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Understanding Resistance to Combination Chemotherapy

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Summary

The current clinical application of combination chemotherapy is guided by a historically successful set of practices that were developed by basic and clinical researchers 50-60 years ago. Thus, in order to understand how emerging approaches to drug development might aid the creation of new therapeutic combinations, it is critical to understand the defining principles underlying classic combination therapy and the original experimental rationales behind them. One such principle is that the use of combination therapies with independent mechanisms of action can minimize the evolution of drug resistance. Another is that in order to kill sufficient cancer cells to cure a patient, multiple drugs must be delivered at their maximum tolerated dose – a condition that allows for enhanced cancer cell killing with manageable toxicity. In light of these models, we aim to explore recent genomic evidence underlying the mechanisms of resistance to the combination regimens constructed on these principles. Interestingly, we find that emerging genomic evidence contradicts some of the rationales of early practitioners in developing commonly used drug regimens. However, we also find that the addition of recent targeted therapies has yet to change the current principles underlying the construction of anti-cancer combinatorial regimens, nor have they made substantial inroads into the treatment of most cancers. We suggest that emerging systems/network biology approaches have an immense opportunity to impact the rational development of successful drug regimens. Specifically, by examining drug combinations in multivariate ways, next generation combination therapies can be constructed with a clear understanding of how mechanisms of resistance to multi-drug regimens differ from single agent resistance.

The origins and continued use of cancer combination chemotherapy

The overwhelming majority of cures in cancer chemotherapy have come from the application of conventional cytotoxic chemotherapies. While some cytotoxic agents have been the product of serendipity, and some the product of large scale screening, others were part of the first wave of “rational” targeted therapies that were developed in the 1940’s and were specifically aimed at targeting cancer cells on the basis of the nutritional properties that made them distinct from normal cells (Chabner and Roberts, 2005; Wall and Wani, 1995). The history of these early successes illustrates a unique combination of serendipity and insight that has led to the development of potentially curative regimens for numerous forms of cancer. It is this early success that still guides current clinical practice and clinical trials.

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In 1946 Goodman and Gilman published a landmark study in cancer chemotherapy. Nitrogen mustard agents that were serendipitously discovered to induce drastic lymphoid cell depletion upon accidental or wartime exposure were shown to produce remarkable responses in human tumors from a variety of tissue origins (Goodman et al., 1984). Two years later Sidney Farber took a more rational target based strategy for anti-cancer drug discovery. He reasoned that if folate deficiency inhibited normal hematopoiesis and the addition of folates accelerated leukemia in children, then anti-folates would make a promising anti-leukemia drug. In his landmark 1948 paper, Farber (Farber and Diamond, 1948) observed the first true remissions in a disease where the time from diagnosis to death was often measured in days. For the purpose of context, it is interesting to note the similarities between Farber's anti-folates and current targeted therapies. Like early clinical trials with EGFR and BRAF inhibitors, these early reports documented some striking but short lasting remissions in a subset of patients.

Combination genotoxic chemotherapy

In 1942, Luria and Delbruck's fluctuation analysis (Luria and Delbruck, 1943), which combined an experimental and mathematical modeling framework, showed that heritable resistance to viruses was derived from pre-viral-exposure genetic variation in a bacterial population. Later, Newcombe (Newcombe and Hawirko, 1949) extended this finding to chemotherapy in bacteria, and in 1952, Law extended it to the resistance to anti-folates in *in vivo* mouse models of cancer (Law, 1952b). Taken together these experiments suggested to early chemotherapy researchers that there might be a benefit to giving drugs in combination (Law, 1952a; Skipper et al., 1954). If a drug provided a resistance rate of 1/m and a second statistically independent (non-cross resistant) drug provided resistance of 1/n then co-resistance would occur in 1/m*n cells.

In 1958, citing the above rationales, and the successful creation of combination therapies for tuberculosis, Emil Frei III published the first randomized control trial of a combination therapy in cancer (Frei et al., 1958). It established clear combination efficacy over the efficacy of the single substituent agents. In the mid 1960's, Howard Skipper showed that in experimental mouse models, as few as one cancer cell could give rise to lethal disease, and that chemotherapy followed a logarithmic killing model. Specifically, the same dose killed the same proportion of cells, regardless of the total disease burden (Skipper et al., 1964). This suggested to Skipper and others that to have any chance of curing a cancer, a physician would have to administer as large of a tolerable drug dose as is possible to the patient.

The rapid success of Emil Frei's 1958 trial, advances in supportive care, and Howard Skipper's principals for curing experimental mouse models all led to the highly successful but not experimentally controlled adoption of the 4-drug VAMP regimen (Freireich EJ, 1964). Coupled with care that was able to ameliorate the side effects of therapy and effectively dose even higher cumulative drug doses into leukemia patients, the VAMP regimen was the first big step towards the large and potentially curative regimens that we have today. It is important to note that this type of study (in which combinations of drugs are combined at maximally tolerated doses) was not able to specify the mechanism of increased efficacy following increased drug dose, nor the minimization of the outgrowth of resistance, but it can and did demonstrate improved efficacy. It was this bold, but less carefully controlled strategy that led to the rapid and successful adoption of combination chemotherapy for many cancers, and by 1973 (DeVita and Schein, 1973) had revolutionized the treatment of cancer by finding cures for previously untreatable diseases. Thus, it is interesting to note that the biggest early successes of cancer therapy owe less to systematic controlled trials and more to bold and decisive attempts to cure very sick patients.

These early and impressive successes fostered a manner of thinking about care that still largely dictates current clinical practice in combination therapy, namely that randomized combinations of chemotherapeutics can identify select combinations that improve the number of patients that show a durable response. Many clinical trials using a similar process are still constructed in attempts to improve the current standard of care (Conroy et al., 2011; Pirker et al., 2009). Next, we will examine current genomic evidence for what makes cells resistant to combination therapy in light of the early rationales underlying combination regimens.

The clinical efficacy of combination therapy and its acquired resistance

The initial hypotheses behind the first combination therapy regimens suggested that: 1) The maximum possible cumulative dose of drug should be given, and 2) Independent drugs with non-overlapping mechanisms of action are important for minimizing the probability of therapeutic resistance. The ideas about resistance have only been tested in the context of cross resistance following the selection of single drug resistant cell lines in Luria-Delbruck fluctuation tests (Luria and Delbruck, 1943). However, the genomic evidence in relapsed cancers following clinical combination treatment in multiple systems suggests that this microbiology inspired rationale, while true in the context of infectious diseases like tuberculosis and HIV (Almeida Da Silva and Palomino, 2011; Durant et al., 1999), is very different in the context of relapsed human cancers that are initially sensitive to combination chemotherapy. In order to think about how systems biology might inform drug selection and the clinical trials process, we must consider how current clinical combinations fail in patients (Figure 1).

