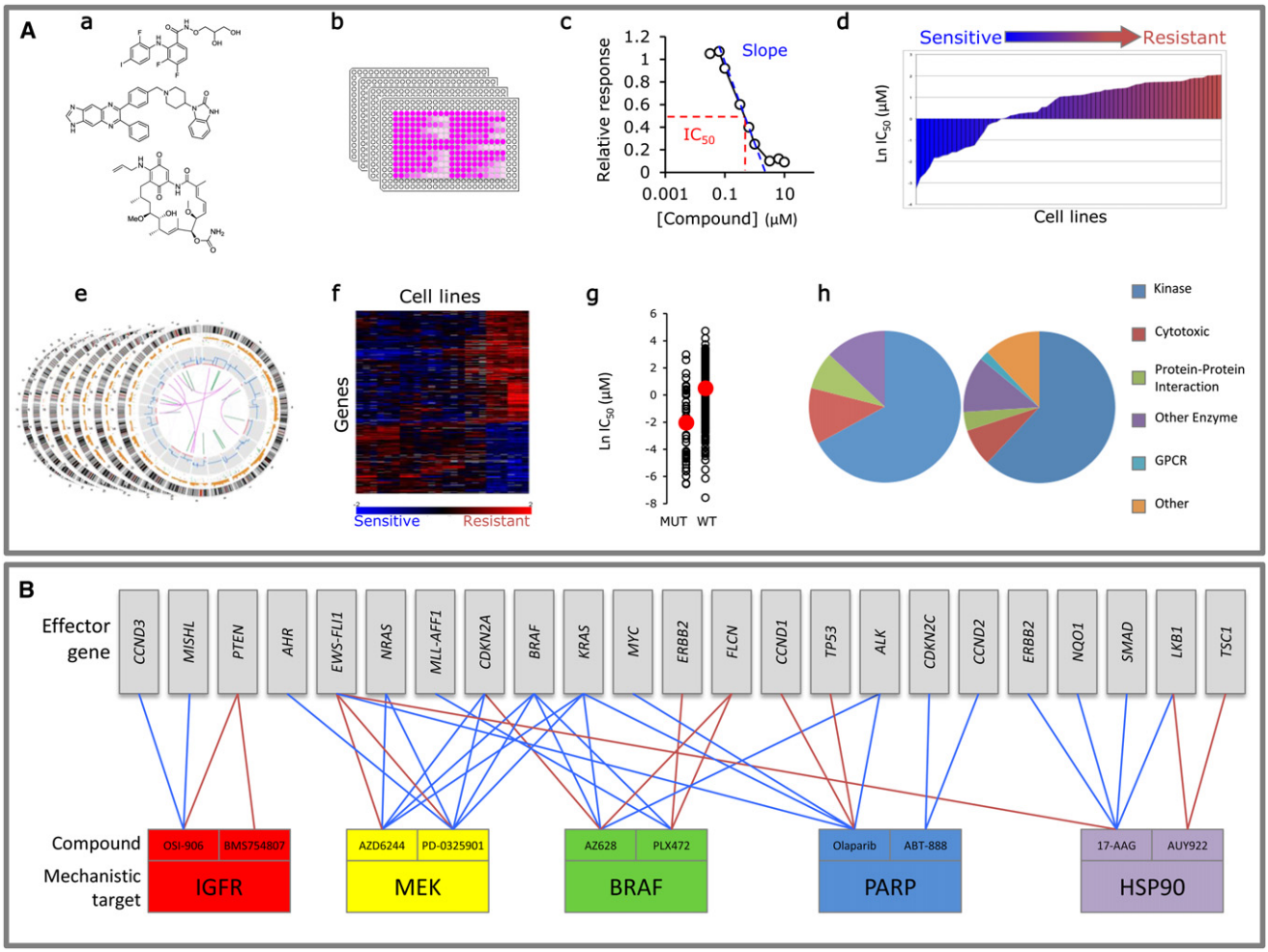

Figure 1. Systematic Large-Scale Pharmacologic and Genetic Profiling of Extensive Human Cancer Cell Line Panels

