Combination chemotherapies have been a mainstay in the treatment of disseminated malignancies for almost 60 y. The development of these regimens in the absence of any knowledge of cancer genetics was a remarkable achievement. However, although our knowledge of the genetic drivers of cancer and the mechanisms of drug action has increased dramatically over the past 30 y, this information has yet to inform the clinical use of chemotherapy substantially. We hypothesize that part of this failure is due to the absence of mechanistic information about how drugs in regimens interact to promote combination effects. Here, we describe a tractable strategy to examine cytotoxic drug interactions. We use this approach to show that commonly used drug regimens function by minimizing the effect of genetic diversity on therapeutic response, whereas, in contrast, less used synergistic drug combinations amplify the impact of patient genotype on component drug efficacy. These data highlight the fundamental challenge in implementing personalized cancer therapy for malignancies with existing multiragent regimens.

Recently, numerous “signature-based” approaches for characterizing the mechanisms of action of small molecules have been developed (1–4). These approaches seek to use indirect high-resolution assays to compare the relative behavior of new drugs relative to well-established small molecules. In doing so, common mechanisms can be inferred from common signatures. However, although these signatures have demonstrated utility with regard to single-drug mechanisms, none have been used to examine how complex regimens of cancer chemotherapies combine to exert their effects. Here, we use RNAi-based “functional signatures,” along with complementary informatics tools, to examine drug combinations in matched pairs of cancer cells with defined alterations. RNAi signatures consist of patterns of drug sensitivity and resistance conferred by a set of shRNAs on the response of cells to a set of cytotoxic agents (Fig. P1). Such signatures have previously been shown to be of sufficient resolution to predict single-drug mechanisms of action for diverse classes of chemotherapeutics in current clinical use (5). These predictions are generated by statistically comparing test compound RNAi signatures with a training set that contains reference signatures for most classic cytotoxic chemotherapies, as well as newer agents like inhibitors of HSP90 and histone deacetylases. In the present study, we extend these signatures to drug combinations. This approach seeks to bring to combination therapy what the knowledge of biochemical targets has brought to single-drug therapy. Most notably, a mechanistic understanding of the evolution of resistance. Additionally, this approach seeks to create a statistical and experimental definition of “combination drug mechanisms of action.” Combination therapies might be hypothesized to interact in two general ways: (a) one agent may simply reinforce the action of another agent, or (b) the two drugs may combine to exert effects that are distinct from either individual compound. Correspondingly, the combination drug shRNA signature would either (a) resemble that of one individual drug or (b) exhibit a distinct pattern of shRNAs that confer drug resistance or sensitivity with respect to the latter possibility, a combination signature could be distinct from that of the individual component drugs in one of at least three ways: (i) It could average, or “homogenize,” individual drug signatures; (ii) it could mimic a compound not present in the combination; or (iii) it could adopt an entirely novel (neomorphic) signature. To extend our functional genomic signature-based framework to combination drug dosing, we created shRNA signatures of resistance or sensitivity in response to combinations of drugs that were controlled for dose level effects. We show here that some highly synergistic drug combinations can resemble the more potent signature of a single drug. For example, the combination of 17AAG (an Hsp90 inhibitor) plus taxol (a spindle poison) yielded a signature similar to that of taxol alone. Conversely, unlike these highly synergistic combinations, commonly used drug regimens average extant single-drug variations in therapeutic response. For example, the suppression of DNAPKCs levels yielded sensitivity to doxorubicin (a topoisomerase II poison) and resistance to chlorambucil.
(an alkylating agent) but had no consequence in the face of a combination drug dosing. Thus, DNAPK status is relevant to the drug response to single agents but loses relevance in response to combination treatment. Further support for these findings is as follows: (i) Combinatorial effects could be extended to large unbiased shRNA libraries; (ii) when combined to form multidrug regimens, averaging combinations form averaging regimens that homogenize genetic variation in spontaneous mouse models of cancer; and (iii) the averaging effect extends to clinical genomics datasets, where mRNAs lose measurement resolution as regimens gain greater diversity.

Although further studies will be required to establish the broad applicability of this work, our findings reveal a surprising simplicity in the statistical and experimental definitions of “combination drug mechanisms of action”: Either drug A potentiates the mechanism of drug B or drug A plus drug B produces distinguishable yet averaging effects. This “averaging” of the impact of genetic variation, although critical in the absence of genetic stratification, is incompatible with personalized medicine. Conversely, use of drug combinations in which one drug potentiates the action of another further necessitates the identification of biomarkers that underlie the success or failure of the potentiated drug, resulting in an intensified need for personalized treatment. Understanding and designing regimens based on these simple modes of action has the potential to enhance fundamentally the effective use of combination therapies.