The mutational analysis of leukemia suggests multidrug resistant cell states

Examining pre and post treatment matched patient samples is critical to identifying the nature of acquired drug resistance in cancer. One of the first studies to analyze recurrent genomic alterations in cohorts of matched pre-treatment and post relapse patients performed copy number analysis on acute lymphoblastic leukemia samples (Mullighan et al., 2008). Mullighan and colleagues showed two striking results that speak to both the historic rationales for combination therapy and current studies on therapeutic resistance. First, the majority of relapse clones following conventional combinatorial chemotherapy represent low frequency variants in the pre-treatment tumor that were progenitors of the dominant diagnosis clone. Second, the majority of alterations that dominated at relapse did not include direct alterations in the known biochemical targets of common therapeutics that were used for leukemia treatment. Rather, the most common alterations tended to affect B-cell development, changes that might be hypothesized to promote generally drug resistant cell states or homing to developmental niches which promote therapeutic resistance. In a later study by the same group, the sequencing of selected exons in matched pre- and post treatment samples also identified recurrent mutations in B-cell developmental pathways, as well as in CREBBP and genes that induce transcriptional states that correlate with drug resistance (Mullighan et al., 2011). Finally, most recently, matched pre and post-treatment AML samples revealed mutations in genes that were not related to the direct drug targets of frontline chemotherapeutic action (Ding et al., 2012). Though these studies employed single measurement methodologies (sequencing or copy number analysis), taken together they suggest that relapsed leukemia treated with multi-agent regimens develop resistance profiles that favor the development of a multi-drug resistant cell state. This state appears to be broad in its definition, but includes the alteration of apoptotic, epigenetic, and developmental cell states. Thus, resistance to multi-drug regimens does not appear to occur via the combination of mutations conferring resistance to component parts of a regimen.

Resistance to targeted therapy is pathway specific

Perhaps one of the most exciting aspects of emerging *targeted* therapies is that resistance to these agents is both easily identifiable and qualitatively distinct from drug resistance to combination regimens. Specifically, resistance to these agents frequently arises as a direct drug-target alteration affecting therapeutic response. For example, following the treatment of chronic myelogenous leukemia (CML) with Abl kinase inhibitor Imatinib, it was noted that resistant cells harbored a spectrum of resistance mutations in the BCR-Abl kinase domain. Importantly, cells harboring some of these resistance mutations remained sensitive to a distinct multi-kinase inhibitor, dasatinib. While still competitively inhibiting BCR-Abl, dasatinib was found to have improved efficacy over imatinib in treating leukemia with most BCR-Abl kinase domain mutations (Shah et al., 2004; Talpaz et al., 2006).

But how might this discovery be used improve the development of effective drug combinations? Surprisingly, when used in a temporally distinct manner, these two kinase inhibitors, whose spectrum of resistance mutations are somewhat distinct can be alternatingly dosed over the course of the disease to stave off resistance, as long as compound mutations or single mutations causing resistance to both drugs are not present. Furthermore, several recent studies suggest that cells bearing the T315I resistance mutation (a mutation that confers resistance to both kinase inhibitors) can be treated with enhanced efficacy by using combination therapy. For example, cells expressing BCR-Abl T315I are sensitive to the combination of dasatinib with an aurora kinase inhibitor (Shah et al., 2007). Additionally, allosteric inhibitors that bind to the myristate-binding groove of the Abl protein were recently developed, that in combination with kinase domain targeted therapies, are capable of reducing T315I positive disease in tumor bearing mice (Zhang et al., 2010). Finally, combinations of conventional cytotoxic drugs (L-asparaginase and dexamethasone) with BCR-Abl inhibitors can overcome T315I-mediated resistance in a mouse model of BCR-Abl positive acute lymphoblastic leukemia (Boulos et al., 2011). All of these strategies illustrate attempts to create next generation combination regimens that incorporate agents to resensitize tumors to targeted therapeutics. Thus, unlike conventional chemotherapeutic regimens, some regimens involving targeted agents seek to restore individual component-specific activity - a situation that would presumably select again for single agent resistance.

While these BCR-Abl-related studies are the most advanced efforts to rationally build clinical regimens to circumvent targeted resistance, similar investigations into the modes of targeted resistance in the hedgehog pathway in medulloblastoma confirm the prevalence of on-target pathway mutations (Yauch et al., 2009) in response to targeted therapy. Studies in EGFR inhibitor-treated lung cancers have identified amplifications in the Met receptor tyrosine kinase, an example of a parallel pathway activation mechanism inducing resistance to targeted therapy (Turke et al., 2010). Furthermore, studies in BRAFV600E treated melanoma have identified downstream mutations in Mek (Wagle et al., 2011). This data suggests that these resistance mechanisms (mutations directly in the pathways of drug action or parallel to drug action in drug sensitive cancers) can be employed in response to numerous targeted therapeutics. With this in mind, there is increasing interest in the use of these agents in combination regimens. This rationale is highly similar to the original rationale for the first combo regimens, i.e. that the addition of independent drugs blocks targeted therapeutic resistance. Just like the initial regimens, in the context of combination therapy, it will be important to consider whether the therapeutic combinations adopted are actually able to shift mechanisms of therapeutic resistance, or whether they increase the effective amount of killing, or both. These data will be critical in understanding and preventing resistance that might develop in response to combinatorial therapy involving new “targeted” therapeutics.

Clinical drug resistance to conventional chemotherapies can also be pathway specific

The comparison between conventional regimen resistance and targeted therapeutic resistance is obscured by the fact that currently regimens contain multiple drugs that are not specifically targeted at oncogenic pathways, and because conventional therapeutics are almost always dosed in combination. This makes it difficult to directly compare the clinical mechanisms of drug resistance following single cytotoxic therapy in clinical cohorts. In spite of this, some pre-clinical evidence suggests that many classic chemotherapeutic agents, when dosed in isolation, can promote very similar modes of resistance as targeted therapies.

Traditional chemotherapeutic agents are often generally thought of as pleiotropic; however, this is not an accurate description of all cytotoxic chemotherapies. While nitrogen mustards and cisplatin are highly chemically reactive, and nucleoside analogs can incorporate into DNA and RNA, other drugs such as methotrexate, camptothecin, doxorubicin, and dexamethasone (Bledsoe et al., 2002; Matthews et al., 1977; Pommier et al., 2010; Staker et al., 2002) make direct and specific contacts with their enzymatic targets. An examination of the preclinical literature suggests that in the case of camptothecin, doxorubicin and methotrexate, resistance to these agents can result in direct modifications to their respective drug targets (Hashimoto et al., 1996). Though many selection experiments have also revealed the role of P-Glycoprotein (P-gp) and drug efflux in multi-drug cross resistance (a topic discussed at greater length in the next section), if P-gp is inhibited and anthracycline resistance is selected for, the resistance profile can revert back to a target dependent (topoisomerase I or topoisomerase II alpha) mechanism of drug resistance (Beketic-Oreskovic et al., 1995; Zander et al., 2010). This is in contrast to the clinical picture of multidrug resistance portrayed above. Thus, in the absence of direct evidence, it is interesting to speculate that the direct target mediated resistance to classic chemotherapeutics is difficult to select for in the face of current clinical combination therapy regimens, and that this is due to the combination of classic chemotherapeutic agents, and not the nature of the agents themselves.

Functional mechanisms of clinical efficacy in combination therapy

If a property of combination therapy in human cancers is that it selects for multi-drug resistant cell states, the distinctions between infectious disease and cancer may be indicative of the complex regulation of drug metabolism and cell death in mammalian cells (Letai, 2008). A well-described mechanism for the development of such a multi-drug resistant state is the overexpression of P-gp (Borst, 2012). This pump can mediate the efflux of numerous drugs used in combinatorial regimens, including anthracyclines, taxanes, and Vinca alkaloids (Szakacs et al., 2006). Surprisingly, however, while there is substantial evidence that elevated drug transport can mediate drug resistance in model systems, there is only limited evidence for a role of these transporters in chemoresistance in human tumors.

More recently it has been shown that in patient clinical samples and primary tissues, the “proximity” to the apoptotic threshold in cancer cells, as measured by the sensitivity of the mitochondrial cytochrome c release to pro-apoptotic peptides, is correlated with therapeutic response and the size of the therapeutic window that a drug can achieve. Thus, the proximity of cancer cells to the apoptotic threshold may be a key determinant of the therapeutic responsiveness of mammalian cancers (Ni Chonghaile et al., 2011). If maximizing the therapeutic dosing across the apoptotic spectrum increases the effective dose of drug and maximally activates apoptosis as a therapeutic response, then it suggests that intrinsic resistance to targeted therapies may be due to the fact that they haven’t been formulated into regimens sufficiently potent to bypass the apoptotic threshold. Curiously, however, while

cell death is the ultimate objective of cancer therapies, apoptotic pathway alterations are not a common mechanism underlying the evolution of drug resistance (Borst et al., 2007). Thus, commonly predicted mechanisms of multi-drug resistance (drug efflux, apoptosis, etc…) are rarely identified in multi-drug resistant tumors.

These data highlight a conundrum in modern therapeutics. Commonly used drug combinations lack clearly defined mechanisms of resistance, thus it is difficult to stratify cohorts of patients based on the status of individual genes (Abramson and Shipp, 2005). Thus, for many malignancies, we are committed to the use of these regimens as first line therapies, even if they have a substantial failure rate – a fact that explains the continued use of these regimens 60 years after their inception. Conversely, targeted agents have defined mechanisms of resistance, but have limited durable efficacy as single agents or when combined with agents that reinforce single drug action. Thus, given that cancer evolves resistance to multi-drug regimens in a manner that is distinct from antibiotic or antiviral resistance, it is critical to develop strategies to understand the genetic determinants of the response of cancer cells to combinatorial chemotherapeutics. In other words, we need to understand mechanisms of multi-drug resistance in order to begin to deviate from decades-old drug regimens. Here, we will describe emerging approaches to model multi-drug action.

Predicting phenotypic response to drug action in mammalian cells

Cancer cells exist in a multivariate landscape (Kreeger and Lauffenburger, 2010), involving both oncogene and tumor suppressor signaling as well as signaling from the tumor microenvironment. Similarly, drugs activate and inhibit cellular pathways, and there is significant complexity to drug response. A variety of modeling approaches, each requiring different levels of molecular detail, and each uniquely suited to different types of data have begun to make progress in predicting drug effects across different cellular contexts. Predictive *in vitro* models of cellular systems may be effective ways to predict the cellular context in which a small molecule or a combination of small molecules might be active. Though most often employed in the context of targeted therapeutic inhibition, the lessons and methods of these studies can be used to examine diverse cytotoxic combinations.

In well-studied systems with well-characterized pathways, it is possible to use differential equations of biochemical reactions to describe the mechanisms by which pro/anti-apoptotic signals are conveyed in biochemical networks. This approach has demonstrated that even non-oncogenic signaling proteins can be targets for drug intervention. Schoerberl and colleagues showed that Erb3 inhibition decreases AKT phosphorylation across a broad range of initial conditions (Schoeberl et al., 2009). The utility of Erb3 inhibition across a range of initial conditions demonstrates the power of *in silico* modeling to identify drug targets that have limited context-dependence. Importantly, from a methodological perspective, to make a useful model, all biochemical parameters do not have to be known *a priori*. They can be estimated by first discovering highly sensitive species in the set of biochemical reactions. Then, using simulated annealing, many parameters can be fit to cell line data. With these fit parameters incorporated, a novel understanding of signaling network function can emerge (Chen et al., 2009). While these approaches have proven their effectiveness for targeted therapeutic discovery, they remain underutilized for conventional cytotoxic therapeutics. However, similar types of models of DNA damage have been built to describe other phenomena (Toettcher et al., 2009), and may be used in the future to inform the therapeutic efficacy for conventional therapies.

When biochemical reactions are less described, higher levels of model abstraction can be used to understand the effect that biochemical reactions have upon cell outcome, even if the reactions are not modeled explicitly. Using partial least squares regression modeling in

mammalian cells, Janes et al. suggested that, given TNF-alpha induced cell death and opposing growth factor stimuli, kinase pathway interventions could be accurately predicted in a colon cancer cell line (Janes et al., 2005; Janes et al., 2004). Impressively, this method incorporated enough of the signaling network context that it allowed for the prediction of apoptotic responses in diverse cell lines of epithelial origin (Miller-Jensen et al., 2007). In isogenic models of RAS signaling, Kreeger et al. showed that a similar multi-pathway model could incorporate diverse effects on cell death that were mediated by different RAS proteins to accurately predict apoptotic response (Kreeger et al., 2009). To examine questions of kinase inhibitor specificity and off target effects, Kumar et al. showed that models incorporating multivariate descriptions of signaling responses could accurately predict cellular outcomes in the presence of promiscuous kinase inhibitor activity (Kumar et al., 2008). Together these studies show that regression based models can incorporate cell type and oncogene specific network influences to estimate drug on and off target effects that contribute to therapeutic action.

While these efforts focused on predicting specific drug effects on particular species in multivariate models, drugs often have a broader spectrum of biochemical and genetic effects. This spectrum requires a drug-centric approach, utilizing profiling methods and high level statistical modeling that can simultaneously assess multiple relevant cellular effects, and predict the most relevant alterations for cellular phenotypes.

Multivariate signatures characterizing drug action

In order to understand the effects of combinations of drugs, we first have to understand single drug effects in a multivariate manner. Beyond simple drug-target biochemical interactions, most small molecules have many biochemical effects and genetic interactions; they act promiscuously on a variety of cellular enzymes and processes (Xie et al., 2012). When considered in the context of large-scale genetic screens, these same molecules harbor a diverse array of genetic interactions (Bartz et al., 2006; Doles and Hemann, 2010; Whitehurst et al., 2007). Comprehensive characterization of small molecules should help provide mechanistic information on how similar two drugs effects are, and can help to identify the cellular backgrounds in which they will show greater efficacy.

Biochemical Signatures

Most compounds exert multiple biochemical effects in cellular systems. In order to examine the pleiotropy of the enzymatic effects induced by several families of anticancer agents, many groups have begun to examine the systematic biochemical profiles of drug action. Using recombinant protein libraries, compound Kd’s can be systematically measured for hundreds of kinase domains. This binding information can yield profiles of affinities that broadly describe kinase inhibitor specificity. When plotted on phylogenetic trees of kinase sequence similarity, selectivity profiles of kinase inhibitors can be easily visualized (Karaman et al., 2008). Because kinase inhibitors are competitive inhibitors of ATP binding, a recent effort has extended the analysis of the selectivity of kinase inhibitors to the measurement of kinase activity in the presence of high ATP concentrations to more closely approximate cellular effects. This allows for the clustering of drugs based upon their relative kinase activity and the discovery of new drug targets/functions for known inhibitors (Anastassiadis et al., 2011). Together these approaches offer broad biochemical readouts of kinase inhibitor action, and allow for the biochemical classification of kinase inhibitors.

Beyond kinase activity, proteomics approaches in cell lysates can be used to couple quantitative mass spectrometry with conventional biochemical characterization approaches. Using non-specific kinase or histone-deacetylase beads as a capture reagent, proteomic signatures that are capable of semi-comprehensively assessing inhibitor function at various

concentrations are used to produce inhibitor signatures of phosphorylation/de-acetylation inhibition (Bantscheff et al., 2007; Bantscheff et al., 2011). Together, these biochemical approaches offer a variety of strategies for comprehensively profiling the biochemical effects of small molecules in an attempt to understand specificity and mechanism of action.

Genetic signatures

While drugs have typically been characterized biochemically, and this approach can elucidate a spectrum of enzymatic effects, these biochemical characterizations often lack functional information about how a drug is actually causing cytotoxicity within a cancer cell.

The first efforts towards large-scale drug characterization in mammalian cell lines were performed on the NCI-60 panel of cell lines. This panel was created in an attempt to accelerate drug discovery for the treatment of therapeutically intractable solid tumors that did not benefit from the first generation of combination regimen building. As a consequence of work with this set of cell lines, a large amount of screening data on thousands of novel compounds was generated by the NCI in the 1980's (Paull et al., 1989). Upon characterizing and examining the profiles of activity for diverse compounds across cell lines, the NCI proposed the COMPARE approach. COMPARE sought to rank-order lists of drugs with correlated patterns of response across the entire NCI-60 panel of cell lines. Following the annotation of the NCI-60 panel with genetic and biochemical data, wide-ranging efforts were undertaken to align and cluster the cell line response data with the molecular characterization data (Scherf et al., 2000; Weinstein et al., 1997) using the discovery algorithm. These efforts highlighted the potential importance of the p53 tumor suppressor and the P-glycoprotein efflux pump in predicting cellular response to many commonly used cytotoxic compounds. Recently, utilizing the NCI data and microarray expression data for the NCI 60 cell lines, the Theordescu group has suggested that it is possible to use the NCI-60 data to predict drug response in completely distinct cell lines and cancer subtypes using the CONEX algorithm (Lee et al., 2007). However, while thousands of compounds have been screened in the NCI-60 cells, and comprehensive genomic data exists, it remains difficult to mine and interpret the data that are generated via these efforts. Additionally, concerns exist as to the relevance of these cell lines to actual human tumors.

In 2006, a large microarray compendium, termed the connectivity map, was generated to allow developers of novel compounds to query a large database of reference compounds for relationships to an investigational compound or a disease state (Lamb et al., 2006). These searches have been suggested to not only identify a compounds' mechanism of action (Hieronymus et al., 2006), but also allow for the querying of target signatures to produce predictions for therapeutic intervention (based on the assumption that opposing drug gene expression patterns should effectively cancel out pathogenic disease states) (Wei et al., 2006). Finally, this work has been used by other groups to reposition drugs with similar molecular profiles but distinct indications (Dudley et al., 2011; Sirota et al., 2011). Importantly, while these efforts have produced new proofs of concept, the signatures that are generated can be very difficult to interpret from a functional perspective. Furthermore, the published efforts to validate predictions often focus on "top-ranked compounds" and have not at this point rigorously validated the predictive depth of these signature-based queries.

Recently in mammalian cells, functional gene perturbation using targeted RNA interference or chemical perturbations has been used to examine the differential sensitivity of cells to drugs (Jiang et al., 2011; Wolpaw et al., 2011). Both of these methods focus on targeted feature sets that are capable of discriminating between drugs that act upon distinct subsets of biology. While the chemical approach has added resolution over non-apoptotic forms of cell

death, the shRNA-based approach allows for quantitative boundaries and predictions to be made using an algorithmic add-on to conventional supervised machine learning approaches. The strength of both of these approaches is that they detail specific functional relationships that alter cellular responses to a given compound.

In silico approaches

Large distinctions exist between efforts that seek to model drug mechanisms of action *in silico* using datasets of clinical information, since these contain diverse data from clinical practice. Campillos et al. started with a database of side effects from the unified medical language system (UMLS) and used it to build a common side effects drug interaction network. They identified sets of compounds with modest structural similarity, but high correlations in overall side effect profiles. Finally, by using *in vitro* biochemical assays, they confirmed common target binding (Campillos et al., 2008). While this method cannot attribute a specific side effect to a specific target, nor prove a functional role for the interaction, it is the only method that uses actual clinical data to classify drugs by their mechanism of action. Furthermore, in the future, this approach may be extended to predict the mechanisms of particular side effects. It is also interesting to speculate that common side effects profiles would demarcate “bad” combinations in a potential regimen, and that early dismissal of combinations with close network proximity might reduce drug toxicity.

The field of signature-based prediction has developed numerous approaches to simultaneously characterize drug action beyond cell death. Signatures of drug action can tell us about inhibitor selectivity/off target effects, transcriptional response similarity, function and toxicological action. All of these indications are valuable, but if used together they might be combined in the drug development process to simultaneously characterize investigational compounds and promote their more rational and safe use.

Systems perspectives in combination therapy

There are many innovative perspectives on how systems/network biology might inform targeted cancer therapy, (Erler and Linding, 2010; Fitzgerald et al., 2006; Pawson and Linding, 2008). A major articulated goal is to identify activated pathways that are druggable, compensatory pathways that account for single targeted treatment failure, or combinations of network nodes that give greater than additive benefits. Furthermore, mass action kinetic models in well-studied pathways (EGFR) have been suggested to be potentially capable of identifying combinations of molecular species with synergistic effects (Fitzgerald et al., 2006). This is most often considered in the context of specific kinase inhibitors targeted against oncogenic pathways. However, many cancers are currently treated with cytotoxic clinical regimens that have diverse cure rates (Abeloff, 2008), and some that respond well to therapy initially but rapidly develop resistance (Abeloff, 2008). Less often considered (Tentner et al., 2012) is the value of systems biology in examining classical chemotherapeutics. Chemotherapeutics activate downstream pathway signaling following treatment. Oncogene and tumor suppressor networks are known to alter similar signaling molecules as a result of oncogenic transformation. For instance: given a particularly responsive patient population, it may be desirable to use two drugs with the same mechanism of action but non-overlapping toxicity. Furthermore, kinase inhibitors are often neither specific nor free of side effects (Kantarjian et al., 2012), and, as such, signatures of their effects and models of their action could help guide regimen building - even if the assumptions present in modeling efforts (inhibition of one key node) are not valid. Applying systems and network biology to characterize the global alterations affecting therapeutic response in the face of chemotherapy should promote the appropriate – or even optimal - use of current clinically used drugs.

Towards combination drug signatures

To integrate systems/network biology into the future of combination regimens, network approaches will have to delineate altered pathways and understand how combinations of drugs will interact with those pathways. If combination chemotherapy in cancer tends to select for drug resistant cell states, a couple of hypotheses might account for this effect. The first is that resistance to multiple drugs in a combination regimen is mediated by downstream network effects that are common to all drugs (a common effects/non independent action hypothesis), and second, combinations may co-opt distinct sub-networks downstream of drug targets to create combination specific effects. Understanding these distinctions will be critical to future regimen building. A good way to test these conflicting hypotheses and examine them with next generation therapeutics will be to develop combination therapy signatures. While this is an attractive and potentially useful idea, no current methods for signature-based drug prediction as detailed above have specifically addressed how, relative to single agents, combinations of drugs might function. This may be due to technical and/or conceptual limitations of certain approaches, but combination signatures will be critical to regimen design.

In examining combination network “signatures”, combinations may be a sum of their component drug networks, they may reinforce single component drug networks, or they may act on sub-networks that are not utilized by single drugs (combination off target effects). All of these effects may be desirable in different personalized medicine contexts with a diverse range of prior knowledge. For example, if a combination of drugs reinforces a single component drug network (one drug potentiates the effect of another), it is best administered to a patient population that is responsive to that single drug mechanism. While this would require single drug sensitizing networks to be well described, it may reveal combinations of drugs that increase the therapeutic window for a single component compound - while also increasing the on-target effects to which a given patient is known to be susceptible.

If a combination of compounds results in an averaging of their component sensitivities and resistances, will drug-specific genetic dependencies be cancelled out? If so, this suggests a striking paradox: namely, could a genetic interaction in a single component drug case be rendered unimportant in the context of a combination? This possibility is an intriguing concept that will have to be explicitly investigated, because, although it would suggest the minimization of resistance to one drug, it would also minimize sensitivity to another. This dichotomy in a combination regimen, one drug sensitizing while the other promoting resistance to a particular legion would have a strong benefit in the absence of any genetic knowledge - a type of drug hedge betting. This averaging of sensitivities and resistances may reflect a requirement inherent in clinical trials – that efficacy must be demonstrated across genetically diverse cohorts. Of course, such homogenization would also come at the cost of optimal regimens for individual patients.

Final thoughts

Understanding the network biology of single and combination drugs could guide clinical practice across a diverse spectrum of knowledge concerning tumor pathology and genetics. In the absence of any information as to the underlying genetic networks driving tumor progression in the patient, broadly acting combinations that independently utilize diverse sub-networks may form an optimal therapeutic strategy. Conversely, in an attempt to administer drug combinations with particularly potent combination action (i.e. synergy), it may be possible to identify the signatures of sub-networks that are functionally important for that drug combination and design companion diagnostics to target the right combination to the right patient. In fact, it may absolutely essential to identify such critical sub-networks

prior to the administration of “synergistic” therapies. Drug synergy has historically been a terrible predictor of *in vivo* combinatorial efficacy. This could be due the strong context-dependence of synergistic efficacy. In other words, “synergistic” therapies may absolutely require biomarkers that stratify optimal patient populations prior to the construction of clinical trials. Finally, in the presence of extensive pathological information, we may not only be able to pick the right combination for the right patient, i.e. match drugs to information about the bulk portion of a patient’s tumor, but identify resistant subpopulations in heterogeneous tumors before they dominate a tumor and dose drug combinations that might minimize the outgrowth of these subpopulations.

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References

Abeloff, MD. Abeloff’s clinical oncology. 4. Philadelphia: Churchill Livingstone/Elsevier; 2008.

Abramson JS, Shipp MA. Advances in the biology and therapy of diffuse large Bcell lymphoma: moving toward a molecularly targeted approach. Blood. 2005; 106:1164–1174. [PubMed: 15855278]

Almeida Da Silva PE, Palomino JC. Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs. J Antimicrob Chemother. 2011; 66:1417–1430. [PubMed: 21558086]

Anastassiadis T, Deacon SW, Devarajan K, Ma H, Peterson JR. Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity. Nature biotechnology. 2011; 29:1039–1045.

Bantscheff M, Eberhard D, Abraham Y, Bastuck S, Boesche M, Hobson S, Mathieson T, Perrin J, Raida M, Rau C, et al. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. Nature biotechnology. 2007; 25:1035–1044.

Bantscheff M, Hopf C, Savitski MM, Dittmann A, Grandi P, Michon AM, Schlegl J, Abraham Y, Becher I, Bergamini G, et al. Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes. Nature biotechnology. 2011; 29:255–265.

Bartz SR, Zhang Z, Burchard J, Imakura M, Martin M, Palmieri A, Needham R, Guo J, Gordon M, Chung N, et al. Small interfering RNA screens reveal enhanced cisplatin cytotoxicity in tumor cells having both BRCA network and TP53 disruptions. Mol Cell Biol. 2006; 26:9377–9386. [PubMed: 17000754]

Beketic-Oreskovic L, Duran GE, Chen G, Dumontet C, Sikic BI. Decreased mutation rate for cellular resistance to doxorubicin and suppression of mdr1 gene activation by the cyclosporin PSC 833. Journal of the National Cancer Institute. 1995; 87:1593–1602. [PubMed: 7563202]

Bledsoe RK, Montana VG, Stanley TB, Delves CJ, Apolito CJ, McKee DD, Consler TG, Parks DJ, Stewart EL, Willson TM, et al. Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. Cell. 2002; 110:93–105. [PubMed: 12151000]

Borst P. Cancer drug pan-resistance: pumps, cancer stem cells, quiescence, epithelial to mesenchymal transition, blocked cell death pathways, persisters or what? Open Biol. 2012; 2:120066. [PubMed: 22724067]

Borst P, Jonkers J, Rottenberg S. What makes tumors multidrug resistant? Cell Cycle. 2007; 6:2782–2787. [PubMed: 17998803]

Boulos N, Mulder HL, Calabrese CR, Morrison JB, Rehg JE, Relling MV, Sherr CJ, Williams RT. Chemotherapeutic agents circumvent emergence of dasatinib-resistant BCR-ABL kinase mutations in a precise mouse model of Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood. 2011; 117:3585–3595. [PubMed: 21263154]

Campillos M, Kuhn M, Gavin AC, Jensen LJ, Bork P. Drug target identification using side-effect similarity. Science. 2008; 321:263–266. [PubMed: 18621671]

Chabner BA, Roberts TG Jr. Timeline: Chemotherapy and the war on cancer. Nature reviews Cancer. 2005; 5:65–72.

Chen WW, Schoeberl B, Jasper PJ, Niepel M, Nielsen UB, Lauffenburger DA, Sorger PK. Input-output behavior of ErbB signaling pathways as revealed by a mass action model trained against dynamic data. Mol Syst Biol. 2009; 5:239. [PubMed: 19156131]

Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardiere C, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. The New England journal of medicine. 2011; 364:1817–1825. [PubMed: 21561347]

DeVita VT, Schein PS. The use of drugs in combination for the treatment of cancer: rationale and results. The New England journal of medicine. 1973; 288:998–1006. [PubMed: 4348752]

Ding L, Ley TJ, Larson DE, Miller CA, Koboldt DC, Welch JS, Ritchey JK, Young MA, Lamprecht T, McLellan MD, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature. 2012; 481:506–510. [PubMed: 22237025]

Doles J, Hemann MT. Nek4 status differentially alters sensitivity to distinct microtubule poisons. Cancer research. 2010; 70:1033–1041. [PubMed: 20103636]

Dudley JT, Sirota M, Shenoy M, Pai RK, Roedder S, Chiang AP, Morgan AA, Sarwal MM, Pasricha PJ, Butte AJ. Computational repositioning of the anticonvulsant topiramate for inflammatory bowel disease. Sci Transl Med. 2011; 3:96ra76.

Durant J, Clevenbergh P, Halfon P, Delgiudice P, Porsin S, Simonet P, Montagne N, Boucher CA, Schapiro JM, Dellamonica P. Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. Lancet. 1999; 353:2195–2199. [PubMed: 10392984]

Erler JT, Linding R. Network-based drugs and biomarkers. J Pathol. 2010; 220:290–296. [PubMed: 19921715]

Farber S, Diamond LK. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid. The New England journal of medicine. 1948; 238:787–793. [PubMed: 18860765]

Fitzgerald JB, Schoeberl B, Nielsen UB, Sorger PK. Systems biology and combination therapy in the quest for clinical efficacy. Nature chemical biology. 2006; 2:458–466.

Frei E 3rd, Holland JF, Schneiderman MA, Pinkel D, Selkirk G, Freireich EJ, Silver RT, Gold GL, Regelson W. A comparative study of two regimens of combination chemotherapy in acute leukemia. Blood. 1958; 13:1126–1148. [PubMed: 13596417]

Freireich EJ, K M, Frei E III. Quadruple combination therapy (VAMP) for acute lymphocytic leukemia of childhood. In Proceedings of the American Association for Cancer Research. 1964

Goodman LS, Wintrobe MM, Dameshek W, Goodman MJ, Gilman A, McLennan MT. Landmark article Sept. 21, 1946: Nitrogen mustard therapy. Use of methyl-bis(beta-chloroethyl)amine hydrochloride and tris(beta-chloroethyl)amine hydrochloride for Hodgkin's disease, lymphosarcoma, leukemia and certain allied and miscellaneous disorders. By Louis S. Goodman, Maxwell M. Wintrobe, William Dameshek, Morton J. Goodman, Alfred Gilman and Margaret T. McLennan. Jama. 1984; 251:2255–2261. [PubMed: 6368885]

Hashimoto MW, Nikaido O, Kobayashi N, Chang CC, Trosko JE, Mori T. A comparison of the propensity for gene amplification between near-tetraploid and near-diploid V79 clones resistant to 150 nM methotrexate. Carcinogenesis. 1996; 17:389–394. [PubMed: 8631121]

Hieronymus H, Lamb J, Ross KN, Peng XP, Clement C, Rodina A, Nieto M, Du J, Stegmaier K, Raj SM, et al. Gene expression signature-based chemical genomic prediction identifies a novel class of HSP90 pathway modulators. Cancer Cell. 2006; 10:321–330. [PubMed: 17010675]

Janes KA, Albeck JG, Gaudet S, Sorger PK, Lauffenburger DA, Yaffe MB. A systems model of signaling identifies a molecular basis set for cytokine-induced apoptosis. Science. 2005; 310:1646–1653. [PubMed: 16339439]

Janes KA, Kelly JR, Gaudet S, Albeck JG, Sorger PK, Lauffenburger DA. Cue-signal-response analysis of TNF-induced apoptosis by partial least squares regression of dynamic multivariate data. J Comput Biol. 2004; 11:544–561. [PubMed: 15579231]

Jiang H, Pritchard JR, Williams RT, Lauffenburger DA, Hemann MT. A mammalian functional-genetic approach to characterizing cancer therapeutics. Nature chemical biology. 2011; 7:92–100.

Kantarjian HM, Shah NP, Cortes JE, Baccarani M, Agarwal MB, Undurraga MS, Wang J, Ipina JJ, Kim DW, Ogura M, et al. Dasatinib or imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: 2-year follow-up from a randomized phase 3 trial (DASISION). Blood. 2012; 119:1123–1129. [PubMed: 22160483]

Karaman MW, Herrgard S, Treiber DK, Gallant P, Atteridge CE, Campbell BT, Chan KW, Ciceri P, Davis MI, Edeen PT, et al. A quantitative analysis of kinase inhibitor selectivity. Nature biotechnology. 2008; 26:127–132.

Kreeger PK, Lauffenburger DA. Cancer systems biology: a network modeling perspective. Carcinogenesis. 2010; 31:2–8. [PubMed: 19861649]

Kreeger PK, Mandhana R, Alford SK, Haigis KM, Lauffenburger DA. RAS mutations affect tumor necrosis factor-induced apoptosis in colon carcinoma cells via ERK-modulatory negative and positive feedback circuits along with non-ERK pathway effects. Cancer research. 2009; 69:8191–8199. [PubMed: 19789336]

Kumar N, Afeyan R, Kim HD, Lauffenburger DA. Multipathway model enables prediction of kinase inhibitor cross-talk effects on migration of Her2-overexpressing mammary epithelial cells. Molecular pharmacology. 2008; 73:1668–1678. [PubMed: 18349105]

Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, Lerner J, Brunet JP, Subramanian A, Ross KN, et al. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science. 2006; 313:1929–1935. [PubMed: 17008526]

Law LW. Effects of combinations of antileukemic agents on an acute lymphocytic leukemia of mice. Cancer research. 1952a; 12:871–878. [PubMed: 13009674]

Law LW. Origin of the resistance of leukaemic cells to folic acid antagonists. Nature. 1952b; 169:628–629. [PubMed: 14929259]

Lee JK, Havaleshko DM, Cho H, Weinstein JN, Kaldjian EP, Karpovich J, Grimshaw A, Theodorescu D. A strategy for predicting the chemosensitivity of human cancers and its application to drug discovery. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:13086–13091. [PubMed: 17666531]

Letai AG. Diagnosing and exploiting cancer's addiction to blocks in apoptosis. Nature reviews Cancer. 2008; 8:121–132.

Luria SE, Delbruck M. Mutations of Bacteria from Virus Sensitivity to Virus Resistance. Genetics. 1943; 28:491–511. [PubMed: 17247100]

Matthews DA, Alden RA, Bolin JT, Freer ST, Hamlin R, Xuong N, Kraut J, Poe M, Williams M, Hoogsteen K. Dihydrofolate reductase: x-ray structure of the binary complex with methotrexate. Science. 1977; 197:452–455. [PubMed: 17920]

Miller-Jensen K, Janes KA, Brugge JS, Lauffenburger DA. Common effector processing mediates cell-specific responses to stimuli. Nature. 2007; 448:604–608. [PubMed: 17637676]

Mullighan CG, Phillips LA, Su X, Ma J, Miller CB, Shurtleff SA, Downing JR. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science. 2008; 322:1377–1380. [PubMed: 19039135]

Mullighan CG, Zhang J, Kasper LH, Lerach S, Payne-Turner D, Phillips LA, Heatley SL, Holmfeldt L, Collins-Underwood JR, Ma J, et al. CREBBP mutations in relapsed acute lymphoblastic leukaemia. Nature. 2011; 471:235–239. [PubMed: 21390130]

Newcombe HB, Hawirko R. Spontaneous Mutation to Streptomycin Resistance and Dependence in Escherichia Coli. J Bacteriol. 1949; 57:565–572. [PubMed: 16561734]

Ni Chonghaile T, Sarosiek KA, Vo TT, Ryan JA, Tammareddi A, Moore Vdel G, Deng J, Anderson KC, Richardson P, Tai YT, et al. Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy. Science. 2011; 334:1129–1133. [PubMed: 22033517]

Paull KD, Shoemaker RH, Hodes L, Monks A, Scudiero DA, Rubinstein L, Plowman J, Boyd MR. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. Journal of the National Cancer Institute. 1989; 81:1088–1092. [PubMed: 2738938]

Pawson T, Linding R. Network medicine. FEBS letters. 2008; 582:1266–1270. [PubMed: 18282479]

Pirker R, Pereira JR, Szczesna A, von Pawel J, Krzakowski M, Ramlau R, Vynnychenko I, Park K, Yu CT, Ganul V, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. Lancet. 2009; 373:1525–1531. [PubMed: 19410716]

Pommier Y, Leo E, Zhang H, Marchand C. DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chemistry & biology. 2010; 17:421–433. [PubMed: 20534341]

Scherf U, Ross DT, Waltham M, Smith LH, Lee JK, Tanabe L, Kohn KW, Reinhold WC, Myers TG, Andrews DT, et al. A gene expression database for the molecular pharmacology of cancer. Nature genetics. 2000; 24:236–244. [PubMed: 10700175]

Schoeberl B, Pace EA, Fitzgerald JB, Harms BD, Xu L, Nie L, Linggi B, Kalra A, Paragas V, Bukhalid R, et al. Therapeutically targeting ErbB3: a key node in ligandinduced activation of the ErbB receptor-PI3K axis. Sci Signal. 2009; 2:ra31. [PubMed: 19567914]

Shah NP, Skaggs BJ, Branford S, Hughes TP, Nicoll JM, Paquette RL, Sawyers CL. Sequential ABL kinase inhibitor therapy selects for compound drug-resistant BCRABL mutations with altered oncogenic potency. The Journal of clinical investigation. 2007; 117:2562–2569. [PubMed: 17710227]

Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science. 2004; 305:399–401. [PubMed: 15256671]

Sirota M, Dudley JT, Kim J, Chiang AP, Morgan AA, Sweet-Cordero A, Sage J, Butte AJ. Discovery and preclinical validation of drug indications using compendia of public gene expression data. Sci Transl Med. 2011; 3:96ra77.

Skipper HE, Schabel FM Jr, Wilcox WS. Experimental Evaluation of Potential Anticancer Agents. Xiii. On the Criteria and Kinetics Associated with “Curability” of Experimental Leukemia. Cancer Chemother Rep. 1964; 35:1–111. [PubMed: 14117037]

Skipper HE, Thomson JR, Bell M. Attempts at dual blocking of biochemical events in cancer chemotherapy. Cancer research. 1954; 14:503–507. [PubMed: 13190531]

Staker BL, Hjerrild K, Feese MD, Behnke CA, Burgin AB Jr, Stewart L. The mechanism of topoisomerase I poisoning by a camptothecin analog. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:15387–15392. [PubMed: 12426403]

Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. Nature reviews Drug discovery. 2006; 5:219–234.

Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, Cortes J, O’Brien S, Nicaise C, Bleickardt E, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. The New England journal of medicine. 2006; 354:2531–2541. [PubMed: 16775234]

Tentner AR, Lee MJ, Ostheimer GJ, Samson LD, Lauffenburger DA, Yaffe MB. Combined experimental and computational analysis of DNA damage signaling reveals context-dependent roles for Erk in apoptosis and G1/S arrest after genotoxic stress. Mol Syst Biol. 2012; 8:568. [PubMed: 22294094]

Toettcher JE, Loewer A, Ostheimer GJ, Yaffe MB, Tidor B, Lahav G. Distinct mechanisms act in concert to mediate cell cycle arrest. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:785–790. [PubMed: 19139404]

Turke AB, Zejnullahu K, Wu YL, Song Y, Dias-Santagata D, Lifshits E, Toschi L, Rogers A, Mok T, Sequist L, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell. 2010; 17:77–88. [PubMed: 20129249]

Wagle N, Emery C, Berger MF, Davis MJ, Sawyer A, Pochanard P, Kehoe SM, Johannessen CM, Macconaill LE, Hahn WC, et al. Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2011; 29:3085–3096. [PubMed: 21383288]

Wall ME, Wani MC. Camptothecin and taxol: discovery to clinic--thirteenth Bruce F. Cain Memorial Award Lecture. Cancer research. 1995; 55:753–760. [PubMed: 7850785]

Wei G, Twomey D, Lamb J, Schlis K, Agarwal J, Stam RW, Opferman JT, Sallan SE, den Boer ML, Pieters R, et al. Gene expression-based chemical genomics identifies rapamycin as a modulator of MCL1 and glucocorticoid resistance. Cancer Cell. 2006; 10:331–342. [PubMed: 17010674]

Weinstein JN, Myers TG, O'Connor PM, Friend SH, Fornace AJ Jr, Kohn KW, Fojo T, Bates SE, Rubinstein LV, Anderson NL, et al. An information-intensive approach to the molecular pharmacology of cancer. Science. 1997; 275:343–349. [PubMed: 8994024]

Whitehurst AW, Bodemann BO, Cardenas J, Ferguson D, Girard L, Peyton M, Minna JD, Michnoff C, Hao W, Roth MG, et al. Synthetic lethal screen identification of chemosensitizer loci in cancer cells. Nature. 2007; 446:815–819. [PubMed: 17429401]

Wolpaw AJ, Shimada K, Skouta R, Welsch ME, Akavia UD, Pe'er D, Shaik F, Bulinski JC, Stockwell BR. Modulatory profiling identifies mechanisms of small molecule-induced cell death. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:E771–780. [PubMed: 21896738]

Xie L, Kinnings SL, Bourne PE. Novel computational approaches to polypharmacology as a means to define responses to individual drugs. Annual review of pharmacology and toxicology. 2012; 52:361–379.

Yauch RL, Dijkgraaf GJ, Alicke B, Januario T, Ahn CP, Holcomb T, Pujara K, Stinson J, Callahan CA, Tang T, et al. Smoothened mutation confers resistance to a Hedgehog pathway inhibitor in medulloblastoma. Science. 2009; 326:572–574. [PubMed: 19726788]

Zander SA, Kersbergen A, van der Burg E, de Water N, van Tellingen O, Gunnarsdottir S, Jaspers JE, Pajic M, Nygren AO, Jonkers J, et al. Sensitivity and acquired resistance of BRCA1;p53-deficient mouse mammary tumors to the topoisomerase I inhibitor topotecan. Cancer research. 2010; 70:1700–1710. [PubMed: 20145144]

Zhang J, Adrian FJ, Jahnke W, Cowan-Jacob SW, Li AG, Iacob RE, Sim T, Powers J, Dierks C, Sun F, et al. Targeting Bcr-Abl by combining allosteric with ATP-bindingsite inhibitors. Nature. 2010; 463:501–506. [PubMed: 20072125]

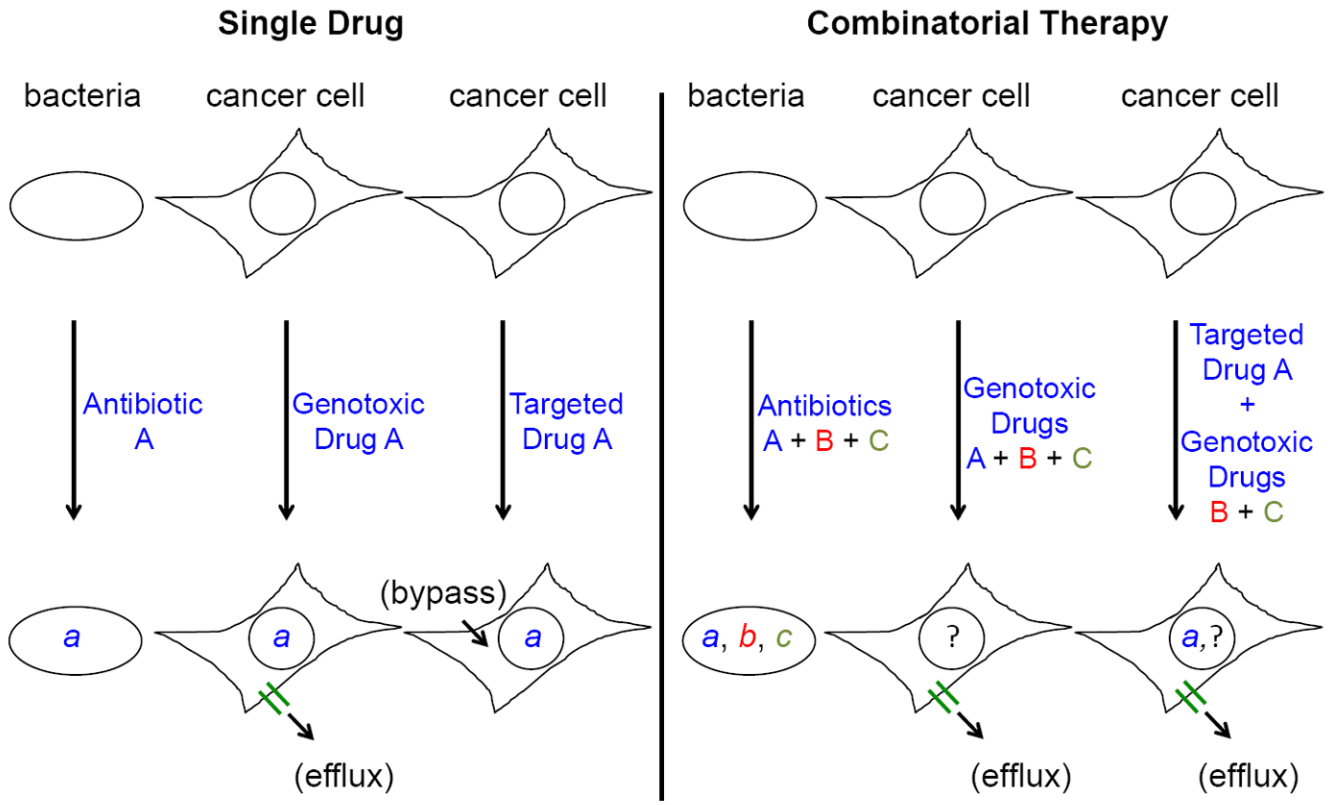

Figure 1.

A diagram showing the relationship between single and combinatorial therapy and the development of drug resistance. In response to single agent treatment of bacteria or tumor cells, whether targeted or “cytotoxic”, drug target alterations or, perhaps, drug efflux, can mediate therapeutic resistance. Here lower case letters indicate specific mutations in drug target genes. Conversely, in response to combinatorial therapy, bacteria evolve specific resistance mechanisms to each agent, while mammalian cells evolve resistance to targeted therapeutics, reinforcing alterations that restore target activity as well as mechanisms of multi-drug (target non-specific) resistance.

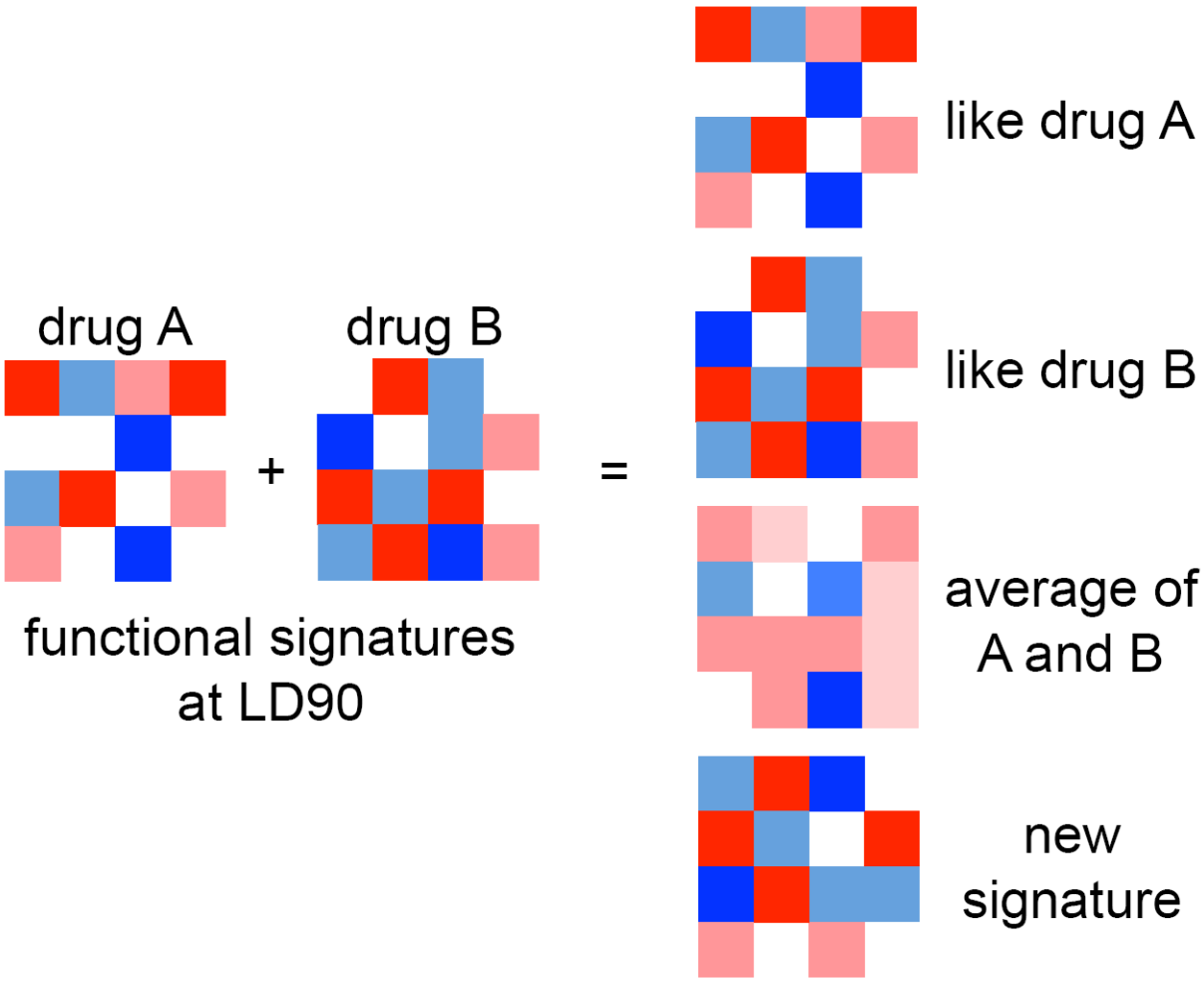

Figure 2.
A diagram showing potential computational solutions revealed by the addition of two drug "signatures". Combination treatments may reveal signatures representative of one of the component signatures. In this case, the drug combination would be expected to act like one of the parental drugs – merely at a lower dose. Alternatively, the combination signature may be an average of component signatures, in which global genetic dependencies are reduced or homogenized. Finally, a combination signature may deviate completely from component signatures – representing a neomorphic affect achieved by the drug combination.