(A) Schematic of the workflow for screening human tumor cell line panels to identify factors that influence response to small-molecule drugs or tool compounds. A library of compounds (a) was screened in microplate format (b) against a diverse panel of cancer cell lines using an eight- to nine-point dose-response curve (c) from which sensitivities were calculated either as the concentration causing half-maximal inhibition of growth (IC_{50}) or from the slope of the responsive part of the curve. This generated a response profile across the cancer cell line panel for each compound (d). Garnett et al. (2012) profiled 130 agents against 275–507 cancer cell lines using a nine-point dose-response following 72 hr continuous exposure to compounds and assessed sensitivity by cell fixation and staining with a fluorescent DNA-binding dye as the end-point. Barretina et al. (2012) assessed 24 compounds across a panel of 479 tumor cell lines with an eight-point dose-response and measured total ATP using a luciferase-based assay following 72–84 hr continuous exposure. The sensitivity profile was then correlated with genomic features (e), gene expression patterns (f), or oncogenic mutations (g) using statistical methods. Both groups used the same chip-based methodology to determine single nucleotide polymorphism status and gene copy number. Garnett et al. (2012) profiled expression of 14.5k genes using oligonucleotide-based microarray technology. Barretina et al. (2012) profiled more genes with a later version of the same system. Point mutation status was determined by capillary sequencing of all coding exons for 64 genes frequently mutated in cancer by Garnett et al. (2012) or by targeted massively parallel sequencing of 1651 protein coding genes by Barretina et al. (2012) (h) Distribution of mechanistic classes of tested compounds in Barretina et al. (2012) (left) and Garnett et al. (2012) (right). (B) Examples of targeted drugs and corresponding effector genes identified in the two studies to be associated with sensitivity (blue lines) or resistance (red lines).

of panobinostat sensitivity, in agreement with the majority of clinical responses to panobinostat and other HDAC inhibitors being in hematological cancers.

Overall, these two important new studies firmly establish the value of systematic large-scale automated human cancer cell panel profiling for discovering cancer cell vulnerabilities, defining the cellular mechanism of action of small-molecule inhibitors, and identifying predictive biomarkers. The analytical power of the panels will grow as more compounds are profiled, including the important need to confirm biological associations using compounds of diverse chemotypes that hit the same target (Workman and Collins, 2010) and will also increase as the depth of genetic annotation rises, with the planned inclusion of the sequencing data for the coding exons of all ~22,000 human genes (Caponigro and Sellers, 2011; Garnett and McDermott, 2012). Additional cancer cell line annotations, including epigenomic, proteomic, and metabolomic features, would add further mechanistic and predictive power. Future iterations of the screens could involve use of more physiological growth conditions and longer incubation times.

Thus patient-predictive human cancer cell panels are set to become an

increasingly powerful platform for systematic mechanistic studies and especially for biomarker discovery. This, in turn, will facilitate the development and widespread application of personalized cancer medicine.

REFERENCES

Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A.A., Kim, S., Wilson, C.J., Lehar, J., Kryukov, G.V., and Sonkin, D. (2012). Nature *483*, 603–607.

Brenner, J.C., Ateeq, B., Li, Y., Yocum, A.K., Cao, Q., Asangani, I.A., Patel, S., Wang, X., Liang, H., Yu, J., et al. (2011). Cancer Cell *19*, 664–678.

Brenner, J.C., Feng, F.Y., Han, S., Patel, S., Goyal, S.V., Bou-Maroun, L.M., Liu, M., Lonigro, R.J., Prensner, J.R., Tomlins, S.A., and Chinnaiyan, A.M. (2012). Cancer Res. *72*, 1608–1613.

Caponigro, G., and Sellers, W.R. (2011). Nat. Rev. Drug Discov. *10*, 179–187.

Garnett, M.J., and McDermott, U. (2012). Drug Discov. Today *17*, 188–193.

Garnett, M.J., Edelman, E.J., Heidorn, S.J., Greenman, C.D., Dastur, A., King, L.W., Greninger, P., Thompson, R., Luo, X., Soares, J., et al. (2012). Nature *483*, 570–575.

Kelland, L.R., Sharp, S.Y., Rogers, P.M., Myers, T.G., and Workman, P. (1999). J. Natl. Cancer Inst. *91*, 1940–1949.

Weinstein, J.N., Myers, T.G., O'Connor, P.M., Friend, S.H., Fornace, A.J., Jr., Kohn, K.W., Fojo, T., Bates, S.E., Rubinstein, L.V., Anderson, N.L., et al. (1997). Science *275*, 343–349.

Workman, P., and Collins, I. (2010). Chem. Biol. *17*, 561–577.

Yap, T.A., and Workman, P. (2012). Annu. Rev. Pharmacol. Toxicol. *52*, 549–